

# Antihyperglycemic Activity of 70% Ethanolic Extract of Kecombrang (*Etlingera elatior*) Stems in Alloxan-Induced Male Sprague Dawley Rats

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## ABSTRACT

Hyperglycemia is a condition characterized by elevated blood glucose levels and is closely associated with diabetes mellitus. The long-term use of synthetic antihyperglycemic drugs may cause adverse effects, thereby encouraging the exploration of natural products as alternative therapeutic candidates. Kecombrang (*Etlingera elatior*) is known to contain secondary metabolites with potential antihyperglycemic activity. This study aimed to evaluate the antihyperglycemic activity of 70% ethanolic extract of kecombrang stems in alloxan-induced male Sprague Dawley rats. The extract was prepared by maceration using 70% ethanol, followed by phytochemical screening. A total of 25 male Sprague Dawley rats were divided into five groups: a negative control group receiving 0.5% Na-CMC, a positive control group receiving glibenclamide at a dose of 5 mg/kg body weight, and three extract-treated groups receiving kecombrang stem ethanol extract at doses of 100, 200, and 300 mg/kg body weight. Hyperglycemia was induced using alloxan, and treatments were administered orally once daily for 21 days. Fasting blood glucose levels were measured after induction and on days 7, 14, and 21 of treatment. The results showed that the extract contained flavonoids, alkaloids, saponins, tannins, and triterpenoids. Administration of kecombrang stem extract reduced fasting blood glucose levels in all extract-treated groups compared with the negative control. On day 21, the extract doses of 100, 200, and 300 mg/kg body weight reduced blood glucose levels to  $72.4 \pm 10.90$ ,  $63.0 \pm 6.04$ , and  $65.6 \pm 4.83$  mg/dL, respectively, while the positive control showed  $68.8 \pm 2.39$  mg/dL. Statistical analysis indicated that all extract-treated groups differed significantly from the negative control but were not significantly different from the positive control. These findings suggest that 70% ethanolic extract of kecombrang stems has potential antihyperglycemic activity in alloxan-induced rats. However, further studies are required to clarify its dose-response relationship, safety profile, and mechanism of action.



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## Keywords:

*Etlingera elatior*; Extract; Kecombrang; Antihyperglycemic; Sprague Dawley Rats

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## 1. Introduction

Hyperglycemia is a condition characterized by elevated blood glucose levels above normal values, which is the primary hallmark of diabetes mellitus (DM). DM is a chronic metabolic disease whose prevalence continues to rise and has become a global health issue. According to PERKENI, the number of people with DM in Indonesia is projected to increase from 10.7 million in 2019 to 13.7 million by 2030 [8]. Suboptimal management of DM can lead to various chronic complications, such as cardiovascular disease, nephropathy, neuropathy, and retinopathy [17].

Diabetes therapy generally uses synthetic drugs to control blood glucose levels. However, long-term use can cause various side effects, such as gastrointestinal disturbances, headaches, and the risk of hypoglycemia [17]. Therefore, the development of natural compounds as alternatives for antihyperglycemic therapy has become a growing research focus [15]. One plant with potential as an antihyperglycemic agent is kecombrang (*Etlingera elatior*). This plant is known to contain various secondary metabolites, such as flavonoids, phenolics, alkaloids, saponins, and tannins, which act as antioxidants and have the potential to lower blood glucose levels [4],[6],[10],[18]. Previous studies have reported antidiabetic activity in kecombrang leaf extracts [1],[5]. However, research on the antihyperglycemic activity of the kecombrang stem is still very limited.

The selection of kecombrang stems in this study was based on their abundant availability and their relatively low utilization compared to the leaves and flowers. Additionally, kecombrang stems are reported to contain phenolic compounds and flavonoids that have the potential to provide antihyperglycemic effects [6],[15]. Thus, the utilization of kecombrang stems has the potential to enhance the plant's overall utility while serving as an alternative raw material source for the development of herbal antidiabetic drugs. To date, most studies on the antihyperglycemic activity of kecombrang have focused on the leaves [1],[5], while scientific data regarding the effectiveness of kecombrang stem ethanol extract in lowering blood glucose levels remains limited. Furthermore, information regarding the antihyperglycemic activity of kecombrang stem ethanol extract in Sprague Dawley male white rat models has also not been widely reported [3],[9]. These limitations form the basis for this study. Based on the above, this study aims to test the antihyperglycemic activity of ethanol extract from the stems of kecombrang (*Etlingera elatior*) in Sprague Dawley male white rats induced with hyperglycemia.

## 2. Methods

The equipment used in this study is as follows: Glassware (Pyrex), blender (Maspion), cotton, filter paper (cellulose membrane with a pore size of 0.45  $\mu\text{m}$ ), aluminum foil, analytical balance (KERN), alcohol meter, vacuum rotary evaporator (EYELA), refrigerator (Liebherr Medline), freezer (General Gensui), oven (Memmert), furnace (Thermolyne), porcelain crucibles, weighing bottles, pipettes, parchment paper, evaporating dishes, hot plate (Cimarec), mortar and pestle, vortex mixer (Hettich Eba 20), animal scale (AND GH-202), mouse cages with food and water dishes, oral probes, surgical scissors, syringes (Terumo), glucometers (Autocheck), and glucose test strips (Autocheck).

### Materials

The materials used in this study included kecombrang stems (*Etlingera elatior*), 70% ethanol (Brataco), distilled water (Smartlab), sodium carboxymethylcellulose (Mitra Kimia), alloxan (Sigma-Aldrich), saline solution (0.9% NaCl), glucose (Gulaku), ether

(Sigma-Aldrich), glibenclamide, acarbose (Bayer), 10% FeCl<sub>3</sub>, Mayer's, Dragendorff's, and Liebermann-Burchard's reagents, magnesium powder (Mg), 2N HCl, and dilute ammonia. The test animals used were 2–3-month-old male Sprague Dawley white rats weighing between 150–200 grams.

#### **Preparation of Test Animals**

This study used male Sprague Dawley strain white rats (*Rattus norvegicus*) as test animals. The animals were divided into five groups, each consisting of five replicates. All rats were housed in cages, fed pelleted feed, and provided with sterile drinking water. A seven-day acclimatization period was conducted before treatment began.

#### **Collection and Drying**

The samples collected were kecombrang stems (*Etligeria elatior*) obtained in the Cilogkrang area, Pondok Kahuru Village, Ciomas Subdistrict, Serang Regency. This was followed by the preparation of crude drug. The collected kecombrang stems were separated into stems, leaves, and rhizomes, then washed thoroughly with running water, followed by drying in an oven at 50 °C

#### **Preparation of the Etanol Extract from Kecombrang Stems**

The crude drug powder was then macerated using 70% ethanol. The maceration process was repeated until no further compounds were extracted, as indicated by a clear macerate. The macerate was then filtered using cotton and filter paper, and the resulting filtrate was evaporated using a vacuum rotary evaporator to produce a concentrated extract. The concentrated kecombrang stem extract was then qualitatively tested to detect the presence of alkaloids, flavonoids, saponins, steroids/triterpenoids, and phenols.

#### **Phytochemical screening**

##### **Flavonoid test**

0.5 grams of a 70% ethanol extract of kecombrang stems was placed in a reaction tube, followed by the addition of magnesium powder, and then 5–6 drops of concentrated HCl were added. If a sample produces an orange or orange-yellow color during testing, this indicates the presence of flavonoids in the sample [7].

##### **Alkaloid test**

0.5 grams of 70% ethanol extract of kecombrang stems is placed in a test tube and dissolved in HCl. The solution is then divided into three test tubes. To the first tube, 2–3 drops of Dragendorff's reagent (potassium bismuth) are added; the formation of an orange color indicates a positive result for alkaloids. To the second tube, 2–3 drops of Mayer's reagent (potassium mercury iodide) were added; a white or yellow color indicates the presence of alkaloids. To the third tube, 2–3 drops of Wagner's reagent were added; the formation of a brown color indicates the presence of alkaloid compounds [7].

##### **Tannin test**

0.5 grams of 70% ethanol extract of kecombrang stems were placed in a test tube, followed by the addition of 2–3 drops of 1% FeCl<sub>3</sub> solution. The formation of a blackish-green or blackish-blue color indicates the presence of tannins [7]

##### **Saponin test**

0.5 grams of 70% ethanol extract of kecombrang stems were placed in a test tube, then warm water was added until the entire sample was submerged, and the mixture was vigorously shaken. If foam formed, the solution was allowed to stand for 10 minutes. Next, 2–3 drops of 1% HCl were added. Foam that remained stable indicated the presence of saponin compounds in the sample [7]

### Terpenoid and Steroid test

0.5 grams of 70% ethanol extract of kecombrang stems is placed in a test tube, then dissolved in 0.5 mL of chloroform and 0.5 mL of anhydrous acetic acid is added. Next, 1–2 mL of concentrated H<sub>2</sub>SO<sub>4</sub> was added along the wall of the test tube. The formation of a red or purple ring at the interface between the two solvents indicates the presence of terpenoids, while a bluish-green ring indicates the presence of steroids [7].

### In Vivo Hyperglycemia Testing

A total of 25 male Sprague Dawley strain white rats (n = 25) were used in this study and randomly divided into five treatment groups (n = 5 per group). Prior to the study, the test animals were acclimatized for 7 days and housed in standard cages at a temperature of 22–25°C with a relative humidity of 50–60% and a 12-hour:12-hour light-dark cycle. Test animals were provided with standard chow and water ad libitum during the acclimatization and study periods [3], [9]. Hyperglycemia was induced using alloxan monohydrate dissolved in physiological saline and administered intraperitoneally (i.p.) to the treatment groups at a dose of 150 mg/kg body weight. The alloxan solution was prepared fresh immediately before use to maintain its stability [12], [13]. Prior to induction, test animals were fasted for 12 hours while still having access to drinking water. Fasting was performed because it increases the animals' sensitivity to the diabetogenic effects of alloxan [12]. The mechanism of action of alloxan involves selectively damaging pancreatic  $\beta$ -cells through the formation of free radicals, thereby causing a decrease in insulin secretion and an increase in blood glucose levels [13]. To prevent acute hypoglycemia resulting from insulin release in the early phase following induction, test animals were administered a 5% glucose solution in drinking water for 24 hours post-induction [3]. Fasting blood glucose levels were measured using a glucometer 72 hours after induction. Animals with fasting blood glucose levels >140 mg/dL were considered hyperglycemic and met the criteria for inclusion in the study [12]. After confirmation of hyperglycemia, test animals were administered the treatment orally once daily for 21 days according to their respective groups. Doses of the extract and positive control were expressed in mg/kg body weight. Fasting blood glucose levels (mg/dL) were measured on day 0 (after confirmation of hyperglycemia), day 7, day 14, and day 21 of treatment. Animal allocation into groups was performed using simple randomization. Blood glucose level measurements and data recording were conducted by researchers unaware of the treatment group allocation (single-blind) to minimize observational bias and enhance the validity of the study results.

### Data Analysis

The treatment groups consisted of five groups: a negative control group receiving 0.5% Na-CMC, a positive control group receiving glibenclamide at a dose of 5 mg/kg body weight, and three treatment groups receiving 70% ethanolic extract of kecombrang (*Etlintera elatior*) stems at doses of 100, 200, and 300 mg/kg body weight. Each group consisted of five male Sprague Dawley rats. All preparations were administered orally using an oral gavage at a volume of 10 mL/kg body weight once daily for 21 days.

The antihyperglycemic effect was evaluated based on changes in fasting blood glucose levels. Blood glucose levels were measured before alloxan induction, 72 hours after alloxan induction, and during the treatment period on days 7, 14, and 21. The results were expressed in mg/dL and presented as mean  $\pm$  standard deviation (SD). Data were analyzed using statistical software. Normality was assessed using the Shapiro-Wilk test, while homogeneity of variance was evaluated using Levene's test. Data that were normally distributed and homogeneous were analyzed using one-way analysis of

variance (ANOVA) at each observation time point, followed by the Least Significant Difference (LSD) post hoc test when significant differences were found. A p-value of less than 0.05 was considered statistically significant.

### Research Ethics Code

This study has undergone an ethical review conducted by the Animal Ethics Committee of Muhammadiyah University of Purwokerto. The use of laboratory animals in this study considers aspects of benefit as well as the ethical principles of animal welfare. Therefore, prior to the study, an ethical review of the test animals was conducted with registration number: KEPK/UMP/337/II/2025.

## 3. Results and Discussion

### Extract Characteristics and Phytochemical Profile

The kecombrang (*Etlingera elatior*) stem samples were collected from the Cilogkrang area, Pondok Kahuru Village, Ciomas Subdistrict, Serang Regency. Before extraction, the samples first underwent identification at the Biology Laboratory of Ahmad Dahlan University to confirm the scientific identity of the plant, resulting in registration number: 137/Lab.Bio/B/II/2025 [1]. After drying 15 kg of fresh material in an oven at 50°C, 1.4 kg of dried crude drug was obtained [1].

**Table 1.** Results for Kecombrang Stems

Solvent	Sample Weight (g)	Extract Weight (g)	Yield %
70% Ethanol	800 g	51.3	6.41

Extraction was performed using the maceration method with 70% ethanol as the solvent at a ratio of 1:10 (800 g of dried crude drug in 8 L of solvent). 70% ethanol was chosen for its optimal ability to extract a greater amount of polar metabolites compared to other concentrations [2]. This maceration process yielded 4000 mL of filtrate, which was then evaporated using a rotary evaporator (50°C) and concentrated in a water bath to produce a concentrated extract weighing 51.3 g [1]. The extraction yield obtained was 6.41% (**Table 1**). This yield is considered quite efficient and is higher than that reported in previous studies using high-concentration ethanol (96%), which generally yield lower yields due to limitations in the extraction of polar compounds [14].

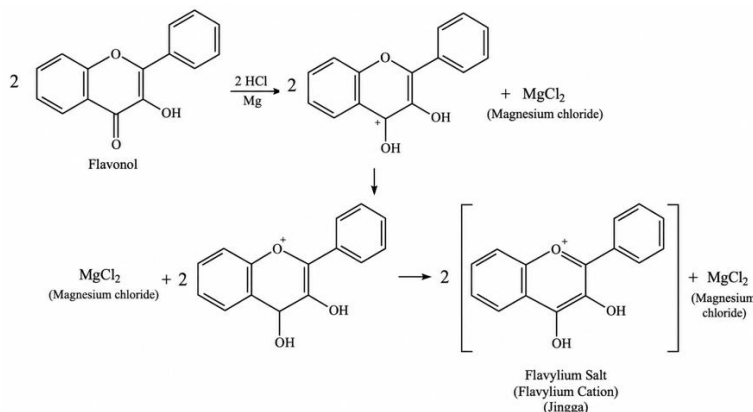
**Table 2.** Results of Phytochemical Screening Tests

Secondary Metabolites	Reagent	Color	Remarks
Alkaloids	Mayer's reagent	White precipitate	+
	Dragendrof's reagent	Red precipitate orange	+
	Wagner's reagent	Brown precipitate light	+
Flavonoids	HCl + Mg strip	Orange - red	+
Saponin	HCl	Stable foam 1 cm	+
Tannin	FeCl <sub>3</sub>	Hijau kehitaman	+
Triterpenoids/Steroids	Anhydrous acetic acid;	Brown ring at the border + solution (+) Triterpenoids	+
	Concentrated sulfuric acid		+

(Liebermann-  
Burchard)

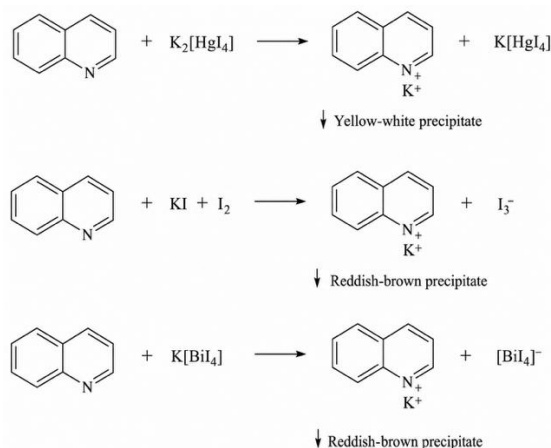
Phytochemical screening was conducted as an initial step to identify secondary metabolites in the concentrated extract of kecombrang stems [11]. Based on the test results shown in **Table 2**, the 70% ethanol extract of kecombrang stems was found to contain flavonoids, alkaloids, saponins, tannins, and triterpenoids [1].

The presence of these compounds underpins the pharmacological efficacy of kecombrang stems as an antihyperglycemic agent [5]. In the flavonoid test, the formation of an orange-red color is caused by the formation of a flavilium complex resulting from the addition of a strong acid (**Figure 1**) [6]. In the alkaloid test, the formation of precipitates with Mayer's, Dragendorff's, and Wagner's reagents occurs via the precipitation of alkaloid cations on nitrogen-based groups with heavy metals, forming insoluble complex salts (**Figure 2**) [13]. Saponins are identified by their ability to reduce water surface tension and form stable foam due to their natural surfactant characteristics [4]. Meanwhile, tannins induce complexation with  $\text{Fe}^{3+}$  ions, producing a characteristic blackish-green color due to their interaction with phenolic groups [7]. In the Liebermann-Burchard test, triterpenoids produce a brownish ring due to the condensation of concentrated sulfuric acid and acetic anhydride, which forms a stabilized carbocation [10].



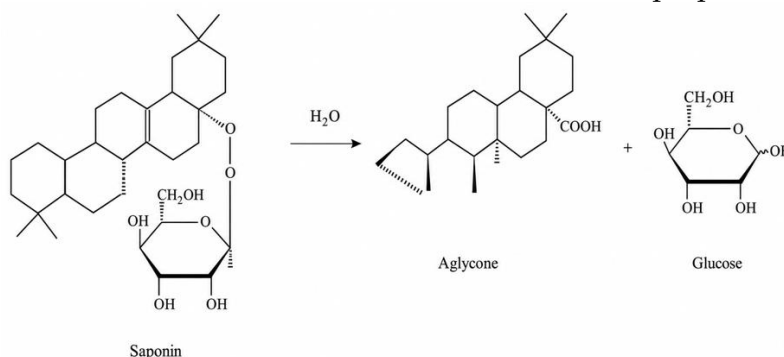
**Figure 1.** Reaction of flavonoids with HCl and magnesium metal [6]

Alkaloid tests use Mayer's reagent ( $\text{K}_2\text{HgI}_4$ ), Dragendorff's reagent ( $\text{KBiI}_4$ ), and Wagner's reagent ( $\text{I}_2/\text{KI}$ ), all of which operate on the principle of precipitating alkaloid cations as insoluble complex salts; this interaction occurs because the nitrogen-based groups in alkaloids form ionic or coordinate bonds with heavy metals in the reagents [18].



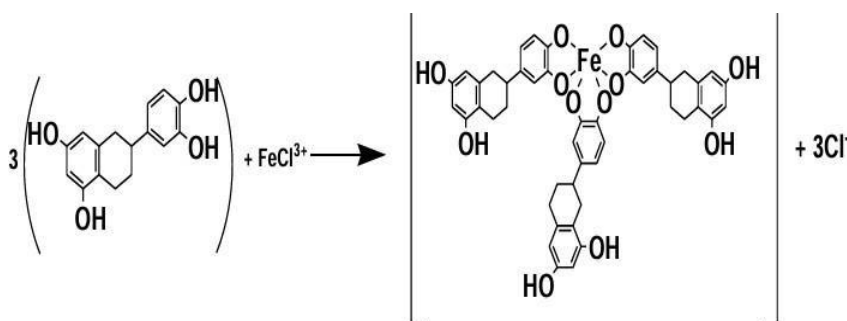
**Figure 2.** Alkaloid reactions with Meyer, Dragendorff, and Wagner reagents [13]

Saponins were tested for their ability to reduce the surface tension of water because their glycosidic structure (a nonpolar sapogenin moiety and a polar sugar chain) forms stable foam that lasts for more than 10 minutes. Saponins were tested for their ability to form stable foam due to their natural surfactant properties (**Figure 3**) [4].



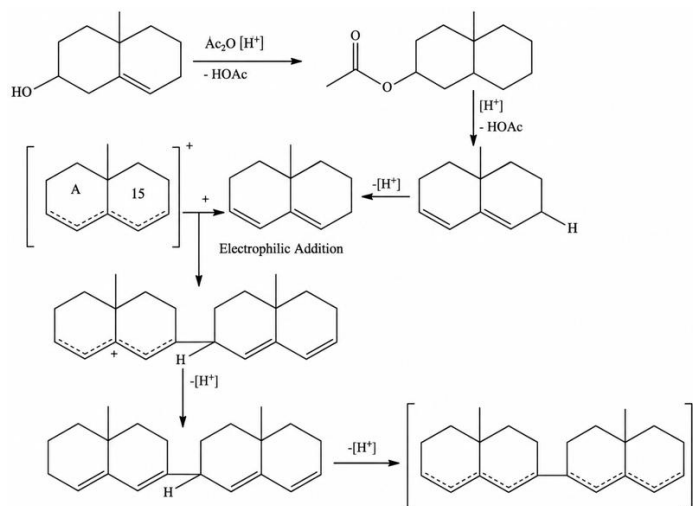
**Figure 3.** Hydrolysis Reaction of Saponins in Water [4]

Tannins are identified by specific color changes, where condensed tannins (flavonoid polymers) produce a dark blue color and hydrolyzable tannins (gallic esters of glucose) exhibit a dark green color, due to the formation of complexes with iron ions ( $Fe^{3+}$ ) that interact with phenolic groups. Tannins exhibit color changes due to complexation with  $Fe^{3+}$  ions (**Figure 4**) [10].



**Figure 4.** Tannin +  $FeCl_3$  Reaction [15]

Meanwhile, the Liebermann–Burchard reaction for triterpenoids and steroids produces a bluish-green or purple color due to the condensation of concentrated sulfuric acid and acetic anhydride, which leads to the formation of a carbocation stabilized by the conjugated cyclic structure, resulting in a characteristic color change [14].



**Figure 5.** Triterpenoid reaction via the Liebermann–Burchard test [10]

### Blood Glucose Levels in All Treatment Groups

The hyperglycemic animal model was established by intraperitoneal administration of 150 mg/kg body weight of alloxan monohydrate after the rats had been fasted for 12 hours [3]. The induction process in this study was performed over three experiments to achieve stable diabetic conditions [1]. The first induction was hindered by a dosage calculation error that resulted in the death of the test animals, while the second induction failed to trigger hyperglycemia due to variations in biological response and pancreatic compensation among the individual animals [1]. Stable hyperglycemia (fasting blood glucose level  $\geq 140$  mg/dL) was fully achieved in the third induction, where there was a significant surge from the initial normal baseline level to above 140 mg/dL [1]. Alloxan selectively damages pancreatic  $\beta$ -cells through the formation of free radicals, thereby causing a drastic decrease in insulin secretion [8], [9].

This study used 25 male Sprague Dawley white rats, divided into five treatment groups, each consisting of five rats. Throughout the study, the condition of the test animals was monitored daily to assess their response to alloxan induction and the administered treatments. Animals that died or did not meet the hyperglycemia criteria (fasting blood glucose level  $>140$  mg/dL after 72 hours of alloxan induction) were excluded from both the treatment phase and statistical analysis. Animal replacement was performed before the treatment phase began to maintain the sample size according to the study design and in accordance with the Animal Ethics Committee's approval. Statistical analysis was conducted using the final sample size that met the inclusion criteria and completed the entire observation period.

During the hyperglycemia induction process, the test animals' responses to alloxan varied. In the first induction experiment, animal deaths occurred due to an error in calculating the alloxan dose; therefore, data from that experiment were not used in the analysis. In the second induction experiment, some animals did not meet the established hyperglycemia criteria (fasting blood glucose level  $>140$  mg/dL), so they

were not proceeded to the treatment phase. The third induction successfully produced hyperglycemia in accordance with the study criteria. To maintain the validity of the study, the final sample size used in the analysis was 25 rats, with five rats in each treatment group.

**Table 3.** Results of the Antihyperglycemic Effect Test of Ethanol Extract of Etlingera Elatior Stems on Alloxan-Induced Sprague Dawley Rats (mean  $\pm$  SD, n = 5)

Treatment Group	Baseline Level (mg/dL)	After Induction Alloxan (mg/dL)	Blood Glucose Level After Treatment			Percentage %
			H7 (mg/dL)	H14 (mg/dL)	H21 (mg/dL)	
Negative control (0.5% Na-CMC)	95.2 $\pm$ 19.56	195.8 $\pm$ 41.03	205.2 $\pm$ 39.10	209.6 $\pm$ 38.76	214.0 $\pm$ 38.10	-9.3
Positive control (Glibenclamide 5 mg/kg body weight)	94.4 $\pm$ 6.91	279.2 $\pm$ 45.41	154.0 $\pm$ 7.56	106.6 $\pm$ 7.40	68.8 $\pm$ 2.39	75.4
100 mg/kg body weight extract	92.4 $\pm$ 11.61	284.0 $\pm$ 45.08	115.4 $\pm$ 7.44	103.8 $\pm$ 9.42	72.4 $\pm$ 10.90	74.5
200 mg/kg body weight extract	86.2 $\pm$ 12.50	311.6 $\pm$ 55.55	146.6 $\pm$ 5.94	98.2 $\pm$ 5.97	63.0 $\pm$ 6.04	79.8
300 mg/kg body weight extract	86.6 $\pm$ 9.71	359.4 $\pm$ 51.37	143.8 $\pm$ 6.46	90.4 $\pm$ 6.47	65.6 $\pm$ 4.83	81.7

Based on the test results presented in **Table 3**, all groups experienced an increase in blood glucose levels after alloxan induction, indicating the successful establishment of a hyperglycemia model in Sprague Dawley rats. Blood glucose levels after induction ranged from 195.8  $\pm$  41.03 mg/dL to 359.4  $\pm$  51.37 mg/dL, higher than the initial glucose levels, which ranged from 86.2  $\pm$  12.50 mg/dL to 95.2  $\pm$  19.56 mg/dL. This condition indicates that alloxan successfully damaged pancreatic  $\beta$ -cells, thereby reducing insulin secretion and causing a significant increase in blood glucose levels [3], [12], [13].

Administration of ethanol extract of kecombrang stems for 21 days showed a gradual decrease in blood glucose levels in all treatment groups. The 100 mg/kg body weight extract group experienced a decrease in blood glucose levels from 284.0  $\pm$  45.08 mg/dL after induction to 72.4  $\pm$  10.90 mg/dL on day 21, with a percentage decrease of 74.5%. In the 200 mg/kg body weight extract group, blood glucose levels decreased from 311.6  $\pm$  55.55 mg/dL to 63.0  $\pm$  6.04 mg/dL, representing a 79.8% reduction. The 300

mg/kg body weight extract group showed the highest percentage reduction in blood glucose levels descriptively, with a decrease of 81.7%. However, this finding should be interpreted cautiously because the differences among the extract-treated groups were not statistically significant. In addition, the day-21 blood glucose level in the 200 mg/kg body weight group was slightly lower than that in the 300 mg/kg body weight group. Therefore, the results indicate that all tested doses of kecombrang stem ethanol extract produced comparable antihyperglycemic activity, rather than confirming 300 mg/kg body weight as the definitive optimal dose.

In contrast, the negative control group, which received only the 0.5% Na-CMC vehicle, showed no improvement in hyperglycemia. Blood glucose levels in this group actually increased from  $195.8 \pm 41.03$  mg/dL after induction to  $214.0$

$\pm 38.10$  mg/dL on day 21, resulting in a negative percentage decrease (-9.3%). These results indicate that the vehicle used lacks antihyperglycemic activity and is unable to improve established hyperglycemia [16]. The antihyperglycemic activity of kecombrang stem extract is believed to be related to its secondary metabolites, namely flavonoids, alkaloids, saponins, tannins, and triterpenoids, which were identified in the phytochemical screening results. Flavonoids are known to enhance insulin sensitivity and reduce oxidative stress in pancreatic  $\beta$ -cells, while saponins and alkaloids play a role in inhibiting glucose absorption and enhancing glucose utilization by peripheral tissues [6], [15]. Additionally, the antioxidant activity of phenolic compounds and flavonoids in kecombrang may help protect pancreatic  $\beta$ -cells from damage caused by free radicals generated during hyperglycemia [5],[15].

#### Comparative Effects Compared to the Positive Control and Dose-Response Trends

The results of the one-way ANOVA analysis (Table 4) indicated a significant difference among the treatment groups ( $p = 0.037$ ). Therefore, the analysis was continued using the LSD post hoc test to identify which groups differed specifically. The results of the post hoc test showed that the negative control group differed significantly from all treatment groups and the positive control group ( $p < 0.05$ ). Conversely, the groups receiving 100 mg/kg body weight, 200 mg/kg body weight, and 300 mg/kg body weight doses of the extract did not differ significantly from the positive control group ( $p > 0.05$ ).

**Table 4.** Comparison of Blood Glucose Levels on Day 21 Among Groups Based on One-Way ANOVA and Post Hoc LSD Tests

Treatment Group	Day 21 Blood Glucose Levels (mg/dL)
Negative Control (NACMC 0.5%)	$214.00 \pm 38.10^a$
Positive Control (Glibenclamide 5 mg/kgBW)	$68.80 \pm 2.39^b$
Extract 100 mg/kgBW	$72.40 \pm 10.90^b$
Extract 200 mg/kg body weight	$63.00 \pm 6.04^b$
300 mg/kg body weight extract	$65.60 \pm 4.83^b$

These results indicate that administration of ethanol extract of kecombrang stems at all doses significantly reduced blood glucose levels compared to the negative control and produced effects comparable to glibenclamide as the standard drug. Although descriptively, the 300 mg/kg body weight dose produced the highest percentage

reduction in blood glucose levels (81.7%), the differences between extract doses were not statistically significant ( $p > 0.05$ ).

This study has several limitations that should be considered when interpreting the findings. First, the hyperglycemic model used in this study was induced by alloxan, which primarily reflects selective pancreatic  $\beta$ -cell damage. Therefore, the results may not fully represent the broader and more complex pathophysiology of diabetes mellitus, particularly insulin resistance and chronic metabolic dysfunction. Second, the study only evaluated fasting blood glucose levels as the main pharmacological outcome, without measuring insulin levels, oxidative stress markers, inflammatory markers, or pancreatic histopathology. These additional parameters are important to clarify the possible mechanism underlying the antihyperglycemic effect of the extract. Third, phytochemical screening was conducted qualitatively; therefore, the specific active compounds responsible for the glucose-lowering effect were not identified or quantified. Fourth, although the extract-treated groups showed significant antihyperglycemic activity, the differences among extract doses were not statistically significant, indicating that the optimal dose could not be conclusively determined. Finally, safety evaluation and toxicity testing were not performed; therefore, further studies are required to assess the dose-response relationship, active compound profile, pancreatic protective effect, and safety margin of kecombrang stem ethanol extract.

#### 4. Conclusion

This study showed that phytochemical screening of 70% ethanolic extract of kecombrang (*Etlingera elatior*) stems indicated the presence of several secondary metabolite groups, including flavonoids, alkaloids, saponins, tannins, and triterpenoids. The extract demonstrated antihyperglycemic activity in alloxan-induced male Sprague Dawley rats, as shown by the reduction in fasting blood glucose levels over 21 days of treatment. All tested extract doses produced significant glucose-lowering effects compared with the negative control and showed statistically comparable effects to glibenclamide. However, the differences among extract doses were not statistically significant; therefore, the optimal effective dose could not be conclusively determined in this study. In addition, because the alloxan-induced model mainly reflects pancreatic  $\beta$ -cell damage, further studies using other diabetes models, broader dose ranges, active compound identification, safety evaluation, and mechanistic analysis are required to further validate the antihyperglycemic potential of kecombrang stem extract.

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#### Conflicts of Interest:

The authors declare no conflict of interest regarding the publication of this paper.

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