

Oral glucose tolerance assay of extract from *Mangifera foetida* L. and *Pandanus amaryllifolius roxb.* leaves



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Abstract. This study examines the potential antidiabetic activity of a combination of *Mangifera foetida* L. and *Pandanus amaryllifolius Roxb.* leaves. *Mangifera* leaves contain a compound called mangiferin, which acts as an antidiabetic and antioxidant. On the other hand, *Pandanus* leaves, are rich in terpenoids and steroids that also possess anti-diabetic effects. An Oral Glucose Tolerance Test (OGTT) was conducted on male mice of the Bahl/c strain to assess the antidiabetic effects. The test involved administering a combination of ethanolic leaf extracts from *Mangifera foetida* L. and *Pandanus amaryllifolius Roxb.* in a 1:1 ratio. The study followed a pre-test design with a control group, comprising six treatment groups, each consisting of four male mice. The groups were described as follows: Group I was served as the normal group without any treatment. Groups II-IV received single extracts or combinations of extracts at doses of 62.5 mg/200g BW, 125 mg/200g BW, and 250 mg/200g BW. Group V acted as the positive control and was given oral glucose and glibenclamide at a dose of 0.09 mg/200g BW. Group VI was served as the negative control, receiving oral glucose and distilled water. The group that exhibited the highest percentage of decreased blood sugar levels was the one given oral glucose at a dose of 0.078g/20g BW of mice, along with the combination of *Mangifera foetida* L. and *Pandanus amaryllifolius Roxb.* leaves extract (1:1) at a dose of 35 mg/20g BW. This group demonstrated a decrease of 71.985 ± 4.858 in blood glucose levels. The ANOVA analysis confirmed that the percentage decrease in blood sugar levels was significantly different from the positive control, indicating a higher effectiveness of the combination treatment in reducing blood glucose levels than the positive control.

Keywords: blood glucose level, *Mangifera foetida* L. leaves, oral glucose tolerance test, *Pandanus amaryllifolius Roxb.* leaves.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by elevated blood glucose levels, known as hyperglycemia [1]. This condition can occur due to insufficient insulin production or ineffective insulin utilization by the body [2]. According to the World Health Organization (WHO) data from 2017, the global number of people living with diabetes mellitus was 425 million, projected to increase to 629 million by 2045 [3]. In 2019, Indonesia ranked 7th among the top 10 countries with the highest number of diabetes cases, with 10.7 million individuals affected. Notably, Indonesia is the only country in Southeast Asia on this list, indicating its significant contribution to the prevalence of diabetes in the region [4].

Currently, there are several approaches to treating diabetes mellitus, including the administration of synthetic Oral Antidiabetic Drugs (OAD) or insulin injections [5]. Glibenclamide (a member of the

sulphonylurea group) is a commonly used OAD by the general public. However, prolonged use of glibenclamide in elderly patients with liver and kidney abnormalities can lead to the side effect of hypoglycemia [1]. These side effects associated with synthetic antidiabetic drugs have prompted research into alternative treatments for individuals with diabetes mellitus, such as exploring the potential of natural plants ingredients.

Mango (*Mangifera foetida* L.) leaves, abundantly found in Indonesia, have emerged as a potential resource for combating diabetes mellitus. As a member of the *Mangifera* genus, mango trees thrive in tropical climates and offer a rich composition of various beneficial compounds, including alkaloids, steroids, flavonoids, polyphenols, tannins, and saponins [6]. Among these compounds, mangiferin is particularly noteworthy as it exhibits numerous pharmacological activities and is considered one of the key phytochemicals in *Mangifera foetida* L. [7]. Mangiferin is known for its antibacterial,



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antifungal [9], and antioxidant properties [10], and it also holds potential as an antidiabetic agent [11]. It is important to note that *Mangifera foetida* L. comprises several species, each with varying mangiferin content. The previous studies have indicated that *Mangifera foetida* L. possesses the highest mangiferin content compared to *Mangifera odorata* G. and *Mangifera indica* L. [12].

In addition to mango leaves, Pandan (*Pandanus amaryllifolius Roxb.*) leaves are also abundant in Indonesia. These leaves contain various secondary metabolites, including flavonoids, tannins, alkaloids, polyphenols, and saponins [13]. The previous studies have demonstrated several beneficial properties of *Pandanus amaryllifolius Roxb.* leaves, such as their potential as an antidiabetic [14], antioxidant [15], anticancer [16], and antibacterial agent [17]. Furthermore, these leaves have been found to contain secondary metabolites that can help reduce blood glucose levels. Specifically, tannins isolated from *Pandanus amaryllifolius Roxb.* leaves have been shown to stimulate glucose metabolism [18]. Additionally, research has indicated that combining extracts from *Mangifera foetida* L. and *Pandanus amaryllifolius Roxb.* leaves can increase flavonoid content and enhance antioxidant activity [19]. The results of previous research also show that using a combination of ethanol extract from *Physalis angulata* and *Aquilaria malaccensis* leaves effectively lowers blood sugar levels [20]. Flavonoids are known for their ability to lower blood glucose levels [3]. These findings suggest that the combination of *Mangifera foetida* L. and *Pandanus amaryllifolius Roxb.* leaf extracts hold potential as a herbal medicine for managing diabetes mellitus.

To explore the potential of using *Mangifera foetida* L. leaves and *Pandanus amaryllifolius Roxb.* leaves as herbal medicines for diabetes, various testing stages are necessary, starting with initial screening for anti-diabetic activity. The initial screening involves conducting tests to evaluate the ability of the extracts to reduce blood glucose levels using the oral glucose tolerance test (OGTT). This method utilizes healthy experimental animals to assess the extract's effectiveness in lowering blood glucose levels [21]. This research aims to investigate the potential of the combination of ethanolic leaf extracts from *Mangifera foetida* L. and *Pandanus amaryllifolius Roxb.* in reducing blood glucose levels in *Mus musculus* (mice) using the OGTT.

METHODOLOGY

Materials

Mangifera foetida L. leaves were sourced from Gambiran Village, Kalisat District, Jember Regency, while *Pandanus amaryllifolius Roxb.* leaves were obtained from Summersari Village, also located in Jember Regency. The samples were authenticated by the Plant Laboratory at the Faculty of Agriculture, Jember Polytechnic. Other necessary ingredients for the study, including glucose, glibenclamide, CMC Na, distilled water, 96% technical ethanol, technical alcohol, and

blood glucose test strips (GlucoDr AGM-2100), were procured from PT. Medisindo Bahana.

Animals

In this study, 25 healthy white male mice Balb/c (*Mus musculus*) were used. The mice had a body weight ranging from 20 to 30 grams and were between 6 to 8 weeks old. They were acclimatized for seven days at the Pharmacology Laboratory, Clinical, and Community Pharmacy Section of the Faculty of Pharmacy, University of Jember. During this acclimatization period, the mice were provided with sufficient food and water on a daily basis. The study was conducted in accordance with ethical guidelines, with ethical clearance number 1157/UN25.8/KEPK/DL/2021 granted by The Ethical Committee of Medical Research at the Faculty of Dentistry, Jember University.

Preparation of Ethanolic Extracts from *Mangifera foetida* L. and *Pandanus amaryllifolius Roxb.* Leaves

The dried *Mangifera foetida* L. leaves and *Pandanus amaryllifolius Roxb.* leaves were weighed individually, with each weighing 200 grams. They were then subjected to maceration using 96% ethanol in a ratio of 1:10 w/v for a period of 3 days [22]. The resulting filtrate was subsequently concentrated by using a rotary evaporator and drying it in an oven at 50°C until a thick, viscous extract was obtained. This extract was carefully stored in a refrigerator until it was ready to be used.

D-Glucose Monohydrate Preparations

A quantity of 2.50 grams of D-glucose monohydrate was weighed and then dissolved in 10 mL of distilled water. The dose of glucose administered to the mice was calculated as 0.195 grams per 20 grams of body weight (BW) for each mouse.

Preparation of 1% CMC Na Suspension and Glibenclamide Suspension

To prepare the sodium carboxy methyl cellulose (CMC-Na) solution, 1 gram of CMC-Na was weighed and sprinkled onto the surface of hot water. The amount of water used was 20 times the mass of CMC-Na to ensure proper swelling, which took approximately 15 minutes. The mixture was then crushed until a mucilage consistency was achieved. Distilled water was added to bring the total volume to 100 mL. This resulting CMC-Na suspension was subsequently utilized to prepare the glibenclamide suspension. Amounted 65.0 mg of glibenclamide was suspended using a 1% CMC-Na solution, resulting in a final suspension volume of 20.0 mL.

Preparation of the Suspensions of Combination Extracts

The viscous combination of ethanolic extract *Mangifera foetida* L. and *Pandanus amaryllifolius Roxb.* was prepared in a 1:1 ratio at doses of 312.5 mgkg⁻¹ BW, 625 mgkg⁻¹ BW, and 1250 mgkg⁻¹ BW (combination extracts). To create the suspension of combination extracts, a dose of each combination extract was mixed with 2 mL of a 1% CMC Na suspension. This process

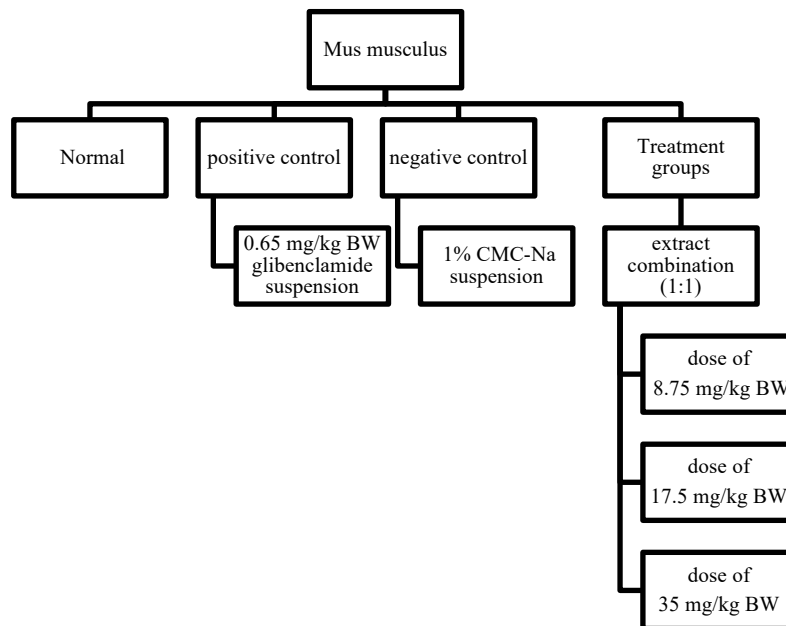


Figure.1. The scheme of the assay

yielded the suspensions of extracts, combining *Mangifera foetida* L. and *Pandanus amaryllifolius Roxb.*

Test Animal Treatment

The mice were randomly assigned to 11 groups, with 5 mice in each group as illustrated in Figure 1. Before commencing the treatment, the mice underwent a fasting period of approximately 18 hours while still being allowed access to drinking water [23]. The mice were subsequently re-weighed, and only those meeting the designated body weight requirements were included in the research [24]. Basal data was collected by extracting blood from a vein. Following this, the experimental animals received their respective treatments. The positive control group received a 0.65 mgkg⁻¹ BW glibenclamide suspension, while the negative control group received a 1% CMC-Na suspension. The test groups were administered a combination of *Mangifera foetida* L. and *Pandanus amaryllifolius Roxb.* extracts in a 1:1 ratio at doses of 312.5 mgkg⁻¹ BW, 625 mgkg⁻¹ BW, and 1250 mgkg⁻¹ BW. Thirty minutes after the sample administration, oral glucose was given to the mice at a dose of 0.975 gkg⁻¹ BW.

Measurement of Blood Glucose Levels

The data collection involved blood sampling from the mice's tails at different time points: 0, 30, 60, 90, 120, 150, and 180 minutes before administering of the samples. The glucose levels in the blood were measured using a glucometer, specifically the EasyTouch®GCU model (please mention the specification of a glucometer) [11]. Blood sampling continued until the 180th minute, as blood glucose levels returned to baseline. The measurements of blood glucose levels were expressed in mg/dL.

RESULTS AND DISCUSSION

Oral Glucose Tolerance Test (OGTT)

The antidiabetic activity testing involved using of the oral glucose tolerance test (OGTT) method. This method aims to evaluate the ability of the test group to restore blood glucose levels to a balanced state after an increase caused by oral glucose administration. The OGTT provides insights into the body's capacity to metabolize blood glucose [25]. It can also determine how well the test material restores blood glucose levels to normal following the administration of a glucose solution. In this study, the reduction in blood glucose levels was assessed by measuring the Area Under Curve (AUC) using the LDDK calculation, which describes glucose concentration in the blood plasma [26]. After randomly grouping the mice, initial blood samples (T₀) were collected to establish the baseline blood glucose levels. Subsequently, the treatment groups received the respective test preparations (carrier, comparator, or test extract) orally based on their assigned groups. After 30 minutes, all groups except the negative control were orally administered a glucose solution equivalent to 3.9 grams per kilogram of body weight of mice. Oral or intraperitoneal administration of glucose can result in a greater increase in plasma insulin compared to intravenous administration, influenced by digestive hormones involved in glucose metabolism. Blood glucose measurements were taken at 30, 60, 90, 120, 150, and 180 minutes following the administration of the glucose solution. A glucometer (EasyTouch®GCU) was used for these measurements. The test was conducted over a 3-hour period, with measurements taken at 30-minute intervals to observe the effect of reducing blood glucose levels. The data collected on blood glucose levels at each time point are presented in Table 1, while the decrease in blood glucose levels over time is illustrated in Figure 2. The AUC₀₋₁₈₀ value was

Table 1. The average blood glucose level per unit of time

Times (minutes)	Blood glucose level (mgdL ⁻¹) ± SD					
	Normal	Negative control	Positive control	Dosage 1 (312.5 mgBW ⁻¹)	Dosage 2 (625 mgBW ⁻¹)	Dosage 3 (1250 mgBW ⁻¹)
0	92,6±7.54	110.4 ± 6.95	108.2 ±13.33	113.4 ±13.76	112.6 ±12.16	110.8 ± 7.53
30	105,4±13.40	233.2 ± 17.08	226.0 ±14.16	271.4 ±21.56	287.6 ±22.84	243.4 ±22.37
60	100,87±8,05	236.4 ± 14.47	98.0 ±14.25	227.4 ±13.43	219.8 ±46.26	160.0 ±28.50
90	94,4±10.98	227.8 ±10.76	79.0 ±13.32	164.4 ±7.54	163.2 ±6.14	116.8 ±15.02
120	83,9±6.48	220.0 ± 9.11	67.0 ±10.70	147.2 ±7.33	141.0 ±17.10	83.4 ±17.56
150	79.67±11.30	212.6 ± 9.34	56.0 ± 5.39	130.8 ±4.92	120.4 ±16.07	74.0 ±16.63
180	73,2±7.65	198.6 ±12.12	57.6 ±9.91	126.4 ±3.85	111.8 ±17.30	72.6 ±17.53

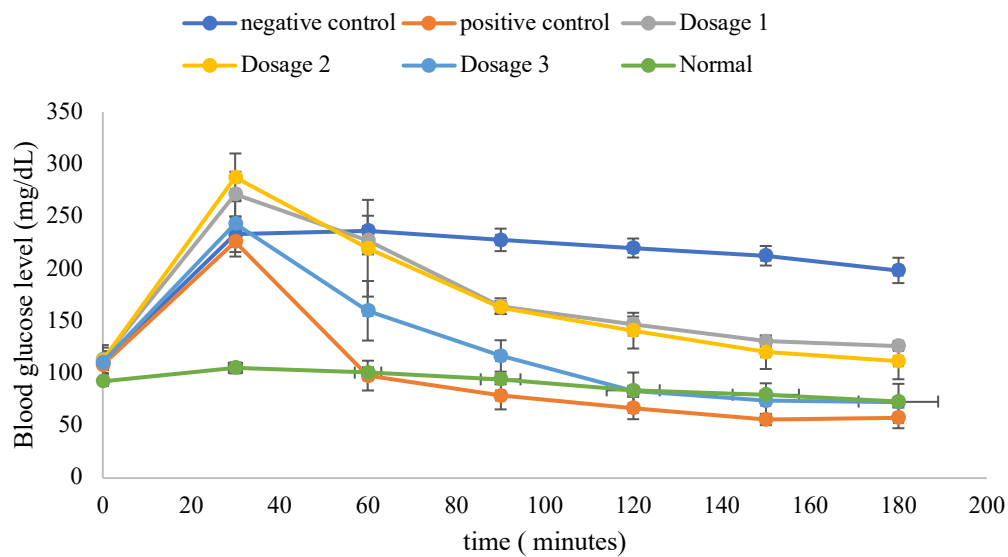


Figure 2. Graph of average blood glucose levels per unit of time.

calculated from the individual data on blood glucose levels. Subsequently, the data was analyzed using ANOVA, followed by the Least Significant Difference (LSD) follow-up test, to determine which group exhibited significantly different values compared to the other groups.

Table 1 illustrates the blood glucose levels observed at different time points. From T0 until T180, all groups exhibited blood glucose levels ranging from 76.6 to 115.4 mgdL⁻¹. At T30, there was a significant increase in blood glucose levels across all groups, ranging from 105.4 to 244.2 mgdL⁻¹. Notably, the average blood glucose levels measured at T0 before the treatment did not show statistically significant differences among the groups. This indicates that the initial blood glucose levels of all mice in the treatment groups were relatively similar. Based on the T0 values obtained, it can be concluded that the mice in all treatment groups had normal glucose levels within the range of 73.2 to 105.4 mgdL⁻¹.

Figure 2 illustrates the maximum increase in blood glucose levels observed at T30. Among the test groups, the highest increase in blood glucose levels was observed in the group receiving dosage 2 (625 mgBW⁻¹), compared to the other groups. Elevated blood glucose levels stimulate the release of insulin by pancreatic β cells, which helps maintain body homeostasis by converting glucose into glycogen, decreasing blood glucose levels. At T60, T90, T120, and T180, there was a decline in blood glucose levels, indicating the elimination of glucose due to the administration of the test preparation. The comparator used in this study was glibenclamide. It was selected based on its mechanism of action, which is similar to the test preparation. Both substances stimulate insulin secretion from the pancreatic Langerhans β cell granules. The results demonstrated that glibenclamide effectively reduced blood glucose levels at the 60th minute, but blood glucose levels increased again by the 180th minute. Statistically, there was a significant difference between the comparator and the positive control group, validating the method and confirming the procedure correctly. The results of the AUC₀₋₁₈₀ values for each group are presented in Table 2.

Table 2. The Average of AUC₀₋₁₈₀ Value

Groups	AUC ₀₋₁₈₀ value ± SD (hours. mgdL ⁻¹)
Normal	31.558 ± 4.059 ^a
Negative control	38.535 ± 1.803 ^b
Positive control	18.267 ± 1.400 ^c
Dosage Extract Combination Suspension 312,5 mg/BW	31.833 ± 1.296 ^d
Dosage Extract Combination Suspension 625 mg/ BW	31.326 ± 2.530 ^d
Dosage Extract Combination Suspension 1250 mg/ BW	23.079 ± 2.384 ^e

The same superscript letter indicates no significant difference between groups using the LSD test ($p < 0.05$) for AUC₀₋₁₈₀ values

Data Analysis

The calculated AUC₀₋₁₈₀ values of the blood glucose levels were analyzed for normality and homogeneity. In both the normality and homogeneity tests for the AUC₀₋₁₈₀ values, all groups showed a p-value greater than 0.05. This indicates that the sample data follows a normal distribution and that the compared sample groups have similar variances or are homogeneous. Subsequently, data analysis proceeded with the One-Way ANOVA test, which yielded a p-value of 0. This result indicates a significant difference in the AUC values among the five treatment groups. Further analysis was conducted using the LSD test, which revealed significant differences in all groups ($p < 0.05$). However, no significant difference was observed between the treatment groups receiving a dose of 312.5 mgkg⁻¹ and 625 mgkg⁻¹ ($p = 0.704$). The LSD test results are presented in Table 2.

Analyzing the data in Table 2, it can be observed that the negative control group exhibited the highest AUC value, in contrast positive control group had the lowest AUC value among all the groups. In the extract test group, the dose of 312.5 mgkg⁻¹ displayed the highest AUC value, followed by the dose of 625 mgkg⁻¹, and the dose of 1,250 mgkg⁻¹ had the lowest AUC value. A lower AUC value indicates a more effective reduction in blood glucose levels [23]. Based on the results of SPSS data analysis, it can be concluded that there is a significant difference between the treatment group and the control group. This indicates that the combination of ethanolic extract of *Mangifera foetida* L. leaves and *Pandanus amaryllifolius* Roxb. leaves have the ability to reduce blood glucose levels in experimental animals. The dose of 1,250 mgkg⁻¹ exhibits an AUC value close to that of the positive control group. On the other hand, the doses of 312.5 mgkg⁻¹ BW and 625 mgkg⁻¹ BW demonstrate a similar ability to decrease blood glucose levels in the experimental animals with the AUC value of 31.833 ± 1.296 and 31.326 ± 2.530 respectively.

The findings of this study suggest that ethanolic extracts from *Mangifera foetida* L. and *Pandanus amaryllifolius* Roxb. leaves can potentially to reduce blood glucose levels. These results align with previous studies indicating that the mangiferin compound found in mangoes can effectively lower blood glucose levels [26]. Mangiferin belongs to the flavonoid group and is classified as a xanthone compound [27]. Mangoes also

contain other beneficial components such as tannins and alkaloids [6]. Similarly, the extract from *Pandanus amaryllifolius* Roxb. leaves also demonstrates the ability to lower blood glucose levels. This is attributed to the presence of bioactive compounds, including flavonoids, tannins, and alkaloids, within the fragrant pandan extract. Flavonoids are known for their antioxidant activity, which can protect against diabetes mellitus by enhancing the function of pancreatic β cells and stimulating insulin secretion [18]. Additionally, flavonoids can inhibit glucose transporters (GLUT-2) in the intestinal mucosa, reducing glucose absorption and maintaining stable blood glucose levels [28]. Tannin compounds are recognized for their ability to inhibit α -glucosidase, delaying glucose absorption after meals [29]. They can also promote glycogenesis, preventing excessive glucose accumulation in the bloodstream. Alkaloid compounds stimulate the hypothalamus, leading to increased secretion of Growth Hormone Releasing Hormone (GHRH). This, in turn, triggers the release of Growth Hormone (GH) from the pituitary gland. Elevated GH levels stimulate the liver to secrete Insulin-like Growth Factor-1 (IGF-1), which reduces gluconeogenesis and consequently lowers blood glucose levels [19].

The combination of extracts from *Mangifera foetida* L. and *Pandanus amaryllifolius* Roxb. leaves have shown promising results in reducing blood glucose levels. In fact, its effectiveness is comparable to that of glibenclamide, which serves as a positive control, and surpasses the negative control. Among the tested doses, 1250 mgkg⁻¹BW dosage exhibited the highest ability to lower blood glucose. These findings highlight the potential of developing the combination of *Mangifera foetida* L. and *Pandanus amaryllifolius* Roxb. leaves extract as an antidiabetic treatment option.

CONCLUSION

The results of this study lead to the conclusion that administering a combination of ethanolic leaves extracts *Mangifera foetida* L. and *Pandanus amaryllifolius* Roxb. in a 1:1 ratio, at a dose of 1250 mgkg⁻¹ BW, effectively lowered blood glucose levels in experimental animals. These results were comparable to the effects of glibenclamide and significantly better than the negative controls, with a notable and meaningful difference.

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