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RESEARCH ARTICLE

Antidiabetes Activity of Ethanol Extract of Pandan Leaves (*Pandanus amaryllifolius* Roxb) in Male White Mice (*Mus musculus*)

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ABSTRACT

Diabetes mellitus is a global health problem with increasing prevalence every year. Pharmacological management of diabetes often causes side effects, so alternative treatments based on natural ingredients are needed that are safer and more effective. This study aims to test the antidiabetic activity of ethanol extract of pandan wangi leaves (*Pandanus amaryllifolius* Roxb) in male mice (*Mus musculus*) that induce alloxan. The method used was a pre- and post-test design with a control group, using glibenclamide as a positive control and 1% Na CMC as a negative control. The extract was given at doses of 200, 400, and 600 mg/kgBW for 9 days, and blood glucose levels were measured on days 3, 6, 9, and 12. The results showed that pandan wangi extract contains flavonoids, polyphenols, alkaloids, and tannins, and is able to significantly reduce blood glucose levels ($p \leq 0.05$). A dose of 600 mg/kgBW showed the highest decrease in blood sugar levels by 26%, more effective than glibenclamide (10%). In conclusion, the ethanol extract of pandan wangi leaves has the potential as an effective alternative therapy in lowering blood sugar levels in diabetes mellitus.

Keywords: Alloxan, antidiabetic, diabetes mellitus, *Pandanus amaryllifolius* Roxb

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Introduction

Diabetes mellitus is a global health problem with an increasing incidence every year. Diabetes mellitus is a metabolic disease characterized by hyperglycemia due to impaired secretion or insulin function [1]. In Indonesia, factors that contribute to the high prevalence of diabetes mellitus include genetic factors, environment, lifestyle, and delays in treatment due to erroneous diagnosis or non-compliance with therapy [2]. Data from the International Diabetes Federation [3], shows that Indonesia is ranked seventh in the world with 10.7 million people with diabetes. This figure is expected to continue to increase to 13.7 million by 2030.

Management of diabetes mellitus can be done through non-pharmacological and pharmacological therapy. Non-pharmacological therapy includes regular exercise, regulating carbohydrate intake, choosing foods with a low glycemic index, and good psychological control [4]. Meanwhile, pharmacological therapy is carried out by administering oral hypoglycemic drugs, such as glibenclamide from the sulfonylurea group, which functions to increase insulin secretion and lower blood glucose levels [1]. However, the use of hypoglycemic drugs often causes unwanted side effects, so alternative therapies based on safer and more effective natural ingredients are needed [5].

One of the natural ingredients that has the potential as an antidiabetic agent is pandan wangi leaves extract (*Pandanus amaryllifolius* Roxb). Pandan wangi leaves are known to contain flavonoid compounds that can lower blood glucose levels by inhibiting GLUT-2 (glucose transporter isoform 2), a glucose transporter protein found in the intestinal membrane [5]. Previous research by Gantoro *et al.* [6] showed that a pandan wangi leaves extract at a dose of 600 mg/kg BW or its effects on alloxan-induced male mice. This study aims to examine the antidiabetic activity of ethanol extract of pandan wangi leaves in male white mice (*Mus musculus*). Unlike previous studies that used metformin as a positive control and were carried out for 15 days, this study will use glibenclamide as a positive control with an observation period of 12 days. It is hoped that the results of this study can provide scientific information regarding the potential of ethanol extract of pandan wangi leaves as an alternative treatment for diabetes mellitus that is safer and more effective.

Materials and Methods

Materials

The tools used in this study were glassware, stirring rods, porcelain cups, glucometers, glucotest strips, 3 mL injection syringes, oral syringes (sondes), analytical scales, rotary evaporator (BUCHI Rotavapor R-205®, Germany) parchment paper, blender (Philips®), filter paper, spatulas (Pyrex®), knives, scissors, mice drinking bottles, test animal cages, aluminum foil, trays, ovens (memmert®), droppers, volume pipettes, ball filers, and mesh no. 44.

The materials used in this study were samples of pandan wangi leaves, 96% ethanol (Merck®), Mayer's reagent, 0.5 N hydrochloric acid, 3% ferric chloride ($FeCl_3$), sodium hydroxide (Merck®), distilled water, Dragendorff's reagent, 1% $FeCl_3$, MG powder, chloroform (Merck®), ammonia (Merck®), sulfuric acid (Merck®), Wagner's reagent, 1% Na CMC, 5 mg glibenclamide preparation, 1 g alloxan preparation, and cotton.

Methods

1. Preparation

The samples that have been determined in the laboratory of IAIN Syekh Nurjati Cirebon No.31/In.08/LB.1.1/PP.009/3/2024 are dried using an oven at a temperature of 40 C. After drying, 1 kg of samples are ground and macerated using 3 L of 96% ethanol solvent (1:3). The extract obtained from the maceration results is concentrated using a water bath. Then, the thick extract is tested for its phytochemical content.

2. Phytochemical Test

a. Polyphenols

Pandan wangi leaf extract was weighed to 0.10 g, then, 5 mL of distilled water was added and boiled for 5 minutes. The mixture was filtered until the filtrate is obtained. After that, 5 drops of 1% $FeCl_3$ was added to the filtrate. The presence of polyphenol compound was indicated by a change in color to blue green to black.

b. Flavonoids

A concentrated extract of pandan wangi leaves of 0.1 g was added with 2-3 mL of ethanol solution. The mixture was heated and added with magnesium powder and 2 mL of concentrated HCl. The presence of flavonoid compound was indicated by a change in color to red, yellow, or orange [7].

c. Alkaloids

Identification of alkaloids was carried out by adding Mayer, Dragendorff, and Wagner's reagents. About 0.5 g of concentrated extract was added into 2 mL of concentrated HCl, then filtered. The filtrate obtained was divided into three parts then added Mayer, Dragendorff, and Wagner's reagents [7].

d. Tannins

A concentrated extract of 0.1 g was dissolved in 5 mL of hot water and added with 2-3 drops of 5% $FeCl_3$ solution. The presence of tannin content was indicated by a change in color to blue or blackish green [7].

3. Measurement of Blood Sugar Levels

The researcher has previously obtained a letter of ethical approval for the treatment of animal testing with the number srat 229/KEPK/EC/VII/2024 from the health research ethics commission of the faculty of pharmacy, YPIB University. This study is an experimental study with a pre and post test with control group design by testing the treatment group of glibenclamide (comparator), 1% Na-CMC and pandan wangi extract doses of 2, 4, 8 and 12 mg/kgBW given to diabetic white mice after being induced by alloxan 24 mg/kgBW. Initial blood glucose levels (Pretest) were measured on the third day after alloxan was given. After ensuring that there was an increase in blood glucose to the specified limit, a test treatment intervention was given and blood sugar levels were measured again on days 6, 9 and 12 to obtain data on the decrease in blood sugar levels (Postest). Then a statistical test was carried out with a one-way ANOVA test and continued with a paired sample t-test statistical test to see significant differences between treatment groups with a data confidence level of 95% ($\alpha = 0.05$).

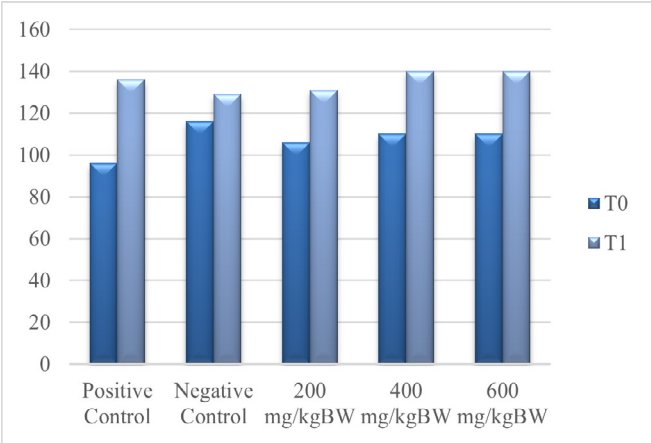
Result

The result of this study obtained the yield obtained was 10.9%. Phytochemical test on the thick extract of pandan wangi leaves obtained polyphenol, flavonoid, alkaloid, and tannin compounds, this can be seen in **Table 1**. The administration of pandan wangi leaves extract (*Pandanus amaryllifolius* Roxb) to test animals induced by alloxan on day 1, and blood glucose levels were observed on day 3. The following are the results of blood glucose level observation data after alloxan induction in each group. **Table 1.** Result of phytochemical test

Content	Reagent	Test Results	Result of References	+/-
Polyphe-nol	1% $FeCl_3$	Blue green to black color	Formation of blue green to black color	+
Flavonoid	Magnesium and HCl	Red color in the ethanol layer	Formation of red color on the ethanol layer	+
Alkaloid	Meyer	White precipitate	Formation of white sediment	+
	Dragendorff	Red precipitate	Formation of red sediment	+
	Wagner	Brown precipitate	Formation of brown sediment	+
Tannin	1% $FeCl_3$	Dark blue or blackish green color	Formation of dark blue or blackish green color	+

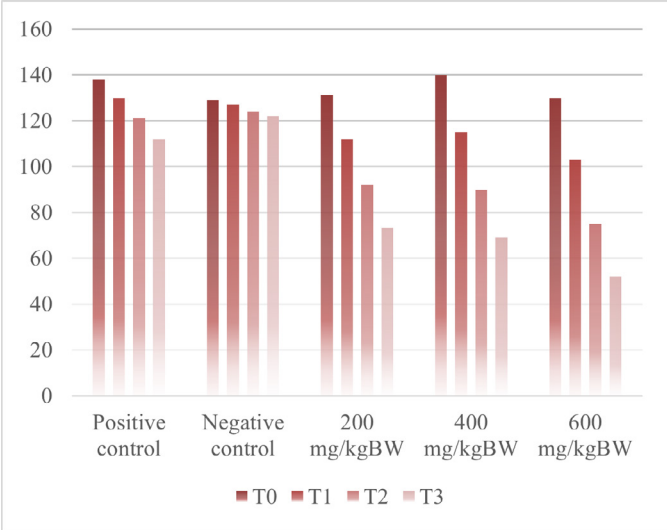
Pandan wangi leaves were showed in **Figure 1**. Administration of pandan wangi leaves extract was carried out for 9 days after alloxan induction. Blood glucose levels were taken on days 6, 9, and 12. **Figure 3** showed a graph of the results of the decrease in blood sugar levels after administration of pandan wangi leaves extract experienced a decrease in blood glucose levels.

Figure 1. Pandan wangi leaves (*Pandanus amaryllifolius* Roxb) (Personal documents, 2024)



T0: Initial glucose level
T1: Glucose levels after Alloxan induction

Figure 2. Graph of measurement results of increase in blood glucose levels after being induced by Alloxan



T0: Glucose levels after Alloxan induction
T1: 6th Glucose Level
T2: 9th Glucose Level
T3: 12th Glucose Level

Figure 3. Graph results of blood glucose level measurements in animals after being given treatment

Discussion

The % yield value obtained from 1000 g of fragrant pandan leaf powder obtained in this study was 10.9 %. The yield value is what indicates the amount of secondary metabolites. According to the Indonesian Herbal Pharmacopoeia, the yield requirement is not less than 10%. This can be stated that the yield results are in accordance with Indonesian herbal pharmacopoeia regulations. Phytochemical tests were carried out to obtain information on secondary metabolites contained in the extract of pandan wangi leaves. The compounds tested in this study were polyphenols, flavonoids, alkaloids, and tannins.

In the polyphenol test of ethanol extract of pandan wangi leaves, the extract was added with 1% $FeCl_3$, the result was blue-green to black [8]. This is because of the reaction between the phenol hydroxyl functional group and 1% $FeCl_3$. According to Asra & Azni [9], in the phenol test, the formation of blackish-green phenol in the extract after adding $FeCl_3$ was because the phenol compound reacted with Fe^{3+} ions to form a blackish-green complex compound. The formation of a red color in pandan wangi leaves extract indicates the presence of flavonoid compounds with the addition of magnesium and HCl powder to reduce the benzopyrone core contained in the flavonoid structure so that a flavilium salt is formed. Magnesium and HCl powder react to form H_2 gas [10].

Flavonoids lower blood glucose by inhibiting the enzymes α-glucosidase, maltase, and α-amylase. According to Hidayah & Anggarani [11], flavonoids increase glucose uptake in muscles through GLUT-4 regulation and act as antioxidants that protect pancreatic cells from damage. According to Oktavia *et al.*, [12] alkaloids have been shown to have the ability to regenerate β-pancreatic cells, namely by capturing free radicals that can damage β cells due to type 1 diabetes. In addition, the mechanism of alkaloid compounds can stimulate the sympathetic nerves in the pancreas so that it can increase insulin secretion [13].

In this study, the test animals were grouped into several groups, namely the positive control group, negative control, dose group 200 mg/kgBW, 400 mg/kgBW, and 600 mg/kgBW. All groups were induced by alloxan for 1 day. All groups were induced by alloxan for 1 day. On the third day, the treatment group experienced an increase in blood glucose levels. Alloxan works by forming reactive oxygen which damages insulin receptors and pancreatic β cells, so that insulin production is disrupted. As a result, blood glucose cannot be absorbed and converted into energy, causing glucose levels to increase [14].

Glibenclamide was used as a positive control and was suspended with 1% Na CMC. The suspension was chosen because glibenclamide has low solubility in metal chloride, ethanol, and methanol, and is almost insoluble in water. The drug used in this study was glibenclamide at a dose of 5 mg. Glibenclamide is an oral antihyperglycemic drug of the sulfonylurea group that works by stimulating insulin secretion in pancreatic β cells. This is in accordance with the condition of test animals with alloxan that have experienced partial pancreatic β cell damage so that there is still insulin production in small amounts [15].

Glibenclamide given to test animals will undergo an absorption process or absorption into the bloodstream. If given orally, glibenclamide will be absorbed through the gastric and intestinal mucosa. The rate of drug absorption can be influenced by several factors, including the size of the drug, dosage form, solubility of the drug, dose used, physicochemical properties, method of drug administration, length of contact with the surface of the digestive tract, and the method of diffusion of the drug [1].

The pharmacodynamics of the drug glibenclamide involve stimulation of granules in pancreatic β-cells through ATP-sensitive K ion channels on their membranes. After this channel opens, ions entering the β-cells will trigger the release of insulin [16]. Research conducted by Kaban & Putri [13] showed that water extract of pandan wangi leaves has the ability to lower blood glucose levels and repair damage to pancreatic tissue because it contains tannins, alkaloids, flavonoids, and polyphenols. Water extract of pandan wangi leaves has the potential to lower blood sugar levels.

Flavonoid compound content functions as an antidiabetic by inhibiting glucose reabsorption in the kidneys and increasing glucose solubility in the blood, so that glucose can be more

easily excreted through urine [17]. Alkaloids can stimulate the sympathetic nervous system (sympathomimetic) which results in increased insulin secretion. The mechanism of action of alkaloids in lowering blood sugar is by increasing glucose transport in the blood, inhibiting glucose absorption in the intestine, stimulating glycogen synthesis and inhibiting glucose synthesis and inhibiting the enzyme glucose 6-phosphatase, fructose 1,6 - bisphofatase which is an enzyme that plays a role in gluconeogenesis, and increasing glucose oxidation through glucose 6-phosphate dehydrogenase [18]. The effectiveness of pandan wangi leaf extract in lowering blood glucose levels is with a dose concentration of 200 mg/kgBW, 400 mg/kgBW and 600 mg/kgBW, glibenclamide as a positive control and 1% Na CMC as a negative control. In this study, a dose of 600 mg/kgBW was more effective in lowering blood glucose levels in mice. This is in line with Khairiyani's [19] study that administering a dose of 600 mg/kgBW was better at lowering blood glucose levels. In this study, glibenclamide used as a positive control showed low effectiveness in lowering blood glucose levels. This is due to the mechanism of action of alloxan which can damage pancreatic β cells, so that the pancreas is unable to produce insulin [20]. This is not in line with Utami's [21] study that glibenclamide lowers blood glucose levels from day 6 to day 12.

In this study, the Paired Sample T-Test aims to determine the difference in blood glucose levels before and after treatment based on the data in graph 2 and 3, it can be stated that on the probability results with a Significance value (Sig.) (2-tailed) of $0.000 \leq 0.05$, it shows that H1 is accepted and H0 is rejected, which means there is a significant difference.

There is a difference in the Paired Sample T-Test, then the N-Gain Score test is carried out to see the effectiveness of each dose of pandan wangi leaf extract. In this study, the dose concentration of 600mg/KgBW has a better effectiveness in reducing blood glucose levels than the positive control using glibenclamide. This is because the phytochemical compound, namely flavonoids found in pandan wangi leaf extract, can reduce blood glucose levels.

Conclusion

Based on the results, it can be concluded that pandan wangi leaf extract (*Pandanus amaryllifolius* Roxb) effectively reduces blood glucose levels in alloxan-induced male white mice (*Mus musculus*), with the 600 mg/kgBW dose showing the highest effectiveness (26%), outperforming both lower doses and the positive control (glibenclamide). This suggests its potential as a natural alternative for diabetes treatment.

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