

## **The Effectiveness of Ethyl Acetate Extract of Sungkai Leaves (*Peronema Canescens* Jack.) Against Blood Glucose Levels in Alloxan-Induced Male Mice**

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### **Abstract**

This research aims to test antidiabetic using alloxan induction on mice test animals. The type of research used is experimental, which includes sampling, sample determination. Extract preparation with ethyl acetate solvent, extract characteristics test and simplicia, extract and simplicia screening test. Antidiabetic testing was carried out using male mice as test animals. Screening tests for the presence of alkaloids, flavonoids, tannins, saponins, steroids, and glycosides for the anti-diabetic test of Sungkai leaves were carried out using alloxan induction in mice. With initial KGD 90-120 mg/dl. Extract concentrations were 100 mg/kg, 200 mg/kg and 300 mg/kg with negative control CMC Na 0.5% and metformin positive control 65 mg/kg. The most effective decrease in mice blood glucose on days 3, 7, 10 and 14 was at an extract concentration of 300 mg/kg bw with a decrease of 114 mg/dl. Based on the results of the ANOVA statistic  $F_{count} 208.813 > F_{table} 2.87$  so  $H_0$  is rejected, there is a significant decrease in blood sugar in mice. The results of the anti-diabetic test of the ethyl acetate extract of Sungkai leaves at a dose of 300 mg/kg body weight had an anti-diabetic effect on male mice after alloxan induced on the 14th day with an average of 114 being the most effective in reducing the male mice's KGD.

**Keywords:** Antidiabetic, *Peronema Canescens* Jack, Blood Sugar Levels, Male Mice

### **Introduction**

The development of an increasingly modern era has many positive and negative impacts on health problems. Nowadays lifestyle, especially diet, greatly affects one's health. Unhealthy eating patterns include a diet high in carbohydrates with a high glycemic index and high in fat, consumption of foods with low fiber content is one of the causes of a disease that continues to increase in the number of sufferers every year, namely Diabetes Mellitus (DM). DM, better known as diabetes, is a metabolic disorder characterized by blood sugar levels (Ministry of Health RI. 2020. Infodatin 2020 Diabetes Mellitus Center for Data and Information Ministry of Health RI., 2020a).

DM is a metabolic disease characterized by the inability of the pancreas to effectively produce insulin. Because this disease can persist in the long term, it is classified as a chronic disease. Based on the cause, DM is classified into four types, including type 1 DM, type 2 DM, other types of DM and gestational DM. Antidiabetic is an activity provided by certain compounds that can treat diabetes (Ministry of Health RI. 2020. Infodatin 2020 Diabetes Mellitus Center for Data and Information Ministry of Health RI., 2020b).

DM as a global problem continues to increase in prevalence from year to year both in the world and in Indonesia. Based on data from the International Diabetes Federation (IDF) the global

DM prevalence in 2021 is estimated at 537 million people, increasing to 643 million in 2030 and 783 million in 2045 (IDF, 2021). According to IDF data, Indonesia is currently in fifth place with 19.47 million people with diabetes. With a population of 179.72 million, this means that the prevalence of diabetes in Indonesia is 10.6%. The 2018 Riskesdas report stated that the prevalence of DM in North Sumatra Province was 1.39% (69,517 people) (Basic Health Research (Riskesdas), 2018).

Currently, there are still many medicinal plants that grow wild and have not been utilized. One of the medicinal plants that can be used is Sungkai (*Penorema canescens* Jack.). The Sungkai plant (*Penorema canescens* Jack.) is often also referred to as teak sabrang, ki sabrang, sungkai, or sekai, belonging to the Verbenaceae family P. house barrier. Sungkai leaves contain secondary metabolite compounds including alkaloids, flavonoids, phenolics and tannins (Ramadenti et al., 2017).

Sungkai leaves (*Penorema canescens* Jack.) have many benefits, namely as antibacterial, antioxidant, antipyretic, immune booster, and antidiabetic (Ibrahim et al., 2021). Traditionally this plant is used by the Dayak tribe in East Kalimantan as medicine, including cold medicine, fever medicine, worm medicine, used as a bath for women after giving birth and mouthwash to prevent toothache (Ningsih & Ibrahim, 2013). Based on several research reports, it was found that plants containing the chemical content of flavonoids can be used as anti-diabetics (Alusinsing, G., Bodhi, W., Sudewi, 2014).

The content of flavonoids can reduce blood sugar levels by inhibiting GLUT2 in the intestinal mucosa resulting in a decrease in glucose absorption from the intestine (Brahmachari, 2011). The ethanol extract of Sungkai leaves has effectiveness as an antidiabetic and a dose of 350 mg/kg has a significant effect on glibenclamide. Metformin is used as a comparison for the alloxan induction test because the mechanism of action does not depend on the presence of functioning  $\beta$ -pancreatic cells (Prameswari, 2014). Alloxan is one of the chemical compounds used in the induction of diabetes in animals for research. Alloxan can be used intravenously, subcutaneously and intraperitoneally (TWS, 2018).

## **Methods**

This research method was carried out in an experimental laboratory. The study was conducted at the Pharmacology Laboratory of the Helvetia Health Institute in July-September 2022. The tools used in this study included laboratory glassware, blender, aluminum foil, glucometer and glucometer strips, drying cupboard, mortar and stamper, analytical balance, oral sonde, rotary evaporator, water bath, and 1 ml syringe, furnace. oven, hot plate. The materials used in this study include plant materials and chemicals. The plant material used is sungkai leaves (*Peronema canescens* Jack.). The chemicals used were aquadest, ethyl acetate, alloxan, 500 mg Metformin tablets, and 0.5% CMC Na.

## **Research procedure**

Sungkai leaves (*Peronema canescens* Jack.) which have been collected as much as 5 kg. Then wet sorting to separate impurities or other foreign materials and weighing, then washing is carried out to remove soil and other impurities attached to the simplicia material, weighed, then sliced (1-3 mm) to facilitate drying. After that, the drying process is carried out in a drying cupboard to obtain simplicia that is not easily damaged, so that it can be stored for a longer time, then in a blender to obtain simplicia powder (Ditjen POM, 1995).

The ethyl acetate extract of Sungkai leaves was prepared using the maceration method with a ratio (1:10). As much as 700 grams of simplicia powder was put into a glass vessel, poured with 5250 mL (75 parts) of ethyl acetate, covered with aluminum foil and left for 5 days protected

from light, while occasionally stirring. After 5 days the extract is filtered with filter paper to produce filtrate and residue. The residue is then soaked again with 1750 mL (25 parts) of ethyl acetate then the container is covered with aluminum foil and left for 2 days while stirring occasionally, after 2 days the sample is filtered to produce filtrate and residue. Filtrate one and two are mixed together and then evaporated using a rotary evaporator at a temperature of no more than 40°C until a thick extract is obtained. The thick extract obtained was stored in a place protected from sunlight (DepKes, 1979).

Simplicia characteristics include macroscopic and microscopic examination of simplicia, determination of water content, determination of total ash content, determination of acid-insoluble ash content, determination of ethanol-soluble extract content, and determination of water-soluble extract content. Phytochemical screening includes analysis of alkaloids, flavonoids, tannins, saponins, steroids, triterpenoids and glycosides analysis. As much as 150 mg of alloxan monohydrate was put into a 10 mL volumetric flask, dissolved in 0.9% NaCl solution in cold condition. The volume is added up to the mark line (Haryoto & Afifah UN, 2017a).

As much as 0.5 g of CMC Na was sown in a hot mortar containing 10 mL of hot distilled water. Then let stand for 15 minutes until a transparent mass is obtained, then crushed until homogeneous, diluted with warm distilled water and put into a 100 mL volumetric flask then made up to 100 mL with warm distilled water (M., 2007).

As much as 72 mg of metformin tab powder was put into the mortar and added a little 0.5% Na CMC suspension while grinding until a homogeneous volume was made up to 10 mL (Suhendy et al., 2021). Ethyl acetate extract of Sungkai leaves as much as 100 mg, 200 mg and 400 mg respectively were crushed in a mortar and then added 0.5% CMC Na suspension little by little while homogeneously crushed and then diluted with 0.5% CMC Na suspension in a 10 mL volumetric flask (Dan et al., 2021a).

Before the experiment was carried out, the mice were fasted (not eating but still drinking) for 18 hours, then the weight of each mouse was weighed and marked on the tail. Then each mouse was measured fasting blood sugar levels, blood was taken through the vein in the tail which was pierced with a lancet. The blood that comes out is touched to the test strip that has been attached to the glucometer. Left for 5 seconds, the tool measures blood sugar levels automatically. The numbers that appear on the screen of the tool are recorded as blood sugar levels (mg/dL) (Haryoto & Afifah UN, 2017b).

### **Determination of the Effect of Lowering Blood Sugar Levels EEADS**

Twenty-five male mice with a body weight of 20-30 g were randomly divided into 5 groups, each group consisting of 5 mice. Mice that had been fasted for 18 hours were weighed and their fasting blood sugar levels were measured. Then each mouse was induced intraperitoneally with alloxan dose of 150 mg/kg bw.

Mice were given food and drink as usual, their behavior and body weight were observed. The mice's KGD was measured on day 3, mice were considered diabetic if their fasting blood glucose levels were > 200 mg/dL and could be used for testing (Dan et al., 2021b). The research data were analyzed using the One-Way analysis of variation (ANOVA) method using the Statistical Product and Service (SPSS) 25 program with a 95% confidence level.

## Results and Discussion

### Characteristics of Simplisia

Table 1. Characteristics of Sungkai Leaf Simplisia

Parameters	Result
Moisture content	0,44%
Water-soluble juice content	4,1%
Total ash content	1,8%
Insoluble ash content in acids	0,8%
Juice content is soluble in ethanol	3,8%

### Extract Characteristics

Table 2. Characteristics of Sungkai Leaf Extract

Parameters	Result
Acid insoluble ash content	0,39%
Total ash content	0,95%
Moisture content	0,58%

### Phytochemical Screening

Table 3. Sungkai Leaf Phytochemical Screening

Compound	Screening Results	
	Simplisia	Extract
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	+
Saponins	+	+
Terpenoids	-	-
Steroids	+	+
Glycosides	+	+

### KGD Mice Measurement

Table 4. KGD Mice Before Aloksan Induced

Group	Average fasting KGD (mg/dl) $\pm$ SD
CMC 0.5 %	90.6 $\pm$ 3.51
Metformin 65 mg/kg bb	98 $\pm$ 8.69
EEADS 100 mg/kg bb	97 $\pm$ 7.35
EEADS 200 mg/kg bb	95.8 $\pm$ 6.06
EEADS 300 mg/kg bb	94.2 $\pm$ 3.96

Table 5. KGD Mice After Aloksan Induced

Group	Average fasting KGD (mg/dl) $\pm$ SD
CMC 0.5 %	338.4 $\pm$ 17.53
Metformin 65 mg/kg bb	481 $\pm$ 83.85
EEADS 100 mg/kg bb	404.4 $\pm$ 78.16
EEADS 200 mg/kg bb	385.6 $\pm$ 54.67
EEADS 300 mg/kg bb	477.2 $\pm$ 59.96

## Antidiabetic Test with EEADS

Table 6. Average Decrease in KGD Mice

Group	KGD Before induced alloxane (mg/dl)	KGD After induced alloxane (mg/dl)	KGD After Treatment (mg/dl)			
			Day -To			
			3	7	10	14
CMC Na 0.5%	90.6	338,4	364,8	416	484,2	531,4
Metformin	98	481	366,8	250,2	153,8	105
P1	97	404,4	367,2	323	248,6	186
P2	95.8	385,6	303,6	267,4	208,2	162,8
P3	94.2	477,2	402,4	293,8	184,8	114

### Information:

- Possitive Control = Metformin
- Negative Control = CMC Na 0.5%
- P1 = EEADS 100 mg/kg bb
- P2 = EEADS 200 mg/kg bb
- P3 = EEADS 300 mg/kg bb

### EEADS Extract

Sungkai leaf extraction process (*Penorema canescens* Jack.) The extraction method used is the maceration method using ethyl acetate as a solvent. Because this method is easier to implement and does not require specific equipment. The extraction maceration method can be used for both heat-resistant and non-heat-resistant compounds and can be used for compounds that have not been identified. After the extraction process, solvent evaporation will be carried out with a rotary evaporator to obtain a thick extract from Sungkai leaves.

### Characteristics of Simplicia

The simplicia characteristics of Sungkai leaves were carried out on five parameters, namely the determination of water content, water-soluble essence content, total ash content, acid-insoluble ash content, and ethanol-soluble extract content. It can be seen in table 4.1 that it is not listed in the *Materia Medika Indonesia* book so it is not there is a reference in carrying out simplicia characteristics. However, researchers have carried out the characteristics of simplicia by following the standard provisions for working on the correct characteristics of simplicia according to the Indonesian Herbal Pharmacopoeia, edition II. The water content of the sungkai leaves obtained is 0.44%, the purpose of the water content is to provide a minimum limit or range of the amount of water content in the material (RI, 2000).

The water-soluble extract content is 4.1%, the soluble ethanol extract of Sungkai leaf simplicia is 3.8%. Determination of the water content indicates the amount of substances extracted in water solvents such as sugar glycosides, gums, proteins, enzymes, dyes, and organic acids, while the determination of the soluble extract content in ethanol indicates the amount of substances extracted in ethanol solvents such as glycosides, anthraquinones, steroids, flavonoids, chlorophyll, saponins, tannins and those that are dissolved in small amounts, namely fat (Paramita NLPV, Andani NMD, Putri IAPY, Indrayani NKS, 2019).

In Sungkai leaves, the total ash content was 1.8%, the purpose of determining the ash content was to provide an overview of the internal and external mineral content from the initial process to the formation of the extract. Sungkai leaves obtained 0.8% ash content insoluble in acid.

## **Extract Characteristics**

The characterization of the extract was carried out on three parameters, namely the determination of water content, total ash content and acid insoluble ash content. The results can be seen in Table 4.2 by obtaining the value of the content of each parameter 0.39% acid insoluble ash content, 0.95% total ash content and 0.58 water content of Sungkai extract.

## **Phytochemical Screening**

There are chemical compounds that can be used as antidiabetics, namely flavonoids, alkaloids, tannins, saponins and phenols (Antidiabetes et al., 2021b). Tests were carried out on groups of chemical compounds including alkaloids, flavonoids, tannins, saponins, terpenoids, steroids and glycosides. In this test the compounds contained (positive) in simplicia and sungkai leaf extract were alkaloids, flavonoids, tannins, saponins, steroids, and glycosides. Meanwhile, terpenoid compounds were not contained (negative) in simplicia and Sungkai leaf extract.

## ***Antidiabetic Test Using Aloksan Induction Method***

Prior to administration of alloxan, the average KGD values were normal, namely 80-130 mg/dl. From the results of measuring fasting blood glucose levels in 5 groups under normal circumstances, namely the CMC Na 0.5% group of 90.6 mg/dl, positive control of metformin 65 mg/kg body weight of 98 mg/dl, EEADS 100 mg/kg body weight of 97 mg/dl, EEADS 200 mg/kg body weight of 95.8 mg/dl, EEADS 300 mg/kg body weight of 94.2 mg/dl. Then from the 5 groups that had been induced by alloxan an increase in KGD was in the CMC Na 0.5% group, Metformin 65 mg/kg bw, EEADS 100, 200 and 300 mg/kg bw. From the results of alloxan induction at a dose of 150 mg/kg body weight, it can be seen in table 4.5, it was found that the average data in the CMC Na 0.5% group experienced an increase of 338.4 mg/dl, Metformin 65 mg/kg body weight of 481 mg/dl, EEADS 100 mg/kg body weight was 404.4 mg/dl, EEADS 200 mg/kg body weight was 385.6 mg/dl and EEADS 300 mg/kg body weight was 477.2 mg/dl.

In this study alloxan was used at a dose of 150 mg/kg bw with this dose after three days of mice experiencing permanent hyperglycemia (temporary blood glucose levels greater than 200 mg/dl) of more than 90%, therefore all DM mice with blood glucose levels a moment more than 200 mg/dl can be used in this study. Alloxan induction was carried out intraperitoneally with the aim of shortening the induction pathway. The most dominant factor in producing alloxan's diabetogenic properties is the formation of reactive oxygen compounds that occur in pancreatic cells. Several studies reported that alloxan increased the concentration of cytosolic free calcium in pancreatic  $\beta$  cells as a result of several processes, including increased influx of calcium from the extracellular fluid, intracellular mobilization, and reduced calcium loss in the cytoplasm. The ability of alloxan to cause diabetes also depends on the induction pathway, dose, compounds, experimental animals and nutritional status (Cahyaningrum et al., 2019).

Sungkai leaf ethyl acetate extract (*Penorema canescens* Jack.) is able to suppress the increase in blood glucose levels, by preventing the intestine from absorbing the glucose eaten and stimulating the cells of the pancreas gland of the body to produce more insulin, in addition to increasing the deposit of glycogen glucose reserves in the liver. So that the next day it can accelerate the decrease in blood glucose levels. The decrease in blood glucose levels in mice given ethyl acetate extract of Sungkai leaves (*Penorema canescens* Jack.) was caused by the content of flavonoids, saponins and tannins which were identified in the phytochemical screening contained in the ethyl acetate extract of Sungkai leaves (*Penorema canescens* Jack.) (Yuda et al., 2013). Flavonoid compounds play a role in damaging the morphological pancreatic tissue of mice due to DNA alkylation due to the induction of alloxan flavnoids which have a

role as an antioxidant and an aldose reductase inhibitor. Another mechanism by which flavonoids exhibit a hypoglycemic effect is reducing glucose uptake and negating the expression activity of enzymes involved in carbohydrate metabolism. In saponin compounds, by stimulating insulin in the pancreas and increasing insulin activity, the decrease in sugar levels is due to the presence of beta cells which balance homeostasis so that insulin and tannins are released smoothly, increasing glucose absorption and inhibiting adipogenesis, also acting as a chelating agent which can constrict the small intestinal epithelial membrane so that reduces food absorption and consequently inhibits sugar intake and the rate of increase in blood sugar is not too high (Antidiabetes et al., 2021b).

## Data Analysis

Table 7. Statistical Test Results

Decrease in KGD	Sum of Squares	Df	Mean Square	F	Sig.
Day-3	25476,16	4	6369,04	2,553	0,071
Day -7	85291,04	4	21322,76	7,752	0,001
Day -10	349645,44	4	87411,36	105,186	0,000
Day -14	640197,2	4	160049,3	204,813	0,000

The results of measurements of rat blood glucose levels were analyzed statistically using the SPSS version 25 program. The first statistical test to be performed was the normality test which aimed to find out the distribution of data for each test group. Furthermore, a homogeneity test was carried out which aims to determine the presence of homogeneous variants in the data. Data is normally distributed and homogeneous if it has a p-value  $\geq 0.05$ . Furthermore, if the data is normally distributed and homogeneous, then the data is said to pass the One Way ANOVA test. The KGD on the 10th day was 0.184 and the KGD on the 14th day was 0.040, and these values were more than 0.05. The results of the normality test show that not all of the data is normally distributed with a significance value of  $p \geq 0.05$ . Based on the results of the One-Way ANOVA analysis for day 14 it has  $F_{count} = 204.813 > F_{table} = 2.87$  so  $H_0$  is rejected where  $H_0$  has a significant difference.

## Conclusion

In the anti-diabetic test, the ethyl acetate extract of Sungkai leaves at a dose of 300 mg/kg had an effect on the KGD of male mice with a decrease of 114 percent, metformin 65 mg/kg 105 on day 14; The ethyl acetate extract that had an effective reduction in the mice's KGD was the 300 mg/kg bw group on day 14 with an average of 114.

## Suggestion

For further research, it is hoped that they will perform fractions from Sungkai leaves and conduct anti-diabetic testing with different animals.

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