

The Activity of Wungu Leaf (*Graptophyllum pictum* (L) Griff) Extract in Reducing Blood Glucose Level of Hyperglycemic Mice

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ABSTRACT

*Wungu leaf (*Graptophyllum pictum* (L.) Griff) is a plant thought to have potential use in alleviating symptoms of diabetes mellitus. The purpose of the present study was to evaluate the activity of wungu leaf extracts in decreasing blood glucose level of alloxan (200 mg/kg BW)-induced hyperglycemic mice. Extracts of wungu leaf were obtained by macerating with ethanol and then partitioning the extract with diethyl ether, ethyl acetate, and butanol. Each extract obtained was used to treat hyperglycemic mice for 28 days. The results showed that wungu leaf extracts have the ability to decrease the blood glucose level of hyperglycemic mice (dose 50 mg/kg BW). The ethyl acetate extract showed the highest activity, bringing about a decrease of blood glucose of 37.6%. The wungu leaf extract has the potential to be developed as a source of anti-diabetic agents.*

Keywords: *Graptophyllum pictum* (L.) Griff, partition extraction, ethyl acetate

1. INTRODUCTION

Diabetes mellitus is a chronic disease characterized by high blood-glucose levels (hyperglycemia) caused by a decrease of the hormone insulin. The insulin cannot be produced in normal level because of β cells pancreatic damage, and also because of the lack of responsiveness of body cells to insulin. This decrease of insulin function, influences the metabolism of carbohydrates, lipids and proteins (Studiawan and Santosa 2005).

There are many ways which have been taken to control diabetes, ranging from dietary adjustments and regular exercise, to the use of synthetic anti-diabetic drugs or even by insulin injection. The value of synthetic anti-diabetic drugs have been reduced on the market because of the emergence of herbal medicines. This is because of the effects of herbal medicines are not harmful if taken according to the recommended dosage, in addition to their cheaper price compared to the synthetic medicines (Sunarsih *et al.* 2007).

There are some plants which have been studied and have been indicated to have anti-diabetic activity including gods crown, makasar fruit, brotowali, and nony. Some of these plants belong to the family *Acanthaceae*, making other plants of this family likely candidates to have anti-diabetic activity, one of which is the wungu leaf (*Graptophyllum pictum* (L.) Griff). The wungu leaf has been used by people in the healing of various diseases, such as hemorrhoids, ulcers, sore ears and stomachs, as well as facilitating the menstrual cycle for women (Dalimartha 1999).

Several other studies on wungu leaf are related to the effect of the active compounds found in the wungu leaf such as alkaline phosphatase activity (Widyowati 2011), isolation of the main metabolite compounds [*Graptophyllum pictum* (L.) Griff and BSLT (Brine Shrimp Lethality test) (Zuhra and Lenny 2005)], *in vivo* studies of wungu leaf extracts such as wungu leaf estrogenic effects on ovariectomized mice (Suhargo 2005), as well as research on oxytocic and anti-implantation of the wungu leaf (Olagbende-Dada *et al.* 2009).

Research by Nurcholis *et al.* (2011) showed that a water-ethanol extract of the wungu leaf is effective as an inhibitor of α -glucosidase enzyme. The work of Olagbende-Dada *et al.* (2011) also reinforces the wungu leaf as an anti-diabetic agent from the results of studies showing the efficacy of hypoglycemic wungu leaf extracts. Existing studies have not revealed the mechanism of action of metabolite compounds wungu leaf which are effective as an anti-diabetic agent. Therefore this study was conducted to determine the activity of compounds from the medicinal plant wungu leaf in alleviating symptoms of *diabetes mellitus*.

2. MATERIALS AND METHODS

Materials

The leaves of *G. pictum* (L.) Griff were collected from the Conservation and Cultivation Unit of the Biopharmaca Research Center, Bogor Agricultural University. Ethanol 96%, diethyl ether, ethyl acetate, butanol, glibenclamide, alloxan, tween 80, were obtained from pharmacy shops in Bogor, Indonesia. The mice *Sprague-dawley* strain were obtained from Faculty of Veterinary Medicine, Bogor Agricultural University. All of the chemical and the solvents used were of analytical grade.

Extraction Plants Wungu leaf

Extraction was done by maceration with a comparative range of samples and solvents (ethanol 96 %) 1:10 for 1x24 hours, 1 minute during the first 6 hours plaud in a shaker according to the procedures developed by Nurcholis *et al.* (2011). Samples were filtered and the filtrate obtained was collected and evaporated with a rotary evaporator at a temperature of 40°C. The ethanol extract was dissolved in water, shaken and extracted by partitioning with a separating funnel, first with diethyl ether (2x50 mL), followed by ethyl acetate and finally with butanol. Furthermore, each filtrate was evaporated on a rotary evaporator to yield diethylether, ethylacetate, and butanol extracts.

Treatment to the experimental animals

The experimental animals were male mice around 2-3 months-old weighing 140-210 grams. Diabetes was induced by alloxan intraperitoneal injection (200 mg/kg weight). Treatment was divided into 7 groups (A , B , C , D , E , F , G) with the number of mice per group as follow

Table 1 Treatment of mice each group

No.	Group	Treatment
1	A	normal, non-alloxan treatment given 1 mL 5% tween 80
2.	B	positive control, glibenclamide 0.25 mg/kg BW in 1 mL 5% v/v tween 80
3.	C	negative control given 1 mL 5% v/v tween 80
4.	D	ethanol extract 50 mg/kg BW in 1 mL 5% v/v tween 80
5.	E	diethyl ether extract 50 mg/ kg BW in 1 mL 5% v/v tween 80
6.	F	ethyl acetate extract 50 mg/ kg BW in 1 mL 5% tween 80
7.	G	butanol extract 50 mg/ kg BW in 1 mL 5% tween 80

Table 1. The treatment was done over 14 days. Blood glucose was measured with a glucometer on the first day before the treatment and at 1 hour after administration of the treatment on days 4, 7, 10 and 14 (Rauter *et al.* 2009).

Statistical Analysis

The study design used was a completely randomized design using the SAS 9.1 program to analyze blood glucose levels, and followed

by the Duncan’s test, which consists of 7 treatments with each treatment consisting of 4 replicates.

3. RESULTS

Data showed that the wungu leaf extracts have the ability to decrease the blood glucose level of alloxan-induced hyperglycemic mice. The blood glucose levels of each mice group

Table 2 Data on average of blood glucose levels (mg/dL) of each group of mice

Group	day 1	day 4	day 7	day 10	day 14
A	62.2±10.7a	81.5±5.2b	98.7±14.5a	84.7±12.7c	79.0±14.3b
B	59.0±7.8a	173.2±324.0a	138.5±60.7a	85.0±11.2c	75.2± 8.2b
C	55.7±12.0a	141.3±41.6a	138.3±34.2a	130.0±17.5a	117.2±12.4a
D	57.2 ±10.7a	132.3±38.0a	125.0 34.5a	112.0±17.8ab	89.3±26.6ab
E	58.7±16.0a	131.0±36.3a	120.3±29.2a	98.0±4.9bc	91.5±12.6ab
F	55.0±5.8a	130.0±2.16a	117.0 ± 9.2a	82.0±14.5c	81.0 ± 8.8b
G	53.2±11.5a	138.0±53.7a	121.5±33.1a	78.5±18.0c	93.0±29.1ab

Description: The index numbers followed the same letter within a column indicate no significant difference between treatments ($p > 0.05$)

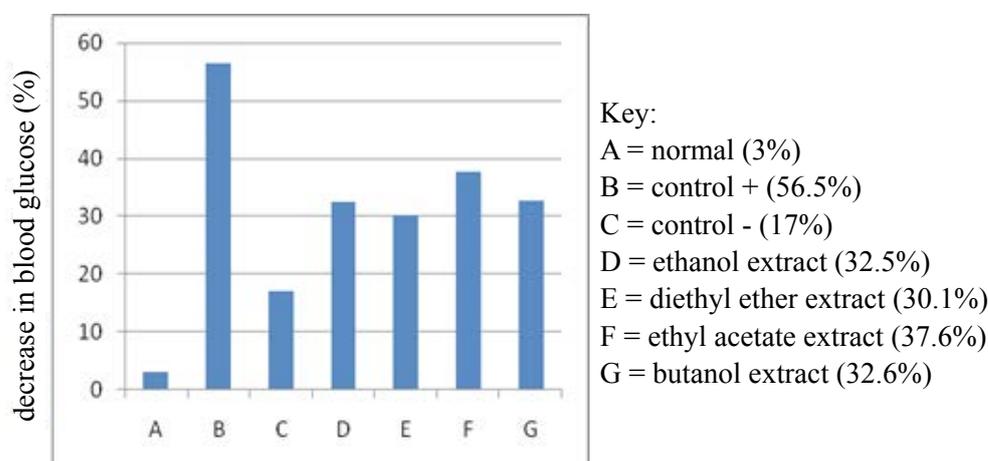


Figure 1 The percentage of glucose-decreased levels in blood of each treatment-group

on day 1, day 4, day 7, day 10 and day 14 are shown in Table 2. The percentage of glucose level decrease of each group is shown in Figure 1. The reduction of blood glucose level due to treatment with wungu leaf extract ranged from 32.5% to 37.6%. The most active extract was the ethyl acetate extract bringing about a decrease blood glucose level of 37.6%.

4. DISCUSSION

From Table 2, blood glucose levels in mice in group A showed normal blood glucose levels. This was due to untreated mice being force-fed with only 1 mL of 5% tween 80. On the first day the blood glucose level was 62.2 ± 10.7 with a 23.6% increase on day 4 and a 17.4% at day 7. While on day 10 it decreased by 16.5%, and it decreased on day 14 by 7.2%. However the group A blood glucose value is still in the normal range.

In group B (positive control) mice 200 mg/kg BW were alloxan-induced and forced fed 0.25 mg/kg BW/day glibenclamide in 5% tween 80. On day 1 of normal blood glucose levels the value was 59.0 ± 7.8 and on day 4 it rose 65.9% after alloxan treatment. This suggests that alloxan had an effect on the mice body marked by the rise of blood glucose which exceeded normal limits. Alloxan works by destroying pancreatic β -cells by inducing the formation of hydroxyl-free radicals which then attack the pancreatic β -cells essential component such as the plasma membrane, lysosomes, mitochondria and DNA and into the early destruction of pancreatic β -cells (Fahri *et al.* 2005)

Blood glucose levels drop back down on days 7, 10, and 14 respectively 20.0%, 62.9%, 13.0%. This is because the provision glibenclamide which can inhibit pancreatic β -cell

damage caused by alloxan. Based on the results of the statistical analysis, the glibenclamide treatment significantly affected blood glucose mice at all times observed according to the fact that glibenclamide works principally as one of a group of sulfonylurea derivative drugs that have the potential of lowering blood glucose levels better than other types of sulfonylurea compounds (Fahri *et al.* 2005).

In group C (negative control) mice were induced with alloxan 200 mg/kg BW and given 1 mL of 5% tween 80/days. Blood glucose levels were 55.7 ± 12.0 on day 1 and increased on day 4 to 60.5%, which was due to alloxan induction. After that the glucose level decreased on day 7, 10, and 14, respectively by 2.1%, 6.3%, 10.9%. This could be caused by environmental conditions that result in stress leading to active movement, thus increasing tissue glucose utilization. This condition causes the blood glucose levels in mice to decrease.

In group D (ethanol extract of 50 mg/kg BW/day in 1 mL of 5% tween 80) on day 1 blood glucose levels were 57.2 ± 10.7 and increased by 56.7% and decreased again on day 7, 10, 14, respectively by 5.8%, 11.6%, 25.4%. At the end of treatment blood glucose levels returned back to normal. The decrease in blood glucose levels is presumably because of the metabolic compounds contained in the wungu leaf extract which are non-toxic alkaloids, glycosides, steroids, and saponins (Dalimartha 1999).

In group E (diethyl ether extract of 50 mg/kg bw / day in 1 mL of 5% tween 80) on day 1 blood glucose levels were 58.7 ± 16.0 , decreasing on day 4 to 55.1% and decreased again on days 7, 10, 14 by 8.8%, 22.7%, 7.1%. This is because the diethyl ether-extracts-effect is thought to contain metabolic compounds. These

metabolites in the mice body play a role in the mice to inhibit pancreatic β -cells from damage.

In group F (ethyl acetate extract 50 mg/kg BW/day in 1 mL of 5% tween 80) on day 1 blood glucose levels were 55.0 ± 5.8 and increased by 57.6% and decreased again on day 7, 10, 14 by 11.1%, 42.6%, 1.23%. The decrease in blood glucose levels at the end of the treatment showed that the extract F can restore the function of the pancreas cell to their initial level, so that insulin can be produced again by the mice body, resulting in constant low blood glucose levels.

In group G (butanol extract of 50 mg/kg BW/day in 1 mL of 5% tween 80) on day 1 blood glucose levels were 53.2 ± 11.5 and an increase of 61.4% on day 4 and again decreased on day 7, 10, 13.5%, 54.7%, and on day 14, blood glucose levels go up by 14.5%. It is predicted that the butanol extract was less stable in the mice pancreas and was not fully functioning to produce insulin, so the blood glucose levels rise again.

Fig 1. shows that wungu leaf extracts can lower blood glucose levels of mice, the most effective extract at the end of each treatment was the ethyl acetate extract with a decrease of blood glucose level of 37.6%, after glibenclamide which caused a 56.5% reduction of glucose level. The second best extract was the butanol extract (32.6% glucose reduction), and the least-well perform extracts were the ethanol extract (32.5% glucose reduction) and the diethyl ether extract causing 30.1% glucose reduction. butanol extract was less stable in the mice pancreas and was not fully functioning to produce insulin, so the blood glucose levels rise again.

These data are supported by the statistical analysis showing that the end of treatment the

blood glucose level was significantly different ($p < 0,05$). This is presumably because of the metabolic compounds contained in the wungu leaf which are more soluble in the solvent ethyl acetate (semi polar) compared to the polar solvents such as butanol, ethanol and the non-polar solvents such as diethyl ether. In conclusion, wungu leaf extract at a dose of 50 mg/kg BW can decrease blood glucose of alloxan induced mice. The best extract is the ethyl acetate extract with an efficiency in decreasing blood glucose of 37.6%.

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