

The Use Extract of Siam Leaf *Eupatorium odoratum* L. as Alternative Material In Lowering Blood Glucose

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Abstract

The use of medicine as oral hypoglycemic and insulin have some limitation, the treatment for diabetics needs more expensive cost relatively because it uses in the long term and can give the unwanted side effect. At the recent time, one of the conducted efforts is by using the plant as alternative medicine. This research aims to know the potential of leaves' extract and to know the effectiveness of siam weed leaves extract's dosage which most effective in lowering the level of blood glucose in mice which is induced by alloxan. On this research, anti-diabetic effect of siam weed leaves was observed to male mice which had been induced by alloxan until the level of blood glucose >200 mg/dl. The given treatment orally was conducted during seven days with three variations of dosage concentration of infused extract, they are 5%, 10% and 20%. Furthermore, it was also used the comparison i.e. infused extract of cinnamon bark with 10% concentration. Data analyse used SPSS 23.0 program with one way ANOVA then was continued by using Duncan test. The research result shows that the siam weed leaves extract with dosage concentration 5%, 10% and 20% can decrease blood glucose level on mice that have suffered hyperglycemia and most effective on 20%. Thus, it can be concluded that siam weed leaves extract have the potential in lowering blood glucose.

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Introduction

Hyperglycemia is the condition where the blood glucose level exceeds normal limits. The increasing of glucose level in blood plasma (hyperglycemia) is one of bases in diagnosis of diabetes mellitus. It is caused by the abnormality of metabolism, specifically the abnormality on carbohydrate metabolism (Wulandari, 2010). The treatment on people with diabetes mellitus needs relative more expensive cost because it uses in long term and can give unwanted side effect. Furthermore, the medicine likes as oral hypoglycemic and insulin have some limitation. Oral hypoglycaemic medicine in sulfonylurea group can cause gastrointestinal disorder such as qualm, diarrhea, stomach-ache, and hyper secretion of gastric acid (Jung *et al.* 2006 in Sakika *et al.* 2015; Dalimartha and Adrian, 2012 in Pasaribu *et al.* 2012). Therefore, it needs the effective medicine, the relative low side effect, the relative

cheap and affordable cost. One of conducted efforts is by using plant as alternative medication. It encourages the amount of research about the plants which have the potential to decrease blood glucose level, one of the plant which has the potential as the lowering of blood glucose is siam weed *Eupatorium odoratum* L.

Siam weed *Eupatorium odoratum* L. is one of the most abundant weed plants. Weed is one of plant pest organism which inhibit the growth, development, and productivity of cultivated plants. The existence of weeds at agriculture area generally gives the negative effect toward the plants, because weeds have the high competitive capacity thus enabling light, CO₂, water, nutrient, and space to grow competition which were used simultaneously (Riry, 2008 in Palijama *et al.* 2012), because of its abundant availability, then it is very potential to be utilized.

Siam weed includes to Asteraceae family, traditionally is used to healing the wounds and infection, headache, diarrhea, astringent, antispasmodic, antihypertensive, anti-inflammation and diuretic (Vital and Rivera, 2009 in Yenti *et al.* 2014). Siam weed leaves contain some main compounds such as tannin, flavonoid, saponin, and steroid. Essentials oil of siam weed leaves has a substance of α -pinene, cadinene, camphora, limonene, β -caryophyllene and candinol isomer (Benjamin, 1987 in Yenti *et al.* 2014). The research of Alisi, *et al.* (2011), in Marianne *et al.* (2014), explained that siam weed has the potential as antioxidant and able to prevent free radicals which is believed as the cause of various degenerative disease and premature aging. And also there was a research which claimed that these siam weed leaves' extract able to be as anti-diabetic (Murthy and Kiran, 2012; Raman *et al.* 2012; Marianne *et al.* 2014). Based on that, then need to be conducted the research about the potential of siam weed leaves' extract to know the bioactive compounds which contained in that plant, thus made as one of alternative in lowering the level of blood glucose.

Materials and Methods

Tools

The tools were used knife/hook, animal raising cage, gloves, drinking water place, analytical balance, blender, jar, mess, oral sonde, mortar and pestle, syringe, syringe 1 cc, stirring rod, spatula, wiper, digital scales, hot plate, stirrer, infused pen, Buchner funnel, sprayers, thin layer chromatography plate, glucometer (Nesco[®]), measuring cup, surgical scissor, measuring flask, vial bottle, alcohol thermometer 10-110 °C, and glass tools (Phyrex).

Materials

The materials which were used are siam weed leaves *Eupatorium odoratum* L. which were obtained from Kassiloe Village, Labakkang District, Pangkep Regency. The material were used aquades, dragendorf reagent, chloride acid (HCl), *CarboxiMetilCellulosa* Na (Na-CMC), standart mice' food, glibenclamide 5 mg, alloxan monohydrate, cinnamon bark *Cinnamomunburmanii*, glucotest stripe, aluminum foil, picric acid, API (Aqua Pro Injection), filter paper, cling wrap, and label. The used test animals are male mice *Balb C* around 18 mice with the age 3-4 months, the average body weight 20-35 gr, healthy and behave normally, which were obtained from Bio pharmacy Laboratory of Pharmacy Faculty, Hasanuddin University. Before used, the mice were acclimatized during 7 days with laboratory condition.

Ethical Clearance

This research had been evaluated and gained the ethical approval recommendation of Medical Research Ethical Committee, Medical Faculty, Hasanuddin University, No.318/H4.8.4.5.31/PP36-KOMETIK/2018, 23th April 2018.

Research framework used complete randomized design with the used treatment group namely:

- K1 : Negative Control (Na-CMC 1%)
- K2 : Positive Control (glibenclamide) 0,083 mg/kgBB
- K3 : Comparison Group (Cinnamon infused extract 10%)
- K4 : Treatment I Group (Siam weed leaves infused extract 5%)
- K5 : Treatment II Group (Siam weed leaves infused extract 10%)
- K6 : Treatment III Group (Siam weed leaves infused extract 20%)

Making Infused Extract of siam weed Leaves

Siam weed leaves were washed and air dried did not under direct sunlight during 5 days until the leaves become brown. Furthermore, the extraction done by using infused method. The process of making extraction that is the sample mashed into powder. After that, it was sifted by using mess in order to gain the powder in the certain subtle degree. After that, it put into the top infused pan then was poured with water as much 100 mL, the bottom of pan was filled with water until full then was heated until the water in the pan boiled. Furthermore, the temperature of extract in the pan was measured by using alcohol thermometer until its temperature gained 90°C. After gained 90°C temperature, waited during 15 minutes, while occasionally steering. The result of infusion was filter while still hot by using Buchner funnel which coated with filter paper until gained the filtrate. The filtrate was the extract of siam weed leaves. The making of infusion's result with concentration 10% then the powder of leaves were weighed was 10 grams then were added water with volume 100 ML., the it will be gained the concentration 10% (Kusumaningrum *et al.* 2013). To make the infusion result with concentration 5% and 20% were made with similar way, by using 5 grams and 20 grams powder of siam weed leaves (Radiansah, 2013).

Phytochemical Test of Infused Extract of siam weed Leaves

After gained the infused extract of siam weed leaves, then conducted phytochemical analysis This phytochemical analysis was conducted in order the contained compounds of infused extract in siam weed leaves can be known. Phytochemical test was based on Harborne (1996) in Halim *et al.* (2015), to know the existence of active compounds such as alkaloid, flavonoid, saponin, and tannin. This phytochemical test was conducted by using tube test and thin layer chromatography plate test which was given reagent appropriate with the wanted compounds to be gained then was seen the occurrence of color change.

Treatment toward Animal Test by Using Alloxan Induced Method

The animals which were used as much 18 male mice in where divided into six groups. Before conducting the test of anti-diabetic effect, the tested animals were fasted during 8 hours. After fasted, the level of blood glucose of all mice was observed to determine blood glucose level of early fasting (GDPO). After that, induced by alloxan in dosage 3,5 mg/kgBBsubcutaneously. Then let it during 3 x 24 hours until occurring the increasing effect of stable blood glucose' level (Marianne *et al.* 2014). Furthermore, was conducted the measurement of fasting blood glucose' level (GDP1), the mice which had been experienced

the increasing of blood glucose' level (>200 mg/dl) were clarified hyperglycemic, in this case, it was ready to be given the treatment. The treatment was given by giving test supply (the infused extract of siam weed leaves) orally (p.o) once per day during 7 days, with the dosage concentration of extract respectively 5%, 10% and 20%. The level of blood glucose (mg/dl) were measured at the early (before treatment), after inducing alloxan (post-alloxan), and the fasting blood glucose' level after 7 days treatment (GDP2) on each group (Marianne *et al.* 2014). The parameter are the occurrence of decreasing blood glucose' level after the animals became diabetics, compared to the negative control (Na-CMC 1%), positive control (glibenclamide) and comparison (infused extract of cinnamon bark). Each of test group used 3 mice.

Examining The Level of Blood Glucose

The level of blood glucose (mg/dl) was determined by using glucometer (Nesco® *Multicheck 1*). Glucometer was activated, and then was put chip and strip on the tool. The blood of mice were taken from the part of their tails then were dropped on the tip of strip and automatically the level of blood glucose will appear on the monitor screen.

Data analysis

Data analysis was processed statistically by using software test *SPSS 23.0 for Windows*. The measurement result of all parameters in this research were analyzed statistically by using one way ANOVA, was conducted the continued test by using Duncan test

Results and Discussion

Phytochemicaltest of infused extract on siam weed leaves

The extract was obtained through infusion method by using water solvent (aquades). The process of extract making observed excessively in temperature and making time to minimalize the damage on the compound which contained in siam weed leaves, specifically on the heating process. Phytochemical analysis was conducted qualitatively to know the compound that has the role as anti-diabetic. The result of photochemistry analysis of infused extract can be seen as the following:

Table 3. Result of extract photochemistry analysis

Numb.	The sort of testing	Reagent	Result	Note
1.	Flavonoid	Sitroborat	Form the green colour	+
2.	Saponin	The heat water + HCl	Form the stable foamas high as 1-3 cm	+
3.	Alkaloid	Dragendorff	Form the red-brown colour	+
4.	Tanin	FeCl ₃	Form the blue colour	+

Note: (+) = contained testing compound

(-) = did not contain testing compound

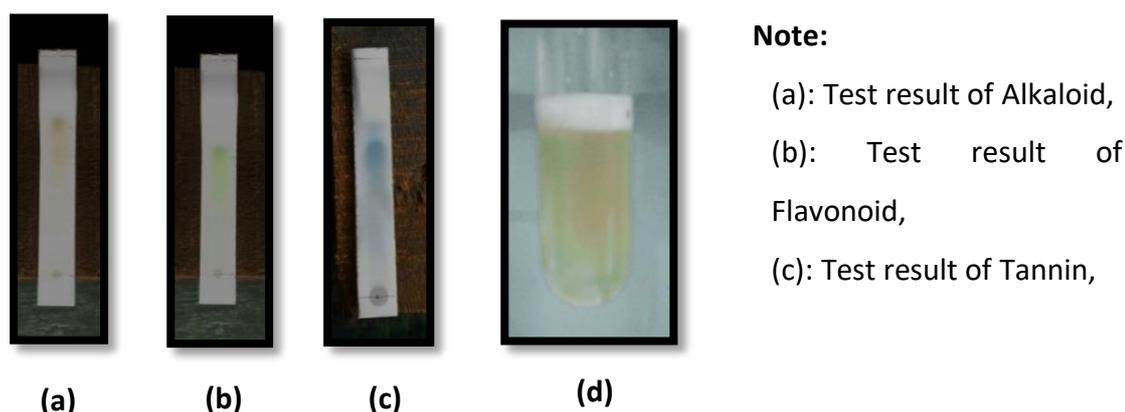


Figure 1. Result of extract phytochemical analysis

According to Prameswari *et al.* (2014), from the fourth compound, alkaloid and flavonoid are the natural active compound which known have hypoglycemic activity. Flavonoid is one of secondary metabolic compound which can be found on the plants tissue, Can has the rule as anti-oxidative compound by giving one of its hydrogen atom (Redha, 2010). Tannin is the component of very complex organics substance, consisted of phenolic compound which hard to be separated and is the secondary metabolite active compound which has some benefits such as astringent, antidiarrheal, antibacterial, and biological antioxidant (Malangngi *et al.* 2012). The function of tannin as astringent can pucker up the epithelium of small intestine's wall thus decreases the absorption of food essence and inhibits the intake of glucose in blood (Prameswari *et al.* 2014). Saponin (triterpenes) based on Kirwanto (2014), it works in similar activity with insulin reaction, thus can take blood glucose into cells. Moreover, according to Prameswari *et al.* (2014), alkaloids compound works by stimulating hypothalamus to increase the secretion Growth Hormone Releasing Hormone (GHRH), thus the secretion of Growth Hormone (GH) on hypophise increased. The high level of GH can stimulate the liver to secrete *Insulin-like Growth Factor-1* (IGF-1). IGF-1 has the effect in inducing hypoglycemia and decreasing gluconeogenesis thus the level of blood glucose and the need of insulin lowered.

The Level Of Blood Glucose After Treatment

Table 4. The influence of giving Infused Extract from Siam Weed leaves Toward lowering the level of blood glucose on male mice

Numb.	Treatment	N	The Average Level of Blood Glucose from Tested Animals (mg/dL)			GDP1-GDP2 (mg/dL)
			GDP0	GDP1	GDP2	
1.	K1	3	74,6	254	238,6	68
2.	K2	3	83,3	213,6	79	134,6
3.	K3	3	76,3	426	137	289
4.	K4	3	96	486,3	296	190,3
5.	K5	3	72,6	222,6	132,3	90,3
6.	K6	3	67,6	382,3	137,6	244,6

Note:

- K1 : Negative Control (Na-CMC 1%)
- K2 : Positive Control (glibenclamyde) 0,083 mg/kgBB
- K3 : Comparison Group (Cinnamon infused extract 10%)
- K4 : Treatment I Group (Siam weed leaves infused extract 5%)
- K5 : Treatment II Group (Siam weed leaves infused extract 10%)
- K6 : Treatment III Group (Siam weed leaves infused extract 20%)
- GDP0 : fasting blood glucose before treatment (mg/dl)
- GDP1 : the early fasting blood glucose after inducing alloxan (mg/dl)
- GDP2 : Fasting blood glucose after 7 days treatment (mg/dl)
- GDP1 – GDP2 : the lowering of blood glucose (mg/dl)

The result of measuring blood glucose' level of mice before and after treatment (table 4), can be drawn into histogram which served the average level of blood glucose of mice, before treatment, after inducing alloxan and also after 7 days of given treatment, as following:

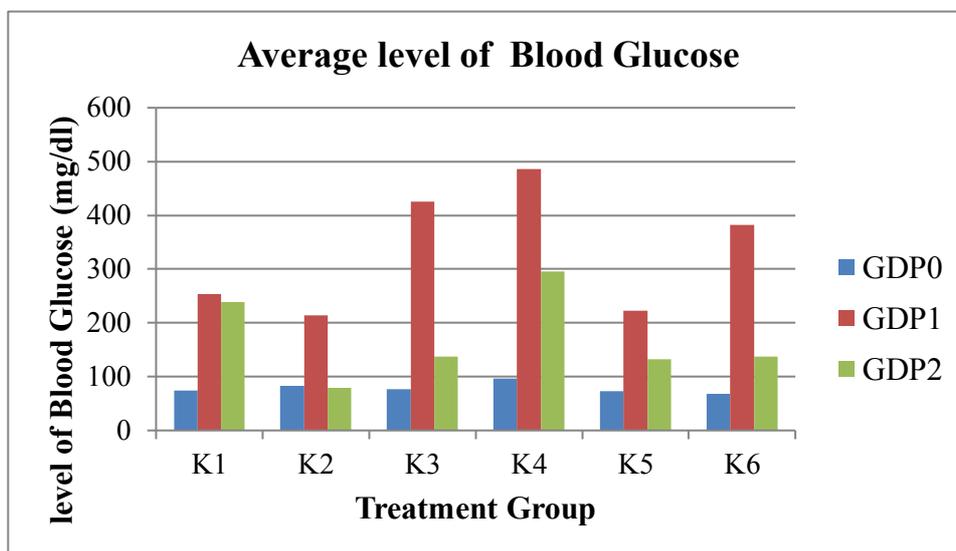


Figure 2. Histogram of Average Level of Mice Blood Glucose

The above histogram shows the existence of different in average level of mice blood glucose on the three times of measurement. GDP0 shows the measurement of early fasting blood glucose' level that is before the treatment, which shows that the average level of fasting blood glucose has enormity which almost similar on each group that still stands at the normal level (<100 mg/dl). The result of measuring GDP1 shows the average level of mice blood glucose begin to increase on each group (>200 mg/dl), which shows the occurrence of hyperglycemic after inducing monohydrate alloxan as diabetogenic agent. The alloxan selectively can damage β cells from Langerhans island with secreted insulin hormone. The work mechanism of alloxan is by putting Ca ion into mitochondria cell which cause the process of cell oxidation disturbed. Damaging β cells of pancreas causes the lowering of insulin secretion (Panjuantiningrum, 2009).

Furthermore, on measurement GDP2 that is after treatment, seen that there are various measurement on each group. The average level of blood glucose on K1 (negative control) still be categorized hyperglycemia. Whereas, on K2 (positive control), K3

(comparison), the treatment on dosage I, II and III of blood glucose' level had been decreased. The average deviation between GDP1 and GDP2 can be seen on the following histogram :

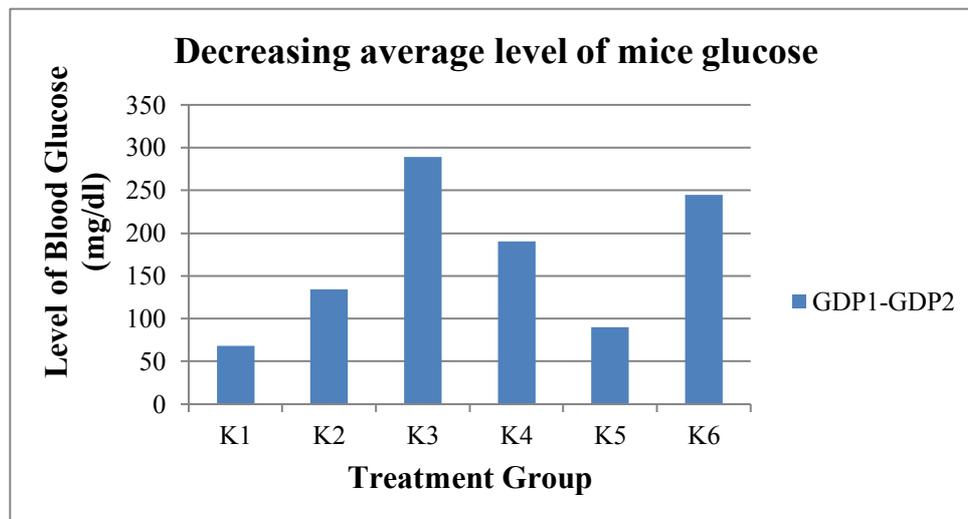


Figure 3. Histogram of decreasing average level of mice glucose (GDP1-GDP2)

Based on above histogram, can be seen the enormity of blood glucose decreasing level on each treatment group. On K1 (negative control) can be known that the occurrence of the lowest blood glucose decreasing level compared to all treatment groups. Whereas on the given treatment of glibenclamide (K2: Positive control), cinnamon extract (K3: comparison), dosage of extract I (K4), dosage II (K5), and dosage of extract III (K6) occurred the large enough decreasing of blood glucose level. This research result shows that by giving infused extract of siam weed leaves can decrease the best blood glucose' level on the concentration 20%. This case can occur because there is antioxidant compound which is had by siam weed leaves. Based on the research which conducted by Marianne *et al.* (2014), that siam weed leaves have antioxidant and cytoprotection activity. Antioxidant activity can ward off free radicals which occur because of giving alloxan, thus the damage of β cells pancreas can be prevented and can repair the function of β cells pancreas so after 7 days of extract giving occurred the decreasing of blood glucose' level on each tested animal.

In accordance with the research which conducted by Vijayaraghavan *et al.* (2013), said that after doing phytochemicals analysis of this siam weed leaves was known that this plant contains various antioxidant compound such as tannin, saponin, flavonoid, beta cyanins, quinones, glycosides, cardioglycosides, terpenoid, phenol, cumarin, steroid, and alkaloid. On this research also was done the phytochemicals test qualitatively and was obtained 4 compounds such as flavonoid, alkaloid, saponin, and tannin. According to Marianne *et al.* (2014), flavonoid is known as inhibition of hydroxy and superhydroxy radicals which can protect the membrane lipid of β cells pancreas toward damaging reaction. According to Widowati (2008), there are some plant mechanisms in lowering blood glucose' level that is the plant has ability as astringent which can precipitate the protein of intestinal mucous membrane and form a layer which protects the intestine, thus can inhibit the glucose' supply and increasing rate of glucose in blood is not too high. Moreover, there is also plant which decreased blood glucose' level by accelerating the secretion of glucose from circulation and by accelerating blood circulation that is through the work of heart and excretion of kidney, thus the secretion rate of urine increased. And there is also plants which accelerate the secretion of glucose through increasing of metabolism or entering into fat deposit. This

process involves pancreas to produce insulin. According to Subroto (2006), there are 5 activities of plant in healing diabetes. There is a plant with activity to inhibit alpha-glycosidase (inhibitor), the plant with activity to stimulate insulin secretion, there is also a plant with activity to repair the function of insulin, there is also plant which has double activities such as to inhibit alpha-glycosidase and to repair the function of insulin, and also a plant with complex activities such as to decelerate food digestion, to decrease the absorption rate of carbohydrate into the liver circulation, to influence the transport of glucose which is mediated by Nitric Oxide (NO) and to modulate the insulin secretion which mediated by NO. Decreasing of blood glucose occurs because of there is antioxidant compound activity which contained in siam weed leaves such as flavonoid, saponin, tannin, and alkaloid.

From the normality test is obtained the significance *Shapiro Wilk* as much 0,536. This result $>0,05$, then data distributed normal. Moreover, on the homogeneity test used *Levene Test*, the data observed was not homogeny, then was transformed thus was obtained significant score as much 0,082 where higher than $>0,05$ that showed there was homogeny variation, therefore the assumption of ANOVA test's requirement had been fulfilled. The result ANOVA test GDP1-GDP2 (Appendix 5), can be known that the result of ANOVA test was gained p-value $> 0,05$ is 0,318 then there is no real influence between treatment because p-value $>0,05$, thus it cannot be continued with post hoc test (Duncan = DMRT).

Conclusions

From the result of research which have been conducted can be concluded that the infused extract of siam weed leaves *Eupatorium odoratum* L. have potential in lowering the level of blood glucose on male mice *Mus musculus* L. with the most effective dosage of concentration on concentration 20%.

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