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Antidiabetics Activity of Koja Bay (*Murraya koenigii*) Leaves Tea Bag

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ABSTRACT

Background: Koja bay leaves *(Murraya koenigii*) which mostly cultivated in Indonesia and the leaves usually used by Aceh people as a flavoring agent for food, has been known to have an antidiabetic effect. In this study, we analyzed the antidiabetic effect of koja bay leaves tea bags which commercially available. **Methods:** The two variant doses of koja bay leaves tea bag (411.11 mg/g body weight and 822.22 mg/g body weight) were administered to 25 male rats which divided into five groups. The antidiabetic activity was determined by measuring blood glucose of rats administered by koja bay leaves tea bag compared to blood glucose of rats in positive control which was given glibenclamide negative control, and normal control. **Results:** The results showed that dose 1 and 2 decreased blood glucose level by 51.27% and 45.17%, respectively, fairly similar to the glibenclamide (63.38%). **Conclusion:** Koja bay leaves tea bag have comparable activity with glibenclamide in reducing the blood glucose level.

***Keywords**: koja bay leaves; tea bag; antidiabetic*

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INTRODUCTION

Herbal as a medicine widely used for generations for a long time ago because of its potential as a preventive and curative treatment. Recently, there is a rise in using medicinal plants as therapeutic drugs due to their phenolic compounds, specifically to flavonoid that could reduce the risk of diseases (Maharaja, 2015). Koja bay leaves *(Murraya koenigii*), is one of the medicinal plants in Indonesia, that the leaves well-known to reduce obesity, diabetes, and hypertension aside as curry flavor by Acehnese people. Previous studies reported koja bay leaves has many pharmacological activities such as antidiabetic, antihypertension, anti-microbial, antidiarrheal, anti-inflammatory, antifungal, antioxidant, and antihyperlipidemia. In 100 g, koja bay leaves contains protein (1%), fat (1%) carbohydrates (16%), fiber (6.4%) minerals (4.2%), calcium (810 mg), phosphorus (600 mg), iron (2.1 mg), carotene (12,600 IU) nicotinic acid (2.3 mg), vitamin C (24 mg), 34 essential oils such as α-pinene (51.7%), β-phellandrene (24.4%), sabbene, β-pinene, β-caryophyllene, limonene, bornyl acetate, terpinen-4-ol, ϒ-terpinene and α-humulene, the alkalies of mahanimbine, girinimbina, carbazole, isomahanimbine, koenimbidine, tannin, flavonol, isoflavones, and some alkene organic compounds.The chemical constituent of mahanimbine which present in *M. koenigii* has been studied for antidiabetic activity in streptozotocin-induced diabetic Swiss mice (Tembhurne et al., 2010; Bhat et al., 2012, Imad et al., 2017). Mahanimbine was shown to decrease the blood glucose levels by enhancing insulin effect through increasing the peripheral glucose uptake or secretion by islets of Langerhans of pancreatic beta cells. Mahanimbine also showed a significant alpha-amylase inhibitory effect compared to acarbose.The glucose-6-phosphate dehydrogenase enzyme level was also increased which leads to a normal glucose uptake (Dineshkumar *et al*., 2010). Khan *et al*., 1995, reported that Koja bay has hypoglycemia effect, however the plant has not been further investigated. Previous research used chloroform for Koja bay leaves extraction, but in this study we used a self made infusion of tea bag Koja bay. Therefore, we studied Koja bay leaves for its effectiveness on reducing blood glucose in alloxan-induced diabetes in an experimental rat model. Alloxan is a toxic glucose analog which selectively destroys insulin-producing cells in the pancreas when administered to rodents and many other animal species, causing an insulin-dependent diabetes mellitus (called "Alloxan Diabetes") with the characteristics are similar to type 1 diabetes in human (Lenzen, 2008).

METHODS

Experimental Animals

Male and female Wistar albino rats weighing about 150±200 g obtained from IPB (Institute Pertanian Bogor) were used for the study and were housed in open-air cages and maintained at 23±20ºC with 12h light/dark photoperiod. They were fed a standard rat pellet diet from IPB and water was provided ad libitum. All the experiments on animals were conducted according to the ethical norms approved by The Ethics Committee of

Table 1. Treatment table

Group	I	II	III	IV	V
Treatment	Normal Control	Negative Control	Positive Control	Dose 1	Dose 2
Acclimatization	Acclimatization for 7 days				
Day 1	-	Induction with alloxan monohydrate			
Day 4	Blood Collection (initial glucose measurement)				
Day 5-11	Given aquadest	Given aquadest	Given glibenclamide	Given infusion koja bay leaves dose 1	Given infusion koja bay leaves dose 2
Day 12	Blood collection (final glucose measurement)				

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Materials

Koja bay leaves (from dried sample), aquadest, testing reactant, bouchardat reactant, dragendorf reagent, meyers reagent, HCL (Brataco), $FeCl_3$ (Brataco), food pellets (IPB), alloxan (Sigma), glibenclamide (Kimia Farma), fructose (Brataco), NaCl (Brataco). Oven, milling, strainer, tea bag, filter paper, feeding tube, disposable spuit, cage, water bath.

Alkaloid Identification of Koja Bay Leaves (Anonim, 1993)

A total of 0.05 g sample was introduced in the test tube, then added 1 ml of HCl 2 N and 9 ml of aquadest, heated over a water bath at 100°C for 2 min, then cooled and filtered. The filter results are removed and divided into two test tubes. The dragendorf and meyers reagent was added to the first and second tubes, respectively for alkaloids identification. The result showed red sediment for dragendorf reaction and white sediment for Meyers reaction.

Flavonoid Identification of Koja Bay Leaves

A total of 0.05 g sample was added by methanol, heated in a water bath at 100 °C and filtered. The filtrate was added by HCl and Mg metal and formed a red color that indicates the presence of flavonoids (Qurrata, 2013).

Saponin Identification of Koja Bay Leaves

A total of 0.05 g sample was added by 10 ml of hot water then cooled and shaken for 10 minutes to form a steady foam for not less than 10 minutes, as high as 1 to 10 cm. One drop of 2 N hydrochloric acid was added into the foam. The presence of saponin in the sample is indicated by the remained foam.

Tannin Identification of Koja Bay Leaves

A total of 0.05 g sample was added by 10 ml of water, boiled in a water bath at 100 °C for 5 minutes, then cooled and filtered. The filtrate was added 1-2 drops FeCl3 1%. If dark blue or dark green color formed, indicated the presence of tannin.

Antidiabetic Activity Test

Alloxan-induced diabetes

Alloxan monohydrate used to induce diabetes by single IP injection (120 mg/kg). After 72 hours injection, the diabetic rats (glucose level>250 mg/dl) were separated and used for the study. Fasting blood glucose level was monitored in blood samples using a glucometer before administration of the drugs.

Preparation of control positive

A total of 5 mg glibenclamide was spread into a mortar that contains 100 ml of aquadest and stirred firmly until it was homogeneous and obtained the solution concentration of 0.05 mg/mL of glibenclamide.

Infusion of Koja Bay Leaves

A total of 2.05 g dried koja bay leaves were soaked with 50 mL of hot water to obtain 41.11 mg/mL concentration. A total of 4.11 g dried koja bay leaves were soaked with 50 mL hot water to obtain concentration 82.22 mg/mL.

Experimental Design

The rats used in this research were 2-3-year-old male Wistar albino rats with body weight between 150-200 gram. All animals were acclimatized for one week and were given standard food. The mice were grouped and weighed according to the experimental design. Group I was normal control where rats were not given treatment and only given drinking water. Group II-V were induced by alloxan through IP injection. After three days of induction, the blood was taken through a vein in the tail

Table 2. Result of phytochemical substances identification

NO	TEST	REAGENTS	RESULT
1	Alkaloid	Meyers reagent	+
2	Saponin	HCl 2N	+
3	Tannin	$FeCl_3$	+
4	Flavonoid	HCl concentrate +Mg	+

Table 3. The effect Koja bay leaves infusion on serum glucose

Parameters		Group I	Group II	Group III	Group IV	Group V
		Normal Control	Negative Control	Positive Control	Diabetic treated with 411.11mg/kg body weight of koja bay leaves infusion	Diabetic treated with 822.22 mg/kg body weight of koja bay leaves infusion
Serum Glucose (mg/dl)	Before	111.4 ± 3.06	393.8 ± 2.66	365 ± 1.06	332.2 ± 0.06	370.4 ± 0.68
	After	102.6 ± 4.25	374.8 ± 3.25	144.2 ± 4.20	159.4 ± 0.25	220.2 ± 1.25

to measure blood glucose levels (initial glucose). In day 5, each group was given the treatments (the treatment table was shown in Table 1). Group IV and V were given test preparation. The positive control (Group III) was given glibenclamide, while the negative control (Group II) and normal control (Group I) was only given standard food and drink. In day 12, all of the mice's blood was taken again to measure the blood glucose level (final glucose). The percentage of inhibitory was measured following the formula:

$$\%\ lowering = \frac{beginning\ level - final\ level}{beginning\ level}\ x\ 100\%$$

Data Analysis

The result data of blood glucose lowering test was analyzed by one-way ANOVA test. The data was then tested with Tukey HSD test to see the difference among dose given.

RESULTS AND DISCUSSION

Antidiabetic Test Results of Koja Bay Leaves

Phytochemical screening results of koja bay leaves showed that the active ingredients contained were alkaloids, flavonoids, saponins, tannins, triterpenoids, and steroids. The screening test result was presented in Table 2. According to several studies, alkaloid and flavonoid compounds play a role in reducing blood glucose, cholesterol, and LDL levels. The alkaloid content found in koja leaves includes mahanimbicine and mahanimbine (Nagappan et al. 2011). In this study, we did not examine the levels of mahanimbicine and mahanimbine contained in koja bay leaves infusions, and those can increase insulin secretion in beta Langerhans cells or increase glucose uptake. The mechanism of action is to induce alpha amylase which inhibits the degradation of carbohydrates to become oligosaccharides and disaccharides. In other studies, it has also been shown that flavonoids that have hypoglycemic effects found in koja leaves are one of the quercetin. The quercetin mechanism is by stimulating insulin secretion from Langerhans island cells through changes in Ca^{2+} metabolism and induction of hepatic glucosidase causing hypoglycemic effects (Fauziah et al. 2014). Mahanimbine and quercetin do not cause hypoglycemia shock in DM research (Dineshkumar et al. 2010; Fauziah et al. 2014).

The results of pharmacological tests on experimental animals (Table 1) showed a decrease in glucose levels in the Group I (normal group) although not significant (from 111.4 mg/ml to 102.6 mg/ml). In the Group II (negative control group), there was an increase in blood glucose but decreased on day 12. The level of glucose in the serum of alloxan-induced diabetic rats was found to be significantly increased compared to control rats. In the Group III (positive control), there was a very significant decrease in blood glucose levels on day 12 from 365 mg/ml to 144.2 mg/ml. Group IV and V (the animal was induced with alloxan then given an infusion of koja leaves orally 411.11 mg/kg and 822.22 mg/kg body weight for 12 days, respectively) showed a decrease in blood glucose levels. The blood glucose level in the Group IV were decreased from 332.2 mg/ml to 159.4 mg/ml, while Group V showed a decrease in blood glucose levels from 370.4 to 220.2 mg/ml. There was statistical difference between the Group II (negative control) and Group III (positive control) ($p<0.05$).

However, none was significant between the Group III with Group IV and V (Table 3). It can be seen that the infusion of koja bay leaves can reduce glucose levels in the blood of alloxan-induced mice.

This study showed a less active results compared to previous studies which using chloroform extract (Vijayanand, 2015). They used 250 mg/kg/bw and 500 mg/kg/bw dose, with a better decrease of blood glucose level (118.5 ± 20.50 and 80.22 ± 03.63). This can be caused by the solvent and method used, which possibly the active substances in Koja bay leaves was extracted better in chlorofom compared to water infusion. Therefore, the water infused-koja bay leaves has a potential hypoglycemic effect in alloxan-induced diabetic mice, although not as good as chloroform extract, but comparable with glibenclamide.

CONCLUSIONS

Koja bay leaves tea bag have comparable activity with glibenclamide in reducing the blood glucose levels.

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