ANTIHYPERGLICEMIA ACTIVITY OF WATER FRACTION, ETHYL ACETATE FRACTION, AND N-HEXANE FRACTION OF SPINACH LEAVES EXTRACT (Amaranthus Cruentus L) ON MALE MICE SWISS WEBSTER STRAIN

Tita Nofianti[1],Muharam P [1], Tresna Asih [1]
School of Pharmacy, STIKes Bakti Tunas Husada Tasikmalaya,
Cilolohan street no 36 Tasikmalaya, Indonesia
Email: titanofianti.wamsu@gmail.com

ABSTRACT

The activity test ofthe water fraction, ethyl acetate and n-hexane fraction of spinach leaves (*Amaranthus cruentus* L) has been conducted using the glucose tolerance method on male mice Swiss webster strain. The dose were water fraction 0.086 g/kg bw, ethyl acetate fraction 0.03g/kg body weight and n-hexane fraction 0.055 g/kg bw. Time measurement of blood glucose levels was conducted every 30 minutes for 120 minutes using a glucometer. The results showed that administration of water fraction, ethyl acetate fraction and n-hexane fraction of spinach leaves can lowering the blood glucose level of white male mice. While the best group that lowering the blood glucose level was ethyl acetate fraction 0,03 g/kg bw when compared to water fraction and n-hexane fraction. It can be concluded that spinach leaves showed antihyperglicemia.

Keywords : *Spinach leaves, Amaranthus cruentus* L., antihyperglicemia

INTRODUCTION

Diabetes mellitus (DM) is a degenerative disease which prevalence is increasing in the world, both in developed countries or developing countries, so it is said that Diabetes has become a global health problem or illness in the community. The World Health Organization estimates that more than 346 million people worldwide have diabetes. This number is likely to more than double by 2030 without intervention. Almost 80% of diabetes deaths occur in low and middle income countries (Siraoka, 2012).

According to WHO, Indonesia ranks 4th in the number of people with diabetes mellitus in the world. In 2006 the estimated number of people with diabetes mellitus in Indonesia there are 14 million people, 50% are aware have it and 30% just camefor treatment on a regular basis (Nabyl, 2012).

The consequences of an increase in the incidence of diabetes is the increasing of health problems due to complications caused. Acute complications may include hypoglycemia, diabetic ketoacidosis, or hyperosmolar coma nonketotik. While the long-term complications include cardiovascular disease, chronic renal failure, and diabetic retinopathy (damage to the retina) (Siraoka, 2012).

Beside as a vegetable spinach plant is also, has long been used as a traditional medicinal plants by rural communities. Generally, people uses pinach as a cure of colon cancer, diabetes, cholesterol, hypertension, and kidney failure. For diabetics, the fiber in spinach leaves can slow the rise in blood glucose levels that occurs drastically and insulin response dole to resorption of carbohydrate does not take place all at once, but gradually (Dalimartha, 2004).

Based on previous studies the ethanol extract of spinach leaves has efficacious as antidiabetic. So we need further studies using the extract fractionation by a polar solvent, semi-polar and non-polar.

MATERIAL AND METHODS

Preparation of Animal Experiments

Experimental animals used were male Swiss Webster mice weighing between 20-30 gram, obtained from farms in the School of Life Sciences and Technology, Bandung Institute of Technology. Mice were then quarantined for 7 days and were divided into five groups, namely the two control groups and three test groups of fractions.

Preparation of Materials Research

Determination: The identity of collected material was confirmedby plant determinationin Herbarium of School ofLife Sciences and Technology, Bandung Institute of Technology.

The collection of materials: Materials used are spinach leaves (*Amaranthus Cruentus* L) parts of the plant used for antidiabetes testing was spinach leaves that have flowered. Spinach leaves obtained from Pakemitan Village, District Cikatomas, Tasikmalaya. Chemicals used in this study is 70% ethanol, PGA, ethyl acetate, n-hexane solution of glucose. Experimental animals used were male Swiss Webster mice weighing between 20-30 per gram mice, obtained from farms in the School of Life Sciences and Technology, Bandung Institute of Technology.

Simplicia Treatment: The materials usedfortestingwerespinach leaves (*Amaranthus cruentus*L). Wet sortation was done fromdirt, thenwashedwith clean and flow wateruntilno dirtremaining.After that, thenchoppedtoobtaina smallpiecethus simplifyingthe process ofdrying. Leaveswhichhave been clean andchopped, dried without being exposedto direct sunlight, thensortedfromforeign substancesthat still attached tothe dryspinachleaves.After thatthenpowdered using ablenderuntilsmoothsimpliciaobtained.

Extraction: Ethanol extract of spinach leaves obtained by maceration method. 1000 grams of dried spinach leaves powder soaked with 70% ethanol at room temperature with stirring a few times. Maceration was done for 3 x 24 hours and every 24 hours was replaced with the new solvent. Liquid extracts were collected, combined and then concentrated using a rotary evaporator to obtain a thick extract. Viscous extract were then fractionated by solvent polar, semi-polar and non-polar solvent with liquid-liquid extraction method (ECC) using a funnel. Viscous extract was fractionated using n-hexane and water at a ratio of 1: 1. N-hexane fraction was separated from water, collected and dried. Water fraction further fractionated again with ethyl acetate. Ethyl acetate fraction was separated from the water fraction, respectively collected and dried. Water fraction, ethyl acetate fraction and n-hexane fraction obtained was then used for antidiabetic activity test.

Phytochemical screening

Phytochemical screening for secondary metabolites are alkaloids, flavonoids, tannins and polyphenols, monoterpenes and sesquiterpenes, steroids and triterpenoids, saponins.

Antidiabeticactivity test of water fraction, etyl acetate fraction and n-hexane fraction of spinach leaves (Amaranthus CruentusL) in Mice

Male mice prior before used, Were fasted for 24 hours, and drink is still given. After that it is divided into 5 groups that were treated as follows: Control (-) was given a 2% solution of PGA orally, the test group is water faction of spinach leaves doses of 0.086g/ kg bw, ethyl acetate fraction of spinach leaves dose of 0.03 g/ kg bw, n-hexane fraction dose of 0.055 g spinach leaves/ kg that were suspended in 2% PGA orally. An hour later all the groups given glucose orally at a dose of 2g/kg bw. Then measurement of blood glucose levels determined at minute 0, 30, 60, 90, and 120 (Phyto Medica Scientific Working Group, 1991).

Data analysis: the method usedto analyze the results of data experiments was SPSS20 statistical method using ANOVA including normality test, homogeneity, and LSD.

RESULT AND DISCUSSION

Extraction and Fractionation

Results of 1000 grams of dried leaves of spinach powder obtained 65.39 grams of viscous ethanol extract with a yield of 6.53%. Fractionation result 22.28 grams of water fraction, 8.31 grams of ethyl acetate fraction, and 14.25 grams of n-hexane fraction.

Phytochemical screening

Phytochemical screening of viscous extract and fractions of spinach leaves (*Amaranthus cruentus L*) can be seen in Table 1.

Table 1. Results of phytochemical screening of viscous extract and fractions of spinach leaves (*Amaranthus cruentus* L.)

	Ethanol Extract	Water Fraction	Ethyl Acetate Fraction	N-hexane Fraction
Flavonoid	+	+	+	-
Polifenol	+	+	+	+
Saponin	+	+	+	+
Kuinon	+	+	+	+

Description: (+) detected (-) not detected

Based on the results obtained that the phytochemical screening and three of ethanol extract fraction (water fraction, ethyl acetate fraction and n-hexane fraction) contains flavonoids, polyphenols, saponins, and quinones. Flavonoids are not contained in the n-hexane fraction because flavonoids are polar compound that has unsubstitute hydroxyl groups so as ethanol, methanol, ethyl acetate, can be used to extract flavonoids from various plant tissues.

Antidiabetic activity test of water fraction, ethyl acetate fraction, n-hexane fraction of Spinach Leaves (*Amaranthus Cruentus*L)

The antidiabetic activity test of spinach leaves fraction with the dose of water fraction 0.086g/ kg bw, ethyl acetate fraction of 0.03g/ kg bw, and the n-hexane fraction 0.055g/kg body weight using a glucose tolerance test results are as follows:

Preliminary results of Blood Glucose Levels

Results of measurements of preliminary blood glucose levels (mg /dL) every 30 minutes during 120 minutes after administration of glucose 2g/ kg from each group of activity test of fractionation of spinach leaves using the glucose tolerance test with variations in dose of fractionation were water fraction 0.086g/kg bw, ethyl acetate fraction 0.055g/ kg bw, and n-hexane fraction 0.03g/ kg bw can be seen in Table 2.

Table 2. Preliminary blood glucose levels (mg /dL) every 30 minutes for 120 minutes after administration of glucose 2g/ kg from each group of antidiabetic activity test of fractionation of spinach leaves (*Amaranthus cuentus* L).

	K (-)	WF	EAF	NHF
0'	164	164	167	160
	175	169	165	171
	179	163	168	173
Average	**172.67**	**172.6**	**166.67**	**168**
30'	172	155	148	157
	170	164	141	156
	176	150	142	153
Average	**172.67**	**156.33**	**143.67**	**155.33**
60'	170	136	133	146
	157	150	138	148
	166	147	127	124
Average	**164.33**	**144.33**	**118**	**139.33**
90'	158	124	109	122
	152	121	103	125
	140	130	117	120
Average	**150**	**125**	**109.67**	**122.33**
120'	134	120	88	119
	120	122	85	104
	140	126	96	111
Average	**131.33**	**122.67**	**89.67**	**111.33**

Description :

K (-) : Blood glucose levels after administration of PGA2% and glucose 2 g / kg body weight.
WF: Blood glucose levels after administration of water fraction 0.086 g / kg bw and glucose 2 g/ kg bw.
EAF : Blood glucose levels after administration of ethyl acetate fraction 0.03 g / kg bw and glucose 2g / kg.
NHF : Blood glucose levels after administration of n-hexane fraction0.055 g /kg bw and glucose 2 g /kg

Relative Blood Glucose Levels

To determine differences in relative blood glucose levels of each test group every 30 minutes during the 120 minutes of blood sampling, can be seen in Table3.

Table 3. Relative blood glucose levels (%) every 30 minutes for 120 minutes after administration of glucose 2 g/ kg of each group of antidiabetic activity test of fractionation of spinach leaves.

	K (-)	WF	EAF	NHF
	100.00	100.00	100.00	100.00
0'	100.00	100.00	100.00	100.00
	100.00	100.00	100.00	100.00
Average	**100.00**	**100.00**	**100.00**	**100.00**
	104.87	94.51	88.62	98.12
30'	97.14	97.04	85.45	91.22
	98.32	92.02	84.52	88.43
Average	**100.11**	**94.52**	**86.20**	**92.59**
	103.65	82.92	80.60	91.25
60'	89.71	88.75	83.63	86.54
	92.73	90.18	75.59	71.67
Average	**95.36**	**87.28**	**79.94**	**83.15**
	96.34	75.60	65.26	76.25
90'	86.85	71.59	62.42	73.09
	78.21	79.75	69.64	69.36
Average	**87.13**	**75.65**	**65.77**	**72.90**
	81.70	73.17	52.69	74.37
120'	68.57	72.18	51.51	60.81
	78.21	77.30	57.14	64.16
Average	**76.16**	**74.22**	**53.78**	**66.45**

In the minute of 0 all treatment groups had blood glucose levels are relatively the same, namely 100%. It is made to changes in blood glucose levels at each test group visible increase or decrease in blood glucose levels in the next minute. The rise and decline can be seen in the figure 1.

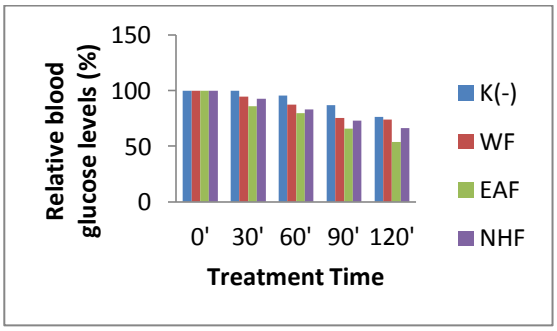

Figure 1. Relative blood glucose levels(%) in all group treatment every 30 minutes for 120 minutes

In the 30th minute the control test group (-) was increase in blood glucose levels. While the other test groups were decreased. Blood glucose levels was highest in the control group (-) as 0.11% of the initial blood glucose levels (100%), then in water fraction group blood glucose levels was decrease 5.48% of the initial blood glucose levels (100%) , ethyl acetate fraction 13.8% of initial blood glucose levels (100%) and n-hexane fraction amounted to 7.41%.

In the 60th minute there is a decrease in blood glucose levels of each test group. In the control group (-) decreased by 4.64% of initial glucose levels (100%). For the three groups of test fraction, blood glucose levels is highest in the test group ethyl acetate fraction of 0.03 g / kg for 20.06% of initial glucose levels (100%) and the lowest occurred in the test group water fraction 0.086 g / kg by 12.72% (from 100%).

In the 90th minute the percentage blood glucose levels were continue to decrease in all test groups. Ethyl acetate fraction of 0.03 g / kg showed the highest percentage of decrease in blood glucose levels as much as 34.32% (from 100%), while the percentage of the control test group (-) was the lowest compared to other test group that was equal to 12.87% (from 100%).

In the 120th minute the decrease of blood glucose levels occured in all test groups: control group (-) amounted to 23.84% of the prelimiary blood glucose levels (100%). Ethyl acetate fraction of 0.03 g / kg showed highest pecentage of decrease compared to the other test group in the amount of 46.22% (from 100%).

Table 4. Average relative of blood glucose levels(%) every 30', 60',90' and 120' minutes

Description	Average relative of blood glucose levels (%)			
	K(-)	WF	EAF	NHF
Average	91,75	86,33	77,13	83,01
±SD	±4,84	±1,35	±0,46	±4,67

In table 4 it can be seen that test group ethyl acetate fraction (EAF) showed the lowest avarage of glucose levels compared to other test groups. While the negative control test group (K-) has the highest average of blood glucose levels that was 91.75% ± 4.84. From the three groups of test fraction, the average, of the lowest glucose levels was the ethyl acetate fraction of 0.03 g / kg body weight of 77.13 ± 0.46% and the water fraction test

group 0.086 g / kg has the highest average of glucose that was 86.33% ± 1.35.

CONCLUSION

Antidiabetic activity test research results polar fraction, semi-polar fraction and non-polar fraction of spinach leaves (*Amaranthus cruentus* L), dose of water fraction 0.086 g/kg, ethyl acetate fraction of 0.03g/kg, and the fraction of n-hexane 0.055 g/kg body weight using a glucose tolerance test showed that the compound contained in the third test group of fraction has antidiabetic effects. While the group that showed the best antidiabetic effects was ethyl acetate fraction of 0.03g/ kg. Further research need to find out the active compounds in spinach leaves (*Amaranthus cruentus* L) as antihyperglicemia and to test the safety when it is used in the long term.

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