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ANTIDIABETIC ACTIVITY OF ETHANOL EXTRACT OF *TRIGONELLA FOENOEM-GRAECUM* L. USING ALLOXAN INDUCTION METHOD

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ABSTRACT

Antidiabetic activity of plants had been widely studied. Diabetes mellitus (DM), commonly known as just diabetes, is a group of metabolic disorders characterized by a high blood sugar level over a prolonged period of time. Symptoms often include frequent urination, increased thirst and increased appetite. If left untreated, diabetes can cause many health complications. The purpose of this study to determine the antidiabetic activity administered orally using alloxan induction of ethanol extract of *Trigonella foenoem-graecum*. Fasting blood glucose was measured on the day before alloxan induced, three days after induction of alloxan, and on the 2nd, 4th, 6th, and 8th after administration of extract using a glucometer. The result showed that sample test has the best effect of antidiabetic activity on decreasing blood glucose level on diabetic rats. It may be concluded that *T. foenoem-graecum* ethanol extract can be used as candidate of antidiabetic treatment.

Keywords: Antidiabetic, Alloxan, *Trigonella foenoem-graecum*, blood glucose

INTRODUCTION

Diabetes Mellitus (DM) is a chronic disease that occurs when the pancreas cannot produce sufficient insulin or the body cannot utilize the insulin produced by the pancreas effectively^[1]. The insulin hormone functions to utilize glucose as a source of energy and fat synthesis, lack of insulin causes glucose to accumulate in the blood (hyperglycemia) so that glucose will be secreted through the urine without being used^[2].

It will be an increase in Diabetes sufferers in Indonesia from 8.4 million in 2000 to 21.3 million in 2030^[3]. In 2000 it was recorded that people with diabetes mellitus in Indonesia reached 8.4 million people. Indonesia is in the fourth position for the country that has the most diabetes mellitus sufferers in the world after India, China, and England. Based on the results of research by Wild et.al., by 2030 people with diabetes in the world will increase by 2 times, including Indonesia. Epidemiologically, people with diabetes mellitus (DM) in Indonesia in 2030 are estimated to reach 21.3 million people. In 2021 Indonesia is ranked 7th in the world in DM ^[4-6].

Treatment of diabetes mellitus is a chronic and lifelong treatment. The use of synthetic drugs for the treatment of diabetes mellitus in the long term can cause unwanted side effects. One alternative is to conduct research on traditional medicines that have a hypoglycemic effect. In 1980 WHO recommended that research be conducted on several plants that can lower blood sugar levels because the use of synthetic drugs is considered unsafe ^[7]

The chemical content of the bitter melon *Momordica charantia* L was flavonoid compounds, phenols, tannins, saponins and sterols/terpenes. The 70% ethanol extract of this fruit at a dose of 110 mg/200 g bw was able to reduce blood sugar levels comparable to gliclazide 1.4 mg/200 g bw ^[8]. Fenugreek seeds contain protoalkaloids, trigonelline (up to 0.37%), flavonoids, sterols, saponins (0.6 – 1.7%), and choline (0.05%) ^[9]. The results showed that the ethanol extract of fenugreek seeds at a dose of 280 mg/200 g BW could reduce blood sugar levels in rats comparable to the oral diabetes drug gliclazide 1.4 mg/200 g BW due to the induction of alloxan tetrahydrate ^[10]. This study reports antidiabetic effect of combination of fenugreek seed and bitter melon extracts.

Methods

Tool

The tools used in this study were a macerator, an analytical balance, a simple grinder (blender), a rotavapor, an evaporating dish, a water bath, a distillation flask, a silica plate, UV 254 and 366 lamps, experimental animal scales, oral probe, syringe, knife cutter, glucose meters, and test strips.

Simplicia

The simplicia materials used were obtained from the Research Agency for Medicinal Plants & Spices, Manoko Research Installation, Lembang, West Java.

Chemical material

The chemicals used were 70% ethanol, toluene, Mayer reagent, Dragendorff reagent, Lieberman-Burchard reagent,

chloroform, ammonia, 2N hydrochloric acid, 1% gelatin, iron (III) chloride, magnesium powder, amyl alcohol, ether, vanillin 10 % in concentrated sulfuric acid, and 5% potassium hydroxide solution, PGA, glucose, distilled water, and glibenclamide tablets.

Experimental Animals

The animals used were white rats (*Rattus norvegicus*) Wistar strain, male sex, 2-3 months old, healthy and have normal activity, body weight between 150-250 grams. Before being used for testing, all mice were adapted for approximately one week and their health was observed.

METHOD

Plant Collection: The simplicia were obtained from the Research Agency for Medicinal Plants & Spices, Manoko Research Installation, Lembang. The material was determined at the Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Padjadjaran University, Jatinagor.

Extraction: The mashed simplicia was extracted by maceration using 70% ethanol solvent separately for 3x24 hours and replaced with a new solvent. The maserate was then concentrated using a rotary evaporator and then evaporation was continued by using a water bath. The viscous extract obtained was calculated for its yield.

Phytochemical

Phytochemical screening was carried out to detect secondary metabolites of alkaloids, polyphenols, tannins,

flavonoids, quinones, saponins, monoterpenoids, sesquiterpenes, steroids and triterpenoids.

Antidiabetic Activity Test: Male white rats were divided into 7 groups (each group consisted of 3 rats), namely normal control, negative control, positive control, and test groups. All rats were measured for initial fasting blood glucose levels first, then alloxan was injected at a dose of 145 mg/kg bw by s.c. (subcutaneous). The rats were allowed to stand for three days while still being fed and drinking. Three days after alloxan induction, fasting blood glucose levels were measured before treatment. All mice used were weighed and then treated as follows:

1. Normal control : PGA 2%
2. Negative control: PGA 2% + alloxan
3. Positive control: PGA 2% + alloxan + glibenclamide 0.45 mg/kg BW
4. Test group 1: PGA 2% + alloxan + test 1 (50 mg/200g BW of bitter melon + 280 mg/200 g BW of fenugreek seeds)
5. Test group 2: PGA 2% + alloxan + test 2 (100 mg/200g BW of bitter melon fruit + 280 mg/200 g BW of fenugreek seeds)
6. Test group 3: PGA 2% + alloxan + test 3 (50 mg/200g BW of bitter melon fruit + 280 mg/200 g BW of fenugreek seeds)
7. Test group 4: PGA 2% + alloxan + test 4 (50 mg/200g BW bitter melon + 560 mg/200 g BW fenugreek seeds)

Each of these preparations was given orally with the same volume of administration (1 ml/200 g BW). The treatment was carried out for 8 consecutive days. Fasting blood glucose

was measured on the day before alloxan induction, three days after alloxan induction and on the 2nd, 4th, 6th, and 8th day of treatment. Blood was obtained by cutting the rat's tail under a lamp, then blood glucose levels were measured using a glucometer.

RESULTS AND DISCUSSION

Results of Collection and Determination of Plants

The results showed that the plants used in the study were bitter melon fruit *M.*

charantia L.) and fenugreek seeds (*T. foenoem-graecum* L.).

Extraction Results

The extraction results can be seen in Table 1.

Phytochemical Screening Results

The results of phytochemical screening on the simplicia of bitter melon, Mahkota Dewa fruit, and fenugreek seeds can be seen in Table 2.

Table 1. Extraction Results of bitter melon and Fenugreek Seeds

Simplicia	Weight of simplicia	Weight of viscous extract	Yields
Bitter melon (<i>M. charantia</i>)	545 gram	155,56 gram	28,54%
Fenugreek seed (<i>T. foenum-graecum</i>)	431,76 gram	87,79 gram	20,33%

Table 2. Phytochemical Screening of Bitter melon and Fenugreek Seeds

Secondary Metabolites	Results	
	Bitter melon (<i>M. charantia</i>)	Fenugreek seed (<i>T. foenum-graecum</i>)
Alkaloids	-	-
Polyphenol	-	+
Tannins	-	-
Flavonoids	+	+
Monoterpenoid and sesquiterpenoid	+	+
Steroid and triterpenoid	-	-
Quinone	-	+
Saponins	+	+

1. Notes: (+) = detected; (-) = not detected

Antidiabetic Activity Test

Testing the antidiabetic activity of the combination of bitter melon extract and fenugreek seeds with variations in the

combined dose in male white rats, the results of measuring the fasting blood glucose levels of rats (mg/dL) are shown in Table 3.

Then the relative fasting blood glucose levels of rats were calculated by comparing the fasting blood glucose levels of rats on the 1st, 2nd, 4th, 6th and 8th days with blood glucose levels on day 0 as shown in Figure 1 The percentage

decrease in antidiabetic activity in the combination of the ethanol extract of bitter melon and fenugreek seeds is shown in Table 6 and made graphically as shown in Figure 2.

Table 3. Average Blood Glucose Levels (mg/dl) Combination of Ethanol Extracts of Bitter melon (*M. charantia*), and Fenugreek Seed in Male White Rats

Time (days)	Normal control	Negative control	Positive control	Test 1	Test 2	Test 3	Test 4
0	61,00	67,00	81,00	62,00	62,00	73,00	66,00
1	57,00	457,00	424,67	393,00	398,67	343,00	329,67
2	80,67	495,67	388,33	406,67	446,67	391,33	271,00
4	58,33	488,33	356,33	238,33	354,00	400,33	376,67
6	74,33	362,67	278,33	192,67	322,33	263,33	285,33
8	54,67	326,00	213,67	171,33	201,33	252,33	299,67
10	55,67	85,67	44,67	189,67	132,00	213,33	199,33

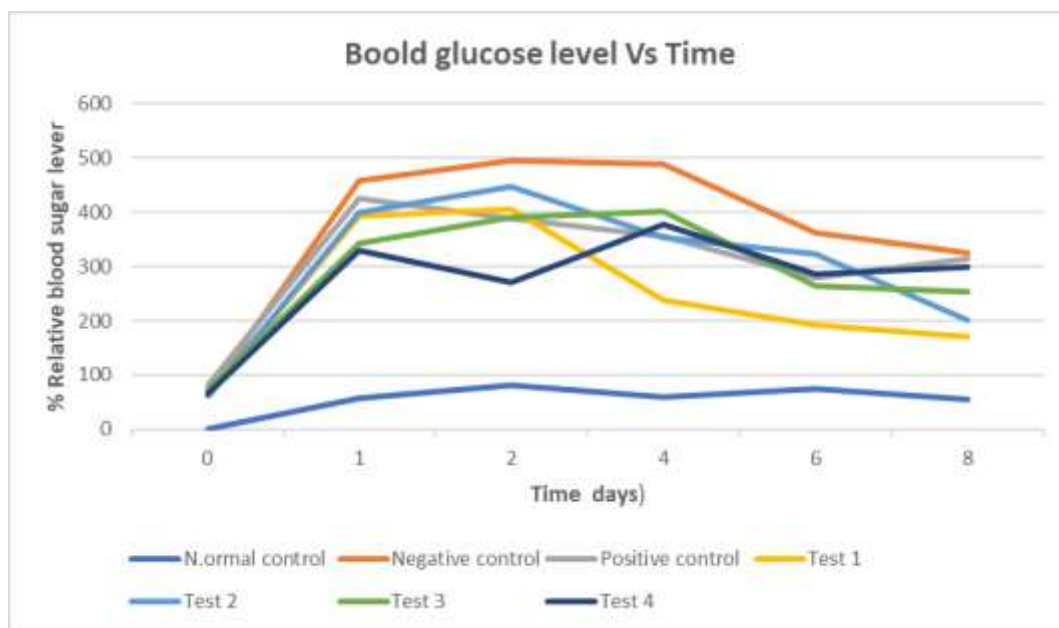


Figure 1. Graph of relative blood glucose levels (%) combination of ethanol extract of bitter melon and fenugreek seeds in male white rats

- Keterangan:**
 Normal control: PGA 2%
 Negative control: PGA 2% + alloxan
 Positive control: PGA 2% + alloxan + glibenclamide 0,45 mg/kg BB
 Test 1: PGA 2% + alloxan + 50 mg/200 g BW bitter mellon extract + 280 mg/200 g BW Fenugreek Seed extract
 Test 2: PGA 2% + alloxan + 100 mg/200 g BW bitter mellon extract + 280 mg/200 g BW Fenugreek Seed extract
 Test 3: PGA 2% + alloxan + 50 mg/200 g BW bitter mellon extract + 280 mg/200 g BW Fenugreek Seed extract
 Test 4: PGA 2% + alloxan + 50 mg/200 g BW bitter mellon extract + 560 mg/200 g BW Fenugreek Seed extract

Whereas, Table 4. shows the percentage decrease in antidiabetic activity of the combination of bitter melon and fenugreek seed ethanol extract in male white rats

Table 4. Percentage of Decreased Antidiabetic Activity Combination of extracts of bitter melon and Fenugreek Seeds in Male White Rats

Time (days)	Decrease in Relative Glucose Levels (%)				
	Positive control	Test 1	Test 2	Test 3	Test 4
1	22,61	8,98	6,50	32,15	23,19
2	35,39	11,61	3,87	27,50	43,66
4	39,51	45,97	24,56	24,59	18,88
6	37,30	39,50	6,81	31,35	14,92
8	42,88	39,92	36,42	26,70	2,64

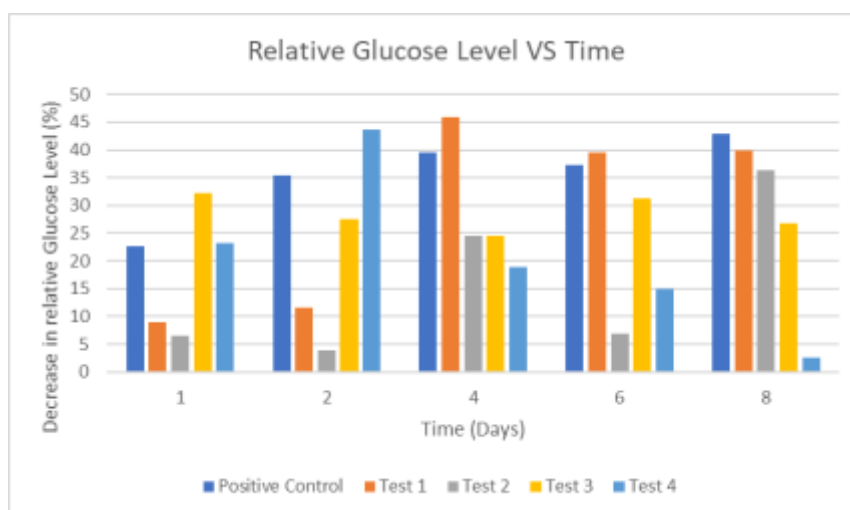


Figure 2. Graph of the percentage decrease in antidiabetic activity of the combination of ethanol extract of bitter melon and fenugreek seeds

Notes:

Positive control: PGA 2% + alloxan + glibenclamide 0,45 mg/kg BW

Test 1: PGA 2% + alloxan + 50 mg/200 g BW bitter melon + 280 mg/200 g BW fenugreek seeds

Test 2: PGA 2% + alloxan + 100 mg/200 g BW bitter melon + 560 mg/200 g BW fenugreek seeds

Test 3: PGA 2% + alloxan + 50 mg/200 g BW bitter melon + 280 mg/200 g BW fenugreek seeds

Test 4 : PGA 2% + alloxan + 50 mg/200 g bitter melon + 560 mg/200 g BW fenugreek seeds

Based on the graph of average blood glucose levels (mg/dL), relative blood glucose levels (%), and the percentage decrease in antidiabetic activity of the combination of ethanol extract of bitter melon and fenugreek seeds both positive control group, test group 1, group test 2, test group 3, and test group 4 gave antidiabetic activity. The extract combination test group showed that the test group 1 with a dose combination of

1:1 (50 mg/200 g BW + 280 mg/200 g BW) gave the highest antidiabetic activity, which was 45.97%.

To find out the significant differences between each treatment group in the activity of lowering blood glucose levels, experimental data were tested using the analysis of variance (Anova) method, the results of which can be seen in Table 5.

Table 5. ANOVA Antidiabetic Activity Combination of Ethanol Extract of Pare Fruit, and Fenugreek Seed in Male White Rats

Variation source	db	JK	KT	F count.	F _{tab}
Treatment	6	861702.97	143617,20	10,72	2,44
Error	28	375070.78	13395,38		
Total	34	1236773.74			

Based on the results of the ANOVA test, it was found that Fcount > Ftable, this means that H0 was rejected so it could be concluded that there was a significant difference between the

treatments given at the significance level (α=0.05). In other words, the combination of the ethanol extract of bitter melon and fenugreek seeds gave a significant difference in reducing fasting blood glucose levels of rats with 95%

confidence. Duncan's follow-up test was used to determine the significant difference between the positive control group, test group 1, test group 4, test group 2, and test group 3 when compared to the negative control group at a significance level of 0.05. It was found that with 95% confidence it was

found that the negative control group was in a different subset with the positive control group, test group 3, and test group 1. Test group 2 and test group 4 were in two subsets, namely a subset of the negative control group and a subset of the positive control group, test group 1, and test group 3. .), bitter melon fruit (*Momordica charantia* L.) and fenugreek seeds (*Trigonella foenum-graecum* L.) test group 2 and test group 4 did not give different effects. Test 3 showed the highest antidiabetic activity compared to negative control, followed by test group 1, test 4, and test 2.

CONCLUSION

The results of this study concluded that the combination of the ethanol extract of bitter melon (*Momordica charantia* L.) and fenugreek seeds (*Trigonella foenum-graecum* L.) gave diabetes activity. Test test 3 followed by test group 1 showed the most effective antidiabetic activity. 1. While test group 4 and test 2 showed antidiabetic activity but the activity was not significant with negative control. It is necessary for further research on Toxicity level of these combination.

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