Antidiabetic activity of *musa acuminata* colla fruit peel (MACFP) ethanol extract in glucose-induced diabetic rats

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Abstract. Diabetes mellitus is a metabolic disorder disease characterized by increased blood glucose levels due to impaired insulin secretion or increased insulin resistance. The study was performed to investigate the blood glucose lowering effect of *Musa acuminata* Colla fruit peel (MACFP) ethanol extract in experimentally induced diabetic rats. 25 rats were divided into five groups, i.e negative control group (0.5% NaCMC), positive control group (glibenclamide), and treatment group of MACFP ethanol extract with dose 250, 375, and 500 mg/kg body weight (mg/kg BW). The blood sample was taken from the lateral vein of the tail, and then blood glucose level was observed on 0, 30, 60, 90, and 120 minutes. The results provide information that MACFP ethanol extract with dose 250, 375, and 500 mg/kg BW have the same effect with a positive control (glibenclamide) in lowering blood glucose level on diabetic rats (p>0,05). MACFP ethanol extract with dose 500 mg/kg BW had the highest percentage of decrease in blood glucose level (42,62%), followed by doses 375 mg/kg BW (37,26%) and 250 mg/kg BW (24,12%).

1 Introduction

Diabetes Mellitus (DM) is a group of metabolic disorder diseases in which blood sugar levels are abnormally high over a prolonged period, that occur due to abnormalities of insulin secretion, insulin work or both [1]. According to the World Health Organization (WHO) in 2016, DM patients in the world's increased from 108 million (1980) to 422 million (2014). International Diabetes Federation (IDF) estimates DM patients in the worlds at 2040 to 642 million. Furthermore, according to the journal of Diabetes Care, DM patients in Indonesia in 2030 is estimated go up from 8.4 million in 2000 to 21.3 million in 2030 [2].

The increasing number of DM patients causes increased use of antidiabetic drugs. Currently, antidiabetic agents from the plant have been widely developed towards phytopharmaca. One of them that will be developed is a plant of Ambon banana (*Musa acuminata* Colla). The part of the plant that is used is the peel of the fruit.

According to Someya (2002), banana fruit peel has higher antioxidant activity than the pulp. This is because the antioxidant compound was more abundant in peel (158 mg/100 g dry wt.) than in pulp (29.6 mg/100 g dry wt.) [3].

The antioxidant compound contained in the *Musa acuminata* Colla fruit peel is flavonoid. Flavonoid has an important role in controlling blood glucose level and prevent complications of diabetes mellitus [4,5]. The results of previous research, water extract of banana fruit peel of Ambon with a dose of 400 mg/kg BW has antidiabetic effect in alloxan induced white rats [6].

Scientific data about the banana fruit peel of Ambon as antidiabetic have not much found, so it is still needed research on the banana fruit peel of Ambon with different methods. This is an attempt to prove that the banana fruit peel of Ambon will be developed as an antidiabetic agent.

2 Materials and methods

2.1 Materials

Musa acuminata Colla, 25 male white rats of Wistar strain (aged 2 to 3 months weighing 150 to 200 g), glucose, NaCMC, ethanol, glibenclamide, glucometer, pellet, and water.

2.2 Licensing of *ethical clearance*

This research using test animals is required to permit ethical clearance to ensure that this test did not use methods that violate animal testing regulations. This permission is submitted to the ethics commission in Faculty of Medicine, University of Jenderal Soedirman.

2.3 Determination of Plants

Plants were obtained on Banjaranyar Village, Sokaraja, Banyumas are determined to find out the true identity of

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the plant being tested. The determination was done at the Laboratory of Plant Taxonomy, Faculty of Biology, University of Jenderal Soedirman.

2.4 Extract Preparation

The banana fruit peel was dried for 2 weeks, then the dried samples are made into powder. 500 grams of simplicia powder soaked with 96% ethanol solvent as much as 3 liters for 3 days with occasional stirring, then filtered with filter paper. The filtrate is collected, then the residue was re-macerated with 96% ethanol solvent up to 3 times. The overall filtrate obtained was evaporated using an evaporator, then evaporated using a water bath at a temperature of 60^{0o}C to obtain a concentrated extract [7].

2.5 Compound Identification

2.5.1 Flavonoid Test (Shinoda Method)

The extract was took 2 g and added enough magnesium powder and 10 drops of 5 M hydrochloric acid. The presence of flavonoids was characterized by the formation of reddish black [8].

2.6. Antidiabetic Test

25 male white rats have fasted for 12-18 hours, subsequently were divided into 5 groups :

Group 1 : Glibenclamide in 0,5% NaCMC with dose of 0,6 mg/kg BW as a positive control (PC)
Group 2 : 0,5% NaCMC as a negative control (NC)
Group 3 : MACFP ethanol extract with a dose of 250 mg/kg BW
Group 4 : MACFP ethanol extract with a dose of 375 mg/kg BW
Group 5 : MACFP ethanol extract with a dose of 500 mg/kg BW

All groups after 30-minute was getting treatment orally, they were given glucose orally with a dose of 6,75 g/kg BW, this was based on preliminary experiments [9,10]. The blood was took from a vein lateral at minute 0; 30; 60; 90; 120 that was calculated after administration of glucose orally and measured blood glucose levels using a *glucometer*.

3 Data Analysis

The results of this study will be analyzed descriptively and statistically. Descriptive analysis was used on the data of compound identification. The data of blood glucose levels calculated the value of AUC_{0-120} and %DBGL (Percentage Decrease of Blood Glucose Level) from each group. Then, the value of AUC_{0-120} was analyzed statistically using *one way ANOVA* if it fulfilled normality and homogeneity of the data requirement.

4 Result and discussion

4.1 MACFP Extraction

Extracts of MACFP were obtained through maceration process using 96% ethanol solvent due to its capability to dissolve almost all substances including polar, semi-polar, and nonpolar substances [11]. The obtained dense extract was 22,84 g with 4,568% of rendemen percentage.

4.2 Compounds Identification

Compounds identification was to determine the presence of flavonoid compound in the extract of MAFCP, it can be seen in table 1. This was because that compound was alleged to be effective in decreasing of blood glucose level.

Table 1. Compounds Identification Result.

Compounds	Reactors	Results
Flavonoid	Mg + dense HCl	+

Notes : (-) = undetected, (+) = detected

4.3 Antidiabetic Test

Table 2. The Average of Blood Glucose Levels**.**

Group	The average of blood glucose level (mg/dl) ±SD				
	0’	30’	60’	90’	120’
I	92 ± 14,4	157,4 ± 20,8	139,8 ± 18,3	113 ± 5,4	77,2 ± 15,6
II	82,4 ± 16,4	285,8 ± 73,3	256,8 ± 71,7	227,2 ± 57,9	205,0 ± 60,4
III	103,6 ± 22,6	238,6 ± 14,3	185,2 ± 14,4	149,6 ± 9,2	135,8 ± 13,7
IV	109,4 ± 17,0	190,6 ± 13,7	151,4 ± 12,7	122,4 ± 14,8	108 ± 11,7
V	78 ± 17,1	183,4 ± 21,2	148,8 ± 24,7	108 ± 9,3	89,8 ± 18,1

Notes :
I : Positive control (glibenclamide)
II : Negative control (NaCMC)
III : MACFP ethanol extract (250mg/kg BW)
IV : MACFP ethanol extract (375mg/kg BW)
V : MACFP ethanol extract (500mg/kg BW)

Measurement of blood glucose levels is started at minutes 0 up to 120 minutes every 30 minutes. Widyaningsih *et al*. (2004) reported normal blood glucose levels ranging from 50-135 mg/dl [12]. Based on table 2, group II (negative control) showed that blood glucose levels were abnormal every time measurement. Furthermore, data of blood glucose levels have calculated the value of Area Under Curve (AUC) from 0 up to 120 minutes (AUC_{0-120}), it can be seen in table 3.

The value of AUC_{0-120} was used as an illustration of antidiabetic effects of MACFP ethanol extract. AUC_{0-120} value is getting smaller, so the effect of antidiabetic is getting bigger. Negative control group has the biggest AUC_{0-120} value and the positive control group has the lowest AUC_{0-120} value. Treatment group with a dose of 500 mg/kg BW has the lowest AUC_{0-120} value, so the effect of antidiabetic is bigger than a dose of 250 mg/kg BW and 375 mg/kg BW (table 3).

AUC_{0-120} values were analyzed statistically. An analysis was done to the value of AUC_{0-120} using *one way ANOVA* because it had been fulfilled normality and homogeneity of data requirement ($p>0,05$). Based on the analysis result, it was discovered that there was a significantly different in each treatments groups, positive and negative control ($p<0,05$). Moreover, an analysis using *LSD test* was conducted to find out which groups were significantly different (the result can be seen in table 4).

Table 3. The Value of AUC_{0-120}.

Group	AUC_{0-120}				
	0-30	30-60	60-90	90-120	Total AUC
I	3741	4458	3792	2853	14844
II	5523	8139	7260	6483	27405
III	5133	6357	5022	4281	20793
IV	4500	5130	4107	3456	17193
V	3921	4983	3852	2967	15723

Notes :
I : Positive control (glibenclamide)
II : Negative control (NaCMC)
III : MACFP ethanol extract (250mg/kg BW)
IV : MACFP ethanol extract (375mg/kg BW)
V : MACFP ethanol extract (500mg/kg BW)

Then, AUC_{0-120} value of each treatment group were calculated percentage of DBGL (Decrease Blood Glucose Levels), it can be seen in table 5. %BDGL is the difference of AUC_{0-120} value from negative control with AUC_{0-120} value from treatment group, then the result is divided by negative control AUC0-120 value, then multiplied by 100% [13]. AUC_{0-120} value is inversely correlated with % DBGL value, so if AUC_{0-120} value is getting smaller, then the value of % DBGL is getting greater.

Table 4. LSD Analysis Result.

Between-group	p-Value	Statistics Test Results
I-II	0,000	Significantly different
I-III	0,218	Not significantly different
I-IV	0,294	Not significantly different
I-V	0,369	Not significantly different
II-III	0,000	Significantly different
II-IV	0,000	Significantly different
II-V	0,001	Significantly different
III-IV	0,843	Not significantly different
III-V	0,794	Not significantly different
IV-V	0,938	Not significantly different

Notes :
I : Positive control (glibenclamide)
II : Negative control (NaCMC)
III : MACFP ethanol extract (250mg/kg BW)
IV : MACFP ethanol extract (375mg/kg BW)
V : MACFP ethanol extract (500mg/kg BW)

Table 5. Percentage of DBGL

Group	%DBGL
Positive control (glibenclamide)	45,83%
MACFP ethanol extract (250mg/kg BW)	24,12%
MACFP ethanol extract (375mg/kg BW)	37,26%
MACFP ethanol extract (500mg/kg BW)	42,62%

Based on table 4, it can be inferred that MACFP ethanol extract has the same effect on the positive control to reduce the blood glucose levels ($p>0,05$). And then, based on table 5 showed that MACFP ethanol extract with dose 500 mg/kg BW had the highest ability to reduce the blood glucose levels as much as 42,62%, followed by doses 375 mg/kg BW (37,26%) and 250 mg/kg BW (24,12%). This is because MACFP ethanol extract has flavonoid compound. Flavonoid is an antioxidant that can play a role in protecting cells of β-pancreatic from damage caused by free radicals (Widowati, 1997) [14]. Effendi (2008) states that flavonoid can stimulate the pancreas to produce more insulin, therefore blood glucose levels in diabetic male white rats can be reduced [15].

5 Conclusion

MACFP ethanol extract with dose 250 mg/kg BW, 375 mg/kg BW and 500 mg/kg BW has the same effect with positive control in lowering blood glucose on male white rats ($p>0,05$).

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