COMPARISON OF ANTIHYPERGLYCEMIC ACTIVITY OF DIFFERENT PARTS OF KLUTUK BANANA (MUSA BALBISIANA COLLA)

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INTRODUCTION

Current development has changed human beings in all aspects, such as lifestyle, which present known as the term of 3 F: fun, fashion and food [1]. These changes impact on changes in the pattern of diseases from infectious towards degenerative diseases such as diabetes mellitus (DM). Especially for developing countries, including Indonesia, which resulted in changes both in lifestyle and diet, which one was the risk factors for DM [2].

According to the International Diabetic Federation (IDF), it is estimated that almost half (49.7%) of all people were living with undiagnosed diabetes in 2017. In addition, about 374 million people have glucose tolerance impaired and it was projected about 21.3 million birth affected by hyperglycemia in pregnancy. In 2017, about 5 million deaths around the world were caused by diabetes. Indonesia ranked 6th in DM and affected 10.3 million people after China, India, United States, Brazil and Mexico [3, 4]. According to data Basic Health Research of the Ministry of Health of Indonesia, DM prevalence increased from 6.9% (2013) to 8.5% (2018). Diabetes mellitus become a burden in cost due to its needed long-life treatment [5].

Therefore, necessary to find other alternative medicine from nature which claimed less adverse reactions. Klutuk banana (M. balbisiana Colla) is a herbal medicine that grows in Indonesia and is mostly consumed by the Indonesian people. This plant has anti-diabetic, anti hyperlipidemia, antioxidant and antibacterial activity [6-9]. Bananas have been classified as one of the rich antioxidant foods [10]. This antioxidant activity has a good role for diabetes mellitus improvement through increase of catalase, decrease of malondialdehyde and increase of insulin level. As known hyperglycemia lead to oxidative stress production and destruct the pancreas [7]. Previous studies showed that the ethanolic extract of flower and stem of M. balbisiana Colla a dose of 250 mg/kg bw inhibited the absorption of glucose in the intestines [6, 7]. However, antihyperglycemic activity of peel and pulp of klutuk banana are still unknown.

MATERIALS AND METHODS

Materials

D-glucose, alloxan, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and sodium carbamoylmethyl cellulose (CMC-Na) were purchased from Sigma Aldrich, chemistry and biotechnology Inc. (St. Louis, Missouri United States). Glibenclamide was purchased from Kimia Farma, Indonesia. Strips test for blood glucose examination was purchased from All Medicus Co., Ltd. The Republic of Korea. Methanol and ethanol analytical grade were purchased from Merck.

Methods

Preparation of plant material

All parts of klutuk banana (M. balbisiana Colla) peel, pulp, seed and flower were obtained from November 2018 at Tasikmalaya, West Java, Indonesia. The identity of the plant was confirmed by the Faculty of Mathematics and Natural Sciences; Department of Biology, Padjadjaran University, with numbers: 156/HB/11/2018. All parts were washed, dried, cut into pieces, then ground. The powder was stored at a dry place for further use.

Preparation of extract

All parts of klutuk banana (M. balbisiana Colla) were extracted by reflux method with 96% ethanol for 3 h and repeated three times. Furthermore, the sample was filtered through filter paper and then vaporated by the rotary evaporator. This extract was kept in the refrigerator at 4 °C for further use.

Phytochemical screening

The phytochemical screening was performed to detect the presence of secondary metabolites such as alkaloid, flavonoid, saponin, steroid/triterpenoid, tannin, quinone, and phenols. Phytochemical screening was performed on crude drug and extracts of different parts of klutuk banana (M. balbisiana Colla) [7].

Preparation of animals

Adult male mice 2-3 mo (23-28 g) were obtained from the Experimental Animal Application and Research Center of Biofarma. The animals were kept at a constant temperature (24±2 °C) with 50% humidity, 12-h light-dark cycles and were allowed ad libitum access to standard mice chow and water. The animals were adapted in one week and divide in 15 groups, 12 treatment groups and 3 control groups. All animals were given 2 g/kg bw glucose monohydrate and blood glucose level was measured every 30 min for 120 min. 15 groups, namely normal control, negative control, positive control (glibenclamide 0.65 mg/kg body weight (bw), and 12 sample groups. All animals were given 2 g/kg bw glucose monohydrate and blood glucose level was measured every 30 min for 120 min.

RESULTS

The results of the oral glucose tolerance test (OGTT) showed that KPE2 (klutuk peel extract 350 mg/kg bw) gave higher activity to decrease blood glucose level compared to the other groups at the minute of 30 (-24.83%; p. 0.00), 60 (-33.93%; p. 0.000), 90 (-46.29%; p. 0.000) and 120 (-35.44%; p. 0.000).

Conclusion

KPE2 extract has very strong antioxidant activity and antihyperglycemic activity at a dose of 350 mg/kg bw.

ABSTRACT

Objective: Banana is a plant that grows in Indonesia and is widely consumed by Indonesian people. One of banana type is klutuk banana (Musa balbisiana Colla). There were many types of researches about klutuk banana for antidiabetic activity; however, antidiabetic activity of its peel and pulp are still unknown.

Methods: The objective of the study was to determine antihyperglycemic activity of different parts of klutuk banana. Animals were divided into 15 groups, namely normal control, negative control, positive control (glibenclamide 0.65 mg/kg body weight (bw), and 12 sample groups. All animals were given 2 g/kg bw glucose monohydrate and blood glucose level was measured every 30 min for 120 min.

Results: The results of the oral glucose tolerance test (OGTT) showed that KPE2 (klutuk peel extract 350 mg/kg bw) gave higher activity to decrease blood glucose level compared to the other groups at the minute of 30 (-24.83%; p. 0.00), 60 (-33.93%; p. 0.000), 90 (-46.29%; p. 0.000) and 120 (-35.44%; p. 0.000).

Conclusion: Klutuk peel extract has very strong antioxidant activity and antihyperglycemic activity at a dose of 350 mg/kg bw.

Keywords: Antihyperglycemic, Musa balbisiana, Antioxidant, Different parts, OGTT

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control groups. Number of animals in each group was calculated using Federer equation. The study was approved by Research Ethics Committee, Padjadjaran University (KEPK UNPAD) with no. 330/UN6. KeP/EC/2019.

**Experimental design**

There were 15 groups and each group contained 5 animals. Normal (CMC-Na 0.5%); negative control (CMC-Na 0.5%); positive control (glibenclamide 0.65 mg/kg bw); 12 sample groups KPE1 (ethanolic klutuk peel extract 175 mg/kg bw), KPE2 (350 mg/kg bw), KPE3 (700 mg/kg bw), KPU1 (ethanolic klutuk pulp extract 175 mg/kg bw), KPU2 (350 mg/kg bw), KPU3 (700 mg/kg bw), KSE1 (ethanolic klutuk seed extract 175 mg/kg bw), KSE2 (350 mg/kg bw), KSE3 (700 mg/kg bw), KFL1 (ethanolic klutuk flower extract 175 mg/kg bw), KFL2 (350 mg/kg bw), and KFL3 (700 mg/kg bw).

**Antidiabetic activity test oral glucose tolerance test (OGTT) method**

The oral glucose tolerance test (OGTT) was applied to evaluate the ability of glucose metabolism regulation, glucose-induced insulin secretion, and glycemic changes. After fasting 12 h previously, all mice were given different treatments depending on the groups, then after 30 min, all groups were orally administered of glucose (2 g/kg bw). The ability of glucose metabolism regulation, glucose-induced insulin secretion, and glycemic changes. After fasting 12 h previously, all mice were given different treatments depending on the groups, then after 30 min, all groups were orally administered of glucose (2 g/kg bw). The glucose concentration was measured from the collected blood tail vein from at 0 (just before glucose administration), 30, 60, 90, and 120-min using glucose oxidase-peroxidase reactive strips and the glucometer (Gluco-Dr Biosensor AGM 2100, All Medicus Co., Ltd, Korea) [12].

**Antidiabetic activity test alloxan-induced method**

Mice were induced using an intraperitoneal injection of alloxan (Sigma, Germany) which was dissolved in normal saline immediately before used [12, 13]. Diabetes was verified in the animal by measuring fasting blood glucose 3 d following alloxan injection. The rats with glucose level more than 200 mg/dL were used for the study [12, 13].

**Antioxidant activity test**

The antioxidant activity was carried out for ethanol peel extracts of klutuk Musa balbisiana Colla, which was assessed by calculating the scavenging effect of free radical DPPH. The diluted test solutions of peel extracts were prepared in methanol. DPPH 30 µg/ml was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solutions. These mixtures were kept in the dark for 30 min and absorbance was measured at λ 516 nm using a UV-Visible spectrophotometer. DPPH solution was used as control [14]. The absorbance was recorded and % inhibition was calculated. IC50 value can be investigated utilizing a calibration curve. The Antioxidant Activity Index (AAI) was determined by dividing final concentration of DPPH with IC50 value.

**Statistical analysis**

All data were expressed as mean±SD. The SPSS package (version 16) was applied in statistical analysis. The comparison between groups was performed by one-way ANOVA, followed by post hoc Tukey [15, 16].

**RESULTS AND DISCUSSION**

Extraction of each part of klutuk banana was carried out by reflux using 96% ethanol. The yield and density of extracts is provided in table 1. The extract was concentrated using a vacuum rotary evaporator. The yield extracts of the present research were 4.31%, 8.18%, 5.10%, and 4.63% for flower, peel, pulp and seed, respectively. Meanwhile, in research by Borah and Das, which used the percolation method using 95% ethanol solvent, the yields extracts were: flower 12.17% and stalk 10.57% [7]. While, the other study stated that the yield of seed extracts was: hexane 0.66%, acetone 3.87%, ethanol 0.55% and aqueous extract 1.0% [17].

**Table 1: Yield and density of extracts**

| Sample   | Yield extract (%) | Density 1% extract (g/ml) |
|----------|-------------------|---------------------------|
| Peels    | 8.18              | 0.83±0.005                |
| Puls     | 5.10              | 0.82±0.006                |
| Seeds    | 4.63              | 0.80±0.002                |
| Flowers  | 4.31              | 0.82±0.005                |

Phytochemical screening was carried out on crude drug and extract of flower, peel, pulp and seed of klutuk banana (M. balbisiana Colla). The results showed that flower, pulp and seed positive for flavonoid, phenols and steroid/triterpenoid (table 2). Saponin was detected only in peel, pulp and seed. In while, tannin was detected only in the flower and peel.

**Table 2: Phytochemical screening of crude drug and extract of M. balbisiana**

| Groups       | Peels Crude drug | Peels Extract | Puls Crude drug | Puls Extract | Seeds Crude drug | Seeds Extract | Flowers Crude drug | Flowers Extract |
|--------------|------------------|--------------|----------------|-------------|-----------------|--------------|-------------------|-----------------|
| Alkaloids    | (-)              | (-)          | (-)            | (-)         | (-)             | (-)          | (-)               | (-)             |
| Flavonoids   | (+)              | (+)          | (+)            | (+)         | (+)             | (+)          | (+)               | (+)             |
| Phenols      | (+)              | (+)          | (+)            | (+)         | (+)             | (+)          | (+)               | (+)             |
| Tannins      | (+)              | (+)          | (-)            | (-)         | (-)             | (-)          | (+)               | (+)             |
| Saponins     | (+)              | (+)          | (+)            | (+)         | (+)             | (+)          | (+)               | (-)             |
| Quinones     | (-)              | (-)          | (+)            | (-)         | (-)             | (-)          | (-)               | (-)             |
| Steroids/triterpenoids | (+) | (+) | (+) | (+) | (+) | (+) | (+) | (+) |

(+) identified, (-) not identified.

The bananas have been known had antidiabetic activities [17-19]. The active compounds of bananas included lupeol, ferulic acid, vanillic acid, trans-cinnamic acid, p-hydroxybenzoic acid, p-coumarate acid, rutin, catechin/epicatechin, chlorogenic acid, gallic acid, caffeic acid, nicotifloreine [20]. Furthermore, flower and stalk of M. balbisiana Colla also had antihyperglycemic, antioxidants and antidiabetic activity by streptozotocin (STZ)-induced type 1 diabetic rats [17, 18, 21]. The active secondary metabolites which were found in these parts such as flavonoids, tannins, saponins, diterpenes, triterpenes, and phenols [7, 8].

The results of antihyperglycemic effect of different parts of klutuk banana (M. balbisiana Colla) were presented in table 3. The percentage of blood glucose level reduction was demonstrated in fig. 1. The parts of M. balbisiana Colla which showed higher antihyperglycemic effect through OGTT method, then continued by using alloxan induced method (table 4).
The glucose tolerance test method was a preliminary test to determine the ability of the ethanolic extracts of different parts of klutuk banana (M. balbisiana Colla). The percentage blood glucose reduction at 30 min showed that KFL2>KPE2 > KPE1> KPL1> KFL1> KPE3> KPU2> KSE1> KSE2> KSE3> KPU3> KPU1. The results demonstrated that KPE1,2,3; KPU1,2,3; KSF1,2,3 and KFL 1,2,3 displayed significant different compared to negative control (P<0.01). At 120 min had that the highest blood glucose reduction was given by KPE2 35.44%, followed by KPE1 37.63% and KPE3 31.65%. These results demonstrated that KPE1,2,3; KPU2; KSE 2,3 and KFL 1,2,3 figured significantly different compared to normal (P<0.01); b: showed significant different compared to the negative control (P<0.01); c: showed significant different compared to a positive control (P<0.01).

Table 3: Antihyperglycemic effect of different parts extracts of M. balbisiana by oral glucose tolerance test method

| Groups | Blood glucose (mg/dl) | Blood glucose reduction (%) |
|--------|-----------------------|-----------------------------|
| Normal Control | 95.2±7.5 | 87.8±9.5  |
| Negative Control (2g/kg bw glucose monohydrate) | 202.8±7.9 | 194.0±12.5 |
| Positive Control (0.65 mg/kg bw glibenclamide) | 162.6±11.6 | 114.6±10.7 |
| KPE1 Klutuk peels extract (175 mg/kg bw) | 139.2±14.4 | 121.0±11.8 |
| KPE2 Klutuk peels extract (350 mg/kg bw) | 134.0±9.1 | 104.2±7.3  |
| KPE3 Klutuk peels extract (700 mg/kg bw) | 148.4±10.3 | 132.6±6.9  |
| KPU1 Klutuk pulps extract (175 mg/kg bw) | 197.0±9.7 | 166.8±11.7 |
| KPU2 Klutuk pulps extract (350 mg/kg bw) | 150.7±10.2 | 145.4±13.5 |
| KPU3 Klutuk pulps extract (700 mg/kg bw) | 180.8±13.8 | 152.0±15.1 |
| KSE1 Klutuk seeds extract (175 mg/kg bw) | 194.0±12.3 | 162.8±13.0 |
| KSE2 Klutuk seeds extract (350 mg/kg bw) | 165.6±11.3 | 155.2±14.9 |
| KSE3 Klutuk seeds extract (700 mg/kg bw) | 168.2±13.7 | 163.6±12.6 |
| KFL1 Klutuk flowers extract (175 mg/kg bw) | 171.2±10.7 | 157.4±10.3 |
| KFL2 Klutuk flowers extract (350 mg/kg bw) | 141.2±10.1 | 121.8±12.6 |
| KFL3 Klutuk flowers extract (700 mg/kg bw) | 145.0±11.8 | 122.0±12.9 |

The reduction of blood glucose levels after treatment by different parts extracts of M. balbisiana Colla showed significant different compared to normal (p<0.01); b: showed significant different compared to the negative control (p<0.01); c: showed significant different compared to a positive control (p<0.01).
The peel extract of *M. balbisiana* Colla which had the highest reduction percentage of blood glucose level, then was continued by using alloxan-induced method. The results showed that KPE2 gave the highest blood glucose reduction (64.12±8.84%) compared to KPE3 (61.81±5.93%) and KPE1 (42.99±9.40%). It was similar to study by Murthy and Felicia which reported that acetone fruit peel extract of *M. sapientum* 200 mg/kg bw, which induced by STZ showed the percentage of decreasing of blood glucose level 63.36% dan 400 mg/kg bw 63.87% [25]. Meanwhile, research by Gosal and Mustarichie stated that ethanol extract of ranggal banana (*Musa troglodytarum* L) 50 mg/kg bw could reduce blood glucose level at 4th day (45.97%), which was induced by alloxan higher than 100 mg/kg bw (24.56%), 150 mg/kg bw (24.5%) and 200 mg/kg bw (18.8%) [26]. The previous study reported that apiforol compound has been isolated from acetone fraction of *M. balbisiana* Colla seed, which has potential antihyperglycemic activity with IC50 83.54 µg/ml and 52.87 µg/ml for α-amylase and α-glucosidase [17].

The peel extract of *M. balbisiana* Colla with dose of 250 mg/kg bw could reduce blood glucose level, which was induced by STZ 30.20% and 25.58%, respectively [7].

The peel extract which gave the highest antihyperglycemic effect was continued by antioxidant activity and performed by DPPH method. The stock solution DPPH 30 µg/ml in methanol was prepared. The method should be verified by using ascorbic acid standard. The klutuk peel extracts 0.5-16 µg/ml were prepared in methanol. By applying calibration curve (percentage of DPPH scavenging activity vs concentration) it can be determined IC50 DPPH of ethanol peel extract was 1.924 µg/ml and it’s AAI 7.80. This AAI>2, therefore it can be classified as very strong antioxidant [27]. Meanwhile, ascorbic acid standard had IC50 DPPH 0.179 µg/ml and AAI DPPH 83.80.

The previous results showed that kepok banana peel extract had higher antioxidant activity, which inhibited 50% oxidation at concentration of 693.15 mg/ml than ambon banana peel extract at concentration of 5000 mg/ml [28]. In addition, kepok peel banana extract showed anticancer activity with cytotoxic activity (IC50)≥250 µg/ml [29]. Furthermore, this study showed that IC50 of DPPH of ethanolic peel extracts from white ambon banana was 0.37 µg/ml. Meanwhile, other results showed that ethanolic peel extracts of raja buah banana, muli banana and ambon lumut banana had IC50 of DPPH scavenging activities were 36.12, 4.39 and 6.91 µg/ml, respectively [30]. The other previous study expressed that IC50 of DPPH scavenging activities of ethanolic peel extracts of tanduk banana, nangka banana and kepok banana was 10.14, 53.05 and 6.22 µg/ml, respectively [31].

### Table 4: Antihyperglycemic effect of peel extracts of *M. balbisiana* by alloxan-induced method

| Groups | Blood glucose concentration (mg/dl) | 3rd Day (After alloxan induced) | 12th Day (Resolve) | Blood glucose reduction (%) |
|--------|-----------------------------------|--------------------------------|-------------------|-----------------------------|
| Negative control (CMC-Na 0.5%) | 90.8±6.38 | 351.2±43.02 | 401.6±26.76 | -50.4±21.10 |
| Positive control (Glibenclamide 0.65 mg/kg bw) | 9.2±8.26 | 345.0±57.06 | 120.6±25.71* | 244.4±44.67* |
| KPE1 Klutuk peel extract (175 mg/kg bw) | 93.6±4.84 | 315.8±62.83 | 180.8±45.29* | 315.0±36.37 |
| KPE2 Klutuk peel extract (350 mg/kg bw) | 9.6±7.09 | 338.0±47.70 | 121.4±36.98* | 216.6±40.95 |
| KPE3 Klutuk peel extract (700 mg/kg bw) | 90.4±5.89 | 345.4±72.24 | 132.6±40.36* | 212.8±44.27 |

*: Showed significant different compared to negative control (p<0.01).

### Table 5: Absorbance and percentage of DPPH scavenging activity of ethanolic peel extract of *M. balbisiana*

| Concentration of *M. balbisiana* (µg/ml) | Absorbance | DPPH scavenging activity (%) |
|-----------------------------------------|------------|------------------------------|
| 0.5 | 0.5021 | 49.19 |
| 1 | 0.5001 | 49.39 |
| 2 | 0.4939 | 50.02 |
| 4 | 0.4804 | 51.39 |
| 8 | 0.4695 | 52.49 |
| 16 | 0.4411 | 55.36 |

IC50 DPPH = 1.9241 µg/ml, AAI = 7.80

This tropical fruit have a strong ability to protect themselves from oxidative stress [6], as a weak source of primary antioxidants but a strong source of secondary antioxidants [7, 32]. Antioxidant compounds in bananas were identified such as ascorbic acid, tocopherol, beta-carotene, phenolic groups, dopamine and gallic acid [33, 34]. Banana peel is an underused by-product that can be processed to obtain flour that is more easily stored for gastrointestinal lumen, especially flavonoids which were in the form of glycosides [30].

**CONCLUSION**

The ethanolic extract of peel, pulp, seed and flower of klutuk banana had antidiabetic activity. However, the klutuk peel extract (350 mg/kg bw) gave the highest antihyperglycemic activity than other test groups. The ethanolic peel extract was very strong antioxidant activity. The klutuk banana peel can be exploited as source of natural anti-diabetes and antioxidant agent.
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AUTHORS CONTRIBUTIONS
All the authors contributed equally.

CONFLICT OF INTERESTS
Authors declare no conflict of interest.

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