Antihyperglycemic Activity of Banana (*Musa nana* Lour.) Peel and Its Active Ingredients in Alloxan-Induced Diabetic Mice

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Abstract. Previous studies have found that banana flowers, leaves, pseudostems, roots, infructescence stalks, and peels (*M. paradisiacal* (Linn.)) have antihyperglycemic effect. The banana peel (*Musa nana* Lour.) might have the antihyperglycemic activity, its antihyperglycemic activity and active ingredients remain to be elucidated. In this paper, fresh banana peel was sequential extraction using solvents with decreasing polarity. Then, the ethyl acetate and petroleum ether extracts (EBP and PBP) were chosen to evaluate the antihyperglycemic activity in alloxan-induced diabetic mice. And the antihyperglycemic activity guided fractionation of compounds. The EBP displayed potent antihyperglycemic activity. lupenone and -sitosterol were separated from EBP. Animal experiments results indicated that lupenone could effective reduce blood glucose of diabetic mice. The banana peel and its ingredient lupenone showed promising antihyperglycemic activity. The banana peel could be utilized as a natural source of antihyperglycemic food, health care or drug and lupenone has potential to develop as an antihyperglycemic drug.

Introduction

Banana is one of people's favorite fruit, but most of its by-product banana peel is not fully utilized. The researches on the peel of banana show that the banana peel is rich in starch, crude protein, crude fat, pectin, cellulose, hemicelluloses, total dietary fibre, and polyunsaturated fatty acids [1, 2], almost all the essential amino acids in banana peel are higher than FAO standard [3]. These studies indicate that banana peel is valuable to develop as a food. In China, The banana peel has been widely used to treat dysentery, cholera, pruritus and hypertension [4].

Recently, several cycloartane triterpenes are isolated from the banana peel [5] and an antihypertensive active ingredient, 7, 8-dihydroxy-3-methyl-isochroman-4-one is isolated from the banana peel [6], Anhwange [7] analyse the contents of potassium, calcium, iron, sodium, manganese, bromine, rubium, strontium and zirconium in the banana peel. Of course, many pharmacological activities of banana peel have also been reported, such as antimicrobial activity, antioxidant activity, mutagenecity [8-10].

Review the literatures, the *Musa sapientum*. L. banana flower [11-14], *Musa* sp. var. elakki bale banana flower [15], *M. sapientum* Linn. stem juice [16], *Musa paradisiacal* stem juice [17], *Musa* sp. var. Nanjangud rasa bale banana pseudostem [18], *M. paradisiacal* (Linn.) banana pseudostem [19], *Musa* spp. ABB Group, Dongguan dajiao unripe (green) bananas [20], *Musa X paradisiaca* Banana leaves [21], *M. paradisiacal* (Linn.) leaves [22], *M. paradisiacal* (Linn.) roots [23], Musa sapientum banana infructescence stalks [24] and *M. paradisiacal* (Linn.) banana peels [19] show a promising anti-diabetic activity. In China, Fruit peel (*Musa nana* Lour.) is very common. Therefore, this material was selected to study its antihyperglycemic potential. In this study, the ethyl acetate and petroleum ether extracts of banana peels were evaluated by animal experiments. Furthermore in order to determine the component(s) responsible for the pharmacological activity, the active ingredients of the banana peel extracts were identified, and the antihyperglycemic activity of the ingredient was also evaluated by animal experiments.

Experimental

Instrumentation and general techniques

NOVA-400MHz superconducting NMR spectrometer (Varian Company), TMS as internal standard; HP-5973 mass spectrometer (Hewlett-Packard); X-4-type melting point apparatus (Beijing Tech Instruments Co., Ltd.); column chromatography on silica gel (200 to 300 mesh) and thin layer chromatography on silica gel H, silica gel GF254 were purchased from Qingdao Haiyang Chemical Co., Ltd. All other chemicals and solvents were analytical grade and used without further purification.

Plant material and reagent

Metformin was produced by Guizhou Tian'an Pharmaceutical Co., Ltd. (Guizhou, China). Alloxan was purchased from Sigma-Aldrich Co., Ltd. (St Louis, MO, USA). Ethyl acetate (EtOAc) and petroleum ether (PE) were produced by Chengdu Kelong Chemical Reagent Factory (Sichuan, China). Absolute ethyl alcohol was purchased from Shanghai Zhenxing Chemcial No.1 Factory (Shanghai, China).Banana peel were collected in June 2013 in Guiyang, China, and confirmed as the peel of banana (*Musa nana* Lour.) by Dr. Xiangpei Wang. All voucher specimens were deposited in the Department of Pharmacognosy, Guiyang College of Traditional Chinese Medicine, China.

Animals

Kunming mice of either sex (15-30g) were obtained from Chongqing Tengxin Biological Technology Co., Ltd. (Chongqing, PR China; qualified number: SCXK Chongqing 2007001). The colony was maintained under controlled conditions of temperature at 23±2 ℃, 60-70 % humidity and a 12-h light-dark cycle. All the animals in the study were cared for and treated humanely according to the national legislations of China, as well as local guidelines. The animal experiments were approved by the Ethics Committee for Animal Experiments of Guiyang College of TCM.

Extraction and isolation

The fresh banana peel was cut into small pieces and then extracted with 95% ethanol (plant material: solvent, 1:6, w/v) five times under soak for 24 h. The extract was concentrated in vacuo at 65℃ to give a brown gum. The brown gum was suspended in distilled water and extracted with petroleum ether (PE) eight times to afford a water layer and petroleum ether extract (PBP). Then the water layer extracted with ethyl acetate (EtOAc) eight times to afford ethyl acetate extract (EBP). The petroleum ether and ethyl ac etate extracts were concentrated in vacuo until no residual reagents. The EBP was further purified yield compounds I and II. The ethyl acetate extract was dispersed in PE and then

submitted to column chromatography on silica gel (200–300 mesh), eluting with PE, and then eluted with EtOAc/PE (100:1), to afford a new compound I (Fig. 1). Another new compound II (Fig.1) was obtained by eluting with EtOAc/PE (10:1).

Spectroscopic and spectrometric data

Compound I: White amorphous powder; mp 170～172℃; EI-MS m/z: 424 [M]+, 409, 381, 367, 245, 219, 189; 1H-NMR (CDCl3, 400 MHz): δ 4.68 (d, 1) , 4.56 (d, 1.68), δ 1.07 (6H, s, 2×CH3), δ 1.67 (s), 1.08 (s), 1.04 (s), 0.96 (s), 0.94 (s).; 13C-NMR (CDCl3, 125 MHz): d 213.4, 150.2, 109.4, 52.2, 49.9, 48.7, 47.1, 46.0, 45.3, 41.6, 41.0, 36.0, 35.4, 33.9, 32.8, 32.7, 31.4, 29.7, 29.2, 28.0, 27.2, 27.0, 25.9, 24.9, 20.2, 19.1, 18.6, 18.3, 17.9, 10.7 [23, 25].

Compound II: white needles; mp 138 ～140℃; EI-MS m/z: 414 [M]+, 396, 381, 329, 303, 233, 259, 43; 1H-NMR (400MHz, CDCl3) δ: 5.36 (d, 4.8), 3.53 (m), 0.68 (s), 0.82 (d, 7.5), 0.84 (d, 7.0), 0.86 (d, 6.5), 0.92 (d, 6.5), 1.01 (s); 13C-NMR (100 MHz, CDCl3) δ: 37.2, 31.6, 71.8, 42.3, 140.7, 121.7, 31.9, 31.9, 50.1, 36.1, 21.1, 28.2, 42.3, 56.7, 24.3, 39.7, 56.0, 12.0, 19.4, 36.5, 18.8, 33.9, 26.0, 45.8, 29.1, 19.0, 19.7, 23.1, 11.8 [26, 27].

Induction of diabetes in Kunming mice and studying of antihyperglycemic effect of EBP, PBP and lupenone

Diabetic mices were developed as described by Ozbek et al [28] and Raafat et al [29] briefly, overnight fasted mices were intravenous injection of alloxan dissolved in sterile cold saline (0.9 %) every 48-h for two times at 220 mg/kg and 200 mg/kg, separately. Fasting glucose levels in the blood samples obtained from the tail of each mice 72 h after the last alloxan injection was measured with One Touch Ultra Glucose Monitor (Lifescan) (OneTouch Ultra (Johnson&Johnson Medical (China) Ltd., China), animals with the serum glucose level above 11.11mmol/L (diabetic) were selected for the following experiments.

In the first test, mice were divided into seven groups, including normal group, a moderately diabetic control group (10 mice per group), two diabetic groups administered EPB orally (6.0 mg/kg and 12.0 mg/kg, 10 mice per group), two diabetic groups administered PBP orally (6.0 mg/kg and 13.0 mg/kg, 10 mice per group), one diabetic group administered metformin (300.0 mg/kg, 10 mice). Blood samples were collected from the tail vein for the measurement of blood glucose at 7 and 14 d after administration.

In the second test, mice were divided into four groups, including normal group, one moderately diabetic control (10 mice per group), one diabetic groups administered lupenone orally (12.0 mg/kg, 10 mice per group), and one diabetic group administered metformin (300.0 mg/kg, 10 mice). Blood samples were collected from the tail vein for the measurement of blood glucose at 14 d after administration.

Statistical analysis

All results are presented as the mean ± S.D. The data were analyzed for statistical significance by one-way ANOVA test. Results were considered significant at $p < 0.05$.

Results and Discussion

Antihyperglycemic effect of EBP and PBP in alloxan-induced diabetic mice

None of the animals died during the experiment. The effects of EBP on blood glucose levels of alloxan-induced diabetic mice were shown in Table 1. On day 7, only EBP (12.0 mg/kg) group can significantly lower blood glucose ($p < 0.05$) compared to diabetes control group. When on day 14, the results showed that all the EBP groups significantly lowered blood glucose levels in diabetic mice ($p < 0.05$). The effects of PBP on blood glucose levels in diabetic mice were also listed in Table 1. The results displayed that the PBP did not reduce the blood glucose levels in alloxan-induced diabetic mice on day 7 and day 14. So EBP was selected to isolate the antihyperglycemic ingredients. Vijai and others (2014) reported that ethanolic extracts and the hexane and chloroform fractions of banana (M. paradisiacal (Linn.)) peels showed promising anti-diabetic activity in STZ-induced diabetic rats, but the anti-diabetic activity of ethyl acetate and petroleum ether extract of banana peel did not evaluate. We found the EBP (*Musa nana* Lour.) had the antihyperglycemic activity, too.

Table1**.** The Antihyperglycemic effect of EBP and PBP in alloxan-induced diabetic mice（n=10）

Groups	Blood glucose(mmol/l)		
	Initial	7 d	14 d
Normal control	4.71±0.89	5.73±0.48	5.20±0.58
Diabetic control	20.28±3.76	13.87±3.83	17.01±4.85
Metformin(300.0 mg/kg)	19.15±4.33	10.61±3.39	12.09±3.47*
EBP(6.0 mg/kg)	18.63±4.25	10.50±4.86	11.41±4.20*
EBP(12.0 mg/kg)	19.39±4.29	10.36±2.97*	12.33±3.75*
PBP(6.0 mg/kg)	19.58±3.44	13.14±3.27	14.91±4.81
PBP(13.0 mg/kg)	19.31±4.66	12.68±3.82	15.17±4.34

The normal control and diabetic control group received 0.2 ml/10g water orally daily. Values are means ± S.D., * $p < 0.05$, ** $p < 0.01$ compared with the diabetic control group.

Identification of the chemical compounds from EBP

Structures of the isolates (Fig.1) were deduced by comparison of their mp, EI-MS, 1H- and 13C-NMR with the literature data, revealed that compoundIis lupenone, compound II is β-sitosterol. Our results represented the first report of isolation of lupenone from banana peel. The β-sitosterol has been yeilded from banana peel reported by Matook S M and Fumio H [30].

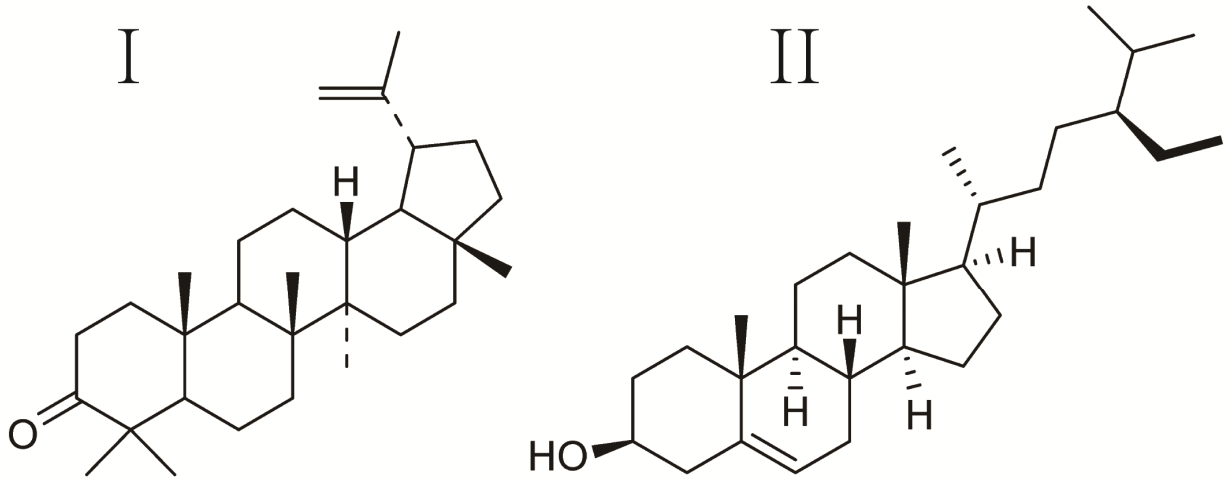

Fig. 1. Chemical structure of the lupenone (I) and β-sitosterol(II)

Antihyperglycemic effect of lupenone in diabetic mice

As the results showed in Table 2, lupenone from EBP had significant antihyperglycemic activity ($p < 0.01$), which showed that lupenone was one of the antihyperglycemic active ingredients of banana peel. According to reports, lupenone can inhibit α -glucosidase (α-Glu) and protein tyrosine phosphatase 1B (PTP1B) activity in vitro [31, 32], the two enzymes are associated with anti-diabetic, and recently study has found that lupenone has the anti-diabetic activity in diabetic sprague-dawley rats [33]. In this study, the results clearly showed that lupenone had a significantly antihyperglycemic activity in alloxan-induced diabetic mice with no animal deaths during the experiment. And Gupta and others [34] reported that β-sitosterol have potential of anti-diabetic and anti-oxidant in streptozotocin-induced experimental hyperglycemia. The lupenone and β-sitosterol yielded from EBP were the antihyperglycemic active ingredients of banana peel. The lupenone also has been isolated from ethyl acetate fraction of Rhizoma Musae (the dried rhizome of *Musa basjoo* Sieb. et Zucc.), the lupenone may exist in Musaceae family.

Table2. The Antihyperglycemic effect of lupenone in alloxan-induced diabetic mice（n=10）

Groups	Blood glucose(mmol/l)	
	Initial	14 d
Normal control	3.84±0.36	3.70±0.38
Diabetic control	15.45±3.16	14.33±2.40
Metformin (300.0 mg/kg)	15.03±3.25	9.10±2.59**
Lupenone (12.0 mg/kg)	13.63±1.68	8.93±3.10**

The normal control and diabetic control group received 0.2 ml/10g water orally daily. Values are means ± S.D., * $p < 0.05$, ** $p < 0.01$ compared with the diabetic control group.

Conclusions

The present study indicated that the EBP and lupenone had significant antihyperglycemic activity. lupenone was first time isolated from banana peel, and the banana peel could be utilized as a natural source of antihyperglycemic food, health care or drug. lupenone have potential to develop as an anti-diabetic drug.

Author Contributions

Dr. Hongmei Wu and Feng Xu contributed equally to this work. They conduct this study. Dr Xiangpei Wang design this study, revising the draft, interpretation of the results, Junjie Hao and Ye Yang analysis the data and revise the draft.

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