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Original Article

Hypoglycemic activity of two anthraquinone derivatives from Juncus setchuensis Buchen

Ying Cai[1*], Rongli Qiu[2*], Yu Lu[1], Chongfa Huang[1], Jing Wang[1], Yongzhang Ji[1], Aiping Wang[1]

[1]No. 454 Hospital of PLA, Nanjing 210002, Jiangsu, China; [2]Nanjing University of Traditional Chinese Medicine, Nanjing 210002, Jiangsu, China. [*]Equal contributors.

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Abstract: Diabetes dramatically impacts human health and quality of life. Substances from medicinal plants could be beneficial in the prevention or treatment of diabetes. Junci setchuensis Buchen (binata), which has been used as a folk medicine for treating diabetes in China, shows obvious hypoglycemic potential. This article focuses on hypoglycemic activity of binata and its main active ingredients. In the present study, hypoglycemic activities of the decoction of Juncus setchuensis Buchen (binata) were confirmed in streptozotocin (STZ)-induced diabetic rats. Further, different compounds were isolated and identified from the active fraction of binata including 2-hydroxy-3-methyl-anthraquinone, 1,8-dihydroxy-6-methoxy-3-methyl-anthraquinone (physcion), stigmasterol, stigmastan-3,6-dione, vanillin, tetracosanoic acid, and masaji heptadecanoic acid. The two anthraquinone derivatives 2-hydroxy-3-methyl-anthraquinone, and physcion were subsequently confirmed to have hypoglycemic activity, suggesting that they were the major active components of binata responsible for anti-diabetic function.

Keywords: Juncus setchuensis Buchen, hypoglycemic activity, active components, anthraquinone derivatives, diabetes

Introduction

Diabetes is an endocrine metabolic disorder that is characterized by hyperglycemia associated with abnormal metabolism of carbohydrates, fats and proteins, causing significant morbidity and mortality due to microvascular and macrovascular complications [1-3]. The disease has been known to affect nearly 10% of the world population and is rapidly increasing worldwide, especially in the developing countries [4, 5]. According to World Health Organization (WHO), the diabetic population is expected to increase up to 300 million by the year 2025 [6]. Currently, antidiabetic therapies including insulin and a variety of oral drugs such as sulfonylureas, biguanides and glinides are often associated with a number of serious complications. Therefore, the search for more effective and safer hypoglycemic agents has recently received considerable attentions.

In the past decades, more than 400 medicinal plants have been reported to be potentially useful in diabetes and used empirically in antidiabetic remedies [7]. The hypoglycemic effects of several herbal medicines from these plants have been confirmed and the associated mechanisms are being studied [8-11]. It has been known that herbal medicines with antidiabetic potential mostly act through either insulin mimetic or secretagogues activities [7]. Junci setchuensis Buchen (binata, **Figure 1**) is the dried aerial part with flowers of the palnt Juncus setchuensis Buchen var. effusoides Buchen. The therapeutic effect of binata on diabetes has been observed and it commonly serves as a traditional folk medicine for diabetes in the boarder area between Jiangsu and Zhejiang provinces in China.

Recently, active ingredients of binata have been isolated and analyzed. In 2009, Wang et al. isolated four new derivatives of 9,10-dihydrophenanthrene, three new phenanthrenes, and three known compounds from the whole plant of Juncus setchuensis [12], which were further identified as n-hexacosanoic acid and β-sitosterol [13]. A total of 13 compounds were identified in phytochemical extracts of Juncus setchuensis including four 9,10-dihydrophenanthrene derivatives, one phenanthrene, one

Figure 1. *Junci setchuensis Buchen* (binata), the dried aerial part with flowers of the palnt Juncus setchuensis Buchen var. effusoides Buchen.

flavonone, two mono-aromatic compounds, one steroid, one anthraquinone and three derivatives of diterpenoid tanshinone [14]. Early studies have shown that the phenanthrene derivatives are important active ingredients of Juncus species, and the 9,10-dihydrophenanthrene derivatives have cytotoxic, antibacterial and antioxidant activities [15, 16]. Currently, there are quite few pharmacological and clinical studies on the antidiabetic effect of binata. In 2012, we used binata to treat early-stage diabetes [17], and found that water extracts from binata effectively reduced blood glucose but increased serum insulin [18]. However, the hypoglycemic activities of the bioactive components of binata have never been previously studied. In the present study, we isolated components of Juncus setchuensis Buchen and evaluated their hypoglycemic effects in both diabetic mice and different cell lines.

Materials and methods

Plant, chemicals, and equipments

Juncus setchuensis Buchen was collected from Jiangsu province in November 2011, and authenticated by Professor Le Wei in Nanjing University of Traditional Chinese Medicine in China. The voucher specimens were deposited in No. 454 Hospital of PLA in Nanjing, China.

Pioglitazone was purchased from Conba Pharmaceutical Co., Ltd. (Hangzhou, China). Streptozotocin (STZ) and α-glucosidase were obtained from Sigma (Louis, MO, USA). RPMI-1640 media was purchased from Gibco (Gaithersburg, MD, USA). Trypsin was purchased from Shenxing Biotechnology Co. Ltd. (Nanjing, China). DMSO was purchased from Guanghua Chemical Co. Ltd. (Guangdong, China). Normal bovine serum (NBS) was obtained from Sijiqin Serum Factory (Hangzhou, China). MTT was purchased from Boao Biotechnology Co. Ltd. (Shanghai, China). Silica gel was purchased from Qindao Marine Chemical Factory (Qindao, China). Mice H4IIE hepatoma cells and 3T3-L1 cells were obtained from ATCC (Manassas, VA, USA). Blood glucose meter was manufactured by Bayer Health Care Co. Ltd.(Wuppertal, Germany). 1H-NMR and 13C-NMR spectra were recorded with Bio-rad Brucker ACF-500 NMR spectrometers (Berkeley, CA, USA). Tri-Carb2910TR liquid flash count (Hewlett-Packard Company (Palo Alto, CA, USA), MK3 microplate reader (Thermo LabSystems, Inc. Philadelphia, PA, United States), MCO-15A CO_2 cell incubator (SANYO Electric Co., Ltd., Osaka, Japan), BX-50 inverted microscope (Olympus Imaging Australia, Macquarie Park, Australia), and Clean Bench (Purification Equipment Factory, Shuzhou, China) were used in this study.

Preparation of reagent and different fractions of binata decoction

1% STZ solution was prepared by dissolving 100 mg of STZ in l0 mL of sodium citrate-citric acid buffer solution at 0°C and protected from light. The solution was fresh made and used within 30 min after preparation. A total of 800 g of binata were extracted with 6400 mL water each time for a total of 3 times. The extracts were combined and concentrated in a vacuum to yield a final concentration of 1 g raw drug/mL. Half of the decoction was deposited in 95% ethanol (a final alcohol concentration of 50%). The supernatant and sediment were respectively concentrated and kept in refrigerator before use.

Isolation and identification of components

Components of binata were isolated and identified as described [19]. Briefly, 30 kg of binata were extracted 3 times with water. The extracts

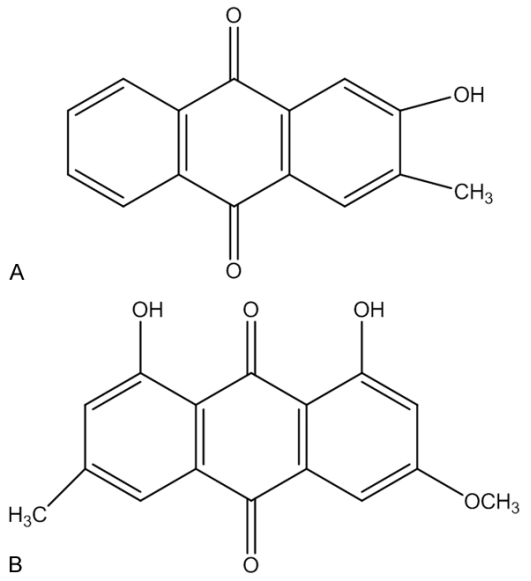

Figure 2. Chemical structure of (A) 2-hydroxy-3-methyl-anthraquinone and (B) 1,8-dihydroxy-6-methoxy-3-methyl-anthraquinone (physcion) isolated from binata.

were concentrated and deposited with 95% ethanol (a final alcohol concentration of 50%). The supernatant was concentrated and subjected to column chromatography with a silica gel (200-300 mesh) column using petroleum ether and ethyl acetate as a gradient eluent system. Silica column chromatography was repeated with petroleum ether-acetone system and methylene chloride-methanol system, respectively. The extracts were sublimated on a Sephadex LH-20 column using methanol as eluent, yielding the following 7 compounds whose structures were elucidated using 1HNMR and 13CNMR. Their chemical data were identical with those reported in the literature: 2-hydroxy-3-methyl-anthraquinone (106 mg, **Figure 2A**) [20, 21], 1,8-dihydroxy-6-methoxy-3-methyl-anthraquinone (physcion 135 mg, **Figure 2B**) [22, 23], Stigmasterol [24, 25], Stigmastan-3,6-dione [26, 27], vanillin [28, 29], (n)-tetracosanoic acid [30], and (n)-heptadecanoic acid [31, 32].

Animals

A total of 130 3-week-old ICR male mice (18±2 g) were housed under standard laboratory conditions (12 h light/dark cycle at a temperature of 20±1°C with food and water available) for 3 days after purchase. All animal experiments were performed strictly in accordance with the international ethical guidelines and approved by the Animal Experimentation Ethics Committee in Nanjing University of Traditional Chinese Medicine, China. The mouse model for type 2 diabetes was constructed as described [33, 34]. Briefly, the 130 mice were randomly divided into two groups: the experimental group (120 mice) and the control group (10 mice). While the basic diet was given to the control group, the experimental group was fed with a high-sugar high-fat diet containing 66.6% basic feed supplemented with 10% lard, 2.5% cholesterol, 1% sodium chlorate, and 20% sucrose for 3 weeks. The experimental group was given a one-time intraperitoneal injection of 1% STZ solution at a dose of 100 mg/kg, and then fed with the high-sugar high-fat diet for 2 weeks. The control group was injected with citric acid-sodium citrate buffer solution at a dosage of 10 mL/kg and fed with basic feed. Only water was allowed 12 h before the fasting blood glucose (FBG) test.

Hypoglycemic activity of different fractions of binata decoction

A total of 80 mice with FBG level above 16.7 mmol/L were selected as diabetic animals and randomly divided into 8 groups of 10 mice each: pioglitazone group (0.2 g/kg/d), low- and high-dose binata decoction group (7.5 and 15 g raw drug/kg/d), low- and high-dose 50:50 ethanol-water extract group (15 and 30 g raw drug/kg/d), as well as low- and high-dose 50:50 ethanol-water precipitate group of binata decoction (15 and 30 g raw drug/kg/d). All drugs were administered orally (P.O.) once per day for 28 days. Blood was collected by an orbital venous plexus, and FBG level of each mouse was tested at week 2 and week 4. Glucose solution was oral administrated at the dosage of 2.5 g/kg at day 28, and blood glucose levels were tested after 0, 0.5, 1, and 2 h, respectively.

In vitro experiments

In vitro experiments were performed as described to determine the hypoglycemic effects of individual components [35].

The inhibitory effect of 2 components on α-glucosidase activity

The blank control group, 2-hydroxy-3-methyl-anthraquinone (300, 100 and 30 μmol/L), phy-

scion (300, 100 and 30 μmol/L) and acarbose group were respectively mixed with 6 mL of 67 mmol/L potassium phosphate buffer (pH 6.8), 0.2 mL of 3 mmol/L glutathione solution, and 0.3 mL of α-D-glucosidase solution (0.04 mg/mL) and incubated at 37°C for 10 min. 0.5 mL of 0.01 mol/L PNPG solution was then added to the mixture for another 20-min incubation. The reaction was terminated by adding 10 mL of 0.1 mol/L Na_2CO_3 solution. The optical density of each group was determined at 400 nm, and the inhibition rate was calculated using the following formula:

Enzyme inhibition rate (%)={[D (λ) blank-D (λ) sample]/D (λ) blank}×100%.

The effect of 2 components on glucose production by H4IIE cells

H4IIE cells were cultured in a petri dish to reach a density of 1.2×10^7 cells/dish and a medium containing 500 nmol/L DEX and 0.1 mmol/L pCPT-cAMP was added on the next day. The obtained cells were subseeded into blank control group, 2-hydroxy-3-methyl-anthraquinone (300, 100 and 30 μmol/L), physcion (300, 100 and 30 μmol/L), and insulin group. The medium were discarded after 5 h and the cells were washed 3 times with PBS. A total of 2 mL of glucose production buffer (DMEM medium containing 5 mmol/L pyruvate sodium and 50 mmol/L lactate sodium without glucose and phenol red) was added to each group. The glucose content in the buffer in each group was measured after 3 h using the glucose oxidase method. Specifically, 1 mL of glucose production buffer was reacted with 0.3 mL of glucose analysis solution at 37°C for 10 min and the optical density was measured at 500 nm.

The effect of 2 components on glucose uptake in fat cells

3T3-L1 cells were induced into fat cells as follows: 3T3-L1 cells were cultured in high glucose DMEM containing 10% fetal calf serum at 37°C with 5% CO_2. After 2 days, the confluency cells were cultured for 3 additional days in high glucose DMEM containing 0.5 mmol/L isobutyl-3-methylxanthine (IBMX), 0.5 μmol/L dexamethasone (DEX), 5 μmol/L insulin and 10% fetal bovine serum (FBS). The obtained cells were incubated for 2 additional days in high glucose DMEM containing 5 μmol/L insulin and 10% FBS. Cells were finally incubated in high glucose DMEM medium containing 10% FBS, and culture medium was changed every 2 days. More than 90% 3T3-L1 cells were induced into fat cells after 8-12 days that were used in subsequent experiment. Mature fat cells were cultured in sugar-free DMEM medium in 24-well plate for 2 h, and divided into a blank control group, 2-hydroxy-3-methyl-anthraquinone (300, 100 and 30 μmol/L), physcion (300, 100 and 30 μmol/L), and insulin group. The plate was incubated at 37°C for 30 min. The medium was discarded and the cells were washed 2 times with PBS. A total of 300 μL of sugar-free DMEM medium containing 2 μCi/mL 3H-labelled 2-deoxyglucose (3H-2-DG) and 0.1 mmol/L 2-DG was added to each well and the plate was incubated at 37°C for 15 min. The reaction was terminated by adding 1 mL of frozen PBS solution containing of 10 mmol/L 2-DG to each well. Cells were washed twice with this solution, smashed with 200 μL of 0.5 mol/L NaOH solution and neutralized with 200 μL of 0.5 mol/L HCl solution. A total of 300 μL of cell lysate were obtained from each well to measure the radiation intensity by the scintillation counting method in order to determine the amount of 3H-2-DG uptaken by the fat cells in each group.

Statistical analysis

All data were analyzed using SPSS statistical package version 11.5. The differences among groups were compared by one-way analysis of variance (ANOVA), followed by post hoc analysis: Fisher's least significant difference (LSD). *P* values smaller than 0.05 were considered statistically significant.

Results

Hypoglycemic activities of different fractions of binata decoction

The inhibitory effect on FBG in diabetic mice: As shown in **Table 1**, a significant ($P<0.01$) increase in FBG was detected in diabetic model controls as compared to the healthy controls throughout the observation period. But the abnormal FBG levels was significantly decreased in the low-, and high-dose BD groups, as well as low- and high-dose 50:50 ethanol-water extract groups ($P<0.01$ or $P<0.05$) as compared to the diabetic controls at 14 d after administration, and further

Table 1. The effect of different fractions of binata decoction on FBG levels in diabetic mice ($\bar{x}$±S.D, n=10)

Group	Dose (g raw drug/kg)	FBG at days after dosing		
		0 d	14 d	28 d
Saline	-	5.28±1.31[2)]	5.93±2.31[2)]	5.54±1.99[2)]
Model	-	19.31±4.39	20.11±3.25	18.74±4.52
Pioglitazone	0.02	20.12±2.81	13.05±2.66[2)]	11.15±4.02[2)]
High-dose BD group	15.0	19.24±3.85	14.21±3.98[1)]	12.75±4.14[2)]
Low-dose BD group	7.5	18.15±4.21	15.56±2.95[1)]	14.57±3.95[1)]
High-dose 50:50 ethanol-water extract group	30	19.35±3.86	13.18±3.46[2)]	12.15±2.34[2)]
Low-dose 50:50 ethanol-water extract group	15	20.63±3.26	16.52±4.15[1)]	15.46±3.51[1)]
High-dose 50:50 ethanol-water precipitate group	30	21.42±4.46	19.42±4.42	18.45±3.45
Low-dose 50:50 ethanol-water precipitate group	15	20.86±3.74	18.82±3.64	18.53±3.74

BD indicates binata decoction. [1)]P<0.05, significantly different compared with the model group. [2)]P<0.01, significantly different compared with the model group.

Table 2. The effect of different fractions of binata decoction on glucose tolerance in diabetic mice ($\bar{x}$ ±S.D, n=10)

Group	Dose (g raw drug/kg)	Blood glucose at hours after orally administrated glucose			
		0 h	0.5 h	1 h	2 h
Saline	-	5.54±1.99[2)]	10.47±2.38[2)]	9.34.±1.35[2)]	7.32±1.24[2)]
Model	-	18.74±4.52	26.05±4.83	24.29±3.56	22.49±3.73
Pioglitazone	0.02	11.15±4.02[2)]	20.51±4.43[1)]	16.23±3.79[2)]	11.73±4.80[2)]
High-dose BD group	15	12.75±4.14[2)]	21.36±4.21[1)]	18.43±4.89[1)]	13.88±4.07[1)]
Low-dose BD group	7.5	14.57±3.95[1)]	22.11±3.53[1)]	20.63±4.62[1)]	15.44±3.24[1)]
High-dose 50:50 ethanol-water extract group	30	12.15±2.34[2)]	21.92±5.34[1)]	17.25±4.45[1)]	12.63±4.37[2)]
Low-dose 50:50 ethanol-water extract group	15	15.46±3.51[1)]	20.34±4.64[1)]	17.57±4.59[1)]	14.57±3.21[1)]
High-dose 50:50 ethanol-water precipitate group	30	18.45±3.45	25.07±3.17	24.82±3.83	21.02±4.87
Low-dose 50:50 ethanol-water precipitate group	15	18.53±3.74	25.24±3.04	23.12±4.49	21.36±2.57

BD indicates binata decoction. Glucose tolerance test was performed on day 28 from the start of administration. [1)]P<0.05, significantly different compared with the model group. [2)]P<0.01, significantly different compared with the model group.

decreased at day 28. In the pioglitazone 0.02 g/kg-administrated group, a significant (P< 0.01) decrease in the FBG level as compared to the diabetic controls was also detected. However, no significant decrease in FBG levels in low- or high-dose 50:50 ethanol-water precipitate group was observed as compared to the diabetic group.

The effect on glucose tolerance in diabetic mice: As shown in **Table 2**, the blood glucose level in the healthy control group increased rapidly after oral administration of 2.5 g/kg glucose. It peaked at 0.5 h and began to gradually decline afterwards. In contrast, the blood glucose level in diabetic model controls remained persistently high throughout the glucose tolerance test. The blood glucose level in the low-, and high-dose BD groups, as well as low- and high-dose 50:50 ethanol-water extract groups was significantly lower (P<0.01 or P<0.05) compared with the diabetic controls at each time points during the test. In the pioglitazone group, the glucose level was also significant (P<0.01 or P<0.05) lower compared with the diabetic model group throughout the test. Nevertheless, the blood glucose level in low- or high-dose 50:50 ethanol-water precipitate group was comparable to that in the model control group.

In vitro experiments

The inhibitory effect of 2 components on α-glucosidase activity: Inhibitory effect of the 2 components 2-hydroxy-3-methyl-anthraquinone and physcion on α-glucosidase activity was evaluated and compared to acarbose, as the positive control (**Table 3**). The optical density in α-glucosidase activity assay in 2-hydroxy-3-methyl-anthraquinone, physcion and acarbose group was significantly lower compared to

Table 3. The inhibitory effect of 2 components of binata decoction on α-glucosidase activity ($\bar{x}$±S.D, n=4)

Group	Dose (μmol/L)	Optical density	Inhibition rate (%)
Blank control		0.425 ±0.031	-
Acarbose	10	0.087±0.062[2)]	79.5
2-hydroxy-3-methyl-anthraquinone	300	0.155±0.085[2),3)]	63.5[3)]
	100	0.223±0.073[2)]	47.5
	30	0.286±0.042[1)]	32.7
Physcion	300	0.169±0.079[2),3)]	60.2[3)]
	100	0.250±0.051[1)]	41.2
	30	0.317±0.093[1)]	25.4

[1)]P<0.05, significantly different compared with the blank group. [2)]P<0.01, significantly different compared with the blank group. [3)]P>0.05, compared with acarbose group.

Table 4. The effect of 2 components of binata decoction on glucose production in H4IIE cells ($\bar{x}$±S.D, n=4)

Group	Dose (μmol/L)	Optical density	Inhibition rate (%)
Blank control		0.192±0.057	-
Insulin	5 U/L	0.105±0.087[2)]	45.3
2-hydroxy-3-methyl-anthraquinone	300	0.116±0.041[2),3)]	39.6[3)]
	100	0. 132±0.063[1)]	31.2
	30	0.157±0.092	18.2
Physcion	300	0.110±0.040[2),3)]	42.7[3)]
	100	0.124±0.084[2)]	35.4
	30	0.146±0.071	24.0

[1)]P<0.05, significantly different compared with the blank group. [2)]P<0.01, significantly different compared with the blank group. [3)]P>0.05, compared with the insulin group.

the blank control group (P<0.01 or P<0.05), suggesting all three agents inhibited the activity of α-glucosidase. Moreover, both 2-hydroxy-3-methyl-anthraquinone and physcion exhibited inhibition activities on α-glucosidase in a dose-dependent manner. It was also worth noting that the inhibition rate in 2-hydroxy-3-methyl-anthraquinone (300 μmol/L) and physcion (300 μmol/L) was comparable to that in the acarbose group (P>0.05).

The effect of 2 components on glucose production by H4IIE cells: We next evaluated the efficacy of 2-hydroxy-3-methyl-anthraquinone and physcion in reducing glucose production by H4IIE rat hepatoma cells through glucose oxidase assay. As shown in **Table 4**, both 2-hydroxy-3-methyl-anthraquinone (300 and 100 μmol/L) and physcion (300 and 100 μmol/L) treatment resulted in significantly lower glucose production by H4IIE cells compared with the blank control (P<0.01 or P<0.05), suggesting their inhibitory effect on liver gluconeogenesis. The inhibition rate of 2-hydroxy-3-methyl-anthraquinone (300 μmol/L) and physcion (300 μmol/L) was 39.6% and 42.7%, respectively, which were slightly lower than that in the insulin group (45.3%, P>0.05).

The effect of 2 components on glucose uptake in 3T3-L1-derived adipocytes: The effect of 2-hydroxy-3-methyl-anthraquinone and physcion on glucose uptake in 3T3-L1-derived adipocytes was assessed by monitoring the radiation intensity of cellular 3H-2-DG. It was shown that 2-hydroxy-3-methyl-anthraquinone (300, 100 and 30 μmol/L) dose-dependently increased glucose uptake compared with the blank control (P<0.01 or P<0.05). Physcion (300 μmol/L) also significantly promoted glucose uptake in the fat cells (P<0.01), although the activity of both components was relatively lower compare to the positive control, insulin (P< 0.05, **Table 5**).

Discussion

Hyperglycemia is one of the most critical problems in patients with diabetes. Therefore, the hypoglycemic effect has been considered as essential function of antidiabetic agents. The screening of effective herbal medicines for diabetes has been based on the evaluation of their hypoglycemic effects [36, 37]. The present study was conducted to access the hypoglycemic activity of binata extract in diabetic rat model.

STZ is a drug commonly used to induce experimental diabetes by selectively destroying B cells and insulin-producing pancreatic endocrine cells [38]. High-fat-diet/low lose of STZ has been widely employed for the induction of type 2 diabetic animal model characterized by hyperglycemia, which is attributable to decreased insulin sensitivity leading to insulin

Table 5. Effect of 2 components of binata decoction on glucose uptake in 3T3-L1-derived adipocytes ($\bar{x}$±S.D, n=4)

Group	Dose (μmol/L)	Radiation intensity	Inhibition rate (%)
Blank control		78.93±25.32	-
Insulin	5 U/L	167.27±39.07[2)]	211.9
2-hydroxy-3-methyl-anthraquinone	300	121.43±10.65[2),3)]	153.9[3)]
	100	103.98±15.87[1)]	131.7
	30	95.54±11.48[1)]	121.0
Physcion	300	104.45±0.040[2),3)]	132.3[3)]
	100	88.42±0.084	112.0
	30	75.32±0.071	95.4

[1)]P<0.05, significantly different compared with the blank group. [2)]P<0.01, significantly different compared with the blank group. [3)]P<0.05,significantly different compared with the insulin group.

resistance [9, 39]. In this study, we established a mouse model of type 2 diabetic by high-fat, high-sugar diet and a one-time STZ intraperitoneal injection, and explored the hypoglycemic effect of different fractions of binata in these diabetic mice.

Generally, a substantial elevation in FBG is one of the major clinical manifestation of diabetes, and the inhibition of FBG increase is considered an indication of hypoglycemic activity of antidiabetic agents. In our study, both water extract and 50:50 ethanol-water extract of binata significantly and dose-dependently reduced the elevated FBG levels in diabetic mice 14 d from the start of administration. The hypoglycemic activities of these binata extracts were further supported by their effect on improving glucose tolerance in diabetic mice after 28 consecutive days of administration. However, from **Tables 1** and **2**, it can be concluded that 50:50 ethanol-water precipitate of binata exhibits no hypoglycemic activity, and thus contains little or very low concentration of active components to improve diabetes. The inhibition of FBG increase and the improvement in glucose tolerance in the present study has been considered to be direct evidences supporting the relatively favorable antidiabetic effects of both water extract and 50:50 ethanol-water extract of binata.

Most reports on antidiabetic activity of medicinal plants have been conducted using crud extracts [7] rather than the pure bioactive components. In our study, we further isolated 7 components from the active fraction of binata and evaluated the hypoglycemic potential of 2 compounds: 2-hydroxy-3-methyl-anthraquinone and physcion in several in vitro assays. Both compounds significantly inhibited the activity of α-glucosidase in a dose-dependent manner. Both compounds also significantly reduced glucose production by H4IIE in glucose oxidase assay. Moreover, treatment of these compounds resulted in good glucose uptake in 3T3-L1-derived adipocytes. Based on these observations, 2-hydroxy-3-methyl-anthraquinone and physcion were identified as the hypoglycemic active components of binata. Although anthraquinone glycosides has been known to cause diarrhea, aglycone has several other biological activities such as antibacterial and anti-inflammatory effects [40]. In 2010, Ye et al. reported that early intervention with rhein significantly improved glucose tolerance and restored early-phase insulin secretion in db/db mice [41]. Further study showed that emodin effectively increased the insulin sensitivity, and decreased FBD levels as well as serum levels of triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) in db/db mice, suggesting that it effectively alleviated the glycolipid metabolic disorder and thus improved blood lipid metabolism in db/db mice [42]. In consistent with these findings in literature, both hypoglycemic active compounds of binata identified in our study were anthraquinone derivatives. Therefore, we speculate that anthraquinone structure could play an important role in improving glucose tolerance and thus might have potential in the treatment of diabetes.

Different mechanisms of anti-diabetic activities of medicinal plants have been extensively described including inhibition of β-galactocidase, α-glucocidase and alpha-amylase [43], renal glucose reabsorption [44], as well as insulin degradative processes [45]. Stimulation of insulin secretion from pancreatic beta cells of islets by increasing the size and number of these cells in the islets has also been reported [46]. Antioxidant activity of antidiabetic plants against oxidative stress that is involved in pan-

creatic β-cell dysfunction has also been reported as a mechanism of action of antidiabetic plants [47]. These finding will be enlightening in future studies on the mechanism of hypoglycemic effect of the two anthraquinone derivatives.

In conclusion, both water extract and 50:50 ethanol-water extract of binata could not only significantly lower FBG level but also improve glucose tolerance in STZ-induced diabetic rats. Furthermore, the potential hypoglycemic activity of binata was found to be attributed to two components: 2-hydroxy-3-methyl-anthraquinone and physcion. To the best of our knowledge, this is the first report that not only scientifically proves the hypoglycemic activity of binata but also identifies its active components. Although future studies have to be carried out on the binata in order to investigate the mechanism behind the hypoglycemic effect of the two compounds, we believe that they are promising antidiabetic substances that will be helpful for the treatment of type 2 diabetes.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Aiping Wang, No. 454 Hospital of PLA, Nanjing 210002, Jiangsu, China. Tel: +86 25 80865135; Fax: +86 25 84451156; E-mail: wangcai1967@163.com

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