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α-Glucosidase inhibition and antihyperglycemic activity of flavonoids from *Ampelopsis grossedentata* and the flavonoid derivatives

Jia Chen, Yuechan Wu, Jianwei Zou, Kun Gao*

School of Biotechnology and Chemical Engineering, Ningbo Institute of Technology, Zhejiang University, Ningbo, 315100, People's Republic of China

Abstract: The dried leaves and stems of *Ampelopsis grossedentata* have been used as a health tea and herbal medicine for hundreds of years in China. The study was aimed at searching for novel *α*-glucosidase inhibitors among the richest components of *A. grossedentata* and their derivatives. Three known major components (**1**-**3**) were isolated by recrystallization process and six new derivatives (**4**-**9**) were obtained by etherification of the bioactive flavonoid. All compounds were evaluated for their inhibitory activities against *α*-glucosidase (from *Saccharomyces cerevisiae*). As a result, compound **9** showed a significant *α*-glucosidase inhibitory activity with IC_{50} value of 9.3 μM and acted as a competitive inhibitor with the value of the inhibition constant (K_i) being 10.3 μM. The oral administration of compound **9** at a dose of 50 mg/kg significantly reduced the post prandial blood glucose levels of normal and streptozotocin (STZ)-induced diabetic mice. Furthermore, compound **9** significantly decreased the fasting blood glucose levels in STZ-induced diabetic mice.

Keywords: *Ampelopsis grossedentata*; flavonoids; *α*-Glucosidase inhibitor; antihyperglycemic activity; health tea.

*Corresponding author. Tel.: +86-574-88229598; Fax: +86-574-88130130.
E-mail address: gaok@nit.net.cn (K. Gao)

1. Introduction

Diabetes mellitus is a chronic metabolic disease, which has become a serious global problem. In 2010 an estimated 285 million people had diabetes and within the next 20 years this number is expected to almost double.[1] Many antidiabetic therapies focus on decreasing the level of blood glucose, for glycemic control is an effective therapy for diabetes.[2, 3] α-Glucosidase, a critical enzyme for the digestion of carbohydrates, catalyzes the cleavage of absorbable monosaccharides, starting from disaccharides and oligosaccharides. Thus, α-glucosidase inhibitors reduce postprandial hyperglycemia by slowing the digestion of carbohydrates in the intestines.[4] Although a number of synthetic medicines are available, drugs of a natural origin have also aroused great interest.[5-8] The consumption of natural and natural analog α-glucosidase inhibitors derived from plant-based foods or supplements offers an attractive strategy to control postprandial hyperglycemia due to their low cost and low incidence of major undesirable side effects.

A. grossedentata belongs to the genus of *Ampelopsis* Michx. (family: Vitaceae) and is a wild plant growing in mountainous areas of southern China. Its dried leaves and stems, called Vine Tea or Mao Yan Mei, have been consumed as a health tea and herbal medicine for hundreds of years.[9] As recorded in the Chinese Materia Medica, vine tea carries out several functions such as clearing away heat, promoting diuresis and blood circulation and removing channel obstructions.[10] Several studies have demonstrated that vine tea possesses many beneficial pharmacological properties including anti-inflammatory,[11] antioxidative,[9, 12] antihypertensive, hepatoprotective and antiviral activities.[13, 14] It is also claimed that drinking the herbal tea of *A. grossedentata* can alleviate the severity of many disorders, such as diabetes and hyperlipidemia.[15] However, to the best of our knowledge, the α-glucosidase inhibition and antihyperglycemic activity of constituents from *A. grossedentata* and their derivatives have yet to be deeply investigated.

In the present paper, work was undertaken to deal with the purification of the major components **1-3** (**Figure 1**) and the structural modification of bioactive component **1** from *A. grossedentata*, to hopefully obtain a better understanding of the potential bioactivity of flavonoids and their analogs.

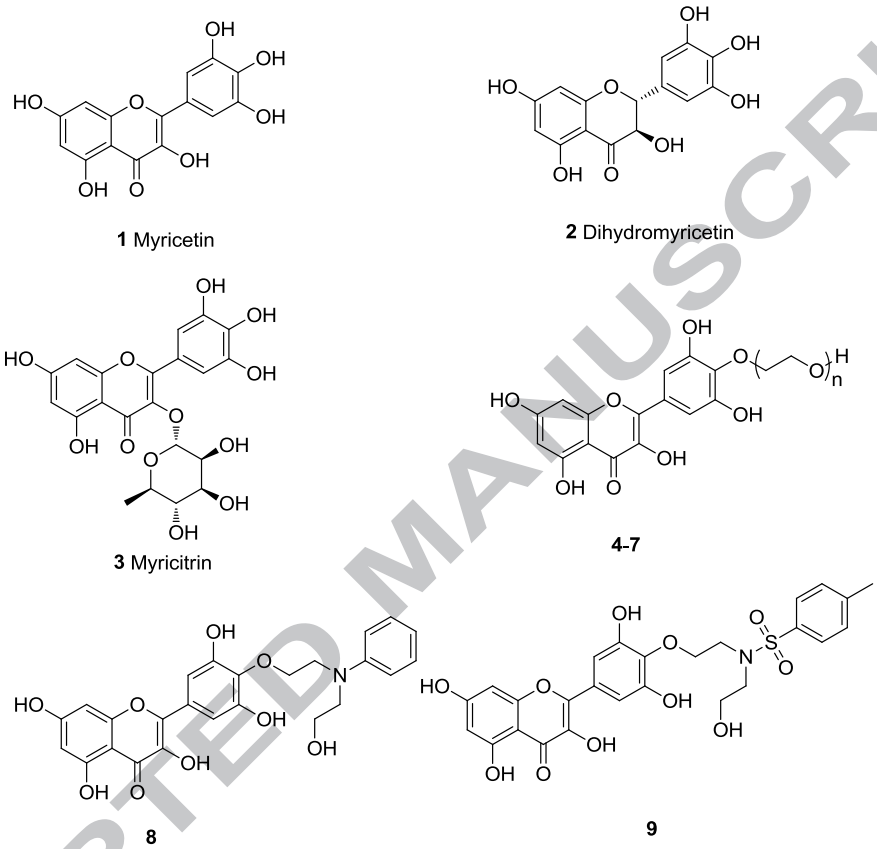

Figure 1. The structures of three known major components **1-3** and six new flavonoid derivatives **4-9**.

2. Results and Discussion

2.1. Chemistry

The chemical structures of compounds **1-9** are reported in **Figure 1**. Three known compounds were recognized as myricetin (**1**), dihydromyricetin (**2**) and myricitrin (**3**) by comparison with literature values.[16]

The synthetic routes for the compounds **4-9** are outlined in **Figure 2**.

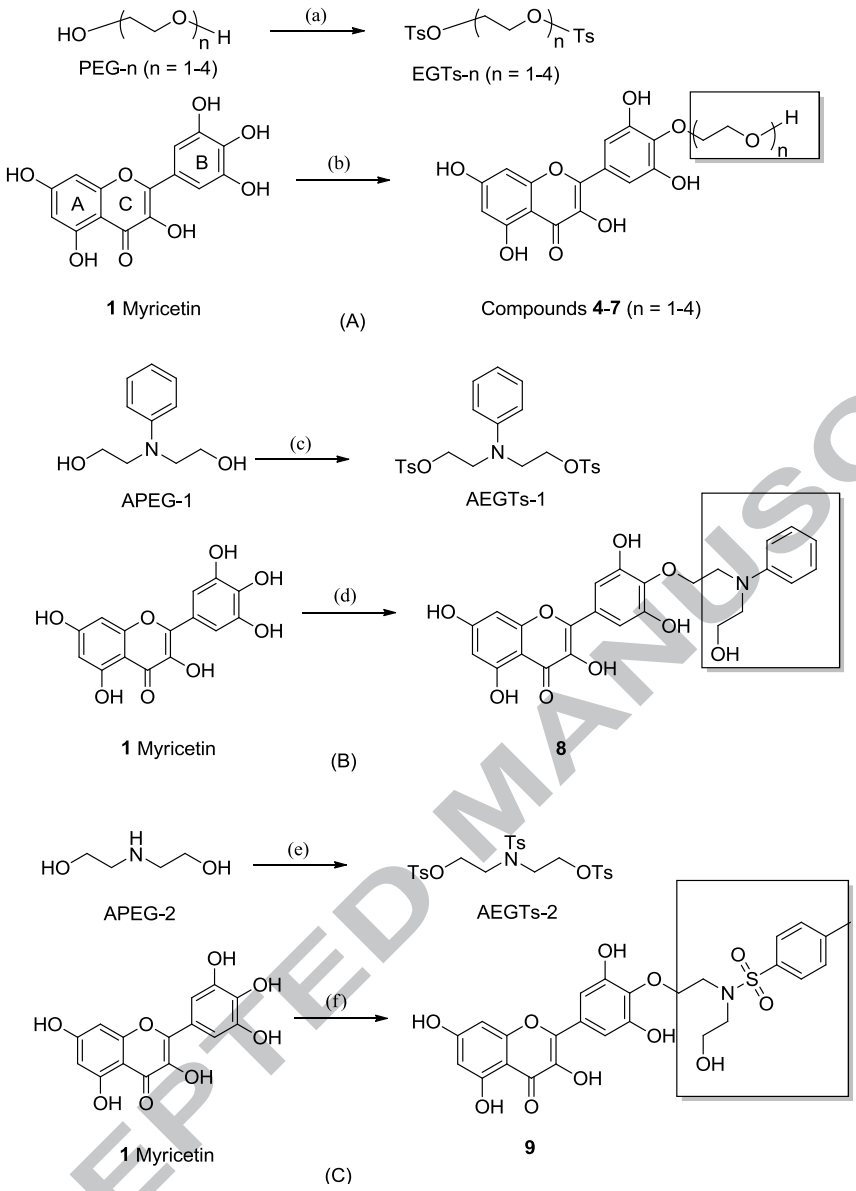

Figure 2. General procedures for the synthesis of new flavonoid derivatives **4**-**9**. Reaction conditions: (a) TsCl, DCM, KOH or Et_3N, 0℃, 2h to 5h; (b) EGTs-n (n = 1-4), K_2CO_3, DMF, 110℃, 3h to 5h; (c) TsCl, DCM, pyridine, 0℃; (d) AEGTs-1, K_2CO_3, DMF, 110℃, 5h; (e) TsCl, DCM, Et_3N, 0℃ to room temperature, 12h; (f) AEGTs-2, K_2CO_3, DMF, 110℃, 3h;

Ethylene glycol ditosylates were prepared from the corresponding polyethylene glycols (PEGs) or amino polyethylene glycol (APEGs) according to previous methods with some modifications.[17, 18] The etherification of myricetin (**1**) was carried out by the method of an improved Williamson ether synthesis using K_2CO_3 as a base in the solvent system of *N*,*N*-dimethylformamide (DMF). The reaction

mixtures were purified by CC or semi-preparative HPLC to give the desired myricetin derivatives **4**-**9**. The purity of the products (**4**-**9**) was analyzed by HPLC.

The structures of **4**-**9** were elucidated on a comprehensive analysis of their spectroscopic data. Compounds **4**-**9** are polar molecules and need a higher temperature at 275 ºC (General experimental procedures) to gasify in the HRESIMS for all the structures of these compounds contain the ethoxyl hydroxyl and five phenolic hydroxyl groups. The conditions for HRESIMS are easy to make ethoxyl hydroxyl group lose a molecule of water; thus only $[M-H_2O+H]^+$ signals for these compounds are observed in the HRESIMS spectra (Supplementary Materials).

Compound **4** has a $[M-H_2O+H]^+$ ion peak at *m*/*z* 345.0602 in the HRESIMS corresponding to a molecular formula $C_{17}H_{14}O_9$ with eleven degrees of unsaturation, being $C_2H_4O_2$ more than myricetin (**1**). The ^{1}H NMR spectrum showed the presence of a hydroxyl proton [δ 12.42 (1H, br s)], two sets of *meta*-phenyl protons assigned to two phenyl groups [δ 7.33 (1H, s), 7.24 (1H, s), 6.43 (1H, s) and 6.20 (1H, s)] and an ethoxyl hydroxyl [δ 4.31 (2H, m) and 4.29 (2H, m)]. The signals of **4** were similar to those of **1** [16], being one ethoxyl hydroxyl group more than myricetin. Accordingly, the ^{13}C NMR spectrum displayed a total of 17 carbon resonances, which were 2 carbon resonances more than myricetin and was also assigned to an ethoxyl hydroxyl group [δ 64.2 and 64.1]. The location of the ethoxyl hydroxyl group at the C-4' position could be determined on the basis of the characteristic downfield shifts of the carbon resonances for C-4' (δ 136.7), when compared to that of myricetin (δ 126.0, C-4').[16] The assignment of ^{1}H NMR and ^{13}C NMR for compounds **5**-**9** was done in the same way by comparison of NMR data with that of myricetin.

2.2. α-Glucosidase inhibition

The α-glucosidase (from *Saccharomyces cerevisiae*) inhibition was assessed according to a slightly modified method of Jeon et al.[19] The IC_{50} values of compounds **1**−**9** are shown in **Table 1**.

Among the major components, compound **1** showed a good α-glucosidase inhibitory activity with an IC_{50} value of 319.3 μM, and other abundant compounds **2** and **3** showed moderate activities with IC_{50} values of 633.9 and 837.7 μM, respectively. The activity of flavonoid **2** being lower than that of **1** revealed that the double band of C ring in the molecular is an essential requirement for the activity. Moreover, the α-glucosidase inhibition of **3** was also less sensitive than that of **1**. The weak activity of **3** could be caused by the absence of the essential hydroxyl group at C ring which may be responsible for the activity.

Table 1 α-Glucosidase inhibitory activity of compounds **1**-**9**.

Compounds	IC_{50} (μM ± SEM)[a]
1	319.3 ± 22.9
2	633.9 ± 24.9
3	837.7 ± 22.0
4	84.5 ± 8.6
5	43.8 ±1.5
6	233.1 ± 12.0
7	463.1 ± 15.8
8	41.1 ± 2.3
9	9.3 ± 0.4
Acarbose	720.3 ± 57.5

[a]IC_{50} values are shown as mean ± SEM from three independent experiments.

Based on a reservation of both the double band and a hydroxyl group of C ring, the natural flavonoid **1** was modified. As a result, new flavonoid derivatives **4**-**7** were obtained. The introduction of some polyethylene glycol in C-4 of the B ring caused an increased activity in the biological assay. The structure-activity relationship among compounds **4**-**7** suggested that the most active flavonoid would display two ethylene glycols, for compound **5** was found to be the most active with IC_{50} value of 43.8 μM. Unfortunately, while the number of introduced ethylene glycols was up to four, a decreased activity in the biological assay ensued, for compound **7** showed a moderate activity with IC_{50} values of 463.1 μM.

Interestingly, the introduction of nitrogen substituted groups including *N*-tosyl group at C-4′ in

flavonoid were also beneficial to the α-glucosidase inhibitory activities. For example, the B ring of compound **8**, replaced by a nitrogen substituted group at C-4′, showed a potent inhibitory activity with an IC_{50} value of 41.1 μM. Additionally, compound **9,** by introducing an *N*-tosyl group in the B ring, was the most active, with an IC_{50} value of 9.3 μM. The result was also supported by a computer-assisted docking (CAD) study, which suggests that it is not only the 8-OH of compound **9** that forms a hydrogen bond with the ASP349 in the active site, but also the *N*-tosyl group of compound **9** that forms hydrogen bonds with the LYS155 and SER244 in the active sites (Supplementary Materials).

2.3. Type of *α*-glucosidase inhibition

To clarify the *α*-glucosidase inhibition mode of compound **9**, which was the analog of the most abundant compound in the extract, Lineweaver−Burk plots were generated.[20] As shown in **Figure 3**, the value of the vertical axis intercept ($1/V_{max}$) remained unchanged with the increase of the concentrations of compound **9**, indicating that compound **9** was a competitive inhibitor. According to Michaelis−Menten kinetics, the value of the inhibition constant (K_i) is 10.3 μM.

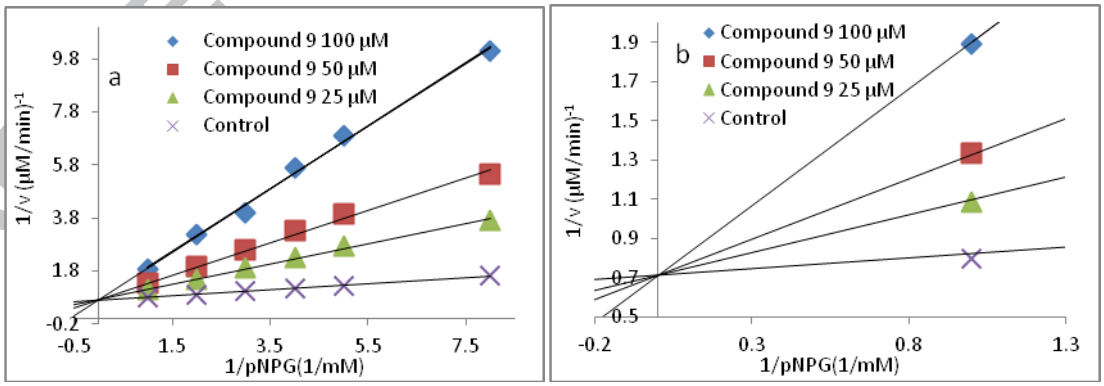

Figure 3. (a) Lineweaver−Burk plots of the reaction of α-glucosidase at different concentrations of substrate and compound **9**. (b) Partially enlarged view of panel A.

2.4. Antihyperglycemic effects of compound 9 on oral sucrose tolerance in normal and STZ-induced diabetic mice.

The OSTT is usually performed to evaluate the efficacy of a drug in inhibiting intestinal *α*-glucosidase *in vivo*.[21] Compound **9** was the most active among the evaluated flavonoids, for it showed a significant *α*-glucosidase inhibitory activity *in vitro*. Thus, compound **9** was further evaluated for its antihyperglycemic effects using an OSTT in both normal and STZ-induced diabetic mice. In comparison to the vehicle, the oral administration of compound **9** at a dose of 100 and 50 mg/kg significantly ($p < 0.05$) reduced the postprandial blood glucose level of normal mice (**Figure 4a**). The antihyperglycemic effect was observed at 30, 60 and 90 min after sucrose loading and was compared to that of acarbose (5 mg/kg). In contrast, the 25 mg/kg dose did not show a significant decrease in glycemia throughout the experiment. The OSTT was repeated in STZ-induced diabetic mice. These results were similar to those for normal mice (**Figure 4b**). According to the results of the OSTT, compound **9** inhibited the activity of intestinal *α*-glucosidase.

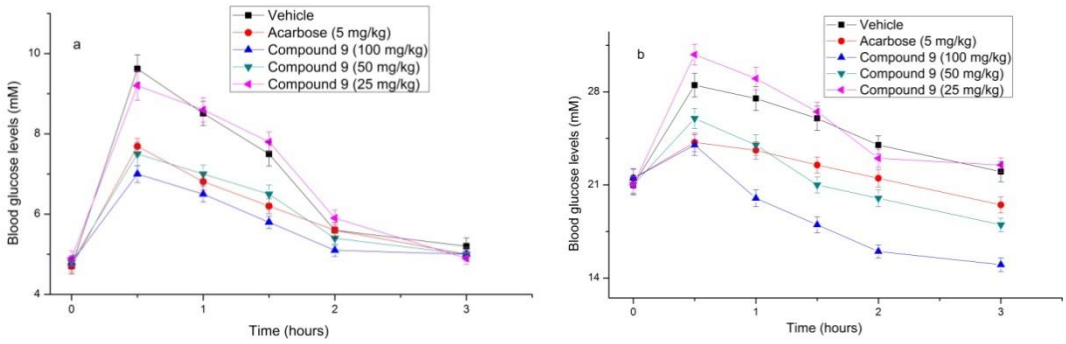

Figure 4. Effects of compound **9** on blood glucose levels in (a) normal and (b) STZ-induced diabetic mice using the OSTT. Data are the means ± SEM for six mice in each group. $p < 0.05$ by one-way ANOVA with a post-hoc test compared to the control.

2.5. Hypoglycemic effect of compound 9 on normal and STZ-induced diabetic mice.

According to previous methods,[22, 23] the hypoglycemic activity of compound **9** was evaluated in both normal and STZ-induced diabetic mice. As shown in **Figure 5**, compound **9**, which had decreased fasting glucose levels in STZ-induced diabetic mice, did not lower the fasting glucose levels in normal

mice. In diabetic mice, the oral administration of compound **9** at doses of 100 and 50 mg/kg significantly decreased the fasting glucose levels when compared to the vehicle treated groups ($p < 0.05$). The dose at 100 mg/kg resulted in a significant decrease in the glucose level at 1.5 h (− 37.0 %), 3 h (− 45.1 %), 5 h (−60.0 %), 7 h (− 65.4 %) and 9 h (− 67.3 %). After an administration of 50 mg/kg of compound **9**, the glucose level decreased by 27.8 % at 1.5 h, 35.9 % at 3 h, 47.3 % at 5 h, 59.6 % at 7 h and 61.5 % at 9 h. Because compound **9** displayed a hypoglycemic effect in diabetic mice but not in normal mice, in contrast with glibenclamide, these results indicate that compound **9** may not act directly via insulin liberation.

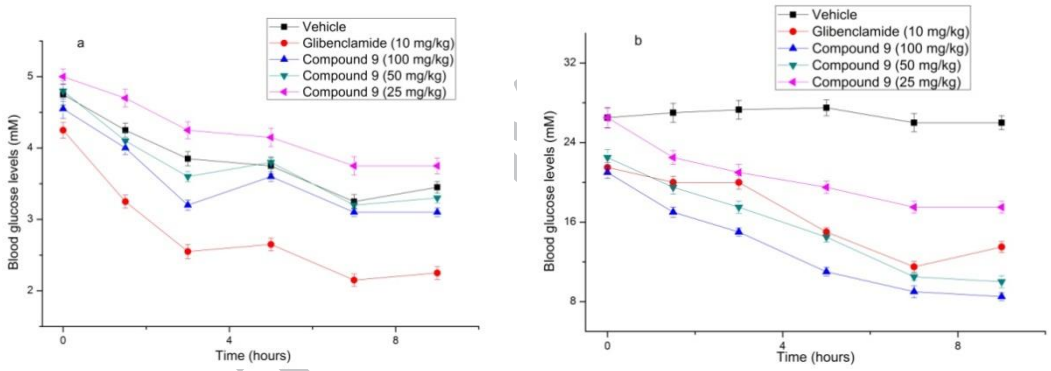

Figure 5. Hypoglycemic effect of compound **9** on (a) normal and (b) STZ-induced diabetic mice. Data are the means ± SEM for six mice in each group. $p < 0.05$ by one-way ANOVA with a post-hoc test compared to the control.

3. Conclusions

In the present study, myricetin (**1**), dihydromyricetin (**2**) and myricitrin (**3**) were major components obtained from leaves and stems of *A. grossedentata*, and flavonoid derivatives (**4**–**9**) were newly semi-synthesized from naturally occurring myricetin. It is particularly noteworthy that compound **9** acted as a competitive α-glucosidase inhibitor with IC_{50} values of 9.3 μM. This is the first report that myricetin analogs showed significant α-glucosidase inhibitory activity. Compound **9** will

enter into the architectural diversity of the antihyperglycemic activity flavonoids family. Moreover, the result suggested that the introduction of ethylene glycol and *N*-tosyl group moieties in myricetin are beneficial for α-glucosidase inhibitory activities. This is also a first-time report on the introduction of ethylene glycol and *N*-tosyl group moieties in naturally occurring myricetin.

The result of Lee's group suggested that the 4'-OH function plays an important role in anti-α glucosidase. [24] While this function is masked in compound **9**, the activity is better in the study, because the α-glucosidase used in the two experiments were not identical, just of 40% sequence similarity (Supplementary Materials); thus it was possible that the interaction mode for compound **9** differed from those reported.

Previous studies suggested that rutin, the analog of compound **9**, improved the chronic hyperglycemia impaired pancreatic β-cell viability and insulinsecretory function.[25] Therefore, the hypoglycemic action of compound **9** probably is involved in the protection of pancreatic β-cell survival and function.

Although compound **9** showed a better α-glucosidase inhibitory activity than that of acarbose, the result of *in vivo* study indicated that acarbose has a stronger hypoglycemic effect than the prepared compound **9**. This might be because many enzyme systems are related to the hypoglycemic effect and acarbose is not the only α-glucosidase inhibitor, there are other enzymes inhibitors too, which also reduce the rate of digestion of complex carbohydrates.[26] Further studies are necessary to demonstrate the mechanisms by which compound **9** decreased the fasting glucose levels in STZ-induced diabetic mice.

4. Materials and methods

4.1. Chemicals and plant materials

All organic solvents used in the study, such as petroleum ether (PE), chloroform ($CHCl_3$), ethyl acetate (EtOAc), methanol (CH_3OH) and *N*,*N*-dimethylformamide (DMF), were of an analytical grade. Methanol-d_4 (99.8%), dimethyl sulfoxide-d_6 (99.9%) and *α*-glucosidase from *Saccharomyces cerevisiae*, *p*-nitrophenyl-α-glucopyranoside (PNPG), streptozotocin (STZ) and acarbose were purchased from Sigma-Aldrich (St. Louis, MO, USA).

The leaves of *A. grossedentata* were purchased from Zhangjiajie Maoyanmei Group Co., Ltd. (Hunan, China).

4.2. Animals

Kunming mice (4−6 weeks old) were obtained from the Experimental Animal Center of Zhejiang Province (China). The use of mice was reviewed and approved by the Ethics Committee for Animal Experimentation of the Ningbo University (Zhejiang, China) and was in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

To induce diabetes, the mice were treated with a single intra-peritoneal injection of streptozotocin (100 mg/kg) dissolved in citrate buffer (pH 4.5) under fasting conditions. The blood glucose level was monitored on day 7 from the tail vein using a one-touch glucometer (Lifescan, Inc., Milpitas, CA). Mice with fasting blood glucose levels of ≥16.0 mmol/L were classified as diabetic mice.

4.3. General experimental procedures

IR spectra were recorded on a Nicolet NEXUS 670 FT-IR spectrometer. UV detections were measured on a Shimadzu UV-260 spectrophotometer. NMR spectra were recorded on Varian Mercury-300BB NMR (300 MHz) and Varian Mercury plus-400 (400 MHz) spectrometers with TMS as an internal standard. High Resolution Electro Spray Ionization Mass Spectroscopy (HRESIMS) data were measured on a Bruker Daltonics APEX II 47e spectrometer and acquired in positive-ion mode, using source voltage of 3.98 kV, capillary temperature of 275 °C, sheath gas flow rate of 2 arb, sweep

gas flow rate of 1 arb. Silica gel (200-300 mesh) and CHP20P MCI gel (75–150 μm, Mitsubishi Chemical Industries, Ltd.) were used for column chromatography (CC). Silica gel GF_{254} (10-40 μm) used for thin-layer chromatography (TLC) were supplied by Qingdao Marine Chemical Factory, Qingdao, P. R. China. Spots were detected on TLC under UV light or by heating after spraying with 5% H_2SO_4 in C_2H_5OH (v/v). Semipreparative and analytical High Performance Liquid Chromatography (HPLC) were carried out on Waters equipment (1525 pump and 2996 photodiode array detector) with a YMC-Pack ODS-A column (250 × 10 mm or 250 × 4.6 mm, 5 μm). The absorbances in the enzymatic assay were determined at 405 nm using a Bio-Rad Model 680 microplate reader.

4.4. General procedures for extraction and isolation of the major components

Air-dried leaves and stems (5.0 kg) of *A. grossedentata* were extracted three times (each for 7 days) with 45% ethanol (40 L) at room temperature. The ethanol filtrates were collected and concentrated under reduced pressure by a rotary evaporator at 40 ºC to dryness, producing 1500 g of ethanol extract. The extract was subjected to CC (D101-macroporous absorption resin) with a gradient of EtOH:H_2O (10:90, 20:80, 40:60, 70:30, 90:1) as eluent, and five fractions (Fr.1-Fr.5) were collected according to TLC analysis. Fr.2 (50 g) was recrystallized from EtOAc:MeOH (4:1) to afford compound **1** (40 g). Fr.3 (500 g) was fractionated using the same procedure (MCI gel CC, eluted with 40 % EtOH in H_2O), leading to the appearance of crude crystals of **2** (450 g), then compound **2** (400 g) was finally obtained by recrystallizing with hot water. The remaining Fr.3 was recrystallized from MeOH to yield compound **3** (10 g).

4.5. General procedures for the synthesis of flavonoid derivatives

4′-O-(2-hydroxy)-ethyl myricetin (**4**): **4** was obtained from myricetin (509 mg, 1.6 mmol), ethylene glycol ditosylate (296 mg, 0.8 mmol), K_2CO_3 (276 mg, 2.0 mmol) and DMF (30 mL), according to the general procedure, as a yellow amorphous powder (262 mg, yield: 45%); UV (MeOH)

λ_{max}(log ε) 364 (5.92), 253 (4.36) nm; IR (K Br) ν_{max}: 3540, 3446, 3334, 2933, 1657, 1617, 1596, 1506, 1102 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.42 (1H, br s, 5-OH), 7.33 (1H, s, H-2′), 7.24 (1H, s, H-6′), 6.43 (1H, s, H-8), 6.20 (1H, s, H-6), 4.31 (2H, m, H-1″), 4.29 (2H, m, H-2″); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 176.0 (C-4), 164.2 (C-7), 160.8 (C-5), 156.2 (C-9), 146.3 (C-3′), 145.8 (C-5′), 143.9 (C-2), 136.5 (C-3), 134.2 (C-4′), 122.7 (C-1′), 108.0 (C-6), 107.8 (C-2′), 103.1 (C-10), 98.3 (C-6), 93.5 (C-8), 64.2 (C-1"), 64.1 (C-2"); HRESIMS *m*/*z* 345.0602 $[M-H_2O+H]^+$ (calcd for $[C_{17}H_{13}O_8]^+$, 345.0605).

4′-O-[2-(2-hydroxy ethoxyl) hydroxy]-ethyl myricetin (**5**): **5** was obtained from myricetin (509 mg, 1.6 mmol), diethylene glycol ditosylate (332 mg, 0.8 mmol), K_2CO_3 (276 mg, 2.0 mmol) and DMF (30 mL), according to the general procedure, as a yellow amorphous powder (236 mg, yield: 36%); UV (MeOH) λ_{max}(log ε) 364 (2.56), 253 (2.40) nm; IR (K Br) ν_{max}: 3397, 2948, 1654, 1021cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.39 (1H, br s, 5-OH), 7.42 (1H, s, H-2'), 7.31 (1H, s, H-6'), 6.43 (1H, s, H-8), 6.20 (1H, s, H-6), 4.38 (2H, br s, H-1"), 4.17 (2H, br s, H-2"), 3.84 (2H, br s, H-3"), 3.39 (2H, br s, H-4"); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 176.1 (C-4), 164.4 (C-7), 160.7 (C-5), 156.3 (C-9), 152.4 (C-3'), 150.6 (C-5'), 145.4 (C-2), 141.0 (C-4'), 136.8 (C-3), 125.7 (C-1'), 112.2 (C-6'), 109.8 (C-2'), 103.0 (C-10), 98.4 (C-6), 93.5 (C-8), 73.1 (C-3"), 72.9 (C-2"), 72.4 (C-1"), 72.1 (C-4"); HRESIMS (positive) *m*/*z* 389.0865 $[M-H_2O+H]^+$ (calcd for $[C_{19}H_{17}O_9]^+$, 389.0867).

4′-O-{2-[2-(2-hydroxy ethoxyl)-hydroxy ethoxyl] hydroxy}-ethyl myricetin (**6**): **6** was obtained from myricetin (509 mg, 1.6 mmol), triethylene glycol ditosylate (367 mg, 0.8 mmol), K_2CO_3 (276 mg, 2.0 mmol) and DMF (30 mL), according to the general procedure, as a brownish amorphous powder (156 mg, yield: 22%); UV (MeOH) λ_{max}(logε): 339 (2.53), 260 (2.41) nm; IR (K Br) ν_{max}: 3410, 2925, 1649, 1197, 1043 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): δ12.67 (1H, br s, 5-OH), 6.70 (2H, s, H-2', 6'), 6.35 (1H, s, H-8), 6.18 (1H, s, H-6), 4.28 (2H, br s, H-1"), 3.97 (2H, d, *J* = 4.4Hz, H-2"), 3.65

(2H, br s, H-6''), 3.32 (2H, br s, H-3''), 3.22 (2H, br s, H-4''), 3.04 (2H, d, *J*=4.4Hz, H-5''); ^{13}C NMR (DMSO-d_6,100 MHz): δ 177.8 (C-4), 165.3 (C-7), 161.4 (C-5), 159.1 (C-9), 156.7 (C-2), 150.3 (C-3',5'), 136.7 (C-4'), 136.0 (C-3), 123.9 (C-1'), 108.3 (C-2',6'), 104.1 (C-10), 98.9 (C-6), 93.8 (C-8), 72.2 (C-5''), 71.8 (C-2''), 69.7 (C-1''), 69.1 (C-3''), 69.0 (C-4''), 68.3 (C-6''); HRESIMS (positive) *m*/*z* 433.1127 $[M\text{-}H_2O+H]^+$ (calcd for $[C_{21}H_{21}O_{10}]^+$, 433.1129).

4′-O-{{2-{2-[2-(2-hydroxy ethoxyl) hydroxy ethoxyl]-hydroxy ethoxyl} hydroxy}}-ethyl myricetin (**7**): **7** was obtained from myricetin (509 mg, 1.6 mmol), tetraethylene glycol ditosylate (402 mg, 0.8 mmol), K_2CO_3 (276 mg, 2.0 mmol) and DMF (30 mL), according to the general procedure, as a yellow amorphous powder (200 mg, yield: 25%); UV (MeOH) λ_{max}(log ε) 345 (2.54), 265 (2.42) nm; IR (K Br) ν_{max}: 3043, 2925, 1655, 1198, 1043 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.64 (1H, br s, 5-OH), 7.06 (2H, s, H-2', 6'), 6.32 (1H, s, H-8), 6.15(1H, s, H-6), 4.27 (2H, br s, H-1''), 4.13 (2H, br s, H-2''), 3.69 (2H, t, *J*=4.8Hz, H-8''), 3.50 (2H, br s, H-3''), 3.45 (2H, t, *J*=4.8Hz, H-4''), 3.37 (4H, m, H-5'', 6''), 3.29 (2H, m, H-7''); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 177.8 (C-4), 165.7 (C-7), 161.3 (C-5), 156.5 (C-9), 156.0 (C-2), 150.1 (C-3', 5'), 137.0 (C-4'), 136.6 (C-3), 124.4 (C-1'), 108.3 (C-2', 6'), 103.7 (C-10), 99.0 (C-6), 93.6 (C-8), 71.5 (C-7''), 71.0 (C-2''), 70.4 (C-1''), 70.2 (C-3''), 70.1 (C-4''), 70.0 (C-5''), 69.5 (C-6''), 69.0 (C-8''); HRESIMS *m*/*z* 477.1389 $[M\text{-}H_2O+H]^+$ (calcd for $[C_{23}H_{25}O_{11}]^+$, 477.1391).

4′-O-(2-*N*,*N*-hydroxy ethyl-phenyl)-ethyl myricetin (**8**): **8** was obtained from myricetin (509 mg, 1.6 mmol), K_2CO_3 (276 mg, 2.0 mmol), *N*,*N*-bis[2-(*p*-toluenesulfonyloxy) ethyl]-*N*-phenylamine (468 mg, 0.8 mmol) and DMF (30 mL), according to the general procedure, as a yellow amorphous powder (398 mg, yield: 52%); UV (MeOH) λ_{max}(log ε) 363 (2.56), 260.4 (2.42) nm; IR (K Br) ν_{max}: 3378, 2925, 2372, 1655, 1594, 1118 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.39 (1H, br s, 5-OH), 7.23 (2H, s, H-2', 6'), 7.13 (2H, t, *J* = 7.8 Hz, H-7'', 9''), 6.74 (2H, d, *J* = 8.3 Hz, H-6'', 10''), 6.36 (1H, s, H-8), 6.18

(1H, s, H-6), 4.09 (2H, t, *J* = 6.8 Hz, H-2''), 3.72 (2H, t, *J* = 6.8 Hz, H-1''), 3.56 (2H, t, *J* = 6.8Hz, H-3''), 3.44 (2H, t, *J*=6.8Hz, H-4''); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 176.0 (C-4), 164.9 (C-7), 160.7 (C-5), 156.3 (C-9), 150.5 (C-3', 5'), 147.9 (C-5''), 145.7 (C-2), 136.7 (C-4'), 136.0 (C-3), 129.1 (C-1'), 125.9 (C-7'', 9''), 115.4 (C-8''), 111.5 (C-6'', 10''), 107.2 (C-2', 6'), 102.8 (C-10), 98.4 (C-6), 93.3 (C-8), 68.7 (C-1''), 58.1 (C-4''), 53.0 (C-3''), 50.3 (C-2''); HRESIMS *m*/*z* 482.1456 $[M-H_2O+H]^+$ (calcd for $[C_{25}H_{24}NO_9]^+$, 482.1446).

4′-O-(2-*N*,*N*-hydroxy ethyl-toluene-4-sulfonyl)-ethyl myricetin (**9**): **9** was obtained from myricetin (509 mg, 1.6 mmol), K_2CO_3 (276 mg, 2.0 mmol), *N*,*O*,*O'*-Tri(toluene-4-sulfonyl)diethanolamine (454 mg, 0.8 mmol) and DMF (30 mL), according to the general procedure, as a light yellow amorphous powder (402 mg, yield: 45%); UV (MeOH) λ_{max}(log ε) 364 (2.56) nm; IR (K Br) ν_{max}: 3397, 2923, 1656, 1160, 1048 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.39 (1H, br s, 5-OH), 7.63 (2H, d, *J* = 8.0Hz, H-6'', 10''), 7.47 (1H, s, H-2'), 7.38 (2H, d, *J* = 8.0Hz, H-7'', 9''), 7.31 (1H, s, H-6'), 6.43 (1H, s, H-8), 6.20(1H, s, H-6), 4.41 (2H, br s, H-1''), 4.23 (2H, br s, H-2''), 3.44(4H, br s, H-3'', 4''), 2.36(3H, s, H-11''); ^{13}C NMR (DMSO-d_6, 100 MHz): δ176.1 (C-4), 164.5 (C-7), 160.7 (C-5), 156.2 (C-9), 152.5 (C-3'), 150.6 (C-5'), 145.2 (C-2), 143.3 (C-8''), 140.9 (C-5''), 136.9 (C-4'), 135.1 (C-3), 129.9 (C-7'', 9''), 126.9 (C-6'', 10''), 126.1 (C-1'), 111.9 (C-6'), 110.2 (C-2'), 103.0 (C-10), 98.4 (C-6), 93.5 (C-8), 73.2 (C-1''), 72.7 (C-4''), 52.2 (C-3''), 51.9 (C-2''), 20.9 (C-11''); HRESIMS *m*/*z* 542.1113 $[M-H_2O+H]^+$ (calcd for $[C_{26}H_{24}NO_{10}S]^+$, 542.1115).

4.6. α-Glucosidase inhibition

The α-glucosidase inhibition was assessed according to a slightly modified method of Jeon et al.[19] The α-glucosidase (0.1 U/mL) and substrate (*p*-NPG, 1.0 mM) were dissolved in potassium phosphate buffer (0.1 M, pH 6.7), and all samples were dissolved in DMSO. The inhibitor (10 μL) was preincubated with α-glucosidase (40 μL) at 37 °C for 10 min, and then the substrate (50 μL) was added

to the reaction mixture. The enzymatic reaction was performed at 37 °C for 30 min and the reaction was then terminated by the addition of Na_2CO_3 (1 M, 100 μL). All samples were analyzed in triplicate with five different concentrations near the IC_{50} values, and the absorbance at 405 nm was determined using a microplate reader. The inhibition percentage (%) was calculated by the following equation: Inhibition (%) = $[(OD_{control} - OD_{control\ blank}) - (OD_{sample} - OD_{sample\ blank})/ (OD_{control} - OD_{control\ blank})] \times 100$.

4.7. Type of *α*-glucosidase inhibition

The mode of inhibition of *α*-glucosidase was investigated with increasing concentrations of substrate (4-nitrophenyl *α*-D-glucopyranoside) and compound **9**. Then, the inhibition type was determined by a Lineweaver−Burk plot according to Michaelis−Menten kinetics. Origin (version 8.0) software was used for plotting the results.

4.8. Oral sucrose tolerance test (OSTT)

The fasting normal and STZ-induced diabetic mice were orally administered with compound **9** (25, 50 and 100 mg/kg of body weight), acarbose (5 mg/kg), or the control and, after 30 min, were given a sucrose solution (3 g/kg of body weight). Each group had six mice. Compound **9** and acarbose were suspended in 0.5 % sodium carboxy methyl cellulose (CMC−Na). The control mice were administered with the same volume of 0.5 % CMC−Na solution. The tail vein glucose concentrations were measured with a glucometer at 0, 0.5, 1.0, 1.5, 2.0 and 3.0 h after the sucrose load.

4.9. Hypoglycemic activity assay

The fasting normal and STZ-induced diabetic mice (n = 6 for each group) were orally administered with compound **9** (25, 50 and 100 mg/kg of body weight), glibenclamide (10 mg/kg), or the control (0.5 % CMC−Na). The tail vein glucose concentrations were measured with a glucometer at 0, 1.5, 3, 5, 7 and 9 h after administration.

4.10. Statistical analysis

The data were expressed as the mean ± standard error of the mean (SEM) and were analyzed using SPSS (version19.0) statistical software (SPSS, Chicago, IL). The statistical significance of the differences ($p < 0.05$) between the mean values of the treatment and control groups were obtained from a one-way analysis of variance (ANOVA) followed by Tukey's or Dunnett's test.

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Supplementary data

HPLC chromatograms, UV, HRESIMS, IR and NMR spectra for compounds **4**-**9** are available.

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Graphical Abstract

α-Glucosidase inhibition and antihyperglycemic activity of flavonoids from *Ampelopsis grossedentata* and the flavonoid derivatives

Jia Chen, Yuechan Wu, Jianwei Zou, Kun Gao*

School of Biotechnology and Chemical Engineering, Ningbo Institute of Technology, Zhejiang University, Ningbo, 315100, People's Republic of China