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Hypoglycemic activity evaluation and chemical study on hollyhock flowers

Yi Zhang [a], Lijun Jin [b], Qiu Chen [c], Zhizhen Wu [b], Yongzhe Dong [a], Lifeng Han [c], Tao Wang [a,*]

[a] Tianjin State Key Laboratory of Modern Chinese Medicine, 312 Anshanxi Road, Nankai District, Tianjin 300193, PR China
[b] Institute of Traditional Chinese Medicine, Tianjin University of Traditional Chinese Medicine, 312 Anshanxi Road, Nankai District, Tianjin 300193, PR China
[c] Key Laboratory of Pharmacology of Traditional Chinese Medical Formulae (Tianjin University of Traditional Chinese Medicine), Ministry of Education, 312 Anshanxi Road, Nankai District, Tianjin 300193, PR China

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ABSTRACT

Hollyhock (*Althaea rosea* (Linn.) Cavan) belongs to *Althaea* genus, Malvaceae family, is a perennial garden plant distributed throughout the world in warm temperate and tropical regions, which was used as anti-diabetes ingredient in traditional Chinese medicine. In the process of our research, ethanolic extract of hollyhock flower (HFE) was found to decrease serum triglyceride and glucose levels significantly in KK-A^y mice after oral administration for 8 weeks. Meanwhile, gene expressions on AMPK, IRS2, PI3K, AKT and GLUT4 in liver were remarkably up-regulated. Three new dihydroflavonol glycosides, named as roseaflavanonolosides A (1), B (2), and C (3), together with two known ones were obtained from HFE. Their structures were elucidated by chemical and spectroscopic methods. Hepatic cell glucose uptake experiment was performed using 2-NBDG as a glucose uptake indicator. At the dosage of 20 μg/mL for 1–5, the glucose uptake increasing level was nearly 30%–40% in HepG2 cells. We partly revealed the glucose metabolism regulation effect may relate to dihydroflavonols in hollyhock flower.

1. Introduction

Hollyhock (*Althaea rosea* (Linn.) Cavan), commonly know as marshmallow plant, or *Alcea rosea*, belongs to *Althaea* genus, Malvaceae family which is a perennial garden plant that grows on the banks of rivers and in salt marshes, and widely distributed throughout the world in warm temperate and tropical regions. The leaves, seeds, roots, and flowers of it were used for internal heat evil treatment (heat-relieve) in certain Chinese regions such as Xinjiang, an autonomous region of northwest China. In traditional Uyghur medicine, hollyhock flowers are regarded as bleed stopping, swell reducing and detoxification.

The chemical and activity researches of hollyhock are rare. Gibberellin glucosides and polysaccharides are the main constituents in shoot apices [1] and seed or leaf [2,3] of this plant, respectively. Meanwhile, phenolic acids [4] and flavonoids [5] are found in flower. Polysaccharides from seeds were reported to have antioxidant activity [2]. Methanolic extract of whole plant could significantly suppress neoplastic cell transformation by inhibiting the kinase activity of the epidermal growth factor receptor [6]. While, methanolic extract of flower showed increasing activities on glucose-6-phosphate dehydrogenase and hydroxysteroid dehydrogenase in the Leydig cells of rats [7].

Because metabolic syndrome is considered as cholesterol, glucose and triglycerides excess accumulations as toxic dampness and heat pathogen in the blood vessels and liver. Heat-relieve herb medicines are important resources for hyperglycemia or hyperlipidemia treatment in traditional Chinese medicine (TCM), which was testified by a lot of research groups.

* Corresponding author. Tel./fax: +86 22 5959 6163.
E-mail address: wangtao@tjutcm.edu.cn (T. Wang).

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For example, *Coptis chinensis* [8], *Momordica charantia* [9], and *Momordica grosvenori* [10] extracts showed significant regulation effects on serum glucose and triglyceride levels.

Though hollyhock flowers were usually used in traditional Uyghur medicine formula for hypoglycemic or hypolipidemic treatment, the active constituents and mechanism researches remain unclear. In this paper, we report the hypoglycemic activity and active constituents' researches for it.

2. Experimental

2.1. General

Optical rotations were obtained on a Rudolph Autopol® IV automatic polarimeter. IR spectra were recorded on a Varian 640-IR FT-IR spectrophotometer. UV spectra were measured on a Varian Cary 50 UV–Vis spectrophotometer. NMR spectra were determined on a Bruker 500 MHz NMR spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C NMR, with TMS as an internal standard. Positive- and negative-ion HRESI-TOF-MS were recorded on an Agilent Technologies 6520 Accurate-Mass Q-Tof LC/MS spectrometer.

Column chromatographies (CC) were performed on macroporous resin D101 (Haiguang Chemical Co., Ltd., Tianjin, China), silica gel (74–149 μm, Qingdao Haiyang Chemical Co., Ltd., Qingdao, China), and ODS (50 μm, YMC Co., Ltd., Tokyo, Japan). Preparative high performance liquid chromatography (preparative HPLC) column, Cosmosil $5C_{18}$-MS-II (20 mm i.d. × 250 mm, Nakalai Tesque, Inc., Tokyo, Japan) were used to purify the constituents.

2.2. Plant material

Hollyhock flowers were collected from Jimusaer County, Xinjiang Uyghur autonomous region, China, and identified by Dr. Xiaoguang Jia at the Institute of Medicinal Plant Development Xinjiang branch, Chinese Academy of Medical Sciences, as dried flower of *Althaea rosea* (Linn.) Cavan. Voucher specimen was deposited at the Institute of Traditional Chinese Medicine of Tianjin University of Traditional Chinese Medicine.

2.3. Extraction and isolation

The dried hollyhock flowers (5.0 kg) were refluxed with 95% EtOH. The solvent was evaporated under reduced pressure, and the 95% EtOH extract (475 g) was given. Then, the extract (380 g) was subjected to D101 macroporous resin CC (bed volume: 3 L) and eluted with H_2O (12 L), 70% EtOH (12 L), and 95% EtOH (9 L), successively. As a result, H_2O (171 g), 70% EtOH (169 g), and 95% EtOH (12 g) eluted fractions were obtained. 70% EtOH eluate (HFE) was used for activity evaluation in KK-A^y mice.

HFE (120 g) was subjected to silica gel CC [$CHCl_3$ → $CHCl_3$–MeOH (100:5, v/v) → $CHCl_3$–MeOH–H_2O (10:3:1 → 7:3:1 → 6:4:1, v/v/v, lower layer)] to yield 12 fractions (Fr. 1–12).

Fraction 3 (9.0 g) was separated by ODS CC [MeOH–H_2O (30:70 → 40:60 → 50:50 → 60:40 → 70:30 → 100:0, v/v)], and 11 fractions (Fr. 3-1–3-11) were obtained. Fraction 3-4 (3.3 g) was purified by preparative HPLC [MeOH–H_2O (40:60, v/v) + 1% HAC] to give (2*R*,3*R*)-dihydrokaempferol (4, 278.8 mg). Fraction 4 (10.0 g) was isolated by preparative HPLC [MeOH–H_2O (40:60 → 45:55 → 50:50, v/v)] to yield 13 fractions (Fr. 4-1–4-13). Fraction 4-4 (638.3 mg) was further separated by preparative HPLC [MeOH–H_2O (40:60, v/v) + 1% HAC] to give roseaflavanonoloside A (1, 25.5 mg). Fraction 4-7 (838.1 mg) was isolated by preparative HPLC [MeOH–H_2O (50:50, v/v) + 1% HAC] and three fractions (Fr. 4-7-1–4-7-3) were yielded. Fraction 4-7-1 (203.2 mg) was further separated by preparative HPLC [CH_3CN–H_2O (16:84, v/v) + 1% HAC] to give roseaflavanonolosides B (2, 16.3 mg) and C (3, 19.1 mg). Fraction 7 (4.9 g) was subjected to ODS CC [MeOH–H_2O (20:80 → 30:70 → 40:60 → 60:40 → 100:0, v/v)] and preparative HPLC [MeOH–H_2O (35:65, v/v) + 1% HAC] to yield (2*R*,3*R*)-taxifolin-4′-*O*-β-D-glucopyranoside (5, 371.5 mg).

2.3.1. Roseaflavanonoloside A (1)

Pale yellow powder. $[\alpha]_D^{25}$ −28.0° (*conc.* 0.93, MeOH); IR ν_{max} (KBr) cm^{-1}: 3443, 2954, 2918, 1731, 1644, 1513, 1463, 1372, 1244, 1166, 1074, 840, and 734; UV λ_{max} (MeOH) nm (log ε): 264 (5.51), 291 (5.12), and 358 (3.44); and CD [MeOH, nm, (Δε)]: 272 (−13.9), and 333 (+4.1). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.01 (3H, s, 6″-COCH_3), 3.20 (1H, dd, *J* = 9.5, 9.5 Hz, H-4″), 3.28 (1H, dd, *J* = 7.0, 9.0 Hz, H-2″), 3.31 (1H, dd, *J* = 9.0, 9.5 Hz, H-3″), 3.62 (1H, m, H-5″), [4.09 (1H, dd, *J* = 6.5, 12.0 Hz), 4.29 (1H, dd, *J* = 2.0, 12.0 Hz), H_2-6″], 4.60 (1H, d, *J* = 11.0 Hz, H-3), 4.94 (1H, d, *J* = 7.0 Hz, H-1″), 5.12 (1H, d, *J* = 11.0 Hz, H-2), 5.86 (1H, br. s, H-8), 5.90 (1H, br. s, H-6), 7.05 (2H, d, *J* = 8.5 Hz, H-3′,5′), 7.45 (2H, d, *J* = 8.5 Hz, H-2′,6′), and 11.91 (1H, br. s, 5-OH); ^{13}C NMR (125 MHz, DMSO-d_6) spectroscopic data, see Table 4; and HRESI-TOF-MS: Positive-ion mode *m/z* 493.1330 $[M + H]^+$ (Calcd for $C_{23}H_{25}O_{12}$, 493.1341).

2.3.2. Roseaflavanonoloside B (2)

Pale yellow powder. $[\alpha]_D^{25}$ −24.8° (*conc.* 1.12, MeOH); IR ν_{max} (KBr) cm^{-1}: 3397, 2922, 1717, 1602, 1570, 1508, 1465, 1370, 1242, 1171, 1083, and 882; UV λ_{max} (MeOH) nm (log ε): 266 (5.54), 291 (5.46), and 359 (4.01); CD [MeOH, nm, (Δε)]: 276 (−14.2), and 331 (+4.3). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.08 (3H, s, 4″-COCH_3), [3.35 (1H, m, overlapped), 3.50 (1H, dd, *J* = 2.0, 11.5 Hz), H_2-6″], 3.36 (1H, m, overlapped, H-2″), 3.52 (1H, dd, *J* = 9.0, 9.5 Hz, H-3″), 3.61 (1H, m, H-5″), 4.60 (1H, d, *J* = 11.5 Hz, H-3), 4.65 (1H, dd, *J* = 9.5, 9.5 Hz, H-4″), 5.02 (1H, d, *J* = 7.5 Hz, H-1″), 5.12 (1H, d, *J* = 11.5 Hz, H-2), 5.86 (1H, br. s, H-8), 5.90 (1H, br. s, H-6), 7.08 (2H, d, *J* = 8.5 Hz, H-3′,5′), 7.45 (2H, d, *J* = 8.5 Hz, H-2′,6′), and 11.90 (1H, br. s, 5-OH); ^{13}C NMR (125 MHz, DMSO-d_6) spectroscopic data, see Table 4; and HRESI-TOF-MS: Positive-ion mode *m/z* 515.1157 $[M + Na]^+$ (Calcd for $C_{23}H_{24}O_{12}Na$, 515.1160).

2.3.3. Roseaflavanonoloside C (3)

Pale yellow powder. $[\alpha]_D^{25}$ −25.0° (*conc.* 1.01, MeOH); IR ν_{max} (KBr) cm^{-1}: 3440, 2922, 1718, 1644, 1601, 1514, 1460, 1373, 1074, and 839. UV λ_{max} (MeOH) nm (log ε): 266 (5.31), 289 (5.30), and 362 (4.08); and CD [MeOH, nm, (Δε)]: 285 (−11.1), 325 (+3.9). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.03 (3H, s, 6″-COCH_3), 3.20 (1H, dd, *J* = 9.5, 9.5 Hz, H-4″), 3.29 (1H, dd, *J* = 7.5, 9.0 Hz, H-2″), 3.33 (1H, dd, *J* = 9.0, 9.5 Hz, H-3″), 3.60 (1H, m, H-5″), [4.11 (1H, dd, *J* = 6.5, 11.5 Hz), 4.29 (1H, dd, *J* = 1.5, 11.5 Hz), H_2-6″], 4.48 (1H, d, *J* = 11.0 Hz, H-3), 4.76 (1H, d, *J* = 7.5 Hz, H-1″), 5.00 (1H, d, *J* = 11.0 Hz, H-2), 5.77 (1H, br. s, H-8), 5.81 (1H, br. s, H-6), 6.88 (1H, dd, *J* = 2.0,