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Original Article

Flavonoids from *Capsella bursa-pastoris* and their hepatoprotective activities *in vitro*

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ABSTRACT

Two new flavonoids (**1** and **2**), named 4′,7-dihydroxy-5-hydroxymethyl-8-prenylflavonoid and 4′,7-dihydroxy-5-hydroxymethyl-6,8-diprenylflavonoid, together with seven known flavonoids (**3**–**9**) were isolated from the aerial parts of *Capsella bursa-pastoris* (L.) Medik., Brassicaceae, for the first time. The chemical structures of the purified compounds (**1**–**9**) were identified by their spectroscopic data and references. Moreover, compounds (**1**–**9**) were evaluated for their hepatoprotective activities against D-galactosamine induced toxicity in WB-F344 cells by using a MTT colorimetric method. As a result, compounds **2**, **3**, **6**, and **9** (10 μM) exhibited moderate hepatoprotective activities.

Introduction

Capsella bursa-pastoris (L.) Medik. is an annual or biennial herb belonging to the Brassicaceae family, and used as a popular vegetable in Chinese folk. Meanwhile, *C. bursa-pastoris* has always been served for a medicinal plant to threat conjunctivitis, vomit, metrorrhagia, and hydropsy (Wang et al., 2014). Diverse groups of biological activities are reported to be present in the different plant parts of *C. bursa-pastoris* which possessed anti-tumor (Kelko et al., 1976), anti-inflammatory (Yue et al., 2007), anti-oxidant (Zhang et al., 2008), anti-microbial (Yang et al., 2010), and anti-hypertensive (Huang et al., 2005) activities. Previous phytochemical investigations of *C. bursa-pastoris* exhibited the Q2 presence of amino acids (Xu et al., 2004a,b), flavonoids (Song et al., 2009), alkaloids (Pan, 2006), and essential oils (Gao and Zhou, 2009).

After consulting a large number of references, we found that there have few reports on hepatoprotective activities of *C. bursa-pastoris*, which prompted us to study its hepatoprotective effect. We carried out a bioassay-guided investigation of *C. bursa-pastoris* in order to evaluate its hepatoprotective activity. As a result, nine compounds (**1**–**9**), including two new compounds, named 4′,7-dihydroxy-5-hydroxymethyl-8-prenylflavonoid (**1**) and 4′,7-dihydroxy-5-hydroxymethyl-6,8-diprenylflavonoid (**2**), were isolated and identified from the active fractions (EtOAc fraction) of *C. bursa-pastoris*. The known compounds (**3**–**9**) were obtained from *C. bursa-pastoris* for the first time. All the compounds (**1**–**9**) were evaluated for their hepatoprotective activities, which were tested against D-galactosamine induced toxicity in WB-F344 cells by using the MTT colorimetric method (Ma et al., 2014).

Materials and methods

Plant material

C. bursa-pastoris (L.) Medik., Brassicaceae, was harvested from Nanyang, Henan province, China, in March 2014. This plant was identified by Dr. Su Zhang of Wuyang Weisen Biological Medicine Co., Ltd,. A voucher specimen (No. JC-201403) has been deposited in Nanyang Normal University.

Extraction and isolation

The dried aerial parts of *C. bursa-pastoris* (11 kg) were extracted with 70% EtOH (17 l ×3) three times, each time for 30 min. The extract was concentrated by rotary evaporator under reduced

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pressure resulting in a black extractum (1.1 kg). The combined extracts were successively partitioned with petroleum ether, EtOAc, and *n*-butanol to yield three parts: petroleum ether extract (108.5 g), EtOAc extract (237 g), and *n*-butanol extract (320.7 g). It was found that the EtOAc extract exhibited potential hepatoprotective activity according to bioassay-guided investigation. Therefore, the EtOAc part was subjected to column chromatography (silica gel, 100–200 mesh) and eluted with a solvent of petroleum ether/EtOAc (10:1, 6:1, 3:1, 1:1, 1:2) to obtain five fractions: A (28.4 g), B (33.6 g), C (48.1 g), D (40.4 g), and E (47.5 g), respectively.

The fraction B was fractionated on column chromatography (silica gel, 200–300 mesh) and eluted with petroleum ether/EtOAc (9:1 → 6:1 → 4:1, v/v) to obtain three sub-fractions: B-a, B-b, B-c, separately. The separation of B-b (9.05 g) was chromatographed on silica gel (100–200 mesh, 200–300 mesh) and Sephadex LH-20, repeatedly, yielded **3** (11.56 mg), **6** (9.68 mg), and **9** (10.21 mg). Similarly, the fraction C was applied to a silica gel CC and eluted with petroleum ether/EtOAc (5:1 → 3:1 → 1:1, v/v) to give three sub-fractions: C-a, C-b, C-c, separately. These sub-fractions were chromatographed over Sephadex LH-20 and silica gel CC (100–200, 200–300 mesh) eluting with suitable mobile phases, yielded **1** (8.05 mg), **2** (9.32 mg), **5** (11.36 mg), and **7** (13.20 mg). The fraction D was separately purified by MPLC (30–100% MeOH–H_2O), silica gel (200–300 mesh), and Sephadex LH-20 (MeOH in H_2O, 95%), and yielded **4** (9.55 mg) and **8** (12.25 mg).

4′,7-dihydroxy-5-hydroxymethyl-8-prenylflavonoid (1): pale yellow powder; mp 131.5–132.8 °C; UV (MeOH) λ_{max}: 206, 255, 288, and 337 nm; IR ν_{max} 3429.8, 2952.4, 1657.8, 1608.5, 1357.4, and 1004.2 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data see Table 1; HR-ESI-MS: *m/z* 375.6853 $[M+Na]^+$ (calcd. for $C_{21}H_{20}O_5Na$, 375.6851).

4′,7-dihydroxy-5-hydroxymethyl-6,8-diprenylflavonoid (2): pale yellow powder; mp 135.1–136.7 °C; UV (MeOH) λ_{max}: 206, 258, 286, and 335 nm; IR ν_{max} 3430.3, 2950.7, 1657.2, 1605.8, 1359.1, and 1003.6 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data see Table 1; HR-ESI-MS: *m/z* 443.1562 $[M+Na]^+$ (calcd for $C_{26}H_{28}O_5Na$ 443.1568).

General experimental procedures

The UV and IR spectra were measured by Australia GBC UV-916 spectrophotometer and Nicolet 5700 FT-IR spectrometer with KBr pellets, separately. The melting points were measured on WRX-4 microscopic melting point apparatus (Shanghai Suoguang Electric Technlogy Co., Ltd, China) which was uncorrected. The 1D & 2D NMR spectral data were run on Bruker-400 with TMS as internal standard (Ma et al., 2015). The HR-ESI-MS data were measured by Agilent 1100 series LC/MSD ion trap mass spectrometer. MPLC was carried out on a BUCHI Sepacore spectrometer with DAD detector (BUCHI Labortechnik AG, Switzerland) (Ma et al., 2013). Column chromatography was performed on silica gel (100–200, 200–300) mesh (Qingdao Yuminyuan Silica-Gel Reagent Factory, Qingdao, China), and Sephadex LH-20 (Amersham Pharmacia Biotech Co., Ltd., Tokyo, Japan).

Hepatoprotective assay

The hepatoprotective activities of compounds (**1**–**9**) were evaluated for using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay in WB-F344 rat hepatic epithelial stem-like cells according to the procedure described previously (Feng et al., 2013; Xu et al., 2004a,b). The WB-F344 cell lines were fostered with 3% fetal calf serum, penicillin (100 units/ml), and 100 units/ml streptomycin in 5% CO_2 at 37 °C in Dulbecco's modified eagle medium (DMEM). They were put the 96-well microplate and precultured for 24 h. The fresh medium (200 μl) containing bicyclol and test samples were added and the cells were cultured for 1 h (Hsiao et al., 2013). The cultured cells were measured for cytotoxic effects which exposed to 40 mM D-galactosamine for 24 h. The medium was replaced for the serum-free medium (0.5 mg/ml MTT) for 3.5 h incubation. After removing of the medium and adding DMSO (150 μl/well) into the microplate, the formazan crystals were redissolved. At last, the optical density (OD) was measured at 492 nm by a microplate

Table 1
^1H NMR (400 MHz, DMSO-d_6), ^{13}C NMR (100 MHz, DMSO-d_6) and key HMBC correlations of compounds **1**–**2**.

No.	1			2		
	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
1	–	–	–	–	–	–
2	–	164.5	–	–	164.3	–
3	6.75(s)	104.2	C-4	6.74(s)	104.3	C-4
4	–	180.2	–	–	180.4	–
4a	–	112.2	–	–	112.2	–
5	–	142.6	–	–	142.4	–
5-CH_2	4.58(s)	46.2	C-4a,C-6	4.60(s)	46.1	C-4a,C-6
6	6.78(s)	108.1	C-8	–	116.3	–
7	–	162.0	–	–	160.2	–
8	–	111.8	–	–	118.3	–
8a	–	158.6	–	–	158.1	–
1′	–	123.1	–	–	123.3	–
2′	7.85(d,8.5)	128.6	C-2,C-4′	7.84(d,8.5)	128.7	C-2,C-4′
3′	6.86(d,8.5)	115.7	–	6.86(d,8.5)	115.8	–
4′	–	157.5	–	-	157.7	–
5′	6.86(d,8.5)	115.7	–	6.86(d,8.5)	115.8	–
6′	7.85(d,8.5)	128.6	C-1,C-4′	7.84(d,8.5)	128.7	C-1,C-4′
1″	3.48(d,6.6)	22.8	C-8a, C-3‴	3.50(d,6.6)	22.7	C-7, C-3″
2″	5.20(t,6.6,3.0)	122.7	C-8	5.21(t,6.6,3.0)	122.8	–
3″	–	132.0	–	–	132.2	–
4″-CH_3	1.65(s)	25.1	–	1.66(s)	25.2	–
5″-CH_3	1.71(s)	18.6	–	1.73(s)	18.8	–
1‴	–	–	–	3.48(d,6.9)	22.9	C-8a, C-3‴
2‴	–	–	–	5.19(t,6.9,3.1)	123.0	C-8
3‴	–	–	–	–	133.0	–
4‴-CH_3	–	–	–	1.64(s)	25.1	–
5‴-CH_3	–	–	–	1.70(s)	18.7	–