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Antioxidant acetophenone glycosides from the roots of *Euphorbia ebracteolata* Hayata

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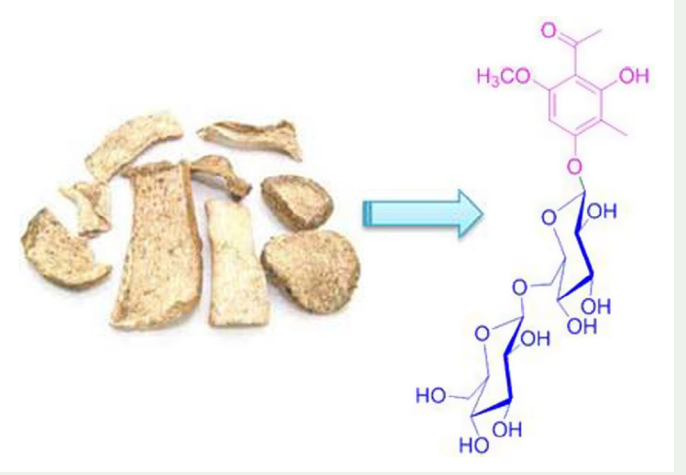
ABSTRACT

A new acetophenone diglycoside, 2,4-dihydroxy-6-methoxy-3-methyl-acetophenone 4-*O*-gentiobioside (**1**), along with six know analogues (**2**–**6**), were isolated from the roots of *Euphorbia ebracteolata*. The structures of these isolates were determined by UV, HRESIMS, 1D and 2D NMR spectral analyses. In addition, all acetophenones showed moderate DPPH free radical scavenging capability, and no cytotoxicity against five human cancer cell lines.



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1. Introduction

Euphorbia ebracteolata Hayata, an herbaceous plant in Euphorbiaceae family, was widely distributed in the central and western regions of China (Zhang et al. 2011). For thousand years or more, the roots of the plant were used to treat cancer (He et al. 2012; Yuan et al. 2016), lymphatic tuberculosis (Zhang 2016), ascites and some other diseases. In addition,

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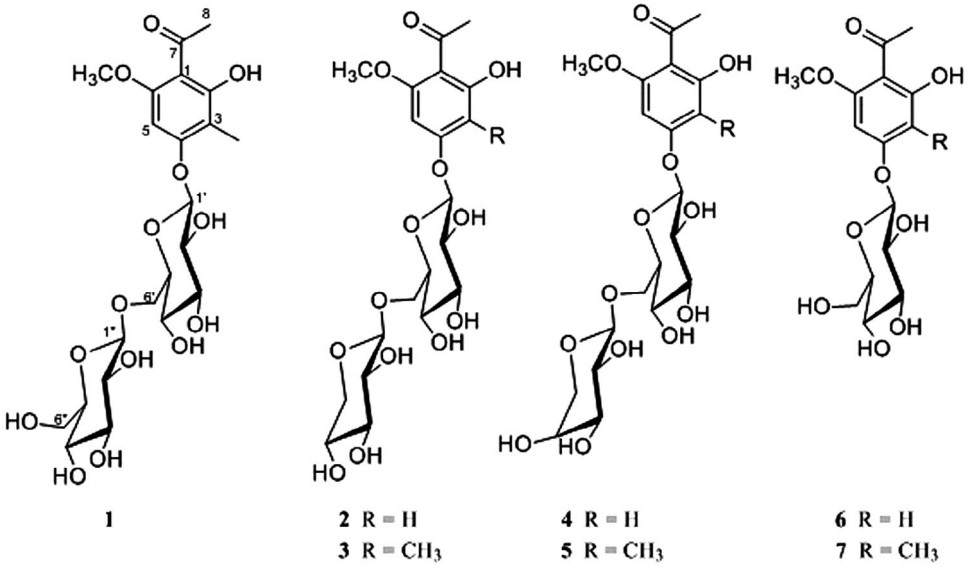

Figure 1. Acetophenone glycosides (**1**–**7**) isolated from the roots of *Euphorbia ebracteolata*.

many types of structures mainly including terpenoids (Wang et al. 2010; Liu et al. 2014), acetophenones (Wang & Ding 1999; Zhang et al. 2010; Delnavazi et al. 2015; Huang et al. 2017) and flavonoids were isolated from *Euphorbia* species. In the present study, a new acetopenone glycoside and six known analogues were isolated from the roots of *E. ebracteolata* and their structures were identified using UV, LC-MS, 1D NMR, 2D NMR (Figure 1). Moreover, their antioxidant and cytotoxic effects were evaluated by DPPH scavenging experiment (Zeng & Shi 2013) and against five human cancer cell lines, respectively.

2. Results and discussion

Compound **1**, obtained as a white amorphous powder, had the molecular formula $C_{22}H_{32}O_{14}$ established by the HRESIMS from its ion peak at *m/z* 543.1689 $[M + Na]^+$ (calcd for $C_{22}H_{32}O_{14}Na$, 543.1792). The UV absorptions λ_{max} 209.6, 287.5 nm of aromatic moiety was observed in the UV spectrum. Analyses of the spectroscopic data of compound **1** suggested the structure to be an acetophenone glycoside analogue (Huang et al. 2017). The ^1H and ^{13}C NMR spectra showed a phloroglucinol unit (δ_C 162.8, 161.4 and 161.0) with *C*-substituents of an acetyl (δ_C 203.3 and 32.9; δ_H 2.58, s, 3H) and of a methyl (δ_C 7.4; δ_H 1.95, s, 3H), as well as *O*-substituents of a methoxyl (δ_C 56.0; δ_H 3.89, s, 3H) and of a disaccharide moiety (δ_C 103.7, 99.6, 69.1 and 60.9; δ_H 5.05, d, J = 7.5 Hz, 4.17, d, J = 7.5 Hz). The free phenolic hydroxyl was positioned on C-2 based on the formation of intramolecular hydrogen bond at δ_H 13.93. The methyl and the glucosyl linkage were ascribed to C-3 and C-4, respectively, as determined by the HMBC correlations between the methyl (δ_H 1.95) with C-2, C-3 and C-4, and between H-1′ (δ_H 5.05) with C-4 (δ_C 161.4). (Figure S1) The remaining methoxyl was consequently assigned on C-6. The glucosyl moiety was elucidated to be *β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside on the basis of the diagnostic NMR data, and supported by the HMBC cross-peak of H-1″/C-6′ and acid hydrolysis experiments. Therefore, the structure of **1** was determined to be 2,4-dihydroxy-6-methoxy-3-methyl-acetophenone 4-*O*-gentiobioside.

According to the previously reports, compounds **2**–**7** were identified as acetophenone glycosides 2,4-dihydroxy-6-methoxy-acetophenone 4-*O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranoside (**2**) (Huang et al. 2017), 2,4-dihydroxy-6-methoxy-3-methyl-acetophenone 4-*O*-*β*-D-xylopyranosyl-(1→6) -*β*-D-glucopyranoside (**3**) (Wang & Ding 1999), 2,4-dihydroxy-6-methoxy- acetophenone 4-*O*-*a*-L-arabinopyranosyl(1→6)-*β*-D-glucopyranoside (**4**) (Huang et al. 2017), 2,4-dihydroxy-6-methoxy-3-methyl-acetophenone 4-*O*-*α*-L-arabinopyranosyl (1→6)-*β*-D-glucopyranoside (**5**) (Wang & Ding 1999), 2,4-dihydroxy-6-methoxy-acetophenone 4-*O*-*β*-D-glucopyranoside (**6**) (Prasad 1999), and 2,4-hydroxy-6-methoxy-3-methyl-acetophenone 4-*O*-*β*-D-glucopyranoside (**7**) (Huang et al. 2017), respectively.

The anti-oxidant effects of acetophenone glycosides (**1**–**7**) have been evaluated using DPPH scavenging assay. All of the isolated compounds displayed anti-oxidant effects with the IC_{50} values ranging from 22.20–36.50 μg/mL (Table 1). Additionally, all of the compounds displayed no cytotoxic activity against five huaman cancer cell lines (MCF-7, A549, Hep-3B, U118 and U87) using MTT assay.

3. Experimental

3.1. General experimental procedures

UV spectra were measured by a JASCO V-650 UV–vis spectrophotometer in methanol. HRESIMS data were obtained using an Agilent 1100 series LC/MSD ion trap mass spectrometer using direction injection. NMR spectra were run in DMSO-d_6 on a Varian Mercury NMR spectrometer. Analytical HPLC data were collected on an UltiMate 3000 instrument (Thermo Scientific dionex). Preparative HPLC was given by an Saipuruisi MH-LC 52 instrument with an Elite UV2300 detector and a SHIMADZU C18 column (250 × 20 mm, 5 μm). Column chromatographic separations were carried out on silica gel H-60 (Qingdao Marine Chemical Group Corporation, Qingdao, China), Macroporous resin D101 (Zhengzhou Qinshi technology Co. Ltd., Zhengzhou, China), Acetonitrile and Methanol were used of chromatographic grade purchasing from Sigma-Aldrich in American. All other solvents were used of chemical grade (Kermel Chemical Co. Ltd., Tianjin, China).

3.2. Plant material

The roots of *E. ebracteolata* were collected in the Bozhou city of Anhui province and identified by Prof Qing-Shan Yang, Anhui University of Chinese Medicine. The specimen was deposited in College of Pharmacy, Dalian Medical University (D-2014–05).

Table 1. The antioxidant activity against DPPH of the isolated compounds **1**–**7**.

Samples	IC_{50} (μg/mL)
Compound **1**	34.62 ± 0.5
Compound **2**	36.20 ± 1.6
Compound **3**	35.63 ± 1.4
Compound **4**	32.33 ± 0.7
Compound **5**	33.17 ± 0.5
Compound **6**	23.23 ± 1.8
Compound **7**	22.50 ± 0.8
Vitamin C	1.57 ± 0.02

Note: Data were presented as means ± SD of three parallel measurements.

3.3. Extraction and isolation

The roots of *E. ebracteolata* (20 kg) were crushed and extracted by 80% aqueous EtOH under Reflux (1.5 h × 3). The EtOH extracts (1300 g) were fractionally extracted with petroleum ether, ethyl acetate, *n*-BuOH successively (10L × 3 times). The *n*-BuOH extract (330 g) were separated by macroporous resin to get three EtOH/water fractions (30% EtOH fraction, 60% EtOH fraction, 90% EtOH fraction). The 30% EtOH fraction (12 g) was dealed with a silica gel column by elution with CH_2Cl_2–MeOH (20:1-1:1) in sequence to give fractions 1–102. Fraction 23 (143 mg) was separated in CH_3CN-0.03% TFA in water (15:85; 8 ml/min; 210, 250 nm) to get compounds **6** (rt. 55 min) and **7** (rt. 59 min). Fraction 59 was isolated firstly by a fully automatic preparative HPLC with CH_3CN-water (10%-30%, 60 min; 10 ml/min; 210, 250 nm), and the sub-fractions were purified by preparative HPLC in CH_3CN-water (0.03% TFA, 15:85; 8 ml/min; 210, 250 nm) to afford compounds **1** (rt. 34 min; 5 mg), **2** (rt. 38 min; 11 mg), **3** (rt. 37 min; 6 mg), **4** (rt. 43 min; 10 mg) and **5** (rt. 41 min; 12 mg).

3.3.1. 2,4-dihydroxy-6-methoxy-3-methyl-acetophenone 4-O-gentiobioside (Compound 1)

A white amorphous powder; $[\alpha]_D^{25}$–15.3 (*c* 0.1 CH_3OH). UV(CH_3CN) λ_{max} 209.6 and 287.5 nm. ^{1}H NMR (600 MHz, DMSO-d_6), δ_H 13.93 (1H, s, 2-OH), 6.35 (1H, s, H-5), 5.05 (1H, d, *J* = 7.5 Hz, H-1′), 4.17 (1H, d, *J* = 7.5 Hz, H-1″), 4.02 (1H, dd, *J* = 11.5, 1.5 Hz, H-6′), 3.89 (3H, s, 6-OCH_3), 3.70 (1H, m, H-5′), 3.64 (1H, brd, *J* = 11.5 Hz, H-6″), 3.60 (1H, dd, *J* = 11.5, 7.0 Hz, H-6′), 3.42 (1H, brd, *J* = 11.5 Hz, H-6″), 3.31 (1H, m, H-2′), 3.31 (1H, m, H-4′), 3.31 (1H, m, H-5″), 3.10 (1H, m, H-3″), 3.03 (1H, m, H-4″), 3.03 (1H, m, H-3′), 2.93 (1H, t, *J* = 8.5 Hz, H-2″), 2.58 (3H, s, 8-CH_3), 1.95 (3H, s, 3-CH_3). ^{13}C NMR (150 MHz, DMSO-d_6), δ_C 203.3 (C-7), 162.8 (C-2), 161.4 (C-4), 161.0 (C-6), 105.7(C-1), 105.1 (C-3), 103.7 (C-1″), 99.6 (C-1′), 90.1 (C-5), 73.0 (C-2′), 76.8 (C-3′), 69.9 (C-4′), 76.8 (C-3″), 76.4 (C-5″), 75.8 (C-5′), 73.5 (C-2″), 69.9 (C-4″), 69.1 (C-6′), 60.9 (C-6″), 56.0 (6-OCH_3), 32.9 (C-8), 7.4 (3-CH_3). HRESIMS: *m/z* 543.1689 $[M + Na]^+$ (calcd for $C_{22}H_{32}O_{14}Na$, 543.1792).

3.4. Hydrolysis of compound 1

Five milligram powder of compound **1** was dissolved in DMSO (The least amount) and participated in a hydrolysis reaction with 10% H_2SO_4 in the 85 °C water bath over 2 h. Next, the mixture was neutralised by the saturated sodium bicarbonate solution. The reaction mixture was extracted by EtOAc, and the water-layer was evaporated *in vacuo* by a rotatory evaporator, the residue was dissolved by DMSO in order to dispose inorganic salt. Subsequently, The DMSO fraction including saccharide was dried by a lyophilizer and next analysed by LC-MS with comparing to standard substance D-glucose (chromatography column: Luna NH_2, 5um, 4.6 mm × 25 cm) (Shi et al. 2009).

3.5. DPPH scavenging assay

The free radical scavenging rates of these samples were detected by DPPH assay as the previous and acknowledged method, which was widely used in the literature for more than 20 years (Apak et al. 2016; William et al. 2017; Zhao et al. 2017). Meanwhile, vitamin C was used as a positive control.

3.6. Cytotoxicity assay

Compounds **1**–**7** were tested for cytotoxicity against MCF-7, A549, Hep-3B, U118 and U87 cell lines by means of the MTT method as described (Wang et al. 2014).

4. Conclusions

The present paper have obtained 7 acetophenone glycosides from the roots of *E. ebracteolata*, including one new compound. On the basis of the structure analysis, the anti-oxidant effects of these isolated compounds have been evaluated *in vitro* using DPPH scavenging bioassay. Compared with the positive control vitamin C, all of the acetophenone glycosides displayed moderate DPPH scavenging effects.

Disclosure statement

No potential conflict of interest was reported by the authors.

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