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Two new phenolic glycosides from the stem of *Zanthoxylum armatum* DC

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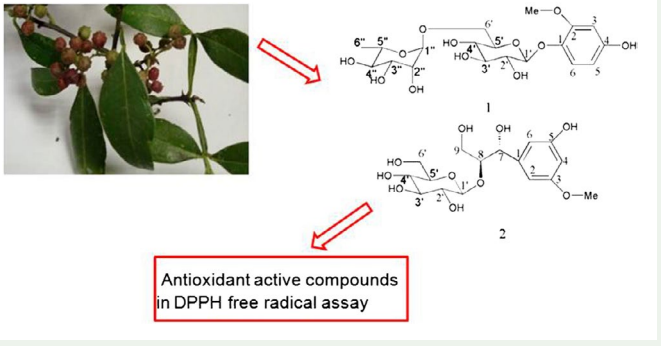
ABSTRACT

Two new phenolic glycoside, 2-methoxy-4-hydroxylphenyl-1-O-α-L-rhamnopyranosyl- (1″ → 6′)-β-D-glucopyranoside. (**1**) and threo-3-methoxy-5-hydroxy-phenylpropanetriol-8-O-β-D-glucopyranoside (**2**), were isolated from the stems of *Zanthoxylum armatum*. The compounds 1 and 2 showed weak scavenging activity in DPPH free radical assay with IC_{50} values of 323 and 114 mM, respectively.



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Zanthoxylum armatum; phenolic glycoside; antioxidant activity

1. Introduction

The genus *Zanthoxylum* is the most widely distributed genus in Rutaceae. It is very well known for its diversified chemistry, particularly by the presence of alkaloids, aromatic and aliphatic amides, and phenylpropanoids (Wang et al., 2015). *Zanthoxylum armatum* DC., distributed almost everywhere in china, is a common specie in the genus, and 3–5 m high of deciduous arbour. Its root, stem, leaf, fruit and seeds were used as herbs, and fruit was also often used as food condiment and aromatic preservatives. Modern pharmacological studies have shown that *Z. armatum* has antispasmodic (Gilani et al. 2010), anti-inflammatory (Guo et al. 2011), antimicrobial (Mehmood et al. 2013), antitumour (Singh et al. 2015),

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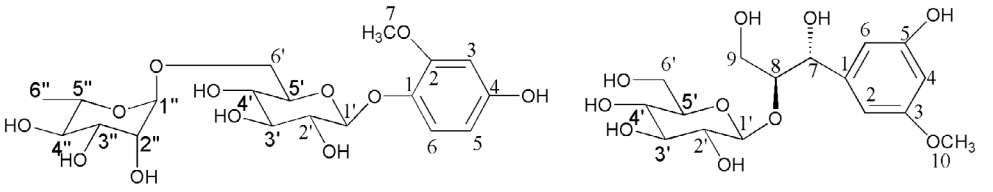

1 2

Figure 1. Chemical structures of compound **1** and **2**.

antidiabetic (Mehmood et al. 2013; Karki et al. 2014), hepatoprotective (Verma & Khosa 2010) and anthelmintic (Kumar et al. 2016) activities.

Most of the previous phytochemical investigations (Bhatt et al. 2015; Guo et al., 2012, 2015, 2017; Samad et al. 2014) have focused on petroleum ether, ethyl acetate and n-butanol fractions of *Z. armatum* extract, while its water fraction of extract was rarely reported. As part of our systematic investigations on chemical constituents from *Z. armatum*, the present study investigated the water fraction of the ethanol extract of *Z. armatum*, resulting in isolation of two new compounds, 2-methoxy-4-hydroxylphenyl-1-*O*-*α*-L-rhamnopyranosyl-(1″→6′)-*β*-D-glucopyranoside (**1**) and *threo*-3-methoxy-5-hydroxy-phenylpropanetriol-8-*O*-*β*-D-glucopyranoside (**2**) (Figure 1).

2. Results and discussion

Compound **1** was obtained as amorphous powder, $[\alpha]_D^{20}$ −58.2° (H_2O). The ESI-MS showed quasimolecular ion peaks at m/z 471 $[M + Na]^+$, indicating a molecular weight of 448. Its molecular formula was determined to be $C_{19}H_{28}O_{12}$, according to the HR-ESI-MS, ^1H and ^{13}C NMR spectroscopic data. The ^1H NMR spectrum of **1** showed the presence of a 1,2,4-trisubstituted benzene ring [δ 6.98 (1H, d, J = 8.8 Hz), 6.58 (1H, d, J = 2.7 Hz) and 6.42 (1H, dd, J = 8.8, 2.7 Hz)] and a methoxy group [δ 3.80 (3H, s)]. In the sugar part of ^1H NMR spectrum, the anomeric proton δ_H 4.88 (1H, d, J = 7.6 Hz) indicated the presence of glucose, while the signal at δ 4.75 (1H, d, J = 1.1 Hz) indicated the presence of rhamnose, which were confirmed by acid hydrolysis and comparison with authentic samples. The stereochemistry of the anomeric carbon of glucose was determined as *β*-configuration according to the coupling constant of the anomeric proton (J = 7.6 Hz) and the chemical shift of C-1′ (101.6). The anomeric carbon of the rhamnosyl residue was determined to be *α* from the ^{13}C NMR chemical shifts of C-3″ and C-5″ (Kasai et al. 1979). The down-field shift of the glucose C-6′ signal (δ_C 66.6) indicated that glucose and rhamnose were linked through a 1 → 6 glycosidic bond. The glycosidic site was unambiguously established by a HMBC experiment in which the long-range correlations between H-1′ (δ 4.88) and C-1 (δ 138.8), H-1″(δ 4.75) and C-6′(δ 66.6) was observed. Significant HMBC correlations were also observed between OCH_3 and C-2. The site of the methoxy group was further deduced from the results of the NOESY spectrum, in which NOE signals were only observed between H-1′ (δ 4.88) and H-6 (δ 6.98). Consequently, the structure of compound **1** was established as 2-methoxy-4-hydroxylphenyl-1-*O*-*α*-L-rhamnopyranosyl-(1″ → 6′)-*β*-D-glucopyranoside.

Compound **2** was obtained as a white amorphous powder. Its molecular formula was determined as $C_{16}H_{24}O_{10}$ by HR-ESI-MS at m/z 399.1261 $[M + Na]^+$. The ^1H NMR spectrum of

2 (Table S1) showed the presence of three aromatic protons [δ 6.94 (1H, s), 6.81 (1H, s), 6.80 (1H, s)] indicating a 1,3,5-trisubstituted benzene ring, two methine protons [δ 4.69 (1H, d, J = 6.8 Hz), 3.89 (1H, m)], a methylene proton [δ 3.55 (1H, dd, J = 12.3, 3.3 Hz), 3.30 (1H, m)], and a methoxy proton [δ 3.76 (3H, s)]. Combining with ^{13}C NMR spectrum together, the characteristic resonances of **2** with a phenylpropanoid moiety and a hexose moiety was suggested. The anomeric proton δ_H 4.38 (1H, d, J = 7.8 Hz) indicated the presence of glucose, which was confirmed by acid hydrolysis and comparison with authentic samples. The stereochemistry of the anomeric carbon of glucose was determined as β-configuration according to the coupling constant of the anomeric proton and the chemical shift of C-1′ (102.8). The glucose position was established by a HMBC experiment, in which the correlation between H-1′ and C-8 was observed. The methoxy group was linked to C-3 by the HMBC correlation between OCH_3 (δ_H 3.76) and C-3 (δ_C 147.2). In addition, the coupling constant ($J_{7,8}$ = 6.8 Hz) in the ^{1}H NMR spectrum indicated a *threo* configuration between H-7 and H-8 (Chang et al. 1999). Through an analysis of ^{1}H-^{1}H COSY, HMQC and HMBC correlations, the compound **2** was determined to be *threo*-3-methoxy-5-hydroxy-phenylpropanetriol-8-*O*-β-D-glucopyranoside.

The results of antioxidant test showed that compounds **1** and **2** displayed some scavenging activity in DPPH free radical assay with IC50 values of 323 and 114 mM, respectively.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a Perkin-Elmer 241 automatic digital polarimeter. UV spectra were determined with a Shimadzu UV-260 instrument. ^{1}H, ^{13}C and 2D NMR spectra were recorded on a Bruker DRX-400 spectrometer (^{1}H 400 MHz and ^{13}C 100 MHz) in D_2O as the internal standard. Chemical shifts are reported in ppm and coupling constants (J) are expressed in Hertz. HR-ESI-MS data were acquired on Bruker APEX 7.0 TESLA FT-MS apparatus (Bruker, Axs Gmbh, Switzerland). Reversed-phase chromatography utilised MCI gel CHP 20P (75–150 μm, Mitsubishi Chemical Industries Co., Ltd.), Cosmosil 75 C_{18}-OPN (42–105 μm, Nacalai Tesque Inc.) columns and TSK gel Toyopearl HW-40F (30–60 μm, Toso Co., Ltd.). TLC was performed on precoated silica gel 60 F_{254} plates (0.2 mm, Merck).

3.2. Plant materials

The stem of *Z. armatum* DC. was collected from Nanning of Guangxi, China, and was identified by associate professor Ze-hao Huang. Voucher specimen (#Z140934) was deposited in school of life science and Engineering, Lanzhou University of Technology, Gansu, China.

3.3. Extraction and isolation

The air-dried stem of *Z. armatum* DC.(20 kg) was successively extracted three times (each for four days) by maceration in 95% ethanol. A residue was obtained after the solvents evaporation under reduced pressure by a rotary evaporator at 50°C. The residue was suspended in water (2L) and extracted three times with petroleum ether, EtOAc and *n*-BuOH at room temperature, successively. The water solvent was evaporated to dryness under reduced

pressure to afford a brown crude extract (83 g). The extract dissolved in 300 mL water was subjected to MCI gel CHP 20P (8 × 60 cm) and eluted with MeOH–H_2O mixtures of decreasing polarity to obtain six fractions as Fr.1 (1.0 L, 100% H_2O), Fr. 2 (1.0 L, 10% MeOH, v/v), Fr. 3 (1.0 L, 30% MeOH, v/v), Fr. 4 (1.0 L, 50% MeOH, v/v), Fr. 5 (1.0 L, 70% MeOH, v/v) and Fr. 6 (1.0 L, 100% MeOH). The Fr. 2 (12.9 g) was separated using Toyopearl HW-40F (6 × 60 cm) with H_2O to give five subfractions (Fr.2A - Fr.2E). The Fr.2A (0.7 g) was further purified by Cosmosil 75 C_{18}-OPN (4 × 30 cm, eluted with H_2O → 10% MeOH) and Toyopearl HW-40F (6 × 60 cm, eluted with H_2O) to yield compound **1** (70.5 mg). Fr. 3 (8.9 g) was chromatographed on Toyopearl HW-40F (6 × 60 cm) with H_2O to obtain five subfractions (Fr.3A - Fr.3D). Fr.3A(0.68 g) was further purified by Cosmosil 75 C_{18}-OPN (4 × 30 cm, eluted with H_2O → 20% MeOH), Toyopearl HW-40F (6 × 60 cm, eluted with H_2O), and Cosmosil 75 C_{18}-OPN (4 × 30 cm, eluted with 10 → 20% MeOH) to yield compound **2** (128.5 mg).

3.3.1. Compound 1

White amorphous powder; $[\alpha]_D^{20}$ −58.2° (*c* 0.10, H_2O); HR-ESI-MS *m/z* 471.1477 $[M + Na]^+$ (calcd for $C_{19}H_{28}O_{12}Na$, 471.1478); ^1H and ^{13}C NMR: see Table S**1**.

3.3.2. Compound 2

White amorphous powder; $[\alpha]_D^{20}$ −22.5° (*c* 0.10, H_2O); HR-ESI-MS *m/z* 399.1261 $[M + Na]^+$ (calcd for $C_{16}H_{24}O_{10}Na$, 399.1261); ^1H and ^{13}C NMR: see Table S**1**.

3.4. Acid hydrolysis

A solution of compounds **1–2** (1 mg each) in 5% HCl (0.5 ml) was heated (90°C) for 2 h. After removing HCl by evaporation in vacuum, the mixture was diluted with H_2O and extracted with EtOAc. The aqueous layer was neutralised with 0.1 M NaOH and sugars were detected by TLC analysis by comparing with authentic sugars: D-glucose and L-rhamnose from **1**, D-glucose from **2**. TLC conditions: $CHCl_3$–MeOH–H_2O (14:6:1), R_f 0.13 (glucose), R_f 0.24 (rhamnose); *n*-BuOH-pyridine-H_2O (6:4:3), R_f 0.37 (glucose), R_f 0.47 (rhamnose) (Çalış & Kırmızıbekmez 2004).

3.5. DPPH radical-scavenging assay

The antioxidant properties were measured using DPPH free radical-scavenging method reported by our research team (Wang et al. 2014). The concentration required for 50% reduction (50% scavenging concentration, IC_{50}) of DPPH was determined graphically. Ascorbic acid was used as a positive control.

4. Conclusion

The chemical investigation of the stems of *Z. armatum* has resulted in the isolation of two new glycosides, 2-methoxy-4-hydroxy-phenol-1-*O*-*α*-L-rhamnopyranosyl-(1″→6′)-*β*-D-glucopyranoside (**1**) and *threo*-3-methoxy-5-hydroxy-phenylpropanetriol-8-*O*-*β*-D-glucopyranoside (**2**).

Supplementary material

Supplementary material relating to this article is available online, alongside Tables S1 and Figure S1–S13.

Disclosure statement

No potential conflict of interest was reported by the authors.

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