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THE ANTIOXIDANT COMPOUNDS FROM *Urtica fissa* FLOWERS

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*Eleven compounds (**1–11**), including two new compounds (**1, 2**), were isolated from the antioxidant portion of the alcoholic extract of* Urtica fissa *flowers. Their chemical structures were elucidated from extensive spectroscopic analysis. The results of activity evaluation indicated that some compounds of phenolic acids (**3, 4**), flavonoids (**7, 9, 10**), and lignans (**1, 6**) possess significant activities of ABTS and DPPH radical scavenging, with EC_{50} values less than 8 μM.*

Keywords: nettle, *Urtica fissa*, flowers, antioxidant activity.

Urtica fissa E. Pritz., a kind of nettle, is an important medical plant widely distributed in China and Vietnam [1]. Its leaves and roots are commonly used for rheumatism treatment, and their chemical constituents and pharmacological activities were investigated in recent years [2]. However, very few researches about its flowers have been reported. In the present study, the phytochemical investigation on the antioxidant portion (EtOAc portion) of the alcoholic extract of *U. fissa* flowers led to the isolation of two new compounds **1** and **2** (Fig. 1), together with nine known compounds rosmarinic acid (**3**) [3], chlorogenic acid (**4**) [4], isoferulic acid (**5**) [3], neourticol B (**6**) [5], kaempferol (**7**) [4], urticol-7-*O*-*β*-D-glucopyranoside (**8**) [6], patuletin-3-*O*-*β*-D-glucopyranoside (**9**) [7], quercetin-3-*O*-*β*-D-glucopyranoside (**10**) [4], and lariciresinol-4,4′-bis-*O*-*β*-D-glucopyranoside (**11**) [8].

Compound **1** was obtained as an amorphous powder. The HR-ESI-MS gave a quasi-molecular ion peak at *m/z* 315.1307 $[M - H]^-$ (calcd as $C_{18}H_{19}O_5$), indicating that the molecular formula of compound **1** was $C_{18}H_{20}O_5$. The ^1H NMR spectrum showed the existence of a 1,4-bisubstituted phenyl group, a 1,3,4-trisubstituted phenyl group, two methoxyl groups at δ 3.81 (3H, s, 2″-OCH_3) and 3.33 (3H, s, 3-OCH_3), a phenolic hydroxyl group at δ 10.56, and a formyl group at δ 9.82 (Table 1).

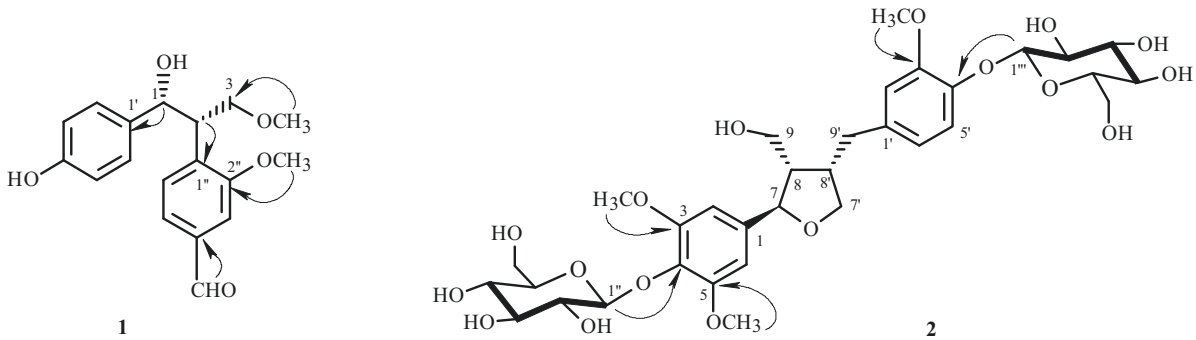

Fig. 1. The chemical structures with key HMBC correlations of compounds **1** and **2**.

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TABLE 1. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) Data of Compound **1** (C_5D_5N, δ, ppm, J/Hz)

C atom	δ_C	δ_H	C atom	δ_C	δ_H
1	71.2	4.59 (1H, d, J = 5.7)	2″	151.8	–
2	52.9	4.36 (1H, m)	3″	108.7	7.35 (1H, d, J = 1.8)
3	69.0	4.03 (1H, dd, J = 6.0, 9.5)	4″	130.6	–
		3.62 (1H, dd, J = 6.0, 9.5)	5″	116.7	6.91 (1H, dd, J = 1.8, 8.3)
1′	132.0	–	6″	126.0	7.54 (1H, d, J = 8.3)
2′, 6′	128.1	7.46 (2H, d, J = 8.4)	3-OCH_3	53.2	3.33 (3H, s)
3′, 5′	116.1	7.23 (2H, d, J = 8.4)	4′-OH		10.56 (1H, br.s)
4′	158.5	–	2″-OCH_3	55.6	3.81 (3H, s)
1″	133.0	–	4″-CHO	193.3	9.82 (1H, s)

TABLE 2. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data of the Compound **2** (DMSO-d_6, δ, ppm, J/Hz)

C atom	δ_C	δ_H	C atom	δ_C	δ_H
	Aglycone moiety			Sugar portion	
1	133.9	–	1″	103.2	4.87 (1H, d, J = 7.2)
2	104.3	6.59 (1H, br.s)	2″	74.6	3.23 (1H, m)
3	152.9	–	3″	77.6	3.25 (1H, m)
4	135.1	–	4″	70.4	3.16 (1H, m)
5	152.9	–	5″	77.5	3.45 (1H, m)
6	104.6	6.59 (1H, br.s)	6″	61.4	3.67 (1H, m)
7	82.3	5.18 (1H, d, J = 4.5)			3.44 (1H, m)
8	52.7	2.61 (1H, m)	1‴	100.6	4.84 (1H, d, J = 7.0)
9	73.1	4.22 (1H, t, J = 7.5)	2‴	73.7	3.21 (1H, m)
		3.90 (1H, t, J = 7.5)	3‴	77.0	3.25 (1H, m)
1′	133.9	–	4‴	70.1	3.15 (1H, m)
2′	113.5	6.82 (1H, d, J = 2.0)	5‴	77.3	3.45 (1H, m)
3′	149.2	–	6‴	61.1	3.68 (1H, m); 3.44 (1H, m)
4′	145.2	–			
5′	115.6	6.98 (1H, d, J = 8.3)			
6′	120.8	6.69 (1H, dd, J = 2.0, 8.3)			
7′	32.6	3.16 (1H, m)			
		2.84 (1H, dd, J = 4.8, 13.2)			
8′	42.9	3.03 (1H, m)			
9′	59.2	4.18 (1H, d, J = 7.1)			
		4.04 (1H, d, J = 10.4)			
3, 5-OCH_3	56.8	3.74 (3H, s)			
3′-OCH_3	56.0	3.74 (3H, s)			

The ^{13}C NMR spectrum and HMBC correlations (Fig. 1) further indicated that the two benzene rings were 4-hydroxyphenyl and 4-formyl-2-methoxyphenyl. A 1-hydroxy-3-methoxypropane group was also deduced from the remaining methines [δ 4.59 (1H, d, H-1) and 4.36 (1H, m, H-2], a methylene [δ 4.03 (1H, dd, H-3a) and 3.62 (1H, dd, H-3b)], and a methoxyl (δ 3.33, 3H, s), according to the ^{1}H–^{1}H COSY and HMBC analysis. The two aromatic rings were connected to the above moiety at C-1 and C-2 according to the HMBC cross peaks between H-1/C-1′ and H-2/C-1″. The configuration was determined to be *erythro* according to the small coupling constant between H-1 and H-2 (J = 5.7 Hz) [9]. Consequently, compound **1** was *erythro*-1-(4-hydroxyphenyl)-2-(4-formyl-2-methoxyphenyl)-1-hydroxy-3-methoxypropane. The absolute configurations 1*R*, 2*S* were further confirmed by the CD spectrum, which exhibited negative Cotton effects at around 230 and 282 nm [10]. Thus, the structure of compound **1** was established to be 1*R*,2*S*-1-(4-hydroxyphenyl)-2-(4-formyl-2-methoxyphenyl)-1-hydroxy-3-methoxypropane.

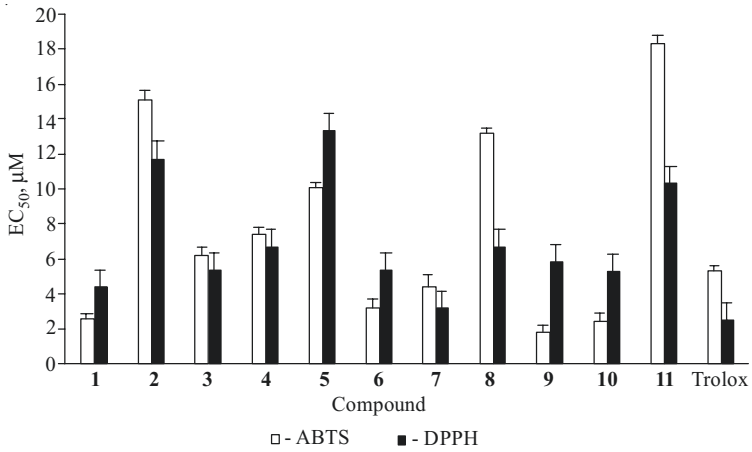

Fig. 2. The antioxidant activities of compounds **1**–**11** (mean ± SD, $n = 3$).

Compound **2**, obtained as a white amorphous powder, was assigned the molecular formula $C_{33}H_{46}O_{17}$ by its HR-ESI-MS data (*m/z* 713.2728 $[M - H]^-$, calcd for $C_{33}H_{45}O_{17}$). The ^1H and ^{13}C NMR data were similar to those of justiciresinol [11] except for the existence of two glucosyl groups (Table 2). Correlations of H-1″ (δ 4.87) with C-4 (δ 135.1) and H-1‴ (δ 4.84) with C-4′ (δ 145.2) in the HMBC spectra further indicated that the glucosyls were linked at C-4 and C-4′. Acid hydrolysis of **2** gave the sapogenin justiciresinol and a sugar fraction. The monosaccharide identified from the sugar fraction by GC analysis was D-glucose. The *β*-anomeric configuration for glucose was judged from their coupling constants (J ≥ 7.0 Hz). Thus, compound **2** was determined to be justiciresinol-4,4′-bis-*O*-*β*-D-glucopyranoside.

The antioxidant functions of all compounds isolated were subsequently evaluated by ABTS and DPPH radical scavenging activity. The results indicated that the two phenolic acids (**3**, **4**), three flavonoids (**7**, **9**, **10**), and two lignans (**1**, **6**) possessed significant antioxidant activities, with EC_{50} values (the effective concentration that resulted in 50% of scavenged radicals) less than 8 μM in the ABTS and DPPH radical scavenging assay (Fig. 2).

EXPERIMENTAL

General Methods. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance DRX-600 spectrometer with a 5 mm $^{13}C/^1H/^{15}N$ TCI CryoProbe. UV spectra were measured on a UV1102 spectrophotometer. Optical rotations were measured on a JASCO P-2000 polarimeter. CD spectra were measured using a JASCO J-815 spectropolarimeter. IR spectra were recorded on a Nicolet 6700 FT-IR spectrophotometer. Semipreparative HPLC chromatography was performed on a Shimadzu LC 2010 AHT liquid chromatography system equipped with an auto-sampler, a UV-Vis detector (detection wavelength 210 nm), and a YMC ODS-AQ column (20 × 250 mm, 5 μm). Optical absorbance was measured using a Bio-Rad 680 microplate reader. Silica gel G (200–300 mesh, Qingdao Haiyang Chemical Co., Ltd.) was used for column chromatography (CC). 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchromancarboxylic acid (Trolox), L-cysteine methyl ester, and 1-trimethylsilylimidazole were purchased from Sigma-Aldrich (St. Louis, USA).

Plant Material. The flowers of *Urtica fissa* were collected from Nanchong City, Sichuan Province (China) in September 2016 and authenticated by one of the authors, Mengyue Wang. A voucher specimen (SJTU 20160923) was deposited in the School of Pharmacy, Shanghai Jiao Tong University.

Extraction and Isolation. The air-dried flowers (430 g) were extracted with 3 L 95% alcohol by reflux three times (2 h each). The extract was combined and completely evaporated under vacuum to give an alcoholic extract (52.4 g). The alcoholic extract was suspended in water (300 L) and then successively partitioned with petroleum ether, CH_2Cl_2, EtOAc, and *n*-BuOH (each 200 mL × 3). The extraction liquids were completely evaporated under vacuum to afford the petroleum ether portion (8.5 g), CH_2Cl_2 portion (4.7 g), EtOAc portion (9.1 g), and BuOH portion (12.2 g). The water layer remaining was dried under vacuum to afford the water layer portion (16.4 g). The portions mentioned above were subjected to antioxidant activity evaluation. The EtOAc portion that showed the best activity (data not given) was subjected to further fractionation.

Part of the EtOAc portion (8.2 g) was fractioned by silica gel CC (4.5 × 60 cm) eluted with CH_2Cl_2–MeOH (100:0 to 75:25) to afford Frs. A–J. Fraction C (647 mg) was purified by silica gel CC eluted with CH_2Cl_2–MeOH (95:5 to 85:15) to

afford Frs. C1–C9. Fraction C2 (52 mg) was further purified by semipreparative HPLC (MeCN–H_2O, 10:90) to afford compounds **3** (23 mg) and **4** (14 mg). Fraction C4 (19 mg) was further purified by semipreparative HPLC (MeCN–H_2O, 25:75) to afford compound **1** (11 mg). Fraction C5 (65 mg) was further purified by semipreparative HPLC (MeCN–H_2O, 10:90) to afford compound **5** (6.1 mg). Fraction G (1.2 g) was purified on silica gel CC eluted with CH_2Cl_2–MeOH (100:0 to 70:30) to afford Frs. G1–G13. Fraction G2 (47 mg) was further purified by semipreparative HPLC (MeCN–H_2O, 30:70) to afford compounds **6** (9.4 mg) and **7** (4.2 mg). Fraction G5 (77 mg) was further purified by semipreparative HPLC (MeCN–H_2O, 25:75) to afford compounds **8** (10 mg) and **9** (23 mg). Fraction G8 (113 mg) was further purified by semipreparative HPLC (MeCN–H_2O, 20:80) to afford compound **10** (39 mg). Fraction I (34 mg) was further purified by semipreparative HPLC (MeCN–H_2O, 15:85) to afford compounds **2** (19 mg) and **11** (7.4 mg).

Compound 1, amorphous powder; $[\alpha]_D^{25}$+0.7° (*c* 0.4, MeOH). UV (MeOH, λ_{max}, nm): 230, 282. CD (MeOH, mdeg): $\Delta\varepsilon_{230}$ nm – 3.7, $\Delta\varepsilon_{282}$ nm –1.2. IR (KBr, ν_{max}, cm^{-1}): 3442, 2928, 2852, 1670, 1616, 1561, 1508, 1466, 1230, 1024. ^1H and ^{13}C NMR data, see Table 1. HR-ESI-MS *m/z* 315.1307 $[M - H]^-$ (calcd for $C_{18}H_{19}O_5$, 315.1311).

Compound 2, amorphous powder; $[\alpha]_D^{25}$–18.1° (*c* 0.31, MeOH). UV (MeOH, λ_{max}, nm): 230, 280. IR (KBr, ν_{max}, cm^{-1}): 3410, 1596, 1512, 1230, 1126, 1073. ^1H and ^{13}C NMR data, see Table 2. HR-ESI-MS *m/z* 713.2728 $[M - H]^-$ (calcd for $C_{33}H_{45}O_{17}$, 713.2735).

Antioxidant Activity Evaluation. The antioxidant activities of the samples were determined by the ABTS and DPPH radical scavenging assay [12]. The scavenging ability of the sample was expressed as EC_{50} values.

Acid Hydrolysis of Compound 2. A solution of compound **2** (11 mg) was hydrolyzed in HCl–MeOH (4:1, 10 mL) at 85°C for 2 h. Then the reaction mixture was diluted with H_2O and extracted with CH_2Cl_2 (10 mL × 2). The CH_2Cl_2 layer was subjected to silica gel CC (CH_2Cl_2–MeOH, 95:5) to give the aglycone as analyzed by NMR. The water layer was neutralized with 5% NaOH solution to give the sugar fraction. The sugar fraction was analyzed by GC. Identification of D-glucose was done by co-injection of the hydrolysate with standard silylated samples.

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