Hepatoprotective and neuroprotective flavanes from the fruits of *Ulmus pumila* L. (Ulmaceae)

Qinge Ma[1,2]*, Rongrui Wei[3]*, Dongli Shang[4], Zhipei Sang[2], Wenmin Liu[2] and Zhongling Cao[2]
[1]College of Pharmacy, Jiangxi Science & Technology Normal University, Nanchang, China
[2]College of Chemistry and Pharmaceutical Engineering, Nanyang Normal University, Nanyang, China
[3]Research Center of Natural Resources of Chinese Medicinal Materials and Ethnic Medicine,
Jiangxi University of Traditional Chinese Medicine, Nanchang, China
[4]Department of Cardiology, Nanyang the First People's Hospital, Nanyang, China

Abstract: Phytochemical study of the EtOAc fraction (active extract) of the fruits of *Ulmus pumila* L. resulted in the isolation of thirteen flavane derivatives, and they were identified by their precise spectral data and literature. All the compounds (1-13) were obtained from the fruits of *U. pumila* L. for the first time. Meanwhile, the compounds (1-13) were assayed for their hepatoprotective and neuroprotective activities, respectively. Compounds 1, 2, 5, 7 and 8 (10μM) exhibited remarkable hepatoprotective activities, and compounds 9, 10, and 13 showed significant neuroprotective activities with IC_{50} values of 4.08, 5.34, and 2.02μM, respectively.

Keywords: *Ulmus pumila* L., flavane, hepatoprotective, neuroprotective.

INTRODUCTION

The fruits of *Ulmus pumila* L. (Ulmaceae) are served as delicious vegetables in folk. They are widely scattered in the warm temperate zone or subtropical zone in the world. The fruits of *U. pumila* L. are not only used as vegetables, but also served for medicinal plants. It was found that the fruits of *U. pumila* L. had the effects of promoting diuresis, relieving stranguria, resolving phlegm, relieving cough (Yu *et al*., 2009), tonifying spleen, normalizing stomac (Huang *et al*., 2014) and nervous breakdown (Rui *et al*., 2007). Modern pharmacology revealed that the fruits of *U. pumila* L. possessed the activities of lowering blood sugar and cholesterol, improving children's growth and development and losing weight (Yu *et al*., 2009). Previous studies on chemical contentients of the fruits of *U. pumila* L. displayed that *U. pumila* L. contained trace elements (Hu *et al*., 2000) and phenolic acids (Huang *et al*., 2014). However, the other constituents of the fruits of *U. pumila* L. were rarely studied and there are no reports on hepatoprotective and neuroprotective activities of the fruits of *U. pumila* L. according to the large number of references, which prompted us to study its further chemical constituents and bioactivities. Based on previous phytochemical and pharmacological investigations of the fruits of *U. pumila* L., we applyed the biological activity to guide separation of the fruits of *U. pumila* L. to assay its pharmacological activities. In this study, the compounds (1-13) were isolated and identified from the fruits of *U. pumila* L. for the first time. Moreover, the compounds (1-13) were evaluated for their hepatoprotective and neuroprotective activities, respectively.

MATERIALS AND METHODS

General

The UV and IR spectra were recorded on an Australia GBC UV-916 spectrophotometer and a Nicolet 5700 FT-IR microscope spectrometer, the optical rotations were measured with a Perkin-Elmer 241 polarimeter at 20°C, and the ^1H and ^{13}C NMR spectra were determined by Bruker-400 with TMS as internal standard (Shang *et al*., 2016). The ESI-MS spectra were recorded on a LTQ Orbitrap XL spectrometer. The HPLC data were measured by using Agilent 1200 series with a DIKMA analytical column (4.6 × 250 mm) packed with C18 (5μm). The column chromatography (CC) was subjected to silica gel (100-200 mesh or 200-300 mesh) and Sephadex LH-20. The TLC was applyed to silica gel GF254 plates and the spots were observed under UV light (254/365 nm) or by spraying with 10% H_2SO_4 in 95% ethanol followed by heating (Ma *et al*., 2016).

Plant material

The fruits of *U. pumila* L. were harvested from Nanyang city, Henan Province, China, in April 2015. They were identified by Dr. Su Zhang of Wuyang Weisen Biological Medicine Co., Ltd,. The voucher specimen (NO.YQ-201504) is deposited in Nanyang Normal University, Nanyang 473061, China.

Hepatoprotective assay

We have used a MTT colorimetric method to assay compounds (1-13) for hepatoprotective activities against D-galactosamine induced toxicity in HL-7702 cells (Ma *et al*., 2014). The HL-7702 cell lines were put in a 96-well

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**Corresponding author:* e-mail: maqinge2006@163.com; weirongrui2011@163.com

micro plate, which were cultivated in Dulbecco's modified eagle medium supplyed with 100 units/mL penicillin, 3% fetal calf serum, and 100 units/mL streptomycin in 5% CO_2 and incubated at 37°C for 24h (Ma *et al*., 2013). After incubating for 3.5 h, the medium was replaced for the serum-free medium (0.5mg/mL MTT) and added DMSO (150μL/well) into the micro plate, then the formazan crystals were redissolved. Meanwhile, the optical density (OD) of compound was recorded on a micro plate reader at 492 nm. At last, the inhibitions of compounds (1-13) were calculated by the following formula for inhibition (%) = $[(OD_{(sample)} - OD_{(control)})/(OD_{(normal)} - OD_{(control)})] \times 100$ (Liu *et al*., 2012).

Neuroprotective assay

We have evaluated the compounds (1-13) for their neuroprotective activities against 6-OHDA-induced cell death in Human neuroblastoma SH-SY5Y cells (Kwon *et al*., 2014). These cells were cultivated in a 96-well plate with the density of 2×10^4 cells/well in 200μL for 24h and treated with 100μM 6-hydroxydopamine and various concentrations of compounds (1-13) for an additional 24 h. The viability of cell was obtained by treatment with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide dissolved in 0.5mg/mL of phosphate-buffered saline at 37°C for 4h. After removing the PBS, the formazan crystals were dissolved using dimethyl sulfoxide. The absorbance was measured by a micro plate reader at 540 nm. The neuroprotective activity against 6-OHDA-induced cell death was calculated by a semi logarithmic graph depicting the relationship between at least four different concentrations of compounds and their percentage effects. Samples were measured in triplicate, and the mean values with standard deviation were used. All the results were typically expressed as IC_{50} with Curcumin as a positive control (Qiu *et al*., 2016).

Extraction and isolation

The dried fruits of *U. pumila* L. (9.0kg) were extracted with 85% EtOH (25L×3, 2.0h each time) and concentrated in vacuum to yield a black residue (0.78 kg). The crude extract was suspended in H_2O and then partitioned with $CHCl_3$, EtOAc, and *n*-Butanol to obtain $CHCl_3$ (87.2g), EtOAc (126.8g), *n*-Butanol (188.1g) extracts, successively. Moreover, all the extracts were tested for their hepatoprotective and neuroprotective activities. As a result, the EtOAc extract exhibited potential hepatoprotective and neuroprotective activities.

The EtOAc fraction was separated by chromatography on silica gel column (100-200 mesh) eluting with petroleum ether: EtOAc (15:1→8:1→6:1→4:1→2:1→1:1, v/v) to afford six fractions (Fr_1-Fr_6). The Fr_2 (21.5 g) was further performed on silica gel column (200-300 mesh) eluting with petroleum ether: EtOAc (10:1→8:1→7:1, v/v) and Sephadex LH-20 column with 100% MeOH, successively and yielded 3 (10.22mg), 5 (9.69 mg), 7 (11.24 mg) and 8 (12.25mg). Similarly, the Fr_3 (33.6 g) was purified on silica gel column (200-300 mesh) eluting with petroleum ether: EtOAc (8:1→6:1→5:1, v/v) and Sephadex LH-20 column with 95% MeOH, successively, and produced 1 (11.63 mg), 2 (9.73 mg), 4 (10.12mg), 6 (8.67mg), 10 (9.67 mg), and 13 (8.89 mg). In the same way, the Fr_4 (28.6 g) was purified by silica gel column (200-300, 100-200 mesh) eluting with petroleum ether: EtOAc (6:1→4:1→3:1, v/v) and Sephadex LH-20 column with 95% MeOH, separately and obtained 9 (8.96mg), 11(11.31 mg), and 12 (10.87 mg) (fig. 1).

RESULTS

Identification of isolated compounds

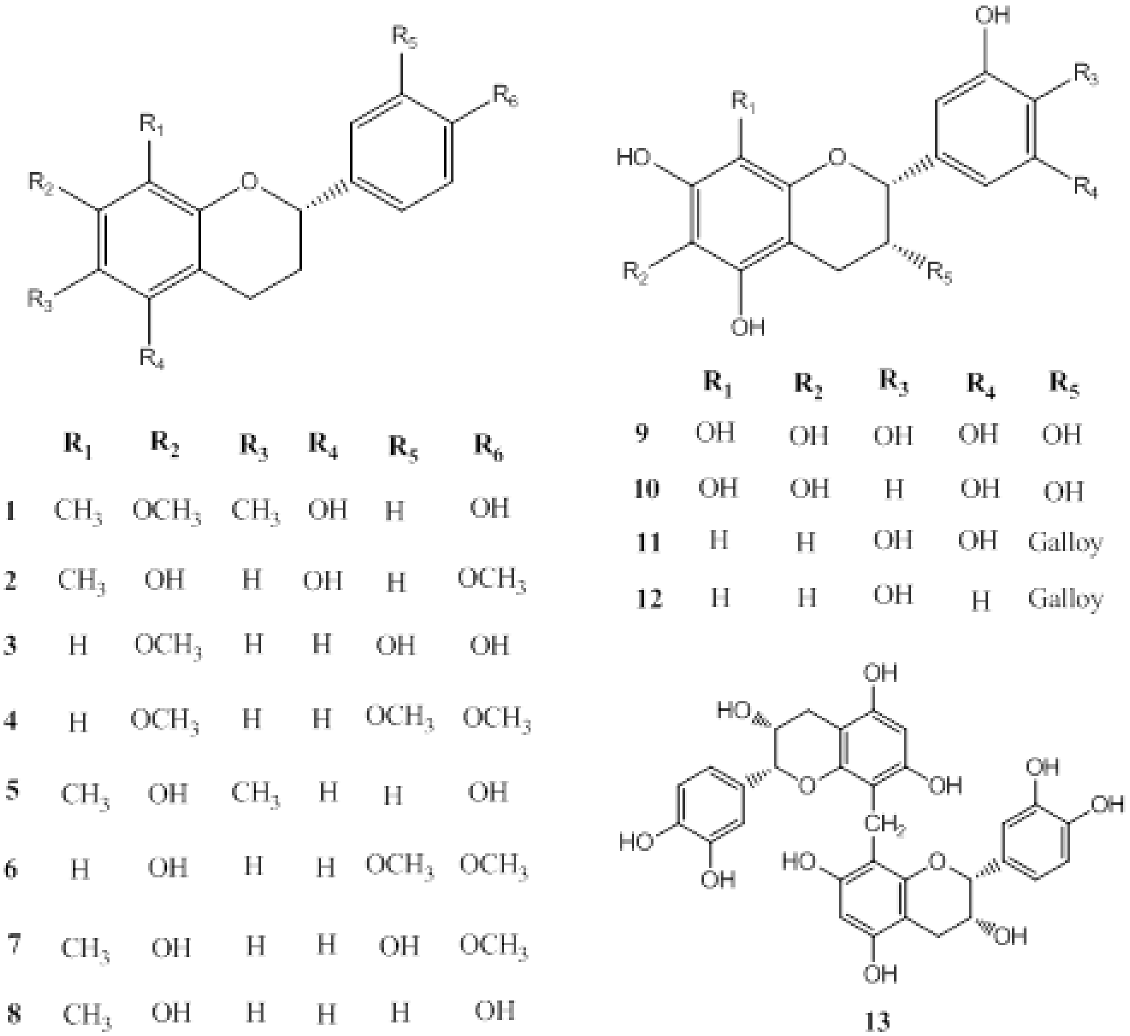
Cambodianins D (1): $[\alpha]^{20}_{D}$ -18.3 (*c* 0.5, CH_3OH); ESI-MS: *m/z* 323.3 $[M+Na]^+$ (calcd. for $C_{18}H_{20}O_4Na$, 323.1); UV (CH_3OH) λ_{max}: 205, 278 nm; IR ν_{max} :3431.8, 1613.0, 1547.9, 1421.1, 1121.3 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm, *J*/Hz, 400 MHz): 4.81 (1H, dd, *J*=10.0, 2.0, H~2), 2.04 (1H, m, H~3a), 1.88 (1H, m, H~3b), 2.67 (2H, m, H~4), 7.13 (2H, d, *J*=8.4 Hz, H~2′/6′), 6.71 (2H, d, *J*=8.4 Hz, H~3′/5′), 1.99 (3H, s, 6~CH_3), 3.58 (3H, s, 7~OCH_3), 1.95 (3H, s, 8~CH_3); ^{13}C NMR ($CDCl_3$, δ, ppm, 100 MHz) 77.2 (C~2), 29.5 (C~3), 19.8 (C~4), 107.0 (C~4a), 151.4 (C~5), 109.2 (C~6), 154.0 (C~7), 151.5 (C~8), 151.6 (C~8a), 133.1 (C~1′), 126.8 (C~2′/6′), 115.0 (C~3′/5′), 156.0 (C~4′), 8.4 (6~CH_3), 59.8 (7~OCH_3), 7.8 (8~CH_3) (Chen *et al*., 2012).

(2S)-5,7-Dihydroxy-4′-methoxy-8-methylflavane (2)

$[\alpha]^{20}_{D}$ -12.8 (*c* 0.4, CH_3OH); ESI-MS: *m/z* 309.1 $[M+Na]^+$ (calcd. for $C_{17}H_{18}O_4Na$, 309.3); UV (CH_3OH) λ_{max}: 236, 285 nm; IR ν_{max}: 3381.6, 2934.5, 1612.1, 1514.6, 1454.3, 1342.9, 1175.5 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm, *J*/Hz, 400 MHz): 4.96 (1H, dd, *J*=10.0, 2.0, H~2), 2.74 (1H, m, H~3a), 1.92 (1H, m, H~3b), 2.64 (2H, m, H~4), 6.05 (1H, s, H~6), 7.31 (2H, d, *J*=8.4, H~2′/6′), 6.80 (2H, d, *J*=8.4, H~3′/5′), 2.06 (3H, s, 8~CH_3), 3.77 (3H, s, 4′~OCH_3); ^{13}C NMR ($CDCl_3$, δ, ppm, 100 MHz) 77.1 (C~2), 29.4 (C~3), 19.5 (C~4), 103.2 (C~4a), 154.0 (C~5), 91.1 (C~6), 155.8 (C~7), 103.2 (C~8), 152.5 (C~8a), 134.3 (C~1′), 127.2 (C~2′/6′), 115.3 (C~3′/5′), 155.1 (C~4′), 7.6 (8~CH_3), 56.5 (4′~OCH_3) (Liu *et al*., 2008).

(2S)-3′,4′-Dihydroxy-7-methoxyflavane (3)

$[\alpha]^{20}_{D}$ -17.6 (*c* 0.3, CH_3OH); ESI-MS: *m/z* 295.3 $[M+Na]^+$ (calcd. for $C_{16}H_{16}O_4Na$, 295.2); UV (CH_3OH) λ_{max}: 221, 283, 288 nm; IR ν_{max} :3562.3, 1621.1, 1585.6, 1168.4 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm, *J*/Hz, 400 MHz): 4.93 (1H, dd, *J*=10.0, 2.0, H~2), 2.16 (1H, m, H~3a), 2.01 (1H, m, H~3b), 2.89 (2H, m, H~4), 6.98 (1H, d, *J*=8.0, H~5), 6.50 (1H, dd, *J*=8.0. 2.5, H~6), 6.48 (1H, d, *J*=2.5, H~8), 6.94 (1H, d, *J*=2.0, H~2′), 6.88 (1H, d, *J*=8.0, H~5′), 6.85 (1H, dd, *J*=8.0, 2.0, H~6′), 3.76 (3H, s, 7~OCH_3); ^{13}C NMR ($CDCl_3$, δ, ppm, 100 MHz) 77.6 (C~2), 29.9 (C~3), 24.5 (C~4), 114.0 (C~4a), 130.0 (C~5), 107.5 (C~6), 159.1 (C~7), 101.5 (C~8), 155.8 (C~8a), 134.6 (C~1′), 113.5



	R_1	R_2	R_3	R_4	R_5	R_6
1	CH_3	OCH_3	CH_3	OH	H	OH
2	CH_3	OH	H	OH	H	OCH_3
3	H	OCH_3	H	H	OH	OH
4	H	OCH_3	H	H	OCH_3	OCH_3
5	CH_3	OH	CH_3	H	H	OH
6	H	OH	H	H	OCH_3	OCH_3
7	CH_3	OH	H	H	OH	OCH_3
8	CH_3	OH	H	H	H	OH

	R_1	R_2	R_3	R_4	R_5
9	OH	OH	OH	OH	OH
10	OH	OH	H	OH	OH
11	H	H	OH	OH	Galloy
12	H	H	OH	H	Galloy

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Fig. 1: Structures of compounds (1-13) isolated from *Ulmus pumila* L.

(C~2′), 143.6 (C~3′), 143.4 (C~4′), 115.4 (C~5′), 118.9 (C~6′), 55.4 (7~OCH_3) (Achenbach *et al*., 1988).

(2S)-7,3′,4′-Trimethoxyflavane (4)

$[\alpha]^{20}_{D}$ -18.1 (*c* 0.4, CH_3OH); ESI-MS: *m*/*z* 323.1 $[M+Na]^+$ (calcd. for $C_{18}H_{20}O_4Na$, 323.3); UV (CH_3OH) λ_{max}: 225, 282, 288 nm; IR ν_{max}: 3541.3, 1615.6, 1583.8, 1164.0 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm, *J*/Hz, 400 MHz): 4.98 (1H, dd, *J* = 10.0, 2.0, H~2), 2.17 (1H, m, H~3a), 2.05 (1H, m, H~3b), 2.83 (2H, m, H~4), 7.01 (1H, d, *J*=8.4, H~5), 6.51 (1H, dd, *J*=8.4, 2.4, H~6), 6.48 (1H, d, *J*=2.4, H~8), 6.99 (1H, d, *J*=2.0, H~2′), 6.88 (1H, d, *J*=7.8, H~5′), 6.97 (1H, dd, *J*=7.8, 2.0, H~6′), 3.77 (3H, s, 7~OCH_3), 3.89 (3H, s, 3′~OCH_3), 3.91 (3H, s, 4′~OCH_3); ^{13}C NMR ($CDCl_3$, δ, ppm, 100 MHz) 77.8 (C~2), 30.0 (C~3), 24.6 (C~4), 113.9 (C~4a), 129.9 (C~5), 107.4 (C~6), 159.1 (C~7), 101.6 (C~8), 155.9 (C~8a), 134.4 (C~1′), 109.4 (C~2′), 149.2 (C~3′), 148.7 (C~4′), 110.1 (C~5′), 118.5 (C~6′), 55.3 (7~OCH_3), 55.8 (3′~OCH_3), 55.8 (4′~OCH_3) (Fu, 2013).

(2S)-4′,7-Dihydroxy-6,8-dimethylflavane (5)

$[\alpha]^{20}_{D}$ -21.6 (*c* 0.5, CH_3OH); ESI-MS: *m*/*z* 293.4 $[M+Na]^+$ (calcd. for $C_{17}H_{18}O_3Na$, 293.2); UV (CH_3OH) λ_{max}: 208, 285 nm; IR ν_{max}: 3423.6, 2859.4, 1616.0, 1566.1, 1104.5 cm^{-1}; ^1H NMR (MeOD, δ, ppm, *J*/Hz, 400 MHz): 4.85 (1H, dd, *J*=10.0, 2.2, H~2), 1.95 (1H, m, H~3a), 1.35 (1H, m, H~3b), 2.64 (2H, m, H~4), 6.61 (1H, s, H~5), 7.25 (2H, d, *J*=8.5, H~2′/6′), 6.79 (2H, d, *J*=8.5, H~3′/5′), 2.13 (3H, s, 6~CH_3), 2.06 (3H, s, 8~CH_3); ^{13}C NMR (MeOD, δ, ppm, 100 MHz) 78.7 (C~2), 31.5 (C~3), 25.9 (C~4), 114.4 (C~4a), 128.7 (C~5), 117.8 (~6), 152.7 (C~7), 113.3 (C~8), 152.8 (C~8a), 134.8 (C~1′), 128.2 (C~2′/6′), 116.1 (C~3′/5′), 157.9 (C~4′), 16.3 (6~CH_3), 8.9 (8~CH_3) (Liu *et al*., 2008).

(2S)-7-Hydroxy-3′,4′-dimethoxyflavane (6)

$[\alpha]^{20}_{D}$ -30.2 (*c* 0.1, CH_3OH); ESI-MS: *m*/*z* 309.1 $[M+Na]^+$ (calcd. for $C_{17}H_{18}O_4Na$, 309.2); UV (CH_3OH) λ_{max}: 225, 280, 287 nm; IR ν_{max}: 3442.3, 1618.3, 1582.9, 1165.8 cm^{-1}; ^1H NMR (MeOD, δ, ppm, *J*/Hz, 400 MHz): 4.94 (1H, dd, *J*=10.0, 2.2, H~2), 2.12 (1H, m, H~3a), 1.99 (1H, m, H~3b), 2.84 (2H, m, H~4), 6.86 (1H, d, *J*=8.2, H~5), 6.32 (1H, dd, *J*=8.2. 2.4, H~6), 6.26 (1H, d, *J*=2.4, H~8), 7.01 (1H, d, *J*=1.8, H~2′), 6.94 (1H, d, *J*=8.0, H~5′), 6.97 (1H, dd, *J*=8.0, 1.8, H~6′), 3.79 (3H, s, 3′~OCH$_3$), 3.79 (3H, s, 4′~OCH$_3$); ^{13}C NMR (MeOD, δ, ppm, 100 MHz) 79.0 (C~2), 31.6 (C~3), 25.6 (C~4), 114.5 (C~4a), 131.1 (C~5), 109.3 (C~6), 157.9 (C~7), 104.1 (C~8), 157.2 (C~8a), 136.5 (C~1′), 111.3 (C~2′), 150.6 (C~3′), 150.3 (C~4′), 112.9 (C~5′), 120.0 (C~6′), 56.6 (3′~OCH$_3$), 56.6 (4′~OCH$_3$) (Fu, 2013).

(2S)-3′,7-Dihydroxy-4′-methoxyl-8-methylflavane (7)

$[\alpha]^{20}_{D}$ -23.7 (*c* 0.3, CH$_3$OH); ESI-MS: m/z 309.2 $[M+Na]^+$ (calcd. for C$_{17}$H$_{18}$O$_4$Na, 309.4); UV (CH$_3$OH) λ_{max}: 218, 286 nm; IR ν_{max}: 3446.1, 1617.1, 1576.4 cm^{-1}; ^{1}H NMR (MeOD, δ, ppm, *J*/Hz, 400 MHz): 4.86 (1H, dd, *J*=10.0, 2.0, H~2), 2.07 (1H, m, H~3a), 1.89 (1H, m, H~3b), 2.82 (2H, m, H~4), 6.64 (1H, d, *J*=8.2, H~5), 6.30 (1H, d, *J*= 8.2, H~6), 6.88 (1H, d, *J*=2.0, H~2′), 6.85 (1H, d, *J*=8.2, H~5′), 6.81 (1H, dd, *J*=8.2, 2.0, H~6′), 2.01 (3H, s, 8~CH$_3$), 3.80 (3H, s, 4′~OCH$_3$); ^{13}C NMR (MeOD, δ, ppm, 100 MHz) 78.6 (C~2), 31.5 (C~3), 25.7 (C~4), 114.1 (C~4a), 127.4 (C~5), 108.5 (C~6), 155.1 (C~7), 112.9 (C~8), 155.3 (C~8a), 136.5 (C~1′), 114.2 (C~2′), 147.6 (C~3′), 148.4 (C~4′), 112.7 (C~5′), 118.5 (C~6′), 8.7 (8~CH$_3$), 56.6 (4′~OCH$_3$) (Gonzalez *et al*., 2004).

(2S)-7,4′-Dihydroxy-8-methylflavane (8)

$[\alpha]^{20}_{D}$ -23.7 (*c* 0.3, CH$_3$OH); ESI-MS: m/z 279.3 $[M+Na]^+$ (calcd. for C$_{16}$H$_{16}$O$_3$Na, 279.4); UV (CH$_3$OH) λ_{max}:226, 278 nm; IR ν_{max} :3449.0, 1618.7, 1579.7 cm^{-1}; ^{1}H NMR (MeOD, δ, ppm, *J*/Hz, 400 MHz): 4.91 (1H, dd, *J*=10.0, 2.2, H~2), 2.08 (1H, m, H~3a), 1.88 (1H, m, H~3b), 2.83 (2H, m, H~4), 6.66 (1H, d, *J*=8.2, H~5), 6.31 (1H, d, *J*= 8.2, H~6), 7.23 (2H, d, *J*=8.4, H~2′,6′), 6.78 (2H, d, *J*= 8.4, H~3′,5′), 2.01 (3H, s, 8~CH$_3$); ^{13}C NMR (MeOD, δ, ppm, 100 MHz) 78.9 (C~2), 31.6 (C~3), 25.9 (C~4), 114.1 (C~4a), 127.4 (C~5), 108.4 (C~6), 155.3 (C~7), 112.8 (C~8), 155.4 (C~8a), 134.9 (C~1′), 128.4 (C~2′), 116.3 (C~3′), 158.2 (C~4′), 116.3 (C~5′), 128.3 (C~6′), 8.6 (8~CH$_3$) (Ioset *et al*., 2001).

2R,3R-3,5,6,7,8,4′-Hexahydroxyflavane (9)

$[\alpha]^{20}_{D}$ -34.8 (*c* 0.4, CH$_3$OH); ESI-MS: m/z 329.0 $[M+Na]^+$ (calcd. for C$_{15}$H$_{14}$O$_7$Na, 329.2); UV (CH$_3$OH) λ_{max}: 228, 280 nm; IR ν_{max}: 3451.6, 1613.6 cm^{-1}; ^{1}H NMR (MeOD, δ, ppm, *J*/Hz, 400 MHz): 4.75 (1H, brs, H~2), 4.08 (1H, brs, H~3), 2.78 (1H, dd, *J*=16.8, 4.4, H~4a), 2.64 (1H, dd, *J*=16.8, 2.4, H~4b), 7.22 (2H, d, *J*=8.5, H~2′,6′), 6.68 (2H, d, *J*=8.5, H~3′,5′); ^{13}C NMR (MeOD, δ, ppm, 100 MHz) 79.9 (C~2), 67.5 (C~3), 29.4 (C~4), 100.1 (C~4a), 158.1 (C~5), 157.9 (C~6), 157.8 (C~7), 157.6 (C~8), 157.5 (C~8a), 131.7 (C~1′), 129.2 (C~2′), 115.9 (C~3′), 157.2 (C~4′), 115.8 (C~5′), 129.3 (C~6′) (Zeng *et al*., 2011).

2R,3R-3,5,6,7,8,3′,5′-Heptahydroxyflavane (10)

$[\alpha]^{20}_{D}$ -31.2 (*c* 0.3, CH$_3$OH); ESI-MS: m/z 345.3 $[M+Na]^+$ (calcd. for C$_{15}$H$_{14}$O$_8$Na, 345.2); UV (CH$_3$OH) λ_{max}: 228, 282, 302 nm; IR ν_{max}: 3447.7, 1612.7, cm^{-1}; ^{1}H NMR (MeOD, δ, ppm, *J*/Hz, 400 MHz): 4.80 (1H, brs, H~2), 4.22 (1H, brs, H~3), 2.85 (1H, dd, *J*=16.8, 4.4, H~4a), 2.75 (1H, dd, *J*=16.8, 2.4, H~4b), 6.75 (1H, m, H~2′), 6.99 (1H, s, H~4′), 6.75 (1H, m, H~6′); ^{13}C NMR (MeOD, δ, ppm, 100 MHz) 79.8 (C~2), 67.6 (C~3), 29.3 (C~4), 100.2 (C~4a), 157.2 (C~5), 157.6 (C~6), 157.8 (C~7), 157.9 (C~8), 158.1 (C~8a), 132.3 (C~1′), 116.0 (C~2′), 145.8 (C~3′), 119.5 (C~4′), 145.6 (C~5′), 115.5 (C~6′) (Zeng *et al*., 2011).

(-)-Epigallocatechin gallate (11)

$[\alpha]^{20}_{D}$ -18.3 (*c* 0.4, CH$_3$OH); ESI-MS: m/z 481.1 $[M+Na]^+$ (calcd. for C$_{22}$H$_{18}$O$_{11}$Na, 481.3); UV (CH$_3$OH) λ_{max}:225, 278 nm; IR ν_{max}:3455.2, 2862.1, 1610.9, cm^{-1}; ^{1}H NMR (MeOD, δ, ppm, *J*/Hz, 400 MHz): 4.93 (1H, brs, H~2), 5.51 (1H, brs, H~3), 2.98 (1H, dd, *J*=16.8, 4.4, H~4a), 2.84 (1H, dd, *J*=16.8, 2.4, H~4b), 5.96 (1H, s, H~6), 5.95 (1H, s, H~8), 6.49 (2H, s, H~2′, 6′), 6.94 (2H, s, H~2″, 6″); ^{13}C NMR (MeOD, δ, ppm, 100 MHz) 78.6 (C~2), 70.0 (C~3), 26.8 (C~4), 99.4 (C~4a), 157.9 (C~5), 96.6 (C~6), 157.8 (C~7), 95.8 (C~8), 157.2 (C~8a), 130.8 (C~1′), 110.2 (C~2′, 6′), 146.7 (C~3′, 4′, 5′), 121.5 (C~1″), 106.8 (C~2″, 6″),146.3 (C~3″, 4″, 5″), 167.6 (C~7″) (Lee *et al*., 1992).

(-)-Epicatechin gallate (12):

$[\alpha]^{20}_{D}$ -16.1 (*c* 0.3, CH$_3$OH); ESI-MS: m/z 465.2 $[M+Na]^+$ (calcd. for C$_{22}$H$_{18}$O$_{10}$Na, 465.1); UV (CH$_3$OH) λ_{max}: 226, 280 nm; IR ν_{max}:3457.8, 2853.7, 1611.3 cm^{-1}; ^{1}H NMR (MeOD, δ, ppm, *J*/Hz, 400 MHz):5.02 (1H, brs, H~2), 5.52 (1H, brs, H~3), 2.98 (1H, dd, *J*=16.8, 4.4, H~4a), 2.83 (1H, dd, *J*=16.8, 2.4, H~4b), 5.95 (1H, s, H~6), 5.94 (1H, s, H~8), 6.91 (1H, d, *J*=1.8, H~2′), 6.79 (1H, d, *J* =8.2, H~5′), 6.81 (1H, dd, *J*=8.2, 1.8, H~6′), 6.94 (2H, s, H~2″, 6″); ^{13}C NMR (MeOD, δ, ppm, 100 MHz) 78.6 (C~2), 70.1 (C~3), 26.9 (C~4), 99.5 (C~4a), 157.9 (C~5), 96.5 (C~6), 157.9 (C~7), 95.9 (C~8), 157.2 (C~8a), 131.4 (C~1′), 115.1 (C~2′), 145.8 (C~3′), 145.9 (C~4′), 116.0 (C~5′),119.5 (C~6′), 121.5 (C~1″), 106.8 (C~2″, 6″),146.3(C~3″, 4″, 5″), 167.6 (C~7″) (Liu *et al*., 2014).

Fangchengenbisflavan A (13)

$[\alpha]^{20}_{D}$ -35.6 (*c* 0.1, CH$_3$OH); ESI-MS: m/z 615.4 $[M+Na]^+$ (calcd. for C$_{31}$H$_{27}$O$_{12}$Na, 615.2); UV (CH$_3$OH) λ_{max}: 215, 280, 302 nm; IR ν_{max}: 3443.6, 2860.3, 1623.1, 1455.8 cm^{-1}; ^{1}H NMR (MeOD, δ, ppm, *J*/Hz, 400 MHz): 4.72 (2H, brs, H~2), 3.83 (2H, brs, H~3), 2.78 (2H, dd, *J*=16.8, 4.4, H~4a), 2.63 (2H, dd, *J*=16.8, 2.4, H~4b), 5.92 (2H, s, H~6), 6.91 (2H, d, *J*=2.0, H~2′), 6.68 (2H, d, *J*=8.2, H~5′), 6.69 (2H, dd, *J* =8.2, 2.0, H~6′), 3.84 (2H, s, 9~CH$_2$); ^{13}C NMR (MeOD, δ, ppm, 100 MHz) 80.4 (C~2), 67.3 (C~3), 29.1 (C~4), 100.5 (C~4a), 155.1 (C~5), 96.7 (C~6), 155.8 (C~7), 106.6 (C~8), 153.7 (C~8a), 131.7 (C~1′), 115.3

Table 1: Hepatoprotective effects of selective compounds against D-galactosamine-induced toxicity in HL-7702 cells[a]

Compounds	Cell survival rate (% of normal)	Inhibition (% of control)
normal	100.0±4.9	-
control	42.7±3.3	-
Bicyclol[b]	58.2±5.2[*]	27.1
1	57.8±1.9[**]	26.4
2	66.4±3.7[*]	41.4
5	52.6±5.8[**]	17.3
7	61.8±2.7[*]	33.3
8	56.5±4.5[**]	24.1

[a]Results were expressed as means ± SD (*n*= 3; for normal and control, *n* = 6); [*]p<0.05, [**]p<0.01, significantly different from control by Student′s *t*-test. [b]Positive control substance.

(C~2′), 145.9 (C~3′), 145.8 (C~4′), 116.0 (C~5′),119.7 (C~6′), 16.7 (9~CH_2) (Liu *et al*., 2014).

DISCUSSION

Hepatoprotective and neuroprotective activitivies

The compounds (1-13) were bioassayed for hepatoprotective activities against D-galactosamine-induced toxicity in HL-7702 cells with bicyclol as the positive control. The inhibition (%) of compounds 1, 2, 5, 7, and 8 were calculated with values of 26.4, 41.4, 17.3, 33.3 and 24.1 (table 1), respectively. The values were expressed as means ± SD and the Student’s t-test was carried out to identify statistical differences between normal (control) and tested samples. The *p* values less than 0.05 or 0.01 were considered as statistically significant. Therefore, compounds 1, 2, 5, 7 and 8 showed remarkable hepatoprotective activities, and compounds 3, 4, 6, 9, 10, 11, 12 and 13 exhibited no hepatoprotective activities compared with the positive control of bicyclol (table 1). Moreover, compounds (1-13) were assayed for neuroprotective activities against 6-OHDA-induced cell death in SH-SY5Y cells with Curcumin served as the reference compound (IC_{50} =6.53μM). According to the results of pharmacological screening, it was found that selective compounds exhibited significant neuroprotective activities with IC_{50} =2.02-5.34μM. Among them, compound 13 showed the strongest neuroprotective activities with IC_{50} value of 2.02μM, respectively. Compounds 9 and 10 with IC_{50} values of 4.08 and 5.34μM, which showed moderate neuroprotective activities. However, compounds 1, 2, 3, 4, 5, 6, 7, 8, 11 and 12 exhibited no neuroprotective activities compared with the standard drug Curcumin with IC_{50} value more than 6.53μM (table 2).

CONCLUSION

In the work, thirteen flavanes (1-13) were isolated and identified from the fruits of *U. pumila* L. for the first time. Moreover, the compounds (1-13) were assayed for hepatoprotective activities against D-galactosamine-induced toxicity in HL-7702 cells and the pharmacological results showed that compounds 1, 2, 5, 7 and 8 possessd remarkable hepatoprotective activities. Furthermore, the compounds (1-13) were evaluated for neuroprotective activities against 6-OHDA-induced cell death in SH-SY5Y cells with curcumin served as the standard drug, and the pharmacological results showed that compounds 9, 10 and 13 showed significant neuroprotective activities with IC_{50} values of 4.08, 5.34, and 2.02μM, respectively.

Table 2: Neuroprotective activities of selective compounds

Compounds	IC_{50} (μM)
9	4.08
10	5.34
13	2.02
Curcumin	6.53

ACKNOWLEDGMENTS

This work was supported by the Scientific and Technological Project of Henan Provincial Department of Science and Technology (NO.172102310552), the Key Scientific Research Project of Colleges and Universities in Henan Province (NO.17A350011), the Special Project of Nanyang Normal University (NO.ZX2014044), the Laboratory Opening Program of Nanyang Normal University (NO.SYKF2016017), and the Scientific and Technological Project of Nanyang (NO.KJGG23).

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