Natural Product Sciences
22(3) : 154-161 (2016)
http://dx.doi.org/10.20307/nps.2016.22.3.154

Chemical Constituents from Leaves of *Pileostegia viburnoides* Hook.f.et Thoms

Xiao Jun Li[1,2], Zu Zhen Liu[3], Kwan-Woo Kim[2], Xiang Wang[1], Zhi Li[1], Youn-Chul Kim[2], Chang Soo Yook[4], and Xiang Qian Liu[1,5,*]

[1]*School of Pharmacy, Hunan University of Chinese Medicine, Changsha, Hunan 410208, China*
[2]*College of Pharmacy, Wonkwang University, Iksan 570-749, Korea*
[3]*Hunan Society of Chinese Medicine, Changsha, Hunan 410008, China*
[4]*School of Pharmacy, KyungHee University, Seoul 130-701, Korea*
[5]*Broad-Ocean Bio-Science and Technique Co., Ltd of Changsha, Hunan Changsha 410205, China*

Abstract – Phytochemical investigation on the leaves of *Pileostegia viburnoides* Hook.f.et Thoms led to the isolation of twenty-five compounds, and their structures were identified as *n*-dotriacontane (**1**), taraxeryl acetate (**2**), friedelin (**3**), epifriedelinol (**4**), canophyllal (**5**), stigmast-4-en-3-one (**6**), stigmasterol (**7**), (24*R*)-5A-stigmastane-3,6-dione (**8**), ursolic acid (**9**), pomolic acid (**10**), umbelliferone (**11**), 4-epifriedelin (**12**), *n*-octatriacontanol (**13**), β-amyrin (**14**), α-amyrin (**15**), taraxerol (**16**), nonadecanol (**17**), friedelane (**18**), arachic acid (**19**), protocatechuic acid (**20**), *n*-pentatriacontanol (**21**), hexadecanoic acid (**22**), vincosamide (**23**), daucosterol (**24**), and skimming (**25**), respectively. To our best knowledge, compounds **1**, **2**, **12**, **13**, **17 - 19** and **21-23** were new within Saxifragaceae family. Compounds **15**, **16**, and **20** were produced from this genus for the first time. Compounds **4**, **14** and **25** were first obtained from species *P. viburnoides* and compounds **3**, **5 - 11**, and **24** were achieved from the leaves of *P. viburnoides* for the first time. Furthermore, the anti-neuroinflammatory activity of these isolates was evaluated.

Keywords – *Pileostegia viburnoides* Hook.f.et Thoms, Triterpenes, 4-epifriedelin, Coumarins, Vincosamide, Chemical constituents, Anti-neuroinflammatory

Introduction

Pileostegia viburnoides Hook.f.et Thoms belongings to Saxifragaceae is mainly distributed in the south of the Yangtze River in China, which has the effect of expelling wind and removing dampness, scattered stasis and relieves pain, removing toxin for detumescence, *etc*. It is the Miao nationality medicinal herbs mainly used to treat rheumatic numbness, rheumatic arthritis, injuries from falls, bone fracture, lumbago due to the kidney deficiency, bleeding caused traumatism and anti-cancer as folk medicine over years.[1,2] In the previous study, 13 compounds including five triterpenoids, three coumarins, four steroids, and one aliphatic amine were isolated from the cane of *P. Viburnoides*.[3] The chemical compositions of essential oils from leaves and stems of *P. viburnoides* were analyzed by GC-MS, respectively.[4,5] Additionally, the anti-inflammatory and analgesic effects of the extracts from stems of *P. viburnoides* were reported.[6,7] In present study, this is the first phytochemical investigation on the leaves of *P. viburnoides* and 25 secondary metabolites were obtained, including eleven triterpenes (**2 - 5**, **9**, **10**, **12**, **14 - 16**, and **18**), two coumarins (**11** and **25**), one alkaloid (**23**), one phenolic acid (**20**), four steroids (**6 - 8** and **24**), and aliphatic series (**1**, **13**, **17**, **19**, **21**, and **22**). Their chemical structures were determined by physicochemical properties and spectroscopic methods. Moreover, the cytotoxicity and inhibition effects of production of NO on lipopolysaccharide (LPS)-induced BV2 microglias of these isolates were investigated.

Experimental

General experimental procedures – Melting points (uncorrected) were measured using a Boetius micro-melting point apparatus. ^{1}H-NMR (400 MHz), ^{13}C-NMR (100 MHz) and 2D-NMR were recorded at room temperature in $CDCl_3$ or CD_3OD using Bruker ACF-500 NMR spectrometer and chemical shifts were given in δ (ppm) value relative to TMS as internal standard. Mass

*Author for correspondence
Xiang Qian Liu, School of Pharmacy, Hunan University of Chinese Medicine, Hunan Changsha 410208, China.
Tel: +86-731-88458240; E-mail: lxq0001cn@163.com

spectra were obtained on MS Agilent 1200 Series LC/MSD Trap Mass spectrometer (EI-MS). Column chromatography was carried out on silica gel (200 - 300 mesh and 100 - 200 mesh, Qingdao Marine Chemical Co., Ltd., Qingdao, China), and Sephadex LH-20 (Merck). RP-TLC was performed on a precoated RP-$18F_{254s}$ (Merck) plate. TLC was conducted on self-made silica gel G (Qingdao Marine Chemical Industry, Qingdao, China) plates and spots were visualized by spraying with 10% H_2SO_4 in ethanol (v/v) followed by heating at 105 ºC.

Plant materials – The leaves of *P. viburnoides* were collected from its natural habitat in Yuanling, Hunan Province, China, in August 2013 and identified by Prof. Xiang Qian Liu, corresponding author of this manuscript. A voucher specimen has been deposited in Herbarium of Hunan University of Chinese Medicine, Hunan, China. (No. 20130830)

Extraction and isolation – The dried, powdered leaves of *P. viburnoides* (8 kg) were extracted with methanol under reflux three times (3×30 L). The solvent was removed under reduced pressure to give a residue (545.0 g), which was suspended in distilled water and successively partitioned with petroleum ether (PE, 60-90), EtOAc and n-BuOH, respectively.

The PE extract (204 g) was subjected to column chromatography (CC) on silica gel eluting with a gradient of PE-EtOAc (100:1 to 1:1, v/v) to give thirteen fractions (Fr.P1-Fr.P13). Compound **1** (100 mg) was achieved from Fr.P2 by decolorization with PE. Fr.P3 was subjected to a silica gel CC, with a gradient PE-EtOAc (50:1 to 10:1, v/v) as the solvent to gain **2** (280 mg). Colorless needle crystal **3** (32 mg) was yielded from Fr.P4 by successive recrystallizations with PE-EtOAc (20:1). Fr.P5 was firstly performed on a silica gel CC, eluted with PE-EtOAc (50:1 to 5:1, v/v), and then purified by recrystallization with PE-EtOAc (10:1) to afford **4** (25 mg) and **5** (20 mg). Compounds **6** (11 mg), **7** (2 g), and **8** (7 mg) were obtained from Fr.P6 by repeated silica gel CC using PE-EtOAc (50:1 to 1:1, v/v) as mobile phase and recrystallization with PE. Fr.P7 was performed on a silica gel CC eluted with PE-EtOAc (50:1 to 1:1, v/v) to afford five subfractions (Fr.P7.1-P7.5). Compounds **9** (8 mg) were isolated from Fr.P7.2 by silica gel CC (PE-EtOAc, 30:1 to 5:1, v/v). Fr.P7.4 was subjected to silica gel CC eluted with PE-acetone (30:1 to 5:1, v/v), and then decolorized by a Sephadex LH-20 CC (MeOH) to give **10** (9 mg). Fr.P8 was chromatographed over silica gel CC with a gradient PE-EtOAc (30:1 to 5:1, v/v) to produce colorless needles **12** (12 mg). Fr.P9 was chromatographed on a silica gel-H CC with PE-EtOAc (30:1 to 5:1, v/v) to give **13** (28 mg), **14** (22 mg), and **15** (18 mg). Compounds **16** (20 mg) and **17** (9 mg) were isolated on silica gel CC from Fr.P10 with PE-EtOAc (gradient, 20:1 to 3:1, v/v) repeatedly. Fr.P11 was subjected to a silica gel column eluted with a gradient of PE-EtOAc (50:1 to 3:1, v/v) to obtain four sub-fractions (Fr.P11.1-P11.4). Compound **18** (27 mg) was yielded from Fr.P11.2 by using silica gel CC eluted with PE-EtOAc (15:1, v/v), and **19** (13 mg) was obtained from Fr.P11.4. Fr.P12 was firstly refractionated on silica gel CC eluted with PE-EtOAc (40:1 to 1:1, v/v) to afford seven sub-fractions (Fr.P12.1-P12.7), of which Fr.P12.2, Fr.P12.3 and Fr.P12.5 were then subjected to silica gel CC to yield **21** (12 mg) and **22** (14 mg), respectively.

The EtOAc extract (44 g) was subjected to column chromatography (CC) on silica gel eluting with a gradient of $CHCl_3$-MeOH (100:1 to 1:1, v/v) to give nine fractions (Fr.E1-Fr.E9). Fr.E2 was purified on silica gel CC eluted with CH_2Cl_2-acetone (100:1 to 5:1, v/v) to afford five subfractions (Fr.E2.1-E2.5). **11** (34 mg) was obtained from Fr.E2.3 by recrystallization with methyl alcohol. Fr.E3 was recrystallized in MeOH, and followed decolorized by a Sephadex LH-20 CC (MeOH) to give **20** (1.5 g). Fr.E5 was firstly subjected to a silica gel CC, with a gradient $CHCl_3$-MeOH (40:1 to 3:1, v/v) as the solvent, and then purified by Sephadex LH-20 (MeOH), finally recrystallized in MeOH to achieve **23** (51 mg). White powder **24** (12 mg) was isolated from Fr.E7 by recrystallization with MeOH. Similarly, White powder **25** (188 mg) was obtained from Fr.E8 by recrystallization and decolorization with methanol.

MTT assay for cell viability – BV2 microglias were maintained at 5×10^5 cells/mL in DMEM medium supplemented with 10% heat-inactivated FBS, penicillin G (100 U/mL), streptomycin (100 mg/L), and L-glutamine (2 mM) and incubated at 37 ºC in a humidified atmosphere containing 5% CO2. Cell viability was determined by adding 100 mg/mL of MTT to 1 mL of a cell suspension (1×10^5 cells/mL in 96-well plates) and incubated for 30 min. The formazan formed was dissolved in acidic 2-propanol, and the optical density was measured at 540 nm.

Nitrite assay – The concentration of nitric oxide (NO) in the conditioned media was determined by a method based on the Griess reaction.[8] An aliquot of each supernatant (100 mL) was mixed with the same volume of Griess reagent (0.1% (w/v) N-(1-naphathyl)-ethylenediamine and 1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid) for 10 min at room temperature. The absorbance of the final product was measured spectrophotometrically at 540 nm using an ELISA plate reader. The nitrite concentration in the samples was determined from a standard

Table 1. ^{13}C-NMR data of triterpenoids **2-5**, **9**, **10**, **12**, **14 - 16**, **18**

Position	**2**	**3**	**4**	**5**	**9**	**10**	**12**	**14**	**15**	**16**	**18**
1	37.9	22.4	16.0	22.4	40.0	39.4	21.9	38.7	38.7	37.9	22.9
2	23.7	41.7	32.5	41.6	28.0	28.1	37.3	27.4	27.2	27.1	41.7
3	81.2	213.5	72.9	213.3	79.8	78.6	216.7	79.0	79.0	79.2	29.9
4	38.1	58.4	49.3	58.3	40.0	39.5	58.9	38.7	38.7	38.8	58.4
5	55.8	42.3	37.8	42.1	56.9	56.2	40.0	55.1	55.1	55.7	42.4
6	18.9	41.4	41.4	41.2	19.6	19.3	37.5	18.3	18.3	18.8	41.5
7	33.3	18.4	17.5	18.2	34.5	33.9	17.9	32.6	32.9	41.5	18.2
8	39.2	53.2	53.4	53.0	40.7	40.7	53.6	38.5	39.9	39.0	53.1
9	49.4	37.6	37.6	37.2	47.4	48.3	37.2	47.6	47.6	49.5	37.7
10	37.7	59.6	61.5	59.4	38.2	37.8	49.6	37.1	36.9	38.0	59.9
11	17.7	35.8	35.8	35.6	24.2	24.3	35.9	23.7	23.3	17.5	35.8
12	36.9	30.6	30.6	30.7	127.0	128.8	30.7	121.7	124.4	33.7	30.7
13	38.1	39.8	39.8	37.8	139.8	139.6	39.9	145.2	139.5	37.6	39.9
14	158.1	38.4	38.4	38.7	42.9	42.2	38.5	41.7	42.0	158.3	38.5
15	117.1	32.5	32.5	28.2	29.3	29.3	32.6	26.1	28.7	117.1	33.0
16	33.9	36.1	36.1	33.5	25.4	26.4	36.2	27.2	26.6	37.7	36.2
17	36.0	30.1	30.1	47.9	47.8	48.1	30.1	32.9	33.7	35.8	30.2
18	48.9	42.9	42.9	36.6	54.5	54.5	42.9	47.2	59.0	48.7	43.0
19	41.4	35.5	35.5	35.1	40.1	73.1	35.5	46.8	39.6	36.7	35.6
20	29.0	28.3	28.3	28.5	40.5	42.6	28.3	31.2	39.6	28.8	28.4
21	35.3	32.9	32.9	30.6	31.8	27.0	32.9	34.7	31.1	33.1	32.6
22	37.6	39.4	39.4	32.4	38.3	38.5	39.4	37.1	41.5	35.1	39.5
23	28.2	6.9	11.8	6.9	29.0	28.7	13.6	28.1	28.1	28.2	7.7
24	16.7	14.8	16.6	14.8	16.0	15.8	23.3	15.6	15.6	15.6	14.3
25	15.6	18.1	18.4	17.3	16.2	16.3	18.2	15.5	15.5	15.6	18.1
26	26.1	20.4	20.3	18.9	17.8	17.4	20.5	16.8	16.8	26.1	20.3
27	21.4	18.8	18.8	20.2	24.1	24.6	18.9	26.1	23.3	21.5	18.9
28	29.9	32.2	32.3	209.3	181.8	179.3	32.2	28.4	28.1	30.0	32.3
29	33.3	35.2	35.2	34.6	17.9	27.2	35.2	33.3	27.4	33.5	35.3
30	30.0	31.9	31.9	29.5	21.7	16.6	31.9	23.5	21.4	30.1	32.0
31	171.1										
32	21.4										

curve of sodium nitrite prepared in phenol red-free DMEM.

Statistical analysis – All values are expressed as the mean ± S.D. Differences between mean values of normally distributed data were assessed with one-way ANOVA (Newman Keuls t-test). Statistical significance was accepted at $P < 0.05$.

***n*-dotriacontane (1)** – Colorless flaky crystal (EtOAc), m.p. 84 - 85 ºC, EI-MS *m/z* 450 $[M]^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.88 (6H, t, $J = 6.8$ Hz, 2×CH_3), 1.26 (30×CH_2).

Taraxeryl acetate (2) – White powder (PE), m.p. 285 - 287 ºC, EI-MS *m/z* 468 $[M]^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.82 (3H, s), 0.86 (3H, s), 0.88 (3H, s), 0.90 (6H, s), 0.95 (6H, s), 1.09 (3H, s), 2.04 (3H, s), 4.46 (1H, dd, $J = 10.0$, 7.0 Hz, H-3α), 5.52 (1H, dd, $J = 8.0$, 3.0 Hz, H-15); ^{13}C-NMR ($CDCl_3$,100 MHz): see Table 1.

Friedelin (3) – Colorless needle crystal (PE), m.p. 232 - 234 ºC; EI-MS *m/z* 426 $[M]^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.72 (3H, s), 0.87 (3H, s), 0.95 (3H, s), 0.99 (3H, s), 1.00 (3H, s), 1.05 (3H, s), 1.18 (3H, s), 0.88 (3H, d, $J = 7.0$ Hz, H_3-23), 2.38 (1H, m, H-4), 2.23 - 2.25 (2H, m, H_2-2); ^{13}C-NMR ($CDCl_3$,100 MHz): see Table 1.

Epifriedelinol (4) – White sand crystal (PE-EtOAc), m.p. 234 - 235 ºC; EI-MS *m/z* 428 $[M]^+$; Liebermann-Burchard reaction was positive. ^{1}H-NMR ($CDCl_3$, 400 MHz), δ: 0.85 (3H, s), 0.93 (3H, d, $J = 7.0$ Hz, H_3-23), 0.94 (3H, s), 0.95 (3H, s), 0.99 (3H, s), 1.00 (3H, s), 1.05

(3H, s), 1.18 (3H, s), 3.71 (1H, m, H-3); ^{13}C-NMR ($CDCl_3$, 100 MHz): see Table 1.

Canophyllal (5) – Colorless needle crystal (PE); m.p. 216 - 217 ºC; EI-MS *m/z* 440 $[M]^+$; L-B reaction was positive. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 9.51 (1H, s, H-28), 0.65 (3H, s), 0.71(3H, s), 0.84 (3H, s), 0.92 (3H, s), 0.98 (3H, s), 1.58 (3H, s), 0.96 (3H, d, J = 7.0 Hz, H_3-23), 2.30 (1H, m, H-4), 2.25 (2H, m, H_2-2); ^{13}C-NMR ($CDCl_3$, 100 MHz): see Table 1.

Stigmast-4-en-3-one (6) – Colorless needle crystal (MeOH); m.p. 159 - 161 ºC; EI-MS *m/z* 412 $[M]^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 5.72 (1H, s, H-4), 1.19 (3H, s, H_3-18), 0.92 (3H, d, J = 6.4 Hz, H_3-26), 0.85(3H, t, J = 8.1 Hz, H_3-29), 0.83 (3H, d, J = 7.2 Hz, H_3-21), 0.81 (3H, d, J = 6.9 Hz, H_3-27), 0.71 (3H, s, H_3-19); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 37.1 (C-1), 35.0 (C-2), 202.6 (C-3), 124.4 (C-4), 175.6 (C-5), 34.3 (C-6), 33.7 (C-7), 37.2 (C-8), 55.7 (C-9), 40.3 (C-10), 22.4 (C-11), 41.3 (C-12), 43.9 (C-13), 57.6 (C-14), 25.5 (C-15), 29.6 (C-16), 57.7 (C-17), 12.7 (C-18), 18.0 (C-19), 37.7 (C-20), 19.6 (C-21), 35.4(C-22), 27.5 (C-23), 47.6 (C-24), 30.7 (C-25), 20.5 (C-26), 19.7 (C-27), 24.5 (C-28), 12.7 (C-29).

Stigmasterol (7) – Colorless needle crystal (MeOH); m.p. 134 - 135 ºC; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 5.36 (1H, s, H-6), 5.16 (1H, dd, J = 15.2, 8.8 Hz, H-22), 4.98 (1H, dd, J = 15.2, 8.8 Hz, H-23), 3.55 (1H, m, H-3); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 37.9 (C-1), 32.5 (C-2), 72.6 (C-3), 43.0 (C-4), 141.5 (C-5), 122.4 (C-6), 32.7 (C-7), 32.6 (C-8), 50.7 (C-9), 37.3 (C-10), 22.4 (C-11), 40.3 (C-12), 42.9 (C-13), 57.6 (C-14), 25.5 (C-15), 29.6 (C-16), 56.7 (C-17), 12.7 (C-18), 19.7 (C-19), 41.2 (C-20), 21.9 (C-21), 139.1 (C-22), 130.1 (C-23), 51.8 (C-24), 32.5 (C-25), 20.2 (C-26), 19.7 (C-27), 26.1 (C-28), 12.9 (C-29).

(24*R*)-5A-stigmastane-3,6-dione (8) – Colorless needle crystal (PE); m.p. 159 - 161 ºC; EI-MS *m/z* 428 $[M]^+$; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.68 (3H, s, H_3-19), 0.80 (3H, d, J = 6.8 Hz, H_3-26), 0.82 (3H, d, J = 6.8 Hz, H_3-27), 0.84 (3H, t, J = 7.6 Hz, H_3-29), 0.92 (3H, d, J = 6.4 Hz, H_3-21), 0.95 (3H, s, H_3-18); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 38.2 (C-1), 39.5 (C-2), 209.6 (C-3), 37.1 (C-4), 57.6 (C-5), 211.3 (C-6), 46.8 (C-7), 38.2 (C-8), 53.6 (C-9), 41.4 (C-10), 21.8 (C-11), 37.5 (C-12), 43.1 (C-13), 56.1 (C-14), 24.1 (C-15), 28.2 (C-16), 56.7 (C-17), 12.7 (C-18), 12.2 (C-19), 36.2 (C-20), 18.8 (C-21), 33.9 (C-22), 26.1 (C-23), 45.9 (C-24), 29.2 (C-25), 20.0 (C-26), 19.1 (C-27), 23.2 (C-28), 12.1 (C-29).

Ursolic acid (9) – White powder (MeOH); m.p. 266 - 267 ºC; EI-MS *m/z* 456 $[M]^+$; Liebermann-Burchard and Salkowski reactions were positive; ^{13}C-NMR (CD_3OD, 150 MHz): see Table 1.

Pomolic acid (10) – White powder (MeOH); m.p. 286 - 289 ºC; EI-MS *m/z* 473 $[M+H]^+$; ^{1}H-NMR (600 MHz, CD_3COCD_3) δ: 0.93 (3H, s, H_3-25), 0.96 (3H, s, H_3-24), 1.00 (3H, d, J = 3.9 Hz, H_3-30), 1.21 (3H, s, H_3-26), 1.24 (3H, s, H_3-23), 1.35 (3H, s, H_3-29), 1.54 (3H, s, H_3-27), 2.54 (1H, s, H-18), 3.16 (1H, dd, J = 4.7, 11.0 Hz, H-3), 5.28 (1H, t, J = 3.5 Hz, H-12); ^{13}C-NMR (150 MHz, CD_3COCD_3): see Table 1.

Umbelliferone (11) – Colorless needle crystal (MeOH); UV 254 nm and 365 nm were blue-fluorescence; EI-MS *m/z* 162 $[M]^+$; ^{1}H-NMR (400 MHz, CD_3COCD_3) δ: 9.55 (1H, s, -OH), 7.89 (1H, d, J = 9.6 Hz, H-4), 7.49 (1H, d, J = 8.4 Hz, H-5), 6.77 (1H, dd, J = 8.4, 1.8 Hz, H-6), 6.70 (1H, d, J = 1.8 Hz, H-8), 6.18 (1H, d, J = 9.6 Hz, H-3); ^{13}C-NMR (100 MHz, CD_3COCD_3) δ: 161.2 (C-2), 111.3 (C-3), 144.4 (C-4), 129.6 (C-5), 113.1 (C-6), 160.4 (C-7), 102.1 (C-8), 155.4 (C-9), 111.2 (C-10).

4-epifriedelin (12) – Colorless needle crystal (PE-EtOAc); m.p. 243 - 244 ºC; EI-MS *m/z* 426 $[M]^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.87 (3H, s, H-25), 0.93 (3H, s, H-24), 0.95 (3H, s, H-29), 1.00 (3H, s, H-30), 1.01 (3H, s, H-26), 1.05 (3H, s, H-27), 1.12 (3H, d, J = 7.3 Hz, H-23), 1.18 (3H, s, H-28), 1.92 (1H, m, H-4); ^{13}C-NMR ($CDCl_3$, 100 MHz): see Table 1.

***n*-octatriacontanol (13)** – White powder; m.p. 85 - 86 ºC, EI-MS *m/z* 550 $[M]^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 3.65 (2H, t, J = 6.8 Hz, H-1), 1.56 (2H, m, H-2), 1.25 - 1.56 (70H, m, 35×CH_2), 0.88 (3H, t, J = 6.8 Hz, CH_3).

β-amyrin (14) – White needle crystal (PE-EtOAc), EI-MS *m/z* 426 $[M]^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.79 (3H, s), 0.83 (3H, s), 0.86 (3H, s), 0.87 (3H, s), 0.95 (3H, s), 0.97 (3H, s), 1.00 (3H, s), 1.13 (3H, s), 3.23 (1H, t, H-3), 5.18 (1H, t, H-12); ^{13}C-NMR ($CDCl_3$, 100 MHz): see Table 1.

α-amyrin (15) – White needle crystal (PE-EtOAc), EI-MS *m/z* 426 $[M]^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.79 (3H, s), 0.83 (3H, s), 0.86 (3H, s), 0.87 (3H, s), 0.95 (3H, s), 0.97 (3H, s), 1.00 (3H, s), 1.13 (3H, s), 3.23 (1H, t, H-3), 5.13 (1H, t, H-12); ^{13}C-NMR ($CDCl_3$, 100 MHz): see Table 1.

Taraxerol (16) – White sand crystal (PE-EtOAc), EI-MS *m/z* 426 $[M]^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 5.65 (1H, dd, J = 8.0, 2.6 Hz, H-15), 3.30 (1H, dd, J = 11.0, 4.0 Hz, H-3), 2.15 (1H, dt, J = 13.0, 3.5 Hz, H-16b), 2.02 (1H, dd, J = 15.0, 3.0 Hz, H-16a), 1.21 (3H, s, H-26), 1.10 (3H, s, H-23), 1.07 (3H, s, H-28), 1.05 (3H, s, H-25), 1.03 (3H, s, H-27), 1.03 (3H, s, H-30), 0.94 (3H, s, H-28), 0.92 (3H, s, H-24); ^{13}C-NMR ($CDCl_3$, 100 MHz): see Table 1.

Nonadecanol (17) – White powder, m.p. 62 - 63 ºC, EI-

MS *m/z* 284 $[M]^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 3.51 (2H, t, *J* = 6.5 Hz, H-1), 1.53 (2H, m, H-2), 1.27 (32H, brs, 16×CH_2), 0.87 (3H, t, *J* = 6.4 Hz, CH_3).

Friedelane (18) – Colorless needle crystal (PE-EtOAc), m.p. > 250 ºC; EI-MS *m/z* 412 $[M]^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.77 (3H, s, H-24), 0.87 (3H, s, H-25), 0.88 (3H, d, *J* = 6.7 Hz, H-23), 0.95 (3H, s, H-30), 1.00 (3H, s, H-29), 1.01 (3H, s, H-26), 1.05 (3H, s, H-27), 1.18 (3H, s, H-28); ^{13}C-NMR ($CDCl_3$, 100 MHz): see Table 1.

Arachic acid (19) – White powder, m.p. 65 - 67 ºC, EI-MS *m/z* 312 $[M]^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 2.36 (2H, t, *J* = 7.2 Hz, H-2), 1.61 (2H, m, H-3), 1.20~1.40 (32H, brs, 16×CH_2), 0.88 (3H, t, *J* = 6.8 Hz, CH_3).

Protocatechuic acid (20) – Colorless needle crystal (MeOH), m.p. 199 - 200 ºC; EI-MS *m/z* 156 $[M]^+$; ^{1}H-NMR (CD_3OD, 400 MHz) δ: 6.80 (1H, d, *J* = 8.0 Hz, H-5), 7.42 (1H, dd, *J* = 2.1, 8.0 Hz, H-6), 7.44 (1H, d, *J* = 2.1 Hz, H-2); ^{13}C-NMR (CD_3OD, 100 MHz) δ: 170.3 (-COOH), 151.5 (C-3), 146.0 (C-4), 123.0 (C-1), 117.7 (C-2), 115.8 (C-6), 123.9(C-5).

***n*-pentatriacontanol (21)** – White powder, m.p. 83 - 85 ºC, EI-MS *m/z* 508 $[M]^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 3.64 (2H, t, *J* = 6.8 Hz, H-1), 1.55 (2H, m, H-2), 1.26 - 1.55 (64H, m, 32×CH_2), 0.88 (3H, t, *J* = 6.8 Hz, CH_3).

Hexadecanoic acid (22) – White powder, m.p. 66 - 67 ºC, EI-MS *m/z* 256 $[M]^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 2.36 (2H, t, *J* = 7.2 Hz, H-2), 1.61 (2H, m, H-3), 1.21 - 1.40 (24H, brs, 12×CH_2), 0.88 (3H, t, *J* = 6.8 Hz, CH_3).

Vincosamide (23) – Colorless needle crystal (MeOH), m.p. 200 - 202 ºC; EI-MS *m/z* 498 $[M]^+$; ^{1}H-NMR (CD_3OD, 400 MHz) δ: 7.45 (1H, d, *J* = 2.4 Hz, H-17), 7.42 (1H, d, *J* = 8.0 Hz, H-9), 7.31 (1H, d, *J* = 8.0 Hz, H-12), 7.07 (1H, td, *J* = 8.0, 1.0 Hz, H-11), 6.98 (1H, td, *J* = 8.0, 1.0 Hz, H-10), 5.52 (1H, m, H-19), 5.50 (1H, d, *J* = 2.0 Hz, H-21), 5.30 (1H, dd, *J* = 16.0, 2.0 Hz, Hb-18), 5.17 (1H, dd, *J* = 10.0, 2.0 Hz, Ha-18), 5.05 (1H, m, Hb-5), 4.93 (1H, m, H-3), 4.70 (1H, d, *J* = 8.0 Hz, H-1'), 3.90 (1H, dd, *J* = 12.0, 2.0 Hz, Hb-6'), 3.70 (1H, dd, *J* = 12.0, 5.2 Hz, Ha-6'), 3.40 - 3.30 (3H, m, H-3', H-4', H-5'), 3.20 (2H, m, H-2', H-15), 2.91 (1H, td, *J* = 4.0, 11.0 Hz, Ha-5), 2.76 (2H, m, H-6), 2.71 (1H, m, H-20), 2.46 (1H, dt, *J* = 12.0, 4.0 Hz, Hb-14), 1.46 (1H, q, *J* = 13.0 Hz, Ha-14); ^{13}C-NMR (CD_3OD, 100 MHz) δ: 166.3 (C-22), 149.3 (C-17), 138.6 (C-13), 134.9 (C-2), 134.2 (C-19), 128.2 (C-8), 122.8 (C-11), 120.8 (C-18), 120.3 (C-10), 119.1 (C-9), 112.0 (C-12), 109.4 (C-16), 109.6 (C-7), 99.9 (C-1'), 97.4 (C-21), 78.3 (C-5'), 78.6 (C-3'), 75.1 (C-2'), 71.8 (C-4'), 63.0 (C-6'), 55.1 (C-3), 44.8 (C-20), 41.5 (C-5), 32.9 (C-14), 27.6 (C-15), 22.3 (C-6).

Daucosterol (24) – White powder (MeOH); m.p. 288 - 290 ºC; L-B reaction was positive; ^{13}C-NMR (CD_3OD, 100 MHz) δ: 36.7 (C-1), 29.1 (C-2), 77.0 (C-3), 39.3 (C-4), 140.4 (C-5), 121.2 (C-6), 31.4 (C-7), 31.4 (C-8), 49.6 (C-9), 36.2 (C-10), 20.6 (C-11), 38.3 (C-12), 41.9 (C-13), 56.2 (C-14), 23.9 (C-15), 27.8 (C-16), 55.5 (C-17), 11.7 (C-18), 19.1 (C-19), 35.5 (C-20), 18.6 (C-21), 33.4 (C-22), 25.5 (C-23), 45.2 (C-24), 28.7 (C-25), 19.7 (C-26), 19.0 (C-27), 22.6 (C-28), 11.8 (C-29), 100.5 (C-1'), 73.5 (C-2'), 76.7 (C-3'), 70.1 (C-4'), 76.8 (C-5'), 61.1 (C-6').

Skimmin (25) – White powder (MeOH); m.p. 219 - 220 ºC; EI-MS *m/z* 324 $[M]^+$; ^{1}H-NMR (CD_3OD, 400 MHz) δ: 8.00 (1H, d, *J* = 9.6 Hz, H-4), 7.60 (1H, d, *J* = 9.0 Hz, H-5), 7.05 (1H, s, H-8), 7.01 (1H, d, *J* = 9.0 Hz, H-6), 6.32 (1H, d, *J* = 9.6 Hz, H-3), 5.02 (1H, d, *J* = 7.6 Hz, H-1'), 3.70 - 3.72 (1H, m, H-6'), 3.41~3.44 (2H, m, H-5', 6'), 3.27 - 3.29 (2H, m, H-2', 3'), 3.16 (1H, m, H-4'); ^{13}C-NMR (CD_3OD, 100 MHz) δ: 160.2 (C-2, C-7), 155.0 (C-8a), 144.2 (C-4), 129.4 (C-5), 113.6 (C-6), 113.2 (C-3), 113.1 (C-4a), 103.2 (C-8), 100.0 (C-1'), 77.1 (C-3'), 76.4 (C-5'), 73.1 (C-2'), 69.6 (C-4'), 60.6 (C-6').

Result and Discussion

Compound **12** was obtained as colorless needles (PE-EtOAc). Liebermann-Burchard reaction was positive. The ^{13}C-NMR spectrum of compound **12** revealed chemical shifts of carbons on C, D, and E rings were very similar to those of friedelin (**3**), but two methyl carbons were found further downfield than friedelin (δ_C 13.6, 23.3 vs δ_C 6.9, 14.8) while one machine was found further upfield (δ_C 49.6 vs δ_C 59.6). HMQC of **12** demonstrated that the proton (δ_H 1.12, 3H) attached to the carbon at δ_C 13.6 was a doublet (*J* = 7.3 Hz), indicating this carbon was assignable to C-23. What's more, HMBC revealed that δ_C 23.3 and 49.6 were assignable to C-24 and C-10, respectively. Based on the above evidences, compound **12** appeared to be the C-4 epimer of friedelin. Therefore, the structure of **12** was determined to be 4-epifriedelin,[9] a compound not commonly reported previously. The structure of **12** was further confirmed by ^{1}H-^{1}H COSY, HMBC, and NOESY techniques.

The structures of other isolated compounds were elucidated according to their spectroscopic data (MS, 1D and 2D NMR) and by comparison with those of literature. They were identified as *n*-dotriacontane (**1**),[10] taraxeryl acetate (**2**),[11] friedelin (**3**),[12] epifriedelinol (**4**),[13] canophyllal (**5**),[14] stigmast-4-en-3-one (**6**),[3] stigmasterol (**7**),[17] (24*R*)-5A-stigmastane-3,6-dione (**8**),[3] ursolic acid (**9**),[15] pomolic acid (**10**),[15] umbelliferone (**11**),[16] *n*-octatriacontanol (**13**),[14] β-amyrin (**14**),[18] α-amyrin (**15**),[18] taraxerol (**16**),[19] nona-

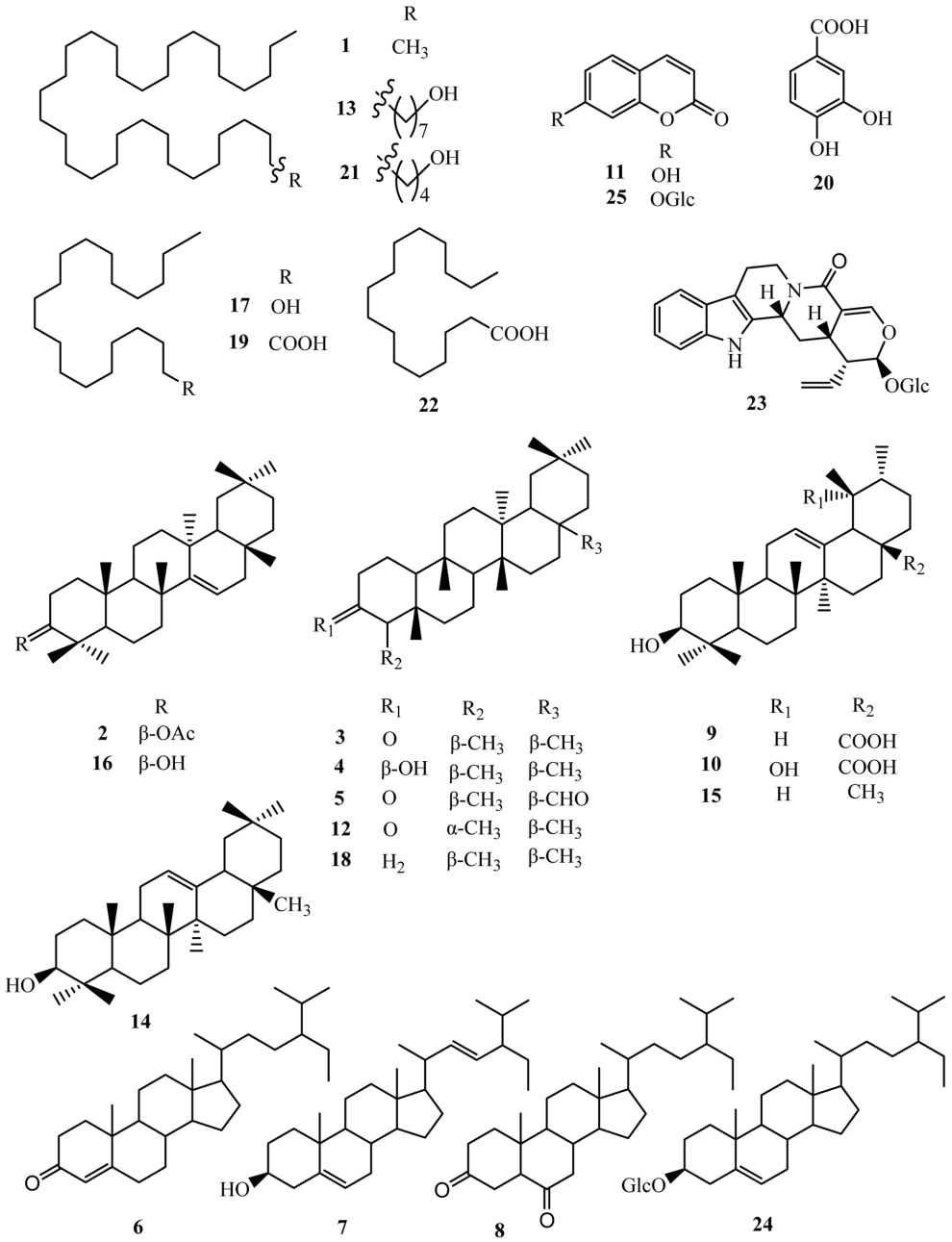

Fig. 1. Chemical structures of the isolated compounds **1 - 25**.

decanol (**17**),[20] friedelane (**18**),[21] arachic acid (**19**),[20] protocatechuic acid (**20**),[21] *n*-pentatriacontanol (**21**),[22] hexadecanoic acid (**22**),[20] vincosamide (**23**),[23] daucosterol (**24**),[24] and skimmin (**25**),[25] respectively (Fig. 1). Among them, compounds **1**, **2**, **12**, **13**, **17 - 19** and **21 - 23** were new within Saxifragaceae family. Compounds **15**, **16**, and **20** were afforded from this genus for the first time. Compounds **4**, **14** and **25** were first obtained from species *P. viburnoides* and compounds **3**, **5 - 11**, and **24** were achieved from the leaves of *P. viburnoides* for the first time.

Moreover, the cytotoxicity and inhibition of production of nitric oxide (NO) of these isolates from *P. viburnoides* were investigated on LPS-stimulated BV2 microglias. As shown in Fig. 2, among tested compounds, **3**, **4**, and **11** showed that the production of NO were down-regulated moderately when the concentration was 80 μM with the inhibition were 46.6%, 45.4%, and 45.4%, respectively. Compound **18** showed significant inhibition of production of NO, however, there was significant effect on cell viability. Other tested compounds showed weak or inactive

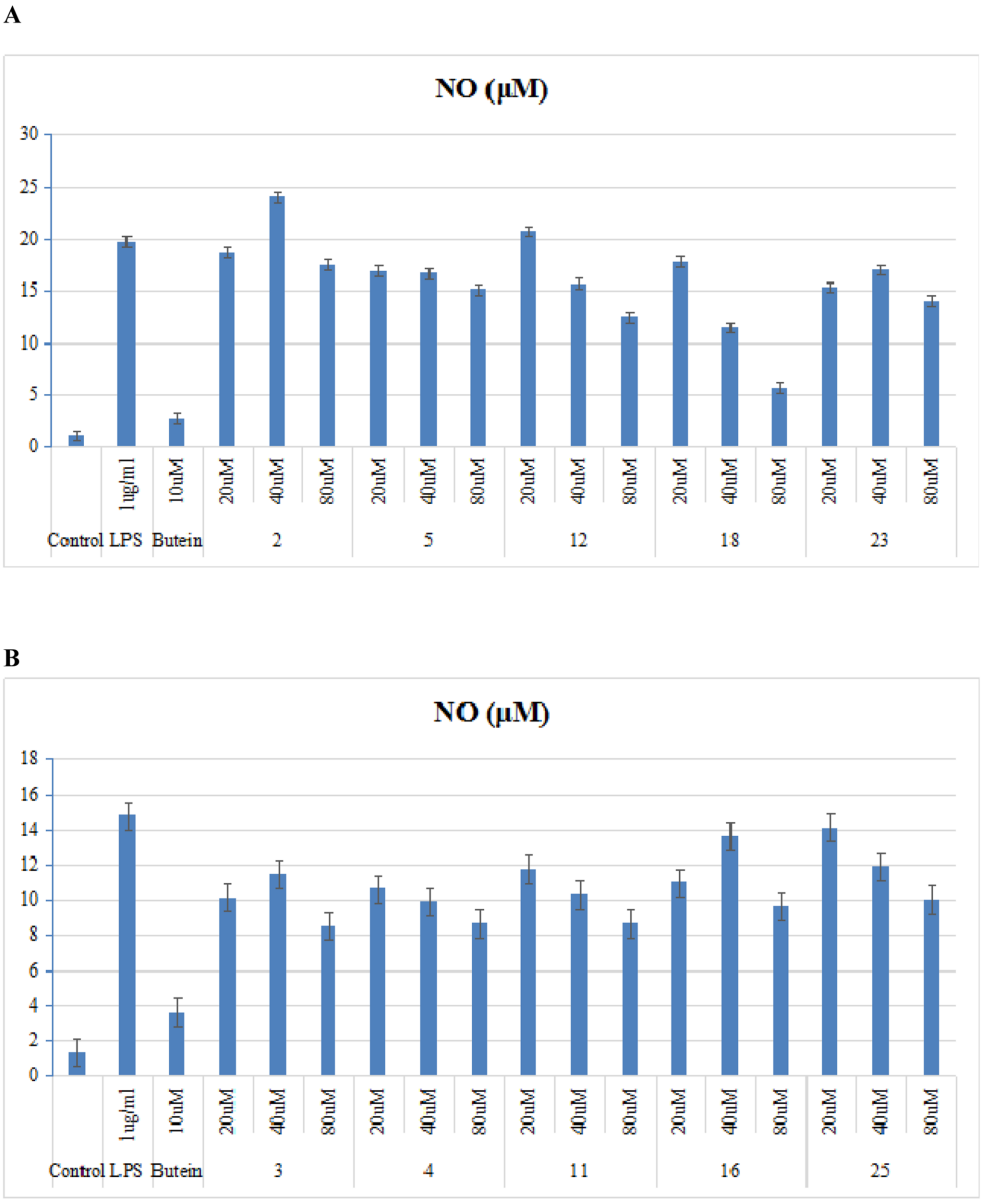

Fig. 2. Effects of selected compounds **2** - **5**, **11**, **12**, **16**, **18**, **23**, and **25** on productions of NO in LPS-stimulated BV2 microglias. Cells were pretreated for 30 min with the indicated concentrations of selected compounds, then stimulated for 24 h with LPS (1 μg/mL). The concentrations of nitrite were determined as described in the Experimental Section. Data represent the mean values of three experiments ± SD.

inhibitory effects on production of NO (inhibition < 40%). The results demonstrated that compounds **3**, **4**, and **11** may possess potential anti-neuroinflammatory activities. Further studies are required to evaluate the anti-neuroinflammatory mechanisms of these active compounds.

Acknowledgments

We are grateful for the financial supported by Traditional Chinese Medicine Scientific Research Projects of Hunan Province (2013136) and Science of Pharmaceutical Analysis of Twelfth Five-Year Key Discipline Program of Hunan University of Chinese Medicine (2012-2) and Science of Chinese Materia Medica of the Key Discipline in Hunan Province (20121).

References

(1) Cai, G. X.; Zhou, S.; Tan, G. B. Hunan Yaowuzhi: Hunan Science and Technology Press: Changsha, **2004**, pp 3360-3361.
(2) Jin, J. D. China's Naturopathy **2001**, *9*, 41.
(3) Li, X. J.; Yuan, Y.; Li, Z.; Liu, X. Q. *Chinese Traditional and Herbal Drugs* **2014**, *45*, 1052-1055.

(4) Yuan, Y.; Liu, X. Q.; Liu, Z. Z.; Zou, Q. P.; Liu, H. Y.; Dai, L. *Journal of Chinese Medicinal Materials* **2011**, *34*, 1894-1897.
(5) Yang, D. D.; Liu, X. Q.; Liu, Z. Z.; Zou, Q. P.; Liu, H. Y.; Dai, L. *Northwest Pharmaceutical Journal* **2012**, *27*, 189-192.
(6) Pan, X. X.; Guo, J. S.; Guo, Z. Y.; Li, Z.; Nie, J. *Lishizhen Medicine and Materia Medica Research* **2012**, *23*, 1920-1921.
(7) Qin, L. H.; Zhong, H. M.; Chen, X. Y.; Wu, Y. F.; Li, Z.; Huang, K. *Chinese Journal of Gerontology* **2014**, *34*, 429-431.
(8) Titheradge, M. A. *Methods Mol. Biol.* **1998**, *100*, 83-91.
(9) Chang, C. W.; Wu, T. S.; Hsieh, Y. S.; Kuo, S. C.; Chao, P. D. *J. Nat. Prod.* **1999**, *62*, 327-328.
(10) Zhang, Q. J.; Yang, X. S.; Zhu, H. Y.; Hao, X. J. *Journal of Guizhou University* (Natural Sciences) **2006**, *23*, 270-271.
(11) Matsunaga, S.; Tanaka, R.; Akagi, M. *Phytochemistry* **1988**, *27*, 535-537.
(12) Leong, Y. W.; Harrison, L. J. *Phytochemistry* **1999**, *50*, 849-857.
(13) Kundu, J. K.; Rouf, A. S.; Hossain, M. N.; Hasan, C. M.; Rashid, M. A. *Fitoterapia* **2000**, *71*, 577-579.
(14) Patra, A.; Chaudhuri, S. K. *Indian J. Chem.* **1989**, *28B*, 376-380.
(15) Sun, L. L.; Zhong, Y.; Xia, H. M.; Zhou, Q.; Jia, L. V. *Chin. Herb. Med.* **2013**, *5*, 1-4.
(16) Chen, I. S.; Lin, Y. C.; Tsai, I. L.; Teng, C. M.; Ko, F. N.; Ishikawa, T.; Ishii, H. *Phytochemistry* **1995**, *39*, 1091-1097.
(17) He, Z.; Zhao, M.; Zong, Y. Y.; Cai, S. X.; Che, Z. T. *Chinese Traditional and Herbal Drugs* **2010**, *41*, 36-39.
(18) Tian, M. Y.; Peng, L. J.; Feng, T. T.; Chen, L.; Zhou, Y. *Journal of Mountain Agriculture and Biology* **2014**, *33*, 089-091.
(19) Yue, J. R.; Feng, D. Q.; Xu, Y. K. *Nat. Prod. Res.* **2015**, *29*, 1228-1234.
(20) Xu, W. Q.; Gong, X. J.; Zhou, X.; Mei, X. P.; Chen, B. B. *Chinese Traditional and Herbal Drugs* **2010**, *41*, 1056-1060.
(21) Ya, Q. K.; Lu, W. J.; Chen, J. Y.; Tan, X. *Journal of Chinese Medicinal Materials* **2010**, *33*, 1254-1256.
(22) Chen, Y.; Wei, H. C.; Wei, Z.; Zheng, X. R.; Zhang, L.; Zhou, Z. *Lishizhen Medicine and Materia Medica Research* **2012**, *23*, 1725-1726.
(23) Erdelmeier, C. A.; Wright, A. D.; Orjala, J.; Baumgartner, B.; Rali, T.; Sticher, O. *Planta Med.* **1991**, *57*, 149-152.
(24) He, Y. Z.; Eric, K. O.; Sivoko, I. P.; Wang, L. N.; Naji, S. A. *Chin. Herb. Med.* **2014**, *6*, 76-79.
(25) Petrul'ová-Poracká. V.; Repčák, M.; Vilková, M.; Imrich, J. *Food Chem.* **2013**, *141*, 54-59.

Received October 26, 2015
Revised February 12, 2016
Accepted February 22, 2016