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NOTE

Two new compounds from the roots of *Ilex pubescens* and their cytotoxic activity

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Abstract A new phenylethanoid, 1-*O*-*β*-D-(4-hydroxyphenyl)-ethyl-6-*O*-vanilloyl-glucopyranoside (**1**), and a new furofuran lignan, (7*R*,7′*R*,7″*S*,8*S*,8′*S*,8″*R*)-4′, 4″-dihydroxy-3,3′,3″,5-tetramethoxy-7,9′:7′9-diepoxy-4,8″-oxy-8,8′-sesquineolignan-7″,9″-diol (**2**), along with five known compounds (**3**–**7**) were isolated from the roots of *Ilex pubescens*. Their chemical structures were elucidated on the basis of extensive spectroscopic analysis, including UV, IR, MS, and NMR experiments. In addition, compounds **2**–**7** were evaluated in vitro for their cytotoxic effects on human HeLa cells; among them, compounds **2**, **3**, **6**, and **7** showed cytotoxic activity against HeLa cells in the test.

Keywords *Ilex pubescens* · Lignans · Phenylethanoid · Cytotoxic activity

Introduction

Ilex pubescens is an evergreen shrub, mainly distributed in the southern regions of China. In traditional Chinese medicine, Mao-Dong-Qing, the dried roots of *I. pubescens*, is used primarily for the treatment of cardiovascular disease and hypercholesterolemia [1]. Pharmacological studies on extracts prepared from the roots of *I. pubescens* demonstrated broad biological properties, such as enlarging blood vessels, decreasing plasma viscosity, inhibiting platelet aggregation, preventing thrombosis, decreasing the excitation of the cardiac conduction system and enhancing anoxia resistance [2–4]. Previous studies on chemical constituents of *I. pubescens* roots and leaves led to the isolation of a series of diverse compounds, including triterpene saponins [5, 6], lignans [7], lignin glycosides [8], phenylethyl alcohol derivatives [9], secoiridoid glucosides [10] and flavonoids [11]. To seek the bioactive constituents, we carried out a phytochemical investigation on the roots of *I. pubescens*, which resulted in two new compounds (**1** and **2**), along with five known compounds (**3**–**7**), which were isolated from this plant for the first time. In this paper, we describe the isolation and structural elucidation of these compounds and their in vitro cytotoxic activity against HeLa cells.

Results and discussion

The EtOH extract of the roots of *I. pubescens* was subjected to silica gel CC, Sephadex LH-20, and reversed-phase HPLC to afford a new phenylethanoid (**1**), and a new furofuran lignan (**2**), together with five known compounds. Compounds **3**–**7** were identified as (7*R*,7′*R*,7″*R*,8*S*,8′*S*,8″*S*)-4′,4″-dihydroxy-3,3′,3″,5-tetramethoxy-7,9′:7′,9-di-epoxy-4,8″-ox-y-8,8′-sesquineolignan-7″,9″-diol (**3**) [12], *erythro*-(7*S*,8*R*)-guaiacylglycerol-*β*-*O*-4′-coniferyl alocohol (**4**) [13, 14], *erythro*-(7*R*,8*S*)-guaiacylglycerol-*β*-4′-coniferyl alocohol (**5**) [13, 14], *erythro*-(7*R*,8*S*)-guaiacylglycerol-*β*-coniferyl aldehyde ether (**6**) [14, 15], and *erythro*-(7*S*,8*R*)-guaiacylglycerol-*β*-coniferyl aldehyde ether (**7**) [14, 15], by comparison of their spectral data with those described in the literature (Fig. 1). Reported herein are the extraction,

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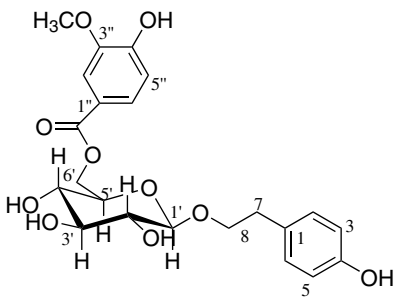
isolation and structure elucidation of the compounds isolated from the roots of *I. pubescens*.

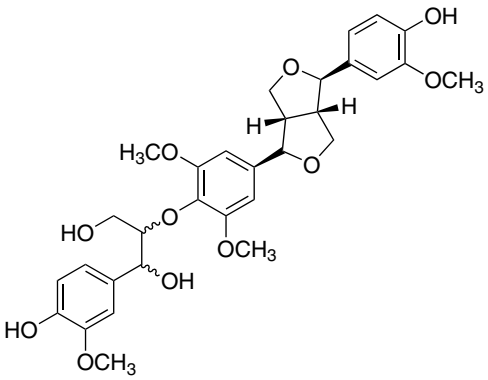
Compound **1** was obtained as a white amorphous powder. Its molecular formula was determined to be $C_{22}H_{26}O_{10}$ according to the positive-ion HRESIMS data at *m/z* 473.1432 $[M + Na]^+$. The IR spectrum of **1** showed characteristic absorptions for hydroxy (3430 cm^{-1}) and aromatic moieties (1639, 1462 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectrum (Table 1) of compound **1** strongly suggested that the compound exhibited typical signals of three fragments, including one *p*-hydroxyphenethyl group [A] [δ_H 2.70 (2H, m), 3.79 (1H, m), 3.60 (1H, m), 6.95 (2H, d, $J = 8.4$ Hz), 6.62 (2H, d, $J = 8.4$ Hz)], one vanilloyl group [B] [δ_H 7.41 (1H, d, $J = 1.4$ Hz), 7.46 (1H, dd, $J = 8.3$, 1.9 Hz), 6.83 (1H, d, $J = 8.3$ Hz), 3.70 (3H, s), δ_C 165.5 (s)], as well as a sugar moiety. In the HMBC spectrum, a long-range correlation from H-7 to C-1/C-2/C-6, from H-8 to C-7/C-1 confirmed the presence of a *p*-hydroxyphenethyl group. Moreover, the HMBC correlation (Fig. 2) from OCH_3-3″ to C-3″, from H-2″ to C-3″/C-4″/C-7″, from H-5″ to C-1″/C-3″/C-4″, from H-6″ to C-2″/C-4″ revealed the presence of vanillyol. The above NMR spectroscopic data were very similar to those of 1-*O*-β-D-(3, 4-dihydroxyphenyl)-ethyl-6-*O*-vanilloyl-glucopyranoside found in the literature [16], except for the reduction of 3-hydroxyphenyl. The location of the other functional groups and NMR data assignments of **1** were determined by HMBC and HSQC spectroscopic analysis. The sugar was identified as D-glucose by optical rotation using chiral detection in HPLC analysis after acid hydrolysis of **1**. The *β*-anomeric configuration of the glucose was determined by the *J* value in the ^{1}H-NMR spectrum ($J_{1',2'} = 7.8$ Hz). The location of the sugar moiety was assigned at C-8 and C-7″ by the observed HMBC correlations from H-1′ to C-8, and H-6′ to C-7″. On the basis of all the evidence obtained, the structure of **1** was defined as 1-*O*-*β*-D-(4-hydroxyphenyl)-ethyl-6-*O*-vanilloyl-glucopyranoside.

Compound **2** was obtained as a yellow oil, and exhibited a quasi-molecular ion peak at *m/z* 583.2189 in negative HREIMS analysis, indicating a molecular formular of $C_{31}H_{36}O_{11}$. The IR spectrum showed hydroxyl (3393 cm^{-1}) and benzene ring (1594, 1517 cm^{-1}) absorptions bands. The ^{1}H-NMR spectrum (Table 1) of **2** showed signals of four aromatic methoxy groups at δ_H 3.76 (3H, s), 3.75 (6H, s), 3.73 (3H, s), along with eight olefinic protons signals at δ_H 6.89 (1H, d, $J = 1.4$ Hz), 6.76 (1H, dd, $J = 8.2$, 1.6 Hz), 6.72 (1H, d, $J = 8.2$ Hz), 6.91 (1H, d, $J = 1.4$ Hz), 6.71 (1H, d, $J = 8.2$ Hz), 6.69 (1H, d,

Fig. 1 Structures of compounds **1**–**7**

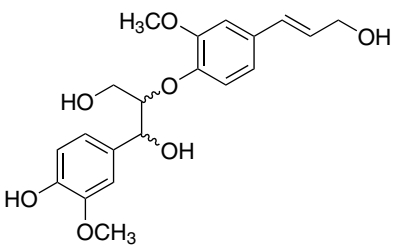


1



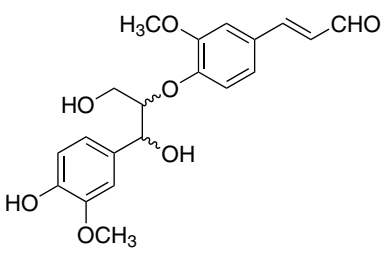
2: 7*R*,7′*R*,7″*S*,8*S*,8′*S*,8″*R*

3: 7*R*,7′*R*,7″*R*,8*S*,8′*S*,8″*S*



4: erythro-7′*S*,8′*R*

5: erythro-7′*R*,8′*S*



6: erythro-7′*S*,8′*R*

7: erythro-7′*R*,8′*S*

Table 1 ^{1}H-NMR and ^{13}C-NMR spectral data of **1** and **2** (δ in ppm)

Position	**1**[a]		Position	**2**[b]	
	δ (H)	δ (C)		δ (H)	δ (C)
1		128.9	1		136.9
2	6.95 (d, 8.4)	129.6	2	6.64 (s)	103.3
3	6.62 (d, 8.4)	115.0	3		152.6
4		155.6	4		134.8
5	6.62 (d, 8.4)	115.0	5		152.6
6	6.95 (d, 8.4)	129.6	6	6.64 (s)	103.3
7	2.70 (m)	34.9	7	4.65 (d, 4.2)	85.1
8	3.60 (m)	70.2	8	3.05 (m)	53.7
	3.79 (m)				
			9	4.16 (m)	71.2
				3.79 (m)	
1′	4.26 (d, 7.8)	103.0	1′		132.2
2′	3.00 (m)	73.4	2′	6.89 (d, 1.4)	110.4
3′	3.15–3.21 (m)	76.5	3′		147.5
4′	3.15–3.21 (m)	70.4	4′		145.9
5′	3.48 (m)	73.7	5′	6.72 (d, 8.2)	115.1
6′	4.20 (dd, 11.6, 1.8)	64.0	6′	6.76 (dd, 8.2, 1.6)	118.6
	4.56 (dd, 11.7, 7.1)				
			7′	4.61 (d, 4.2)	85.1
			8′	3.05 (m)	53.5
			9′	4.16 (m)	71.0
				3.79 (m)	
1″		120.3	1″		133.3
2″	7.41 (d, 1.4)	112.5	2″	6.91 (d, 1.4)	110.9
3″		147.4	3″		147.0
4″		152.0	4″		145.3
5″	6.83 (d, 8.3)	115.2	5″	6.69 (d, 8.0)	114.6
6″	7.46 (dd, 8.3, 1.9)	123.5	6″	6.71 (d, 8.2)	119.4
7″		165.5	7″	4.79 (t, 4.4)	72.1
			8″	4.10 (m)	86.2
			9″	3.40 (m)	59.8
				3.70 (m)	
OCH_3-3′	3.70 (3H, s)	55.4	OCH_3-3	3.75 (3H, s)	56.0
			OCH_3-5	3.75 (3H, s)	56.0
			OCH_3-3′	3.76 (3H, s)	55.5
			OCH_3-3″	3.74 (3H, s)	55.6

[a] ^{1}H NMR at 600 MHz, ^{13}C NMR at 100 MHz, obtained in DMSO-d_6

[b] ^{1}H NMR at 600 MHz, ^{13}C NMR at 150 MHz, obtained in DMSO-d_6

$J = 8.0$ Hz), and 6.64 (2H, s), revealing the presence of two 1,2,4-trisubstituted benzene rings and a 1,2,3,5-tetra-substituted benzene ring. The ^{1}H- and ^{1}C-NMR (Table 1) spectrum showed signals at δ_H 4.65 (1H, d, $J = 4.2$ Hz), 4.61 (1H, d, $J = 4.2$ Hz), 3.05 (2H, m), 4.16 (2H, m), 3.79 (2H, m) and δ_C 85.1 (C-7,7′), 71.2 (C-9), 71.0 (C-9′), 53.7 (C-8), 53.5 (C-8′), suggesting the presence of a furofuran lignan skeleton with 7,7′-diequatorial diayl substitution with different aryl groups. This deduction was further supported by the HMBC correlations from H-7 to C-1/C-2/C-8′/C-9′/, from H-7′ to C-8/C-9/C-2′/C-1′/C-6′, from H-9 to C-8, H-9′ to C-7/C-8′. It also showed signals at δ_H 4.79 (1H, t, $J = 4.4$ Hz), 4.10 (1H, m), 3.70 (1H, m), 3.40 (1H, m) and δc 86.2 (C-8″), 72.1 (C-7″), and 59.8 (C-9″), which are typical signals of the glyceryl moiety of 8-*O*-4′ system neolignan. Furthermore, the HMBC experiment (Fig. 2) showed long-range correlations from H-7″ to C-9″/C-8″/C-2″/C-6″/C-1″; correlations from H-8″ to C-4/C-1″

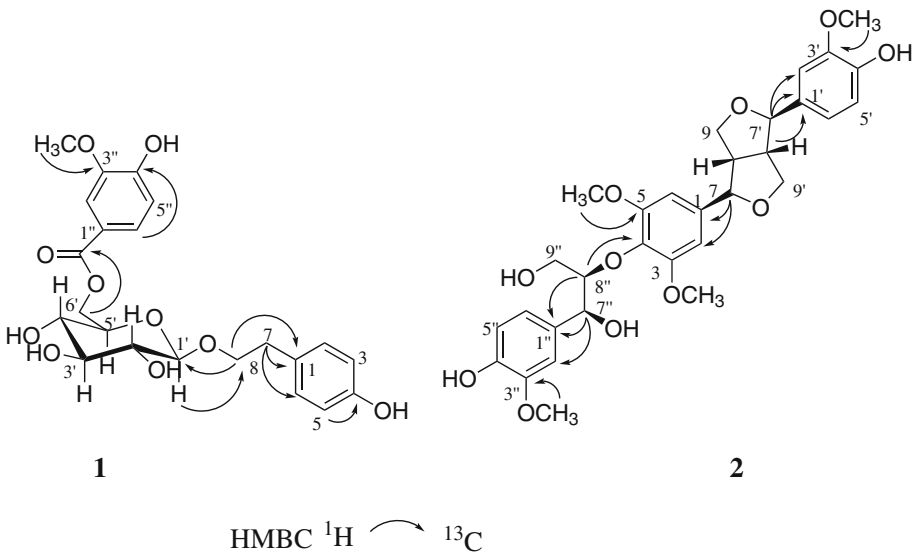


Fig. 2 Selected HMBC correlations of compounds **1** and **2**

confirmed the deduction, revealing that the aryl glycerol portion with C-8″ was linked to C-4 by an ether linkage. Comparison of NMR data of **2** with the known hedyotol C [17] demonstrated that **2** possessed the same planar structure but different absolute configuration between H-7″ and H-8″. The configuration of **2** was supported by CD spectrum, and the ^{1}H-NMR coupling constant $J_{7,8}$, $J_{7',8'}$, $J_{7'',8''}$. The coupling constant of $J_{7,8}$ and $J_{7',8'}$ (4.2 Hz) and the chemical shift for the bridge carbons (C-8/C-8′, 53.7/53.5) indicated that the aryl groups were pseudoequatorial and cis-oriented with H-8 and H-8′ [12, 18]. A negative cotton effect at 278 nm in the CD spectrum revealed that the absolute configuration of the furofuran unit was 7*R*,7′*R*,8*S*,8′*S* [12, 18]. The large and small *J* values for H-7 and H-8 of 8-*O*-4′ neolignan diastereoisomers correspond to the threo form and erythro form, respectively. The small coupling constant ($J = 4.4$ Hz) observed between H-7″ and H-8″ indicated that the aryl glycerol-8″-yloxy moiety was determined to be in the erythro configuration [14]. In addition, the CD spectrum of compound **2** showed negative Cotton effects at 239.5 nm, indicating that the absolute configurations at C-7″ and C-8″ of compound **2** were the 7″*S* and 8″*R* forms [14, 18]. Consequently, the structure of **2** was defined as (7*R*,7′*R*,7″*S*,8*S*,8′*S*,8″*R*)-4′,4″-dihydroxy-3,3′,3″,5-tetramethoxy-7,9′:7′9-diepoxy-4,8″-ox-y-8,8′-sesquineolignan-7″,9″-diol.

Compounds **2**–**7** were tested for their cytotoxic activities against HeLa cells by the MTT assay (Table 2). Compounds **6** and **7** showed mild inhibitory effects, whilst the activities of **2** and **3** were very weak. Compounds **4** and **5** exhibited no significant inhibitory activity, with IC_{50} values higher than 100 μM concentration. The IC_{50} values of **1** were not acquired due to the paucity of the available sample.

Table 2 Inhibitory effects of compounds **2**–**7** isolated from *Ilex pubescens* on HeLa cell ($n = 3$)

Compound	Cytotoxic activity [IC_{50} (μM)]
2	50.60 ± 6.47
3	51.17 ± 8.12
4	>100
5	>100
6	23.88 ± 4.52
7	29.14 ± 3.01
Fluorouracil[a]	19.55 ± 3.47

[a] Positive control

Experimental

General experimental procedures

UV spectra were recorded on an Agilent UV 1100 spectrophotometer. IR spectra were recorded on a Bruker IFS 55 spectrometer. CD spectra were determined with a Bio-Logic Science MOS-450 spectrometer. NMR experiments were performed on Bruker ARX-400 and AV-600 spectrometers. The chemical shifts are stated relative to TMS and expressed in δ values (ppm), with coupling constants reported in Hertz. HRESIMS were obtained on an Agilent 6200 TOF mass spectrometer. Silica gel GF254 prepared for TLC and silica gel (200–300 mesh) for column chromatography (CC) were obtained from Qingdao Marine Chemical Factory (Qingdao, People's Republic of China). Sephadex LH-20 was a product of Pharmacia. RP-HPLC separations were conducted using an LC-10AT VP liquid chromatograph with a Phenomenex Pack ODS-A column (250 × 10 mm, 5 μm, 120 Å) and SPD-10A VP UV/VIS

detector. All reagents were HPLC or analytical grade, and were purchased from Tianjin Damao Chemical Company. Spots were detected on TLC plates under UV light, or by heating after spraying with anisaldehyde-H_2SO_4 reagent.

Plant material

The roots of *I. pubescens* were collected from Bozhou, Anhui province, China, and identified by Professor Jincai Lu, Department of Pharmaceutical Botany, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University. A voucher specimen (AP-20120601) has been deposited in the herbarium of the Department of Natural Products Chemistry, Shenyang Pharmaceutical University.

Extraction and isolation

The dried roots of *I. pubescens* (10 kg) were extracted with 70 % EtOH (90 L × 2 h × 3). The obtained extract was then concentrated under reduced pressure in a rotary evaporator to remove the ethanol, yielding the crude sample extract. Then, the resulting extract was suspended in H_2O (15 L), and partitioned successively with petroleum ether, EtOAc, and *n*-BuOH (15 L × 3). The EtOAc extract (102 g) was subjected to silica gel CC (10 × 80 cm) and eluted with CH_2Cl_2-CH_3OH (100:0, 100:1, 50:1, 20:1, 10:1, 5:1, 1:1 and 0:1 v/v) to obtain six fractions (IE1–IE6). Fraction IE3 (20 g) was subjected to a silica gel column (6 × 80 cm) and eluted with PE-EtOAc (from 30:1 to 0:1) to produce six fractions (IE31–IE36). Fraction IE34 (4.8 g) was further subjected to silica gel CC (3 × 25 cm) and eluted successively with gradient of petroleum ether-actone (10:1–1:1) to afford fractions IE341–IE346. Fraction IE343 was chromatographed over Sephadex LH-20 (CH_2Cl_2/MeOH, 1:1; 1.5 × 30 cm) and then separated by RP-HPLC (50 % MeOH/H_2O, 2 mL/min) to afford compounds **2** (18 mg, $t_R = 39$ min) and **3** (27.6 mg, $t_R = 36$ min). Fraction IE345 was chromatographed over Sephadex LH-20 (CH_2Cl_2/MeOH, 1:1; 1.5 × 30 cm) and then separated by RP-HPLC (45 % MeOH/H_2O, 2 mL/min) to afford compounds **4** (14 mg, $t_R = 49$ min) and **5** (20 mg, $t_R = 53$ min). Fraction IE346 was purified by RP-HPLC (48 % MeOH/H_2O, 2 mL/min) to afford compounds **6** (47 mg, $t_R = 42$ min) and **7** (15 mg, $t_R = 46$ min). IE5 (38 g) was subjected to RP-C_{18} silica gel CC (10 × 80 cm) and eluted with MeOH/H_2O (1:9–8:2) to produce six fractions, and IE54 (100 mg) was purified with Sephadex LH-20 column and RP-HPLC (50 % MeOH/H_2O, 2 mL/min) to give compound **1** (6 mg, $t_R = 19$ min).

Compound **1**: colorless amorphous powder; UV (MeOH) max (log ε) 263.9 (2.94), 220.5 (3.35); IR (KBr) cm^{-1}: 3430, 2920, 2850, 1639, 1462, 1384, 1130; ^{1}H-NMR (DMSO-d_6, 600 MHz) and ^{13}C-NMR (DMSO-d_6, 100 MHz) spectrum data see Table 1; HRESIMS: *m/z* 473.1432 $[M + Na]^+$; calcd for $[M + Na]^+$, 473.1424.

Compound **2**: pale yellow oil; UV (MeOH) max (log ε) 257 (2.74), 230 (2.66); IR (KBr) cm^{-1}: 3393, 1594, 1517, 1463, 1126, 1029; ^{1}H-NMR (DMSO-d_6, 600 MHz) and ^{13}C-NMR (DMSO-d_6, 150 MHz) spectrum data see Table 1; HRESIMS: *m/z* 583.2189 $[M - H]^-$; calcd for $[M - H]^-$, 583.2179.

Acid hydrolysis of compound 1

Compound **1** (3 mg) was dissolved with 2 M HCl (1 mL) and heated for 3 h at 95 °C. After cooling, the reaction mixture was extracted with $CHCl_3$ three times, and the aqueous layer was evaporated repeatedly to dryness with EtOH until neutral. Then, the residue of sugar was dissolved in water (1 mL) and analyzed by HPLC with chiral detection under the following conditions. HPLC was conducted using a Shodex Asahipak NH2P-50 4E column and a JAsco OR-4090 detector, the mobile phase consisted of acetonitrile and water (3:1), the flow rate was kept constant at 0.8 mL/min. The peak shape (positive peak) and retention time (14.9 min) was consistent with D-glucose.

Inhibitory activities on HeLa bioassay

Cytotoxicity for compounds **2–7** was performed in HeLa cells by the MTT colorimetric assay. Briefly, HeLa cells were seeded into 96-well tissue culture plates at a density of 3×10^4 cells/well. The cells were allowed to grow for 24 h and subsequently treated with the test compounds at different concentrations for 48 h. Fluorouracil was used as the positive control while vehicle control DMSO was used as the negative control. After incubation, 10 μL/well MTT was added and further incubated for 4 h. Thereafter, growth medium was removed and the blue formazan product was solubilized in DMSO. Absorbance was measured on a microplate reader at 570 nm. Data are presented as mean ± SD. Each experiment was performed at least three times.

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References

1. Jiangsu New Medical College (1977) Directory of Chinese Materia Medica Shang-hai Scientific and Technological Press. p 441
2. Fujimoto T, Fujimura K, Kuramoto A (1991) Electrophysiological evidence that *g*-lycoprotein IIb-IIIa complex is involved in calcium channel activation on human p-latelet plasma membrane. J Biol Chem 266:16370–16375

3. Lin L-P, Qu W, Liang J-Y (2011) Chemical constituents from the stems of *Ilex pub-escens* var. glabra. Chin J Nat Med 9:176–179
4. Yang ML, Pang PK (1986) The vascular effects of *Ilex pubescens*. Planta Med 4:262–265
5. Li L, He Y-X, Gou M-L, Dai C (2012) Three new triterpenoid saponins from *Ilex pubescens*. J Asian Nat Prod Res 14:1169–1174
6. Zhou Y, Zeng K-W, Zhang J-Y, Li N, Chai X-Y, Jiang Y, Tu P-F (2014) Triterpene saponins from the roots of *Ilex pubescens*. Fitoterapia 97:98–104
7. Yang X, Ding Y, Sun Z-H, Zhang D-M (2006) Studies on chemical constituents from *Ilex pubescens*. J Asian Nat Prod Res 8:505–510
8. Yang X, Ding Y, Zhang D-M (2007) Studies on lignin glycosides in root from *Ilex pubescens*. Chin J Chin Mater Med 32:1303–1306
9. Zhou Y-B, Wang J-H, Li X-M, Fu C-C, Yan Z, Cong Y, Li X (2008) Two new 2-phenylethyl alcohol derivatives and one new lignan derivative from the root of *Ilex pubescens*. Helv Chim Acta 91:1244–1250
10. Yang X, Ding Y, Zhang D-M (2007) Isolation and identification of iridoid glyco-sides from *Ilex pubescens*. Chin J Med Chem 17:173–178
11. Xing X-D, Zhang Q, Feng F, Liu W-Y (2012) Chemical constituents from stems of *Ilex pubescens*. J Chin Med Mater 35:1429–1432
12. Xiong L, Zhu C-Z, Li Y-R, Tian Y, Lin S, Yuan S-P, Hu J-F, Hou Q, Chen N-H, Yang Y-C, Shi J-G (2011) Lignans and neolignans from *Sinocalamus affinis* and their absolute configurations. J Nat Prod 74:1188–1200
13. Li D-M, Huang X-X, Song S-J (2014) Isolation and identification of constituents from the Seeds of *Crataegus Pinnatifida* Bge.II. Acta Chin Med Pharm 42:52–54
14. Huang X-X, Zhou C-C, Li L-Z, Li F-F, Lou L-L, Li D-M, Ikejima T, Peng Y, Song S-J (2013) The cytotoxicity of 8-O-4′ neolignans from the seeds of *Crataegus pinnnatifida*. Bioorg Med Chem Lett 23:5599–5604
15. Lee D-Y, Song M-C, Yoo K-H, Bang M-H, Chung I-S, Kim S-H, Kim D-K, Kwon B-K, Jeong T-S, Park M-H, Baek N-I (2007) Lignans from the fruits of *Cornus kousa* Burg. and their cytotoxic effects on human cancer cell lines. Arch Pharm Res 30:402–407
16. Zheng X-K, Liu Y-B, Li J, Feng W-S (2004) One new phenylethanoid glycoside from *Corallodiscus flabellata*. Acta Pharm Sin 39:716–718
17. Liao J-H, Hu X-J, Yuan C-M, He H-P, Di Y-T (2014) Chemical constituents from the fruits of Capparis masaikai. Nat Prod Res Dev 26:1780–1784
18. Kim K-H, Moon E, Kim S-Y, Lee K-R (2010) Lignans from the tuber-barks of *Colocasia antiquorum* var. esculenta and their antimelanogenic activity. Agric Food Chem 58:4779–4785