Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Flavones and Lignans from the Stems of *Wikstroemia scytophylla* Diels

Jie-Ping Lei, Juan-Juan Yuan, Sheng-Hao Pi, Rui Wang, Rui Tan, Chao-Ying Ma, Tao Zhang[1], He-Zhong Jiang

School of Life Science and Engineering, Southwest Jiaotong University, Chengdu 610031, [1]Department of Oncology, Chengdu Military General Hospital, Chengdu 610083, P.R. China

Submitted: 05-06-2016 Revised: 11-07-2016 Published: 19-07-2017

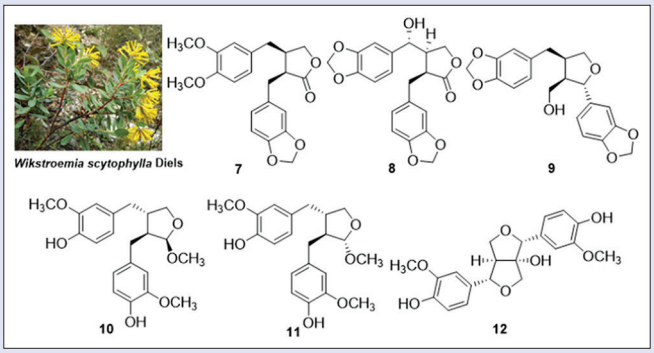
ABSTRACT

Background: The genus *Wikstroemia* has about 70 species, but only a limited number of species have been studied chemically. *Wikstroemia indica* has long been used as a traditional crude drug in China. However, there is no report about the bioactivity of *Wikstroemia scytophylla*. **Objective:** This paper reports the chemical investigation and biological evaluation of the *W. scytophylla*. **Materials and Methods:** The EtOAc extraction of *W. scytophylla* was isolated using chromatographic methods, and the compounds were analyzed by spectroscopic methods. The *in vitro* antitumor activities against five human cancer cell lines were performed according to the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method. **Results:** The chemical investigation of the stems of *W. scytophylla* resulted in the isolation of 12 compounds mainly including one biflavone (1), five flavones (2-6) compounds, and six lignans (7-12), in which compound 8 was a new natural product. Compounds 1 and 7-12 were evaluated for their antitumor activities while these compounds showed weak cytotoxicity with the half maximal inhibitory (IC_{50}) values more than 40 μM. **Conclusion:** All of these compounds were isolated from this plant for the first time, and compounds 2-12 were first reported from genus *Wikstroemia*, in which compound 8 was a new natural product. Compounds 1 and 7-12 exhibited weak antitumor activities (IC_{50} >40 μM). The chemotaxonomic significance of all the isolations was summarized.

Key words: Flavones, lignans, phenolic compounds, *Wikstroemia scytophylla*

SUMMARY

- The chemical investigation of the stems of *W. scytophylla* resulted in the isolation of 12 compounds
- The 12 compounds including six lignans (7-12), in which compound 8 was a new natural product
- The isolated compounds 1 and 7-12 were evaluated for their antitumor activities
- The chemotaxonomic significance of all the isolations was summarized.



Abbreviations used: MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; IC50: Half maximal inhibitory; HL-60: Human leukemia cell line; SMMC-7721: Human hepatocellular carcinoma cell line; A549: Human lung tumor cell line; MCF-7: Human breast cancer cell line; SW480: Human colon cancer cell line; MS: Mass spectrometry; NMR: Nuclear Magnetic Resonance.

Correspondence:
Dr. He-Zhong Jiang,
School of Life Science and Engineering,
Southwest Jiaotong University,
Chengdu 610031, P.R. China.
E-mail: jianghz10@sina.com

DOI: 10.4103/pm.pm_275_16

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

The genus *Wikstroemia* (Thymelaeaceae) comprising approximately 70 species is widely distributed in northern Asia through the Himalayas, Malaysia, Oceania, Polynesia to the Hawaiian Islands.[1] In China, about 44 species have been found mainly in the south of the Yangtze River. *Wikstroemia scytophylla* Diels is regionally distributed in the provinces of Yunnan, Sichuan, and Tibet in China. There is no report about the bioactivity of *W. scytophylla*. *Wikstroemia indica*, the same genus with *W. scytophylla*, has long been used as a traditional crude drug for the treatment of pneumonia, rheumatism, and bronchitis in China.[2]

Previous phytochemical studies on *W. scytophylla*, which has been investigated only by our group, have resulted in the isolation and identification of flavones (including biflavones), coumarins, sesquiterpene, and diterpene esters.[3,4]

MATERIALS AND METHODS

Plant material

The stems of *W. scytophylla* were collected in May, 2015, in Diqing Tibetan Autonomous Prefecture, Yunnan Province, China, and identified by Professor Liang-Ke Song (Southwest Jiaotong University). Voucher specimen (JHZ-201505) was deposited at the Natural Products Chemistry Laboratory, Southwest Jiaotong University, China.

Extraction and isolation

The dried and powdered stems of *W. scytophylla* (5.0 kg) were extracted with 95% EtOH under reflux for three times (3 × 15 L). The extract was concentrated and suspended in water followed by successive partition with petroleum ether (3 × 5 L), EtOAc (3 × 5 L), and *n*-BuOH (3 × 5 L).

Cite this article as: Lei JP, Yuan JJ, Pi SH, Wang R, Tan R, Ma CY, *et al*. Flavones and lignans from the stems of *Wikstroemia scytophylla* diels. Phcog Mag 2017;13:488-91.

The EtOAc extract (380 g) was chromatographed over silica gel (3.8 kg, 200–300 mesh) using a gradient solvent petroleum ether/acetone (100:1-1:1) to afford nine fractions. Fraction 3 (42 g) was subjected to silica gel column using a gradient solvent petroleum ether/EtOAc (20:1-0:1) to afford fractions 3-1 - 3-8. Fraction 3-3 (2.9 g) was separated on Sephadex LH-20 ($CHCl_3$/MeOH 1:1) to give compounds 3 (19 mg) and 7 (15 mg). Fraction 3-4 (14.1 g) gel filtrated on Sephadex LH-20 ($CHCl_3$/MeOH 1:1) to give six subfractions (3-4-1 – 3-4-6). Fraction 3-4-3 (4.4 g) was further purified on repeated RP-18 (MeOH/H_2O, 40%–100%) and Sephadex LH-20 ($CHCl_3$/MeOH 1:1) to give compounds 6 (4 mg), 8 (28 mg), and 9 (143 mg). Fraction 3-5 (22.5 g) gel filtrated on Sephadex LH-20 ($CHCl_3$/MeOH 1:1) to give five subfractions (3-5-1 – 3-5-5). Fraction 3-5-3 (13.7 g) was further purified on repeated RP-18 (MeOH/H_2O, 40%-100%) and Sephadex LH-20 ($CHCl_3$/MeOH 1:1) to give compounds 2 (5 mg) and 4 (12 mg). Fraction 4 (118 g) was chromatographed on silica gel column using a gradient solvent petroleum ether/EtOAc (15:1-0:1) to give nine fractions (4-1 - 4-9). Fraction 4-4 (27.8 g) was further separated on repeated RP-18 (MeOH/H_2O, 40%-100%) and Sephadex LH-20 ($CHCl_3$/MeOH 1:1) to afford compounds 5 (11 mg), 10 (5 mg), and 11 (5 mg). Fraction 4-5 (16.5 g) was separated on silica gel column using a gradient solvent $CHCl_3$/MeOH (30:1-1:1) to give seven fractions (4-5-1 - 4-5-7). Fraction 4-5-1 (302 mg) was further purified on Sephadex LH-20 ($CHCl_3$/MeOH 1:1) to give 12 (2 mg). Further purification of 4-5-6 (1.58 g) was submitted to Sephadex LH-20 ($CHCl_3$/MeOH 1:1) to yield 1 (6 mg).

Cytotoxicity bioassay

The isolated compounds 1 and 7-12 were evaluated for their cytotoxicities against five human cell lines HL-60, SMMC-7721, A549, MCF-7, and SW480, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method[5] with cisplatin (Sigma) and culture solution as positive and negative controls, respectively. Half maximal inhibitory (IC_{50}) values were calculated by Reed and Muench's method.

RESULTS

The structures of compounds 1-12 [Figure 1] were identified as wikstrol B (1),[6,7] apigenin 7-*O*-methyl ether (2),[8] apigenin 5, 7-dimethyl ether (3),[9] luteolin 7, 4'-dimethyl ether (4),[10] 5, 7-di-*O*-methylquercetin(5),[11] 3'-hydroxy-4', 5, 7-trimethoxy-flavone (6),[12] 3-(1, 3-benzodioxol-5-ylmethyl)-4-[(3, 4-dimethoxyphenyl)methyl]dihydro-, (3*S*-cis)-2(3H)-furanone (7),[13,14] 4-[(*R*)-1, 3-benzodioxol-5-ylhydroxymethyl]-3-(1, 3-benzodioxol-5-ylmethyl)dihydro-, (3*S*, 4*R*)-2(3H)-furanone (8),[14,15] (-)-dihydrosesamin (9),[16] phenol, 4, 4'-[[(2*R*, 3*S*, 4*S*)-tetrahydro-2-methoxy-3, 4-furandiyl]bis(methylene)]bis[2-methoxy- (10),[17] 4, 4'-dihydroxy-3, 3', 9-trimethoxy-9, 9'-epoxylignan (11),[17] (+)-1-hydroxypinoresinol (12),[18,19] respectively, by analysis of mass spectrometry and nuclear magnetic resonance (NMR) data, and comparison with those in the literature.

As a continuation of our previous phytochemical investigation[3,4] of *W. scytophylla*, the present study reports a new natural product (8) along with 11 known compounds, including one biflavone (1), five flavones (2-6), and five lignans (7, 9-12). All these compounds were isolated from this plant for the first time, and compounds 2-12 were first reported from the genus *Wikstroemia*. According to the cytotoxicity bioassay results, compounds 1 and 7-12 exhibited weak antitumor activities (IC_{50} >40 μM).

DISCUSSION

The chemical constituents of the family Thymelaeaceae especially the genus *Daphne* and *Aquilaria* are characterized by terpenoids (sesquiterpenoids, daphnane-type diterpene ester), coumarins, flavonoids (flavones, isoflavones, biflavones), and lignans.[20-24] The genus *Wikstroemia* has about 70 species, but only a limited number of species (*W. indica*, *W. chamaedaphne*, etc.) have been studied chemically, and *Wikstroemia* is also characterized by the occurrence of terpenoids, coumarins, flavonoids, and lignans, and the types of these reported compounds are very similar with those in the genus *Daphne*.[3,4,25-27] Compounds 1-12 isolated in the current research were classified as biflavone (1), flavones (2-6), and lignans (7-12). The structural types of these isolates confirm the relationship between *W. scytophylla* and other species of *Wikstroemia*. The phytochemical study suggests that there is a close chemotaxonomic relationship between the genus *Wikstroemia*, *Aquilaria*, and *Daphne* in the family Thymelaeaceae. As flavonoids were widely present in *W. scytophylla* metabolites and isolated as the main type of components in the current study, while terpenoids, coumarins, and lignans were the main bioactive compositions in Thymelaeaceae, lignans could be used as chemotaxonomic markers for the species *W. scytophylla* according to this study. Moreover, it can also be concluded that lignans could be considered as chemotaxonomic markers of the genus *Wikstroemia*. The result of the present study also supports the conclusion of Zhang *et al.*[20] that biflavonoids are characteristic constituents of the Thymelaeaceae family.

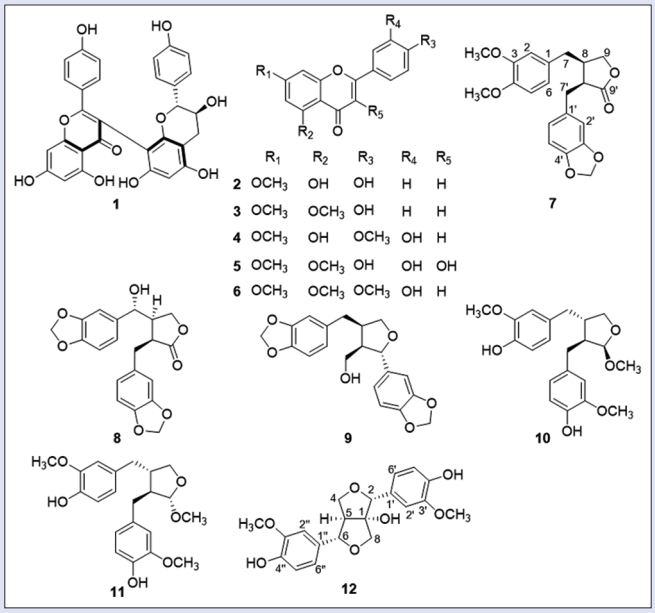


Figure 1: Structures of compounds 1-12 isolated from *Wikstroemia scytophylla*

SUPPLEMENTARY DATA

Flavones and lignans from the stems of *Wikstroemia scytophylla* Diels

^{1}H and ^{13}C NMR spectra of compounds 1-12:

Wikstrol B (1): $C_{30}H_{22}O_{10}$, yellow powder, [α]20 D-31.4° (c = 0.16, MeOH); ^{1}H NMR (400 MHz, CD_3COCD_3) *δ*: 7.56 (2H, d, *J* = 8.8 Hz, H-10", H-14"), 6.86 (2H, d, *J* = 8.8 Hz, H-11", H-13"), 6.83 (2H, d, *J* = 8.6 Hz, H-2', H-6'), 6.72 (2H, d, *J* = 8.6 Hz, H-3', H-5'), 6.38 (1H, d, *J* = 2.4 Hz, H-8"), 6.20 (1H, d, *J* = 2.4 Hz, H-6"), 6.16 (1H, s, H-6), 4.57 (1H, d, *J* = 7.8 Hz, H-2), 3.75 (1H, m, H-3), 2.81 (1H, dd, *J* = 16.0 Hz, 5.2 Hz, H-4a), 2.58 (1H, dd, *J* = 16.0 Hz, 8.4 Hz, H-4b); ^{13}C NMR (100 MHz, CD_3COCD_3) *δ*: 181.2 (s, C-4"), 164.8 (s, C-7"), 163.3 (s, C-2"), 163.3 (s, C-5"), 160.2 (s, C-12"), 158.6 (s, C-8a), 157.7 (s, C-4'), 157.1 (s, C-5), 156.0 (s, C-8a), 154.1 (s, C-7), 131.2 (s, C-1'), 131.1 (d, C-10", C-14"), 129.1 (d, C-2', C-6'), 125.5 (s, C-9"), 115.6 (d, C-11", C-13"), 115.5 (d,

C-3', C-5'), 113.7 (s, C-3"), 104.8 (s, C-4"a), 100.7 (s, C-4a), 99.3 (s, C-8), 99.3 (d, C-6"), 96.2 (d, C-6), 94.1 (d, C-8"), 82.3 (d, C-2), 68.5 (d, C-3), 28.8 (t, C-4).

Apigenin 7-O-methyl ether (2): $C_{16}H_{12}O_5$, yellow powder; ^{1}H NMR (500 MHz, CD_3COCD_3) δ: 7.96 (2H, d, J = 8.6 Hz, H-2', H-6'), 7.13 (2H, d, J = 8.6 Hz, H-3', H-5'), 6.74 (1H, s, H-3), 6.72 (1H, d, J = 1.6 Hz, H-8), 6.32 (1H, d, J = 1.5 Hz, H-6), 3.92 (3H, s, 7-OCH_3); ^{13}C NMR (100 MHz, CD_3COCD_3) δ: 182.5 (s, C-4), 166.4 (s, C-7), 165.2 (s, C-2), 162.9 (s, C-4'), 161.5 (s, C-5), 158.0 (s, C-9), 129.2 (d, C-2', C-6'), 119.5 (s, C-1'), 116.9 (d, C-3', -5'), 104.7 (s, C-10), 103.9 (d, C-3), 98.7 (d, C-6), 93.2 (d, C-8), 56.4 (q, 7-OCH_3).

Apigenin 5, 7-dimethyl ether (3): $C_{17}H_{14}O_5$, yellow needle crystal; ^{1}H NMR (500 MHz, C_5D_5N) δ: 7.97 (2H, d, J = 8.8 Hz, H-2', H-6'), 7.12 (2H, d, J = 8.8 Hz, H-3', H-5'), 6.97 (1H, s, H-3), 6.71 (1H, d, J = 2.0 Hz, H-8), 6.62 (1H, d, J = 2.0 Hz, H-6), 3.77 and 3.75 (6H, 2s, 2×-OCH_3); ^{13}C NMR (125 MHz, C_5D_5N) δ: 182.9 (s, C-4), 166.0 (s, C-7), 164.3 (s, C-2), 163.1 (s, C-4'), 162.8 (s, C-5), 158.2 (s, C-9), 128.7 (d, C-2', C-6'), 115.0 (d, C-3', -5'), 106.0 (s, C-10), 104.8 (d, C-3), 98.7 (d, C-6), 93.0 (d, C-8), 56.0 and 55.6 (q, 7-OCH_3).

Luteolin 7, 4'-dimethyl ether (4): $C_{17}H_{14}O_6$, brown needle crystal; ^{1}H NMR (500 MHz, CD_3COCD_3) δ: 7.59 (1H, d, J = 8.5 Hz, 1.8 Hz, H-6'), 7.52 (1H, d, J = 1.8 Hz, H-2'), 7.14 (1H, d, J = 8.6 Hz, H-5'), 6.73 (1H, d, J = 1.8 Hz, H-8), 6.69 (1H, s, H-3), 6.32 (1H, d, J = 1.7 Hz, H-6), 3.95 and 3.93 (6H, 2s, 2×-OCH_3); ^{13}C NMR (150 MHz, CD_3COCD_3) δ: 183.2 (s, C-4), 166.7 (s, C-7), 165.1 (s, C-5), 163.0 (s, C-2), 158.8 (s, C-9), 151.9 (s, C-4'), 147.9 (s, C-3'), 124.8 (s, C-1'), 119.9 (d, C-6'), 113.8 (d, C-5'), 112.5 (d, C-2'), 106.1 (s, C-10), 104.8 (d, C-3), 98.8 (d, C-6), 93.3 (d, C-8), 56.5 and 56.4 (q, 2×-OCH_3).

5, 7-Di-O-methylquercetin (5): $C_{17}H_{14}O_7$, yellow crystal; ^{1}H NMR (500 MHz, C_5D_5N) δ: 8.10 (1H, d, J = 8.5 Hz, H-6'), 7.23 (1H, s, H-2'), 7.06 (1H, d, J = 8.6 Hz, H-5'), 6.70 (1H, d, J = 1.7 Hz, H-8), 6.33 (1H, d, J = 1.7 Hz, H-6), 3.85 and 3.74 (6H, 2s, 2×-OCH_3); ^{13}C NMR (125 MHz, C_5D_5N) δ: 169.2 (s, C-4), 161.3 (s, C-7), 159.3 (s, C-5), 158.8 (s, C-9), 144.8 (s, C-2, -4), 143.1 (s, C-3'), 124.6 (s, C-3), 120.9 (s, C-1'), 118.0 (d, C-6'), 117.5 (d, C-5'), 114.4 (d, C-2'), 108.7 (s, C-10), 96.1 (d, C-6), 94.9 (d, C-8), 56.4 and 55.6 (q, 2×-OCH_3).

3'-Hydroxy-4', 5, 7-trimethoxy-flavone (6): $C_{18}H_{16}O_6$, brown needle crystal; ^{1}H NMR (500 MHz, C_5D_5N) δ: 8.03 (1H, d, J = 8.5 Hz, 1.8 Hz, H-6'), 7.91 (1H, d, J = 2.3 Hz, H-2'), 7.30 (1H, d, J = 8.6 Hz, H-5'), 7.03 (1H, s, H-3), 6.63 (1H, d, J = 2.2 Hz, H-8), 6.61 (1H, d, J = 2.2 Hz, H-6), 3.80, 3.76 and 3.60 (9H, 3s, 3×-OCH_3); ^{13}C NMR (125 MHz, C_5D_5N) δ: 182.8 (s, C-4), 165.9 (s, C-7), 164.7 (s, C-5), 162.7 (s, C-2), 158.1 (s, C-9), 152.1 (s, C-4'), 148.6 (s, C-3'), 129.0 (d, C-6'), 124.6 (s, C-1'), 118.9 (d, C-5'), 117.0 (d, C-2'), 112.2 (s, C-10), 103.8 (d, C-3), 98.6 (d, C-6), 92.8 (d, C-8), 60.4, 56.5 and 56.4 (q, 3×-OCH_3).

3-(1, 3-benzodioxol-5-ylmethyl)-4-[(3, 4-dimethoxyphenyl)methyl] dihydro-, (3S-cis)-2(3H)-Furanone (7): $C_{21}H_{22}O_6$, colorless oil, [α]20 D+67.8° (c = 0.11, MeOH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 178.5 (s, C-9), 149.0 (s, C-3'), 147.84 (s, C-3), 147.80 (s, C-4'), 146.4 (s, C-4), 131.3 (s, C-1), 130.4 (s, C-1'), 122.2 (d, C-6), 120.6 (d, C-6'), 111.6 (d, C-5'), 111.2 (d, C-5), 109.4 (d, C-2'), 108.1 (d, C-2), 101.0 (t, C-1"), 71.2 (t, C-9'), 55.9 (q, 3'-OCH_3), 55.7 (q, 3-OCH_3), 46.4 (d, C-8), 41.2 (d, C-8'), 38.2 (t, C-7'), 34.7 (t, C-7).

4-[(R)-1, 3-benzodioxol-5-ylhydroxymethyl]-3-(1, 3-benzodioxol-5-ylmethyl) dihydro-, (3S, 4R)-2(3H)-Furanone (8): $C_{20}H_{18}O_7$, colorless needle crystal, [α]20 D+54.7° (c = 0.23, $CHCl_3$); ^{1}H NMR (500 MHz, C_5D_5N) δ: 7.07 (1H, s, H-2'), 6.93 (1H, d, J = 7.8 Hz, H-5'), 6.86 (1H, d, J = 8.0 Hz, H-5), 6.78 (1H, d, J = 7.8 Hz, H-6'), 6.73 (1H, d, J = 7.9 Hz, H-2), 6.01 ~ 5.91 (4H, m, 2×-OCH_2O-), 4.96 (1H, d, J = 4.9 Hz, H-7'), 4.31 ~ 4.20 (2H, m, H-9'), 3.26 ~ 3.04 (3H, m, H-7, H-8), 2.78 (1H, m, H-8'); ^{13}C NMR (125 MHz, C_5D_5N) δ: 179.6 (s, C-9), 148.3 (s, C-3'), 148.1 (s, C-3), 147.3 (s, C-4'), 146.7 (s, C-4), 138.1 (s, C-1'), 132.4 (s, C-1), 123.2 (d, C-6), 119.6 (d, C-6'), 110.3 (d, C-5), 108.4 (d, C-5'), 108.3 (d, C-2), 107.0 (d, C-2'), 101.6 and 101.4 (2t, 2×-OCH_2O-), 74.3 (d, C-7'), 69.7 (t, C-9'), 46.1 (d, C-8), 43.5 (d, C-8'), 35.8 (t, C-7).

(-)-Dihydrosesamin (9): $C_{20}H_{20}O_6$, colorless powder, [α]20 D+21.3° (c = 0.12, MeOH); ^{1}H NMR (400 MHz, $CDC1_3$) δ: 6.80 ~ 6.58 (6H, m, H-2', H-5', H-5, H-6', H-2), 5.91 ~ 5.89 (4H, m, 2×-OCH_2O-), 4.75 (1H, d, J = 6.1 Hz, H-7), 4.00 ~ 3.62 (4H, m, H-9', H-9), 2.80 (1H, dd, J = 13.5 Hz, 5.0 Hz, H-7'b), 2.45 (1H, m, H-7'a), 2.45 (1H, dd, J = 13.4 Hz, 10.8 Hz, H-8), 2.26 (1H, m, H-8'); ^{13}C NMR (100 MHz, $CDC1_3$) δ: 179.6 (s, C-9), 148.3 (s, C-3'), 148.1 (s, C-3), 147.3 (s, C-4'), 146.7 (s, C-4), 138.1 (s, C-1'), 132.4 (s, C-1), 123.2 (d, C-6), 119.6 (d, C-6'), 110.3 (d, C-5), 108.4 (d, C-5'), 108.3 (d, C-2), 107.0 (d, C-2'), 101.6 and 101.4 (2t, 2×-OCH_2O-), 74.3 (d, C-7'), 69.7 (t, C-9'), 46.1 (d, C-8), 43.5 (d, C-8'), 35.8 (t, C-7).

Phenol, 4, 4'-[[(2R, 3S, 4S)-tetrahydro-2-methoxy-3, 4-furandiyl]bis (methylene)]bis[2-methoxy- (10): $C_{21}H_{26}O_6$, colorless oil, [α]20 D+71.4° (c = 0.15, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.82 (1H, d, J = 8.0 Hz, H-5'), 6.77 (1H, d, J = 7.2 Hz, H-5), 6.62 (1H, d, J = 1.6 Hz, H-2), 6.57 (1H, dd, J = 8.0, 1.6 Hz, H-6'), 6.53 (1H, d, J = 1.6 Hz, H-2'), 6.44 (1H, dd, J = 7.2, 1.6 Hz, H-6), 4.74 (1H, d, J = 1.0 Hz, H-9') , 4.04 (1H, t, J = 8.2 Hz, H-9b), 3.83 (3H, s, 3-OCH_3), 3.82 (3H, s, 3'-OCH_3), 3.67 (1H, t, J = 8.2 Hz, H-9a), 3.34 (3H, s, 9'-OCH_3), 2.72 (1H, dd, J = 13.1, 6.9 Hz, H-7'b), 2.57 (1H, m, H-7b), 2.55 (1H, m, H-7a), 2.42 (1H, dd, J = 13.1, 6.9 Hz, H -7'a), 2.15 (1H, m, H-8), 2.15 (1H, m, H-8'); ^{13}C NMR (125 MHz, $CDC1_3$) δ: 146.5 (s, C-4'), 146.3 (s, C-4), 144.0 (s, C-3), 143.8 (s, C-3'), 132.9 (s, C-1'), 132.2 (s, C-1), 121.4 (d, C-6), 121.3 (d, C-6'), 114.3 (d, C-2'), 114.2 (d, C-5), 111.5 (d, C-2), 111.1 (d, C-5'), 105.6 (d, C-9'), 72.3 (t, C-9), 55.9 (q, 3-OCH_3), 55.8 (q, 3'-OCH_3), 54.5 (q, 9'-OCH_3), 52.2 (d, C-8'), 43.3 (d, C-8), 39.3 (t, C-7'), 33.5 (t, C-7).

4, 4'-dihydroxy-3, 3', 9-trimethoxy-9, 9'-epoxylignan (11): $C_{21}H_{26}O_6$, colorless oil, [α]20 D-23.7° (c = 0.14, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.83 (1H, d, J = 8.0 Hz, H-5), 6.81 (1H, d, J = 7.8 Hz, H-5'), 6.68 (1H, dd, J = 7.8, 1.5 Hz, H-6'), 6.67 (1H, d, J = 1.5 Hz, H-2'), 6.64 (1H, d, J = 8.0 Hz, H-6), 6.60 (1H, s, H-2), 4.66 (1H, d, J = 3.6 Hz, H-9'), 3.99 (1H, t, J = 6.6 Hz, H-9b), 3.86 (3H, s, 3-OCH_3), 3.86 (3H, s, 3'-OCH_3), 3.59 (1H, t, J = 6.6 Hz, H-9a), 3.32 (3H, s, 9'-OCH_3), 2.76 (1H, m, H-7'b), 2.73 (1H, m, H-7b), 2.52 (1H, m, H -7'a), 2.46 (1H, m, H-7a), 2.39 (1H, m, H-8'), 2.01 (1H, m, H-8); ^{13}C NMR (125 MHz, $CDC1_3$) δ: 146.5 (s, C-4'), 146.4 (s, C-4), 144.0 (s, C-3), 143.8 (s, C-3'), 132.5 (s, C-1'), 131.6 (s, C-1), 121.6 (d, C-6), 121.2 (d, C-6'), 114.1 (d, C-2'), 114.0 (d, C-5), 111.1 (d, C-2), 110.9 (d, C-5'), 110.1 (d, C-9'), 72.2 (t, C-9), 55.7 (q, 3-OCH_3), 55.7 (q, 3'-OCH_3), 54.7 (q, 9'-OCH_3), 52.4 (d, C-8'), 45.8 (d, C-8), 39.3 (t, C-7'), 38.7 (t, C-7).

(+)-1-Hydroxypinoresinol (12): $C_{20}H_{22}O_7$, yellow powder, [α]20 D+33.6° (c = 0.10, MeOH); ^{1}H NMR (400 MHz, CD_3COCD_3) δ: 7.16 (1H, d, J = 8.0 Hz, H-5'), 7.11 (1H, d, J = 2.0 Hz, H-2'), 7.08 (1H, dd, J = 8.0, 1.9 Hz, H-6"), 6.92 (1H, d, J = 8.0 Hz, H-5"), 6.91 (1H, dd, J = 8.0, 2.0 Hz, H-6'), 6.88 (1H, d, J = 1.9 Hz, H-2"), 4.88 (1H, s, H-2), 4.56 (1H, d, J = 6.8 Hz, H-6), 4.31 (1H, m, H-8a), 3.87 (1H, dd, J = 8.8, 7.5 Hz, H-4a), 3.85 (3H, s, 3-OCH_3), 3.82 (3H, s, 3'-OCH_3), 3.78 (1H, d, J = 8.9, 4.1 Hz, H-4b), 3.70 (1H, m, H-8b), 3.50 (1H, m, H-5); ^{13}C NMR (100 MHz, CD_3COCD_3) δ: 151.8 (s, C-3"), 148.5 (s, C-3'), 147.9 (s, C-4', C-4"), 132.9 (s, C-1"), 129.5 (s, C-1'), 120.5 (d, C-6'), 119.2 (d, C-6"), 115.15 (d, C-5"), 115.06 (d, C-5'), 111.4 (d, C-2'), 110.8 (d, C-2"), 91.1 (s, C-1), 88.4 (d, C-2), 86.6 (d, C-6), 73.7 (t, C-8), 71.4 (t, C-4), 61.8 (d, C-5), 56.2 and 56.1 (q, -OCH_3).

Financial support and sponsorship

This work was supported by the National Natural Science Foundation of China (NSFC) (No. 81374022, 81274184, and 81473337), the research project of the Administration of Traditional Chinese Medicine of

Sichuan (2016Q037), the Fundamental Research Funds for the Central Universities (the Medicine thematic research projects, 2682016YXZT05), the Foundation of Science and Technology Agency of Chengdu (2015-HM01-00311-SF), and the Student Research Training Program (SRTP, 2016089 and 201610613073). The authors are grateful to the members of the analytical group of the Chengdu Institute of Biology, and the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, for the spectral measurements.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Gu CZ. Zhongguo Zhi Wu Zhi (Flora of China). Vol. 52. Beijing: Science Press; 1999. p. 293.
2. Li YM, Zhu L, Jiang JG, Yang L, Wang DY. Bioactive components and pharmacological action of *Wikstroemia indica* (L.) C. A. Mey and its clinical application. Curr Pharm Biotechnol 2009;10:743-52.
3. Jiang HZ, Ma QY, Huang SZ, Liang WJ, Wang PC, Zhao YX. A new tigliane-type diterpene ester from *Wikstroemia scytophylla*. Chem Nat Compd 2012a;48:587-90.
4. Jiang HZ, Ma QY, Huang SZ, Liang WJ, Wang PC, Hu JM, *et al*. A new guaiane-type sesquiterpene with 15 known compounds from *Wikstroemia scytophylla* Diels. Chin J Chem 2012b;30:1335-8.
5. Nian Y, Wang HY, Zhou L, Su J, Li Y, Qiu MH. Cytotoxic cycloartane triterpenes of the traditional Chinese medicine "shengma" (*Cimicifuga dahurica*). Planta Med 2013;79:60-9.
6. Baba K, Taniguchi M, Kozawa M. 3 biflavonoids from *Wikstroemia sikokiana*. Phytochemistry 1994;37:879-83.
7. Liang S, Tang J, Shen YH, Jin HZ, Tian JM, Wu ZJ, *et al*. Biflavonoids from *Daphne feddei* and their inhibitory activities against nitric oxide production. Chem Pharm Bull (Tokyo) 2008;56:1729-31.
8. Zahir A, Jossang A, Bodo B, Provost J, Cosson JP, Sévenet T. DNA topoisomerase I inhibitors: Cytotoxic flavones from *Lethedon tannaensis*. J Nat Prod 1996;59:701-3.
9. Shen T, Jia ZJ, Zheng SZ. Studies on chemical constituents of *Polygonum perforliatum* L. J Asian Nat Prod Res 2007;9:129-33.
10. Ampofo SA, Roussis V, Wiemer DF. New prenylated phenolics from *Piper auritum*. Phytochemistry 1987;26:2367-70.
11. Baez DA, Zepeda Vallejo LG, Jimenez-Estrada M. Phytochemical studies on *Senna skinneri* and *Senna wislizeni*. Nat Prod Lett 1999;13:223-8.
12. Phan MG, Phan TS, Matsunami K, Otsuka H. Chemical and biological evaluation on scopadulane-type diterpenoids from *Scoparia dulcis* of Vietnamese origin. Chem Pharm Bull (Tokyo) 2006;54:546-9.
13. Huang YL, Chen CC, Hsu FL, Chen CF. A new lignan from *Phyllanthus virgatus*. J Nat Prod 1996;59:520-1.
14. Fonseca SF, Campello JP, Barata LE, Ruveda EA. Carbon-13 NMR spectral analysis of lignans from *Araucaria angustifolia*. Phytochemistry 1978;17:499-502.
15. Yamauchi S, Kinoshita Y. Synthesis of *cis*-lactone lignan, *cis-(2S,3R)*-parabenzlactone, from L-arabinose. Biosci Biotechnol Biochem 2001;65:1669-72.
16. Pelter A, Ward RS, Venkateswarlu R, Kamakshi C. Oxidative transformations of lignans – Reactions of dihydrocubebin and a derivative with DDQ. Tetrahedron 1991;47:1275-84.
17. Chen XQ, Li Y, He J, Wang K, Li MM, Pan ZH, *et al*. Four new lignans from *Viburnum foetidum var. foedidum*. Chem Pharm Bull (Tokyo) 2009;57:1129-31.
18. Chang MJ, Hung TM, Min BS, Kim JC, Woo MH, Choi JS, *et al*. Lignans from the fruits of *Forsythia suspensa* (Thunb.) vahl protect high-density lipoprotein during oxidative stress. Biosci Biotechnol Biochem 2008;72:2750-5.
19. Graikou K, Aligiannis N, Chinou IB. Chemical constituents from *Pterocephalus perennis subsp perennis* (Dipsacaceae). Biochem Syst Ecol 2006;34:438-41.
20. Zhang Q, Ye N, Sun WX, Zhang KM, Jiang JQ. Phytochemical investigation of *Daphne giraldii* Nitsche (Thymelaeaceae). Biochem Syst Ecol 2008;36:63-7.
21. Xu WC, Shen JG, Jiang JQ. Phytochemical and biological studies of the plants from the genus *Daphne*. Chem Biodivers 2011;8:1215-33.
22. Li F, Sun Q, Hong L, Li L, Wu Y, Xia M, *et al*. Daphnane-type diterpenes with inhibitory activities against human cancer cell lines from *Daphne genkwa*. Bioorg Med Chem Lett 2013;23:2500-4.
23. Wu Y, Liu C, Li HF, Sun JB, Li YY, Gu W, *et al*. A novel neolignan glycoside from *Aquilaria sinensis*. Biochem Syst Ecol 2014;55:41-5.
24. Wang SC, Wang F, Yue CH. Chemical constituents from the petioles and leaves of *Aquilaria sinensis*. Biochem Syst Ecol 2015;61:458-61.
25. Zhang X, Wang G, Huang W, Ye W, Li Y. Biflavonoids from the roots of *Wikstroemia indica*. Nat Prod Commun 2011;6:1111-4.
26. Khong A, Forestieri R, Williams DE, Patrick BO, Olmstead A, Svinti V, *et al*. A daphnane diterpenoid isolated from *Wikstroemia polyantha* induces an inflammatory response and modulates miRNA activity. PLoS One 2012;7:e39621.
27. Wang GC, Zhang XL, Wang YF, Li GQ, Ye WC, Li YL. Four new dilignans from the roots of *Wikstroemia indica*. Chem Pharm Bull (Tokyo) 2012;60:920-3.