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# Terpenoids from *Ligularia kangtingensis*

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## ABSTRACT

**Background:** *Ligularia kangtingensis*, a species from the genus *Ligularia* (Compositae), is an indigenous plant in Southwest China and more than 20 species in this genus have been used as folk medicines in China. **Objective:** The chemical constituents of the whole plant of *L. kangtingensis* were studied. **Materials and Methods:** The dried whole plants were extracted with ethanol. Its chemical constituents were mainly isolated and purified by silica gel and Sephadex LH-20 column chromatography and their structures were identified on the basis of spectral analysis. **Results:** Twelve known terpenoids, including two monoterpenoids, five sesquiterpenoids and five triterpenoids, were isolated and identified from the whole plant of *L. kangtingensis*. **Conclusion:** All of the 12 known compounds were isolated for the first time from *L. kangtingensis*.

**Key words:** Compositae, *Ligularia kangtingensis*, sesquiterpenoids, terpenoids

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## INTRODUCTION

The genus *Ligularia*, a member from the family compositae, comprises approximately 150 species worldwide and nearly 120 species are distributed in China.[1] The roots and rhizomes of many *Ligularia* plants have long been used as folk medicines for their antibacterial and anti-inflammatory activities in China.[2] The former phytochemical studies on this genus have revealed that it was an important source of terpenoids.[3]

*Ligularia kangtingensis* S. W. Liu is an indigenous plant in Sichuan province, China, and it well adapted to highlands around 4000 m.[4] A literature survey indicated that no phytochemical research was reported, except a study on volatile oil.[5] As a continuation of our phytochemical studies on medicinal plants, herein we report the isolation of 12 known compounds, including two monoterpenoids, namely (3*R*, 4*R*, 6*S*)-3, 6-dihydroxy-1-menthene (11), 5-*p*-methane-1, 2-diol (12), five sesquiterpenoids, namely ligudentatin A (2), liguhodgsonal (3), oplopanone (4), 1$\beta$, 6$\alpha$-dihydroxy-4$\beta$ (15)-epoxyeudesmane (5), 8$\beta$-ethoxyeremophil-3, 7 (11)-diene-8$\alpha$, 12 (6$\alpha$, 15)-diolide (10) and 5 triterpenoids, namely lupeol (1), oleanolic acid (6), ursolic acid (**7**), pomolic acid (8), taraxerol (9) from the whole plant of *L. kangtingensis*.

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## MATERIALS AND METHODS

### General

Nuclear magnetic resonance (NMR) spectra were recorded on Varian Unity 400/54 spectrometer with tetramethylsilane as an internal standard. Column chromatography (CC) was carried out by using silica gel (Qingdao Marine Chemical Industry, 200–300 mesh) and Sephadex LH-20 (GE Healthcare). All the reagents and solvents used for separation and purification were analytical grade and purchased from local firms.

### Plant material

The whole plant of *L. kangtingensis* was collected from Kangding County, Sichuan Province, China, in August, 2010. The plant was identified by Qin-Mao Fang, Institute of TCM Medicinal Resources and Cultivation, Sichuan Academy of Chinese Medicine Sciences. A voucher specimen (No. LK1008) was deposited in the School of Life Science and Technology, University of Electronic Science and Technology of China.

### Extraction and isolation

The air-dried whole plant of *L. kangtingensis* (5 kg) was powdered and extracted three times with 95% EtOH under reflux. The solvents were evaporated in vacuo to yield

ethanol extract, which was suspended in $H_2O$ and then extracted with petroleum ether and EtOAc, respectively. The petroleum ether extract (185 g) was subjected to CC over silica gel (200–300 mesh, 2 kg) and eluted with a gradient solvent system ($CHCl_3$-MeOH, 90:1–2:1) to give 12 fractions (Fr. 1~12). Fr. 2 (1.4 g) was isolated by silica gel chromatography eluted with solvent systems of cyclohexane-EtOAc (18:1) and $CHCl_3$-acetone (600:1) to afford compound 1 (50 mg). Fr. 5 (2.7 g) was separated by silica gel chromatography (petroleum ether-EtOAc, 10:1–6:1) to give eight subfractions (Fr. 5–1 ~ 5–8). Subfraction 5–2 (35 mg) was separated by preparative thin-layer chromatography (TLC) ($CHCl_3$-acetone, 37:2) and purified by Sephadex LH-20 chromatography ($CHCl_3$-MeOH, 2:1) to give compound 2 (5 mg); Subfraction 5–3 (30 mg) was separated by preparative TLC ($CHCl_3$-acetone, 20:1) to give compound 3 (11 mg); Subfraction 5–4 (60 mg) was separated by silica gel chromatography ($CHCl_3$-acetone, 70:1) and purified by Sephadex LH-20 chromatography ($CHCl_3$-MeOH, 2:1) to give compound 4 (7 mg); Subfraction 5–6 (95 mg) was separated by silica gel chromatography ($CHCl_3$-acetone, 55:1) and purified by Sephadex LH-20 chromatography ($CHCl_3$-MeOH, 2:1) to give compound 5 (6 mg). Fr. 7 (750 mg) was isolated by silica gel chromatography (petroleum ether-EtOAc, 9:1; $CHCl_3$-acetone, 50:1) and purified by Sephadex LH-20 chromatography ($CHCl_3$-MeOH, 2:1) to afford compound 6 (11 mg). Fr. 9 (1 g) was isolated by silica gel chromatography eluted with solvent systems of $CHCl_3$-acetone (60:1) and cyclohexane-EtOAc (6.5:1) to afford compound 7 (10 mg). Fr. 10 (3.5 g) was isolated by silica gel chromatography (petroleum ether-EtOAc, 6:1; $CHCl_3$-acetone, 40:1) and purified by Sephadex LH-20 chromatography ($CHCl_3$-MeOH, 2:1) to afford compound 8 (11 mg). The EtOAc extract (38 g) was subjected to CC over silica gel (200–300 mesh, 500 g) and eluted with a gradient solvent system (cyclohexane-acetone, 30:1–1:1) to give 10 fractions (Fr.A~J). Fr. C (1.1 g) was isolated by silica gel chromatography (cyclohexane-EtOAc, 6:1) and recrystallized from cyclohexane to afford compound 9 (20 mg). Fr. D (180 mg) was isolated by silica gel chromatography (petroleum ether-EtOAc, 12:1) and purified by Sephadex LH-20 chromatography ($CHCl_3$-MeOH, 2:1) to give compound 10 (6 mg). Fr. F (2.1 g) was isolated by silica gel chromatography (cyclohexane-EtOAc, 3:2; petroleum ether-acetone, 4:1) to afford subfraction F-1 and F-2. From subfraction F-1 (30 mg), compound 11 (7 mg) was recrystallized with $CHCl_3$. Subfraction F-2 (240 mg) was isolated by silica gel chromatography ($CHCl_3$-acetone, 35:1) and purified by Sephadex LH-20 chromatography ($CHCl_3$-MeOH, 2:1) to afford compound 12 (6 mg).

## RESULTS AND DISCUSSION

Compound 1: White amorphous powder. $^1$H (400 MHz, $CDCl_3$): $d_H$ 4.68, 4.56 (each 1H, br s, $H_2$-29), 3.17 (1H, m, H-3), 1.68, 1.03, 0.96, 0.94, 0.83, 0.79, 0.76 (each 3H, s, 7 × $CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$) [Table 1].

Compound 2: Colorless gum. $^1$H (400 MHz, $CDCl_3$): $d_H$ 7.17 (1H, d, $J$ = 2.8 Hz, H-3), 6.75 (1H, d, $J$ = 2.8 Hz, H-1), 4.77 (2H, br s, $H_2$-12), 3.86 (3H, s, $OCH_3$), 3.17 (1H, dd, $J$ = 17.6, 4.8 Hz, H-6α), 2.85 (2H, m, $H_2$-9), 2.78 (1H, dd, $J$ = 17.2, 11.2 Hz, H-6β), 2.26 (1H, m, H-7), 1.94 (1H, m, H-8α), 1.80 (3H, s, $H_3$-13), 1.62 (1H, m, H-8β). $^{13}$C NMR (100 MHz, $CDCl_3$): $d_C$ 168.1 (C-14), 152.5 (C-2), 149.3 (C-11), 139.4 (C-10), 130.4 (C-4), 130.0 (C-5), 119.3 (C-1), 114.9 (C-3), 109.2 (C-12), 51.9 (OMe), 41.7 (C-7), 32.5 (C-6), 30.2 (C-9), 27.2 (C-8), 20.7 (C-13).

Compound 3: Colorless amorphous solid. $^1$H (400 MHz, $CDCl_3$): $d_H$ 10.25 (1H, s, H-14), 7.17 (1H, d, $J$ = 2.8 Hz, H-3), 6.86 (1H, d, $J$ = 2.4 Hz, H-12), 5.63 (1H, br s, OH),

**Table 1: $^{13}$C-NMR spectroscopic data for compound 1, 6, 7, 8 and 9**

| Position | 1 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|
| 1 | 38.7 | 38.6 | 38.4 | 38.4 | 37.6 |
| 2 | 27.4 | 27.0 | 27.6 | 28.2 | 27.0 |
| 3 | 78.9 | 79.0 | 78.3 | 78.6 | 79.0 |
| 4 | 38.6 | 38.6 | 38.8 | 38.5 | 38.9 |
| 5 | 55.4 | 55.1 | 54.9 | 54.9 | 55.6 |
| 6 | 18.2 | 18.2 | 18.0 | 18.4 | 18.7 |
| 7 | 34.2 | 32.5 | 32.7 | 34.2 | 35.0 |
| 8 | 39.9 | 39.4 | 39.1 | 39.6 | 38.7 |
| 9 | 50.3 | 47.5 | 48.1 | 41.1 | 49.2 |
| 10 | 37.1 | 36.9 | 37.5 | 38.0 | 37.4 |
| 11 | 20.8 | 22.8 | 22.9 | 23.6 | 17.4 |
| 12 | 25.0 | 122.5 | 125.1 | 128.9 | 36.6 |
| 13 | 38.0 | 143.5 | 137.9 | 137.9 | 38.7 |
| 14 | 42.0 | 41.5 | 41.7 | 42.0 | 158.2 |
| 15 | 27.1 | 27.6 | 27.6 | 28.2 | 116.8 |
| 16 | 35.5 | 23.3 | 17.9 | 26.8 | 37.6 |
| 17 | 42.7 | 46.4 | 47.2 | 47.3 | 38.7 |
| 18 | 48.2 | 40.8 | 52.9 | 54.5 | 48.6 |
| 19 | 48.6 | 45.7 | 38.6 | 72.8 | 41.2 |
| 20 | 150.9 | 30.4 | 38.4 | 40.9 | 27.9 |
| 21 | 29.8 | 33.7 | 30.3 | 26.8 | 33.6 |
| 22 | 39.9 | 32.3 | 36.5 | 37.6 | 33.0 |
| 23 | 27.9 | 28.0 | 27.6 | 28.0 | 27.9 |
| 24 | 15.4 | 15.4 | 15.2 | 15.2 | 15.4 |
| 25 | 16.0 | 15.2 | 15.0 | 15.5 | 15.4 |
| 26 | 15.6 | 17.0 | 16.6 | 16.1 | 29.8 |
| 27 | 14.5 | 26.0 | 23.1 | 24.4 | 25.8 |
| 28 | 17.4 | 183.2 | 180.0 | 180.7 | 29.7 |
| 29 | 109.3 | 33.0 | 16.4 | 16.5 | 33.3 |
| 30 | 18.7 | 23.5 | 20.7 | 27.1 | 21.2 |

$^{13}$C-NMR: Carbon-13 nuclear magnetic resonance

4.79 (2H, br d, $J$ = 9.2 Hz, $H_2$-12),3.40 (1H, dd, $J$ = 17.2, 4.8 Hz, H-6α), 1.81 (3H, s, $H_3$-13). $^{13}$C NMR (100 MHz, $CDCl_3$): $d_C$ 192.6 (C-14), 153.5 (C-2), 149.0 (C-11), 139.8 (C-10), 134.6 (C-4), 131.7 (C-5), 121.7 (C-1), 115.6 (C-3), 109.6 (C-12), 41.4 (C-7), 30.7 (C-9), 29.9 (C-6), 27.0 (C-8), 20.7 (C-13).

Compound 4: Colorless gum. $^1$H (400 MHz, $CDCl_3$): $d_H$ 2.64 (1H, ddd, $J$ = 14.2, 9.2, 5.2 Hz, H-3), 2.18 (3H, s, $H_3$-15), 1.19 (3H, s, $H_3$-14), 0.88 (3H, d, $J$ = 6.8 Hz, $H_3$-13), 0.68 (3H, d, $J$ = 6.8 Hz, $H_3$-12). $^{13}$C NMR (100 MHz, $CDCl_3$): $d_C$ 211.5 (C-4), 73.1 (C-10), 56.9 (C-1), 55.6 (C-5), 49.3 (C-7), 46.6 (C-6), 41.9 (C-9), 29.6 (C-15), 29.5 (C-11), 28.5 (C-3), 25.2 (C-2), 22.9 (C-8), 21.9 (C-13), 20.3 (C-14), 15.5 (C-12).

Compound 5: Colorless gum. $^1$H (400 MHz, $CDCl_3$): $d_H$ 3.43 (1H, t, $J$ = 10.0 Hz, H-6), 3.42 (1H, dd, $J$ = 12.4, 4.4 Hz, H-1), 3.21 (1H, dd, $J$ = 3.6, 2.0 Hz, H-15a), 2.77 (1H, d, $J$ = 3.6 Hz, H-15b), 1.65 (1H, d, $J$ = 10.0 Hz, H-5), 0.91 (3H, d, $J$ = 7.2 Hz, $H_3$-12), 0.86 (3H, s, $H_3$-14), 0.80 (3H, d, $J$ = 6.8 Hz, $H_3$-13). $^{13}$C NMR (100 MHz, $CDCl_3$): $d_C$ 78.0 (C-1), 67.5 (C-6), 61.5 (C-4), 51.5 (C-15), 49.6 (C-5), 49.6 (C-7), 41.8 (C-10), 36.7 (C-9), 33.1 (C-3), 29.2 (C-2), 24.9 (C-11), 20.9 (C-13), 17.8 (C-8), 15.8 (C-12), 12.1 (C-14).

Compound 6: White amorphous powder. $^1$H (400 MHz, $CDCl_3$): $d_H$ 5.27 (1H, br s, H-12), 3.21 (1H, m, H-3), 1.25, 1.13, 1.05, 0.98, 0.92, 0.90, 0.90 (each 3H, s, 7 × $CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$) [Table 1].

Compound 7: White amorphous powder. $^1$H (400 MHz, $CD_3OD$): $d_H$ 5.24 (1H, br s, H-12), 3.19 (1H, t, $J$ = 8.0 Hz, H-3), 1.25, 1.26, 1.09, 0.98, 0.93 (each 3H, s, 5 × $CH_3$), 0.95 (3H, d, $J$ = 6.0 Hz, $CH_3$), 0.86 (3H, d, $J$ = 6.4 Hz, $CH_3$). $^{13}$C NMR (100 MHz, $CD_3OD$) [Table 1].

Coumpound 8: White amorphous powder. $^1$H (400 MHz, $CDCl_3$): $d_H$ 5.25 (1H, br s, H-12), 3.28 (1H, m, H-3), 1.17, 1.12, 0.89, 0.81, 0.68, 0.67 (each 3H, s, 6 × $CH_3$), 0.85 (3H, d, $J$ = 6.0 Hz, $CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$) [Table 1].

Compound 9: White needle crystal (cyclohexane). $^1$H (400 MHz, $CDCl_3$): $d_H$ 5.52 (1H, dd, $J$ = 8.0, 2.8 Hz, H-15), 3.18 (1H, dd, $J$ = 10.8, 4.4 Hz, H-3), 1.08, 0.97, 0.94, 0.92, 0.90, 0.81, 0.79 (each 3H, s, 7 × $CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$) [Table 1].

Compound 10: Colorless gum. $^1$H (400 MHz, $CDCl_3$): $d_H$ 6.85 (1H, t, $J$ = 3.0 Hz, H-3), 5.04 (1H, dd, $J$ = 2.8, 1.6 Hz, H-6), 3.53, 3.32 (each 1H, dq, $J$ = 6.0, 4.8 Hz, $H_2$-1′), 2.24 (1H, dd, $J$ = 8.8, 3.2 Hz, H-9a), 2.00 (3H, d, $J$ = 1.2 Hz, $H_3$-13), 1.41 (3H, s, $H_3$-14), 1.21 (3H, t, $J$ = 4.8 Hz, $H_3$-2′). $^{13}$C NMR (100 MHz, $CDCl_3$): $d_C$ 170.5 (C-12), 168.5 (C-15), 152.7 (C-7), 136.9 (C-3), 129.7 (C-4), 128.3 (C-11), 105.1 (C-8), 82.2 (C-6), 59.2 (C-1′), 44.0 (C-5), 35.6 (C-9), 33.0 (C-10), 26.9 (C-14), 21.7 (C-2), 21.5 (C-1), 15.1 (C-2′), 9.1 (C-13).

Compound 11: Colorless needle crystal ($CHCl_3$). $^1$H (400 MHz, $CD_3OD$): $d_H$ 5.46 (1H, s, H-2), 3.90 (1H, br s, H-3), 3.84 (1H, d, $J$ = 9.2 Hz, H-6), 2.10 (1H, m, H-8), 1.76 (3H, s, $H_3$-7), 1.71 (1H, m, H-4),1.58 (1H, m, H-5a), 1.38 (1H, dt, $J$ = 13.2, 4.0 Hz, H-5b), 0.96 (3H, d, $J$ = 7.2 Hz, $H_3$-10), 0.81 (3H, d, $J$ = 6.8 Hz, $H_3$-9). $^{13}$C NMR (100 MHz, $CDCl_3$) $d_C$ 136.9 (C-1), 129.6 (C-2), 69.3 (C-3), 67.9 (C-6), 42.4 (C-4), 29.9 (C-5), 26.3 (C-8), 20.9 (C-9), 20.3 (C-10), 17.1 (C-7).

Compound 12: Colorless gum. $^1$H (400 MHz, $CDCl_3$): $d_H$ 5.71 (1H, dd, $J$ = 10, 2.8 Hz, H-5), 5.61 (1H, dd, $J$ = 10.4, 1.2 Hz, H-6), 3.79 (1H, dd, $J$ = 7.6, 3.2 Hz, H-2), 2.14 (1H, m, H-4), 1.80 (1H, m, H-8), 1.71 (2H, m, $H_2$-3), 1.30 (3H, s, $H_3$-7), 0.93 (3H, d, $J$ = 6.4 Hz, $H_3$-10), 0.92 (3H, d, $J$ = 6.8 Hz, $H_3$-9). $^{13}$C NMR (100 MHz, $CDCl_3$): $d_C$ 133.1 (C-5), 132.5 (C-6), 74.0 (C-2), 71.2 (C-1), 39.5 (C-4), 33.1 (C-8), 30.1 (C-3), 24.5 (C-7), 20.4 (C-10), 20.2 (C-9).

Identification of the 12 compounds isolated [Figure 1] was based on comparison of $^1$H-NMR and $^{13}$C-NMR

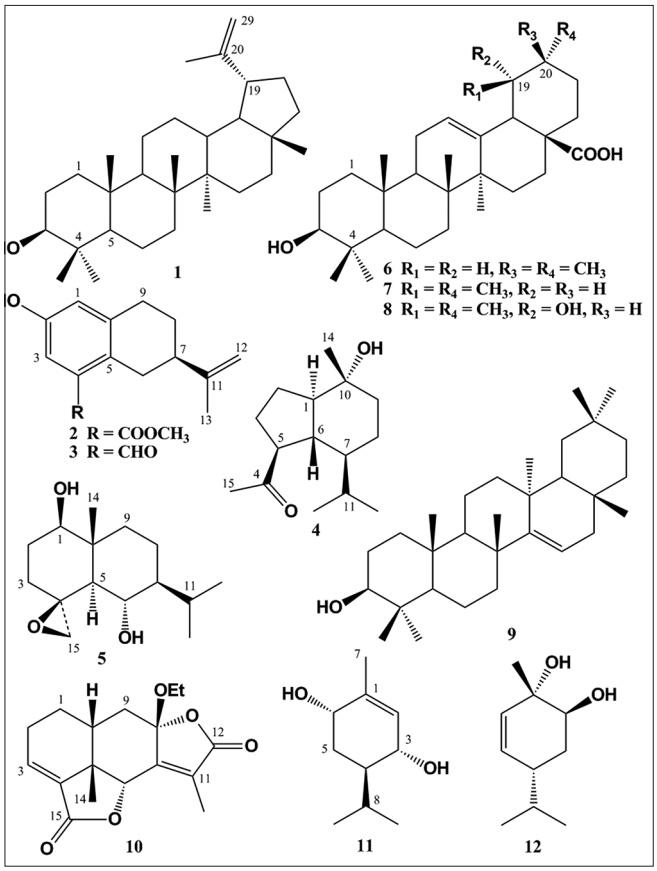


**Figure 1:** Structure of compounds 1–12 isolated from *Ligularia kangtingensis*

data with those reported in literature, and their structures were elucidated as lupeol (1),[6] ligudentatin A (2),[7] liguhodgsonal (3),[8] oplopanone (4),[9] 1$\beta$, 6$\alpha$-dihydroxy-4$\beta$ (15)-epoxyeudesmane (5),[10] oleanolic acid (6),[11] ursolic acid (7),[11] pomolic acid (8),[11] taraxerol (9),[12] 8$\beta$-ethoxyeremophil-3, 7 (11)-diene-8$\alpha$, 12 (6$\alpha$, 15)-diolide (10),[13] (3*R*, 4*R*, 6*S*)-3, 6-dihydroxy-1-menthene (11),[14] 5-*p*-methene-1, 2-diol (12).[15]

## CONCLUSIONS

Terpenoids, especially sesquiterpenoids, are the important secondary metabolites from plants for their diverse bioactivities, and this type of compounds is the major chemical constituent in the genus *Ligularia*. Here, our phytochemical investigation on the whole plant of *L. kangtingensis* has led to the isolation of 12 known terpenoids, including two monoterpenoids, five sesquiterpenoids and five triterpenoids, from this plant for the first time.

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