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Chemical Composition, Antibacterial and Antioxidative Activities of Essential Oil from *Ligularia pleurocaulis*

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The genus *Ligularia* is known as folk medicine for their antibiotic, antiphlogistic and antitumor activities. The essential oil from the whole plant of *Ligularia pleurocaulis* was obtained by steam distillation and its chemical constituents were separated and identified by GC/MS. Antibacterial activities were evaluated by MTT colorimetric assay and antioxidative activity was screened spectrophotometrically through DPPH scavenging. Twenty seven compounds were identified from the essential oil, making up 81.94 % of the total contents. The essential oil showed moderate antibacterial activities against *Escherichia coli* and *Staphylococcus aureus* and weaker antibacterial activities against *Bacillus subtilis*. Meanwhile, its scavenging rate against DPPH was observed as 86.45 % in 40 mg/mL.

Keywords: *Ligularia pleurocaulis*, Essential oil, Antibacterial activity, Antioxidative activity.

INTRODUCTION

The genus *Ligularia* belongs to family Compositae. The roots and rhizomes of many *Ligularia* plants, called "Shanziwan" in traditional Chinese medicine, have been used for a long time as folk remedies for the action of eliminating phlegm and relieving cough[1]. The essential oils of several plants from this genus have already been reported[2-4].

Ligularia pleurocaulis (Franch.) Hand.-Mazz. is an indigenous plant in Northwestern Yunnan and Northwestern Sichuan and adapted to highlands around 4000 m[5]. The essential oil of this plant has not been investigated. Herein we report the chemical constituents of the essential oil from the whole plant of *L. pleurocaulis* and its antibacterial and antioxidative activities.

EXPERIMENTAL

The whole plant of *L. pleurocaulis* was collected from Kangding County, Sichuan Province, P. R. China, in August, 2010. The plant was identified by Qinmao Fang, a researcher from the Institute of TCM Medicinal Resources and Cultivation, Sichuan Academy of Chinese Medicine Science. A voucher specimen (No. LP1008) was deposited in the School of Life Science and Technology, University of Electronic Science and Technology of China.

HP-6890GC/5973MSD GC-MS spectrometry (America Hewlett-Packard Co. Ltd.), Model 680 Microplate Reader (America Bio-Rad); 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma-Aldrich, USA), 3-(4,5-dimethylthiahiazol-2-y1)-2,5-di-phenyltetrazolium bromide (MTT) (Amresco, USA), kanamycin (in sulfate, 98 %, Shijiazhuang Shuguang Pharmaceutical), ascorbic acid (Chengdu Kelong Chemical), others were all from domestic analytical reagent (AR).

Extraction of essential oil: Whole plant of *L. pleurocaulis* (100 g) was coarsely powdered and transferred into a 1000 mL flask with 400 mL water. Then it was submitted to steam distillation process, in a Clevenger's apparatus for 8 h[6]. The collected essential oil was subsequently dried by anhydrous sodium sulfate and stored at 4 °C in refrigerator.

Analysis of essential oil: Gas chromatography condition: HP6890GC/5973MSD GC-MS spectrometry, HP-INNOWax capillary column (50 m × 0.25 mm × 0.25 μm), the temperature program was 60-270 °C at a rate of 10 °C/min and injector temperature was 260 °C. The injected volume was 0.5 μL, helium was used as carrier gas, flow rate 1.2 mL/min; Mass spectrometry: EI ionization mode, 70 eV, scan range 20-450 amu, ion source temperature was 230 °C. Individual components were identified by matching their mass spectra with those of the spectrometer data base.

Antibacterial acitivity: The antibacterial activity of the essential oil was tested against *E. coli*, *S. aureus* and *B. subtilis* using LB medium. Inhibition rate for the essential oil against bacteria were determined by a colorimetric method using the dye MTT according to the reference[7,8]. Essential oil was

dissolved in DMSO to a concentration of 0.625, 1.25, 2.5, 5, 10, 20 mg/mL respectively. Kanamycin and DMSO were used as positive and black control separately. Inhibition rate (%) = [($OD_{570\ mm}$ of the sample-free well-$OD_{570\ mm}$ of wells that contained the sample)/$OD_{570\ mm}$ of the sample-free well] × 100 %.

Antioxidative activity: The antioxidative activity was measured by the DPPH method according to the reference[9,10]. Briefly, a 0.21 mM solution of DPPH in ethanol was prepared. A 10 µL solution of the essential oil in different concentrations (1.25, 2.5, 5, 10, 20, 40 mg/mL) was added to 150 µL of ethanolic DPPH solution. Ascorbic acid and ethanol were used as positive and blank control separately. The change in absorbance at 490 nm was measured at 0.5 h and converted into the percentage of antioxidative activity using the following formula: $[A_0 - (A_1 - A_s)]/A_0 \times 100$, where A_0 is the absorbance of the control solution containing only DPPH, A_1 is the absorbance of the DPPH solution containing samples and A_s is the absorbance of the sample solution without DPPH.

RESULTS AND DISCUSSION

The essential oil obtained by steam distillation gave a yield of 0.251 % (w/w). The spectrum and chemical constituents along with their retention time and percentage obtained from the GC-MS are given in Fig. 1 and Table-1. Twenty-seven compounds were identified by the comparison of their MS spectra with those of data base, comprised 81.94 % of the total essential oil, with predominance of sesquiterpenoids (67.63 %). β-eudesmol (11.76 %), γ-eudesmol (10.98 %) and calarene (7.87 %) were the main constituents identified. These results are consistent with the previous reports in which a large percentage of sesquiterpneoids were identified from the genus *Ligularia*.

TABLE-1
GC-MS DATA OF ESSENTIAL OIL OF *L. pleurocaulis*

Compounds	Retention time (min.)	Area (%)
γ-Selinene	8.03	1.96
9-Methyltetracyclo[7.3.1.0 (2.7).1 (7.11)] tetradecane	8.83	4.69
(–)Isocomene	9.02	3.29
(+)-Sativene	9.81	1.79
β-Caryophyllene	10.08	0.74
3,7-Guaiadiene	10.19	1.59
α-Humulene	11.46	0.79
Eremophilene	12.18	4.59
β-Selinene	12.42	3.98
α-Cedrene	14.03	0.41
β-Oplopenone	19.88	0.85
γ-Eudesmol	20.58	10.98
1,2,3,4,4a,5,6,8a-Octahydro-α, α,4a,8-tetramethyl-2-naphthalenemethanol	21.36	1.51
Eugenol	21.81	2.6
Calarene	22.14	7.87
β-Eudesmol	23.30	11.76
trans-Z-α-Bisabolene epoxide	23.76	0.86
4,8,8-Trimethyl-bicyclo[5.4.1]dodec-3-en-12-one	24.28	2.15
2-Decyne	24.73	4.70
6S-2,3,8,8-Tetramethyltricyclo[5.2.2.0 (1,6)]undec-2-ene	25.32	1.35
2-Isopropyl-4,7-dimethyl-1-naphthol	27.07	4.01
1-Deoxycapsidiol	27.47	0.73
Nerolidol	29.05	1.73
1,3-Dimethyl-2(2,4,6-trimethylphenyl)-1,3-cyclo-pentadiene	29.60	1.27
1-[2,3-Dihydro-2-(1-methylethenyl) benzofuran-5-yl]-ethanone	30.07	1.14
6-Methyl xanthotoxin	31.41	1.60
2-Methyl-4-phenyl-2,3-dihydro-1-benzofuran	34.95	3.00

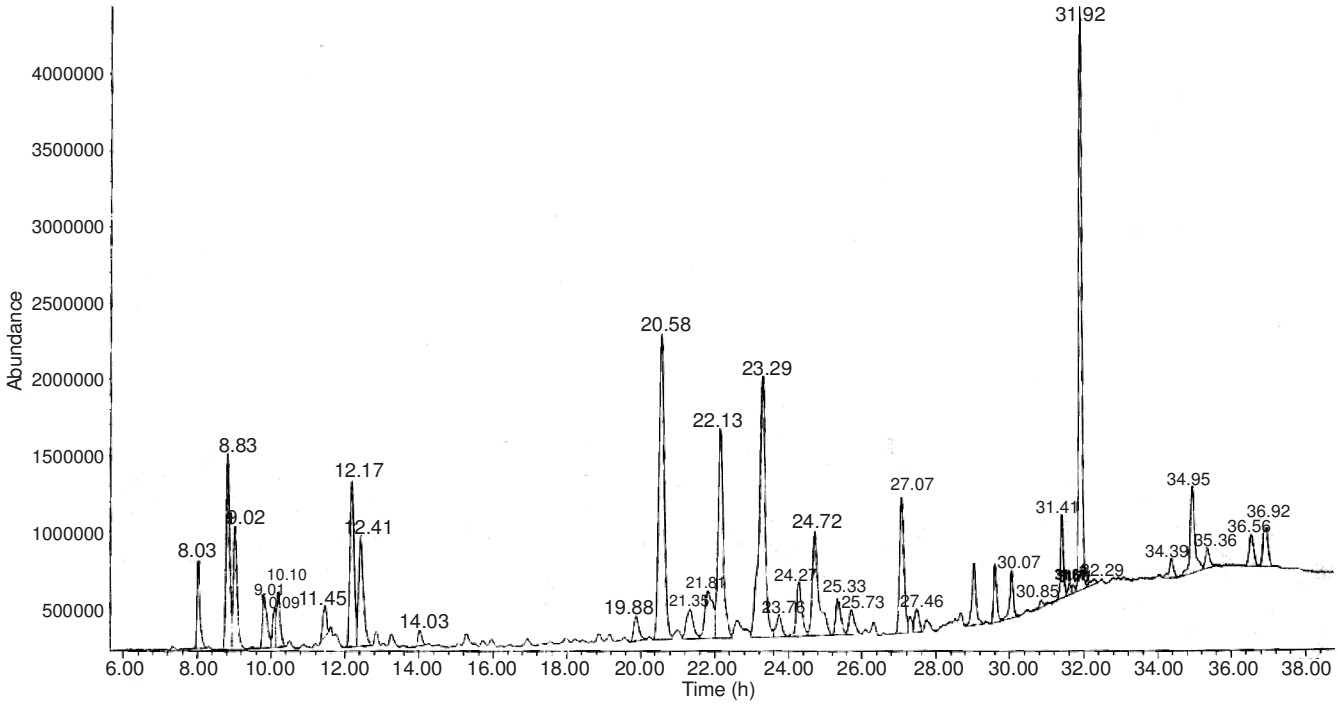

Fig. 1. GC-MS chromatogram of essential oil of *L. pleurocaulis*

The essential oil obtained from *L. pleurocaulis* was tested for its antibacterial activity against *E. coli, S. aureus* and *B. subtilis*. The results (Table-2) indicated that the essential oil had moderate antibacterial activities against *E. coli* and *S. aureus* and weaker antibacterial activities against *B. subtilis*. Several plants from the genus *Ligularia* were used as folk remedies for their antibacterial activities[1] and the results obtained here were consistent with the traditional usage.

TABLE-2
ANTIBACTERIAL ACTIVITY OF
ESSENTIAL OIL OF *L. pleurocaulis*

Concentration (mg/mL)		Inhibition rate (%)		
		E. coli	*S. aureus*	*B. subtilis*
Essential oil from *L. pleurocaulis*	20	47.18	50.78	16.61
	10	37.00	34.74	9.49
	5	17.69	46.87	5.14
	2.5	6.45	7.07	*
	1.25	4.71	0.59	*
	0.625	*	*	*
Kanamycin	0.3	77.83	59.96	12.76

* No results were observed

Free radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. The method of scavenging DPPH free radicals is sensitive, requiring only small amount of samples and allows testing of both lipophilic and hydrophilic samples. The essential oil obtained from *L. pleurocaulis* showed good DPPH free radical scavenging activity (Fig. 2) and the scavenging rate is up to 86.45 % at the concentration of 40 mg/mL. Meanwhile, the scavenging rate for ascorbic acid is 89.91 % at the concentration of 0.12 mg/mL.

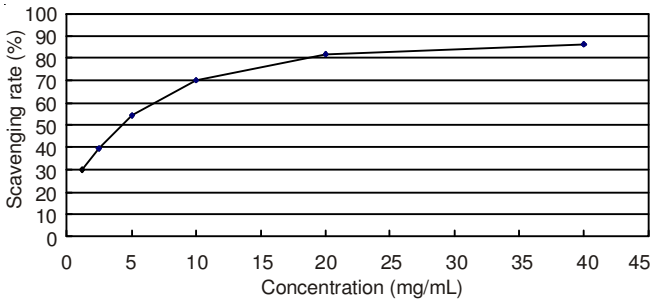

Fig. 2. Antioxidative activity of essential oil of *L. pleurocaulis*

Conclusion

To our best of knowledge, this is the first report on the essential oil of *L. pleurocaulis*. Sesquitepenoids were found to be the major compounds in the GC-MS analysis of this essential oil, which consistent with previous reports. The essential oil from *L. pleurocaulis* showed moderate antibacterial activities and good antioxidative activities in the bioactive test.

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