Journal of Pharmaceutical and Biomedical Analysis 52 (2010) 757–762

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Characterization of homoisoflavonoids in different cultivation regions of *Ophiopogon japonicus* and related antioxidant activity

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ARTICLE INFO

Article history:
Received 10 December 2009
Received in revised form 9 February 2010
Accepted 12 February 2010
Available online 18 February 2010

Keywords:
Ophiopogon japonicus
Homoisoflavonoids
LC–ESI–MS/MS
Antioxidant activity
Chemical profile

ABSTRACT

Homoisoflavonoids were identified in *Ophiopogon japonicus* of two cultivation regions in China: Hang-maidong (grown in Zhejiang province) and Chuan-maidong (grown in Sichuan province). Liquid chromatography (LC), coupled with electrospray ionization (ESI) tandem mass spectrometry (MS), was developed to analyze homoisoflavonoids in these two areas. Based on LC–MS/MS data, 24 homoisoflavonoid compounds—19 in Hang-maidong and 17 in Chuan-maidong—were identified or tentatively characterized. The homoisoflavonoids in the two regions were similar in chemical profile, but distinctive in their combination and ratio. Methylophiopogonanone A and methylophiopogonanone B were the major contributors to the total homoisoflavonoid content. Hang-maidong had a higher total homoisoflavonoid content and better DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical-scavenging activity than that of Chuan-maidong. The total phenolic content showed no significant difference between the two regions. This study allows a clear chemical differentiation of Hang-maidong from Chuan-maidong.

1. Introduction

Ophiopogon japonicus (Chinese name: Maidong) is one of the most frequently used Chinese herbs, whose tubers are considered effective in treating a wide range of disorders, mainly thrombosis, myocardial ischemia, arrhythmias, respiratory disease and hyperglycemia [1–3].

In China, the major cultivation regions of *O. japonicus* are Sichuan and Zhejiang provinces, which are popularly known as Chuan-maidong and Hang-maidong, respectively. They are recorded as two different species in Japan; Hang-maidong is recorded as a derivative of *O. chekiangensis* [4]. They are different in terms of growth region, planting conditions, growth period and processing, but Hang-maidong is generally considered superior [5]. It is well documented that the quantity and quality of herbs grown in different regions are significantly influenced by environmental and growing conditions [6]. Therefore, further study of the characteristics of the products grown in two different areas, as also their pharmacological study and quality evaluation, would be of great significance.

Homoisoflavonoids, reported to be responsible for biomedical activities such as antioxidant activity [7], inhibition of platelet aggregation [8], cough relief [2] and hyperglycemia [3], are one of the main bioactive components of *O. japonicus*. To date, 33 homoisoflavonoids have been isolated from *Ophiopogon* species [9,10]. HPLC fingerprint analysis has demonstrated the difference in the composition of homoisoflavonoids from two regions' products [11,12], HPLC–ESI–MS^n method has been used for the analysis of homoisoflavonoids in Chuan-maidong [13]. However, the compositional difference between the Maidong tubers grown in two different regions remains unclear.

The aim of this study was to characterize the homoisoflavonoid composition of *O. japonicus* from different cultivating areas and to compare their total homoisoflavonoid content. The antioxidant activity and total phenolic content were also determined, and the correlation between them was investigated.

2. Experimental

2.1. Reagents and chemicals

HPLC grade acetonitrile and methanol were produced by Merck (Darmstadt, Germany). Ultrapure water was prepared by Milli-Q system (Milford, MA, USA). 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) was obtained from Sigma–Aldrich (Shanghai, China), the data of elemental analysis showed carbon and nitrogen were 56.2% and 16.6%, respectively. Folin–Ciocalteu's reagent was obtained from Morlab (Shanghai, China). Gallic acid was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China); purity was above 98%. Acetic acid

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doi:10.1016/j.jpba.2010.02.016

(analytical reagent) was purchased from Nanjing Chemical Reagent Factory (Nanjing, China).

Methylophiopogonanone A, methylophiopogonanone B, 5,7,2′-trihydroxy-4′-methoxy-6,8-dimethyl homoisoflavanone, 6-aldehydo-isoophiopogonanone A, methylophiopogonone A, methylophiopogonone B and 5,7,2′-trihydroxy-3′,4′-methylenedioxy-6,8-dimethylhomoisoflavone were isolated and purified from *O. japonicus* and their structures were determined by chemical and spectroscopic methods. The purities of the standards were checked by HPLC-DAD and the results displayed purities of above 95%.

2.2. Sample preparation and extraction

Maidong tubers collected from the two regions were authenticated according to morphological characteristics by the authors. The dried tubers were ground and passed through a standard 60 mesh size.

2.2.1. Preparation of extracts for screening the homoisoflavonoid compounds

Powdered material (1 g) was thoroughly mixed with 25 ml methanol, and then the mixture was ultra-sonicated for 30 min. The extract was concentrated to dryness under reduced pressure and redissolved in water. The obtained solution was partitioned between diethyl ether and water to remove polar components. The diethyl ether layer was dried under reduced pressure and redissolved in 1 ml methanol. The solution was filtered through 0.45 μm membrane before use and a 20 μl aliquot was injected into the HPLC instrument for analysis.

2.2.2. Preparation of extracts for quantification of homoisoflavonoids

The sample solutions for quantitative purpose were prepared as recommended by Ye [14] with some modifications. Material (1 g) was ultra-sonicated with 25 ml chloroform–methanol (1:1) for 30 min and the extract was filtered. The filtrate was evaporated to dryness and the residue dissolved in 5 ml methanol. This solution was filtered through 0.45 μm membrane and 20 μl was injected into the HPLC instrument for analysis.

2.2.3. Preparation of extracts for total phenolic content and antioxidant activity assay

Material (1 g) was ultra-sonicated with 25 ml methanol for 30 min and then centrifuged at 1500 rpm for 10 min. The supernatant was collected for phenolic content and antioxidant activity measurements.

2.3. Identification of homoisoflavonoids

The analyses were performed on a Finnigan Surveyor HPLC instrument (Thermo Electron Corporation, CA, USA) equipped with a diode-array detector (DAD), an autosampler, and a column compartment. The extract, as described in Section 2.2.1, was separated on a 250 mm × 4.6 mm (5 μm) ZORBAX SB-C_{18} column (Agilent, Shanghai, China). The mobile phase consisted of (A) acetonitrile–methanol (90:10) and (B) water containing 0.2% (v/v) acetic acid. Solvent gradient condition was as follows: 41% A for the first 12 min, 44% A at 50 min, 65% A at 73 min, and then held at 65% A for another 22 min. The flow rate was 1 ml/min, and column temperature was set at 30 °C. The DAD detector was monitored at 296 nm, and the on-line UV spectra were recorded in the range of 190–400 nm.

The mass spectrometry detector used was Finnigan TSQ Quantum Ultra triple quadrupole tandem mass spectrometer equipped with an electrospray ionization (ESI) source operating under Xcalibur® 1.1 software. The ESI source was operated in the negative ion mode, as previously described [13]. The optimized parameters in the negative ion mode were as follows: ion spray voltage, 4.5 kV; sheath gas (N_2) pressure, 45 psi; auxiliary gas (N_2) pressure, 10 psi; capillary temperature, 320 °C; source CID, 10 V; collision energy, 25 V. The spectra were recorded over the range of m/z 100–500.

2.4. Determination of homoisoflavonoids

The quantitative analysis was performed on an Agilent 1100 HPLC system (Agilent Technologies, Waldbronn, Germany). The solvent system consisted of acetonitrile–0.2% acetic acid (63:37) at a flow rate of 1 ml/min. The detector was operated at 296 nm, and column temperature was set at 30 °C. The total acquisition time was 15 min.

2.5. Determination of total phenolic content

The total phenolic content was determined by Folin–Ciocalteu method [15] with some modifications. Methanol extract (1 ml), as described in Section 2.2.3, was added to 1 ml of 0.4 N Folin–Ciocalteu reagent; after 5 min, 5 ml sodium carbonate (10%, w/v) was added. The mixture was shaken and diluted with water to a final volume of 10 ml. After incubation for 2 h at room temperature, the absorbance was determined at 760 nm. The results were expressed as milligrams of gallic acid per 100 g of dry weight of plant material.

2.6. Antioxidant activity assay

The antioxidant activity assay was performed by measuring the DPPH radical-scavenging activity following the method of Brand-Williams [16]. A volume of 0.1 ml from appropriate solution of the extract was allowed to react with 3.5 ml of 0.06 mM DPPH methanolic solution for 30 min in the dark at ambient temperature. The absorbance was measured at 517 nm.

The scavenging percentage of DPPH was calculated by the following equation:

$$\text{Scavenging activity } (\%) = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Antioxidant activity was defined as the concentration (mg/ml) of samples necessary to decrease the initial radical concentration by 50% (EC_{50}).

3. Results and discussion

3.1. LC/MS homoisoflavonoid profiles in different cultivating regions

Total ion chromatograms (TIC) of *O. japonicus* from different areas are shown in Fig. 1. A comparative study of this data indicates that the differences in homoisoflavonoid profiles are consistent more in terms of quantity than of quality.

The LC–MS/MS analysis of *O. japonicus* grown in the two regions resulted in the identification of 24 homoisoflavonoids. Seven of them were identified based on the comparison of UV, chromatographic retention time and MS/MS data with those of authentic standards, and the rest based on UV and MS data. Six of them are reported for the first time from *Ophiopogon* species. The details of the identified components are summarized in Table 1 and Fig. 2.

It is interesting to note, from examining the known homoisoflavonoids isolated from *Ophiopogon* species, that there are pairs of analogs with the molecular weight (MW) difference

of 14. Their structures in A and C rings are the same, but differ only in the substitution of methylenedioxyl group at $C_{3'-4'}$ or methoxyl group at $C_{4'}$ in B-ring. In this study, six pairs of peaks, 6 vs. 7, 8 vs. 9, 12 vs. 13, 17 vs. 18, 19 vs. 20 and 15 vs. 16 showed highly contrasting differences in 14 Da. Peak 17 (t_R = 65.82 min) and peak 18 (t_R = 68.30 min) displayed $[M-H]^-$ ion at m/z 341 and 327, respectively. Their HPLC retention time and MS/MS fragments were consistent with those of methylophiopogonanone A and B, respectively. By comparing with the standards, peak 17 was identified as methylophiopogonanone A and peak 18 as methylophiopogonanone B. In HPLC separation, methylophiopogonanone A substituted by methylenedioxyl group at $C_{3'-4'}$ was eluted faster than methylophiopogonanone B with a methoxyl group at $C_{4'}$. The structural correlation between corresponding peaks offers a clue to the structural elucidation.

Peak 6 (t_R = 30.52 min) and peak 7 (t_R = 32.34 min) showed $[M-H]^-$ ion at m/z 357 and 343, respectively. Their UV spectra showed maximum absorption bands at 296 and 291 nm, respectively, which were characterized by saturated C_{2-3} bond homoisoflavonoids [13]. The ion m/z 357 gave the ion at m/z 222, which resulted from the cleavage of C_{3-9} bond. The m/z 222 produced m/z 207 by loss of CH_3 group, suggesting the existence of a methoxyl group in A-ring, and then m/z 207 yielded ion m/z 179 by loss of CO at C-4. In addition, the ion at m/z 153 was observed, which was derived from the simultaneous cleavage of C_2–O and C_{4-11} bonds and further loss of CH_3 group. As the presence of methoxyl group at C-8 could trigger the cleavage of C-ring, the methoxyl group probably took place at C-8. Therefore, peak 6 was tentatively identified as 5,7-dihydroxy-8-methoxy-3′,4′-methylenedioxy-6-methyl homoisoflavanone. Peak 7 displayed ions at m/z 222, 207, 179 and 153 by the same pathway as that of peak 6. Thus, peak 7 was tentatively characterized as 5,7-dihydroxy-8,4′-dimethoxy-6-methyl homoisoflavanone.

Peak 8 (t_R = 36.26 min) and peak 9 (t_R = 39.00 min) displayed $[M-H]^-$ ion at m/z 355 and 341, respectively. The MS/MS spectrum of peak 8 showed ions at m/z 340, 205, 178 and 150. Fragments of

Table 1
Identification of homoisoflavonoids in *Ophiopogon japonicus* from different cultivating regions.

Peak no.	t_R (min)	λ_{max} (nm)	$[M-H]^-$ m/z	MS/MS fragments (relative intensity, %)	Tentative identification	Hang-maidong	Chuan-maidong
1	15.10	288	373	358(2), 328(2), 222(20), 207(20), 183(55), 168(95), 153(100)	5,2′-Dihydroxy-7,8,4′-trimethoxy-6-methyl homoisoflavanone[b]	–	++
2	18.93	286	339	324(70), 311(2), 296(20), 295(100), 237(2)	5-Hydroxy-7,4′-dimethoxy-6,8-dimethyl homoisoflavone[b]	–	+
3	23.49	296	373	207(100), 179(4)	5,7,4′-Trihydroxy-3′,5′-dimethoxy-6,8-dimethyl homoisoflavanone[b]	–	+
4	26.68	296	343	207(100), 192(2), 179(10), 163(1)	5,7,2′-Trihydroxy-4′-methoxy-6,8-dimethyl homoisoflavanone[a]	+	++
5	29.36	295	359	344(25), 329(60), 222(90), 207(100), 169(20), 154(50)	Ophiopogonanone E[b]	+	++
6	30.52	296	357	339(100), 222(25), 207(95), 179(45),153(10)	5,7-Dihydroxy-8-methoxy-3′,4′-methylene-dioxy-6-methyl homoisoflavanone[b]	+	++
7	32.34	291	343	325(23), 310(15), 222(20), 207(100), 179(35), 153(10)	5,7-Dihydroxy-8,4′-dimethoxy-6-methyl homoisoflavanone[b]	+	+
8	36.26	291	355	340(30), 325(5), 205(100), 177(5)	5-Hydroxy-7-methoxy-3′,4′-methylenedioxy-6, 8-dimethyl homoisoflavanone[b]	+	+
9	39.00	284	341	326(5), 311(1), 205(100), 177(10)	5-Hydroxy-7,4′-dimethoxy-6,8-dimethyl homoisoflavanone[b]	+	–
10	39.98	265	355	327(3), 218(35), 205(25), 189(20), 152(2)	5,7,2′-Trihydroxy-3′,4′-methylenedioxy-6, 8-dimethyl homoisoflavone[a]	–	+
11	47.05	291	357	342(2), 327(4), 221 (10), 206(55), 178(25)	5,2′-Dihydroxy-7,4′-dimethoxy-6,8-dimethyl homoisoflavanone[b]	+	–
12	51.77	292	327	205(1), 192(20), 164(20), 136(2)	Ophiopogonanone A[b]	+	+
13	55.63	292	313	192(75), 164(40), 136(5)	5,7-Dihydroxy-4′-methoxy-6-methyl homoisoflavanone[b]	++	+
14	60.11	292	343	328(35), 207(100), 179(25), 151(15), 137(6)	5,7,4′-Trihydroxy-3′-methoxy-6,8-dimethyl homoisoflavanone[b]	+	–
15	62.46	265	339	311(100), 218(25), 205(15), 179(15), 161(5), 151(5)	Methylophiopogonone A[a]	–	++
16	64.35	265	325	310(70), 281(10), 204(5), 179(10), 163(5)	Methylophiopogonone B[a]	+	+
17	65.82	296	341	295(5), 206(25), 178(30), 163(2), 150(5)	Methylophiopogonanone A[a]	+++	+++
18	68.30	296	327	206(90), 178(55), 163(5), 150(5), 135(1)	Methylophiopogonanone B[a]	+++	+++
19	70.80	256	369	354(10), 341(20), 326(5), 219(95), 207(5), 191(75), 192(9), 164(6), 136(1)	Ophiopogonanone D[b]	++	–
20	72.96	256	355	340(12), 327(10), 312(5), 219(100), 206(5), 191(95), 164(8), 136(2)	8-Aldehydo-7-hydroxy-5,4′-dimethoxy-6-methyl homoisoflavanone[b]	++	–
21	80.30	273	353	325(100), 297(25), 204(30), 191(10), 165(8), 150(5)	6-Aldehydo-isoophiopogonone A[b]	+	+
22	82.40	274	355	327(20), 307(5), 281(3), 192(55), 164(10)	6-Aldehydo-isoophiopogonanone A[a]	+	+
23	83.69	283	353	325(55), 297(10), 281(2), 204(2), 191(1), 165(1)	Ophiopogonone C[b]	+	+
24	85.33	274	339	324(1), 311(30), 296(60), 183(16)	6-Aldehydo-isoophiopogonone B[b]	+	+

(+++) High ratio; (++) medium ratio; (+) low ratio or minor; (–) absent compound.
[a] Identification based on UV spectrum, MS/MS fragmentation pattern and chromatographic comparison with standard.
[b] Assignment based on UV spectrum and MS/MS fragmentation pattern.

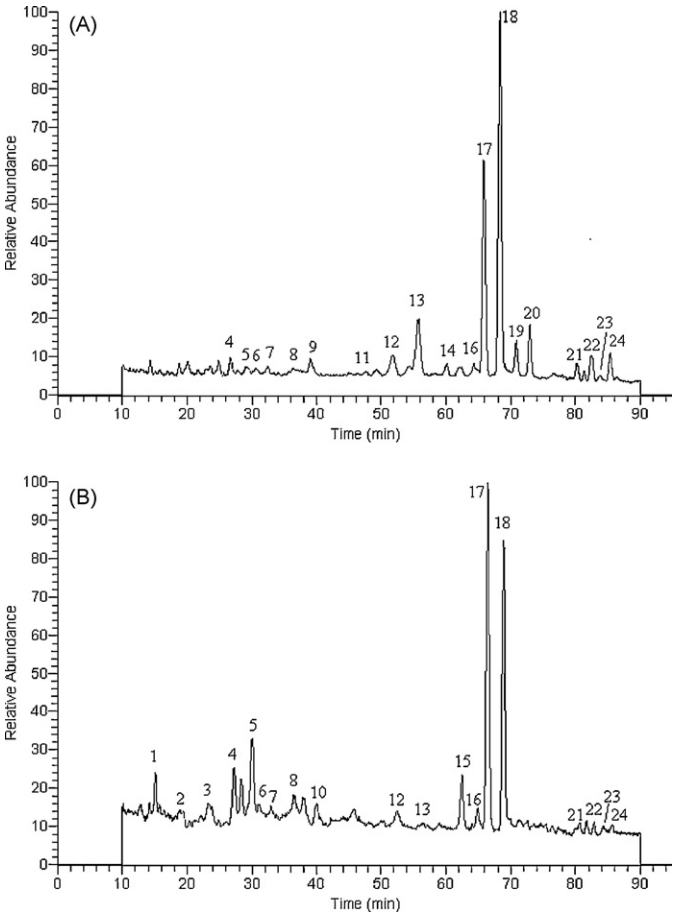


Fig. 1. LC–ESI–MS total ion current profiles in negative ion mode for the ether fraction of Hang-maidong (A) and Chuan-maidong (B). LC running conditions were described in Section 2.3. Conditions for the MS were as follows: ion spray voltage, −4.5 kV; sheath gas (N_2), 45 units; auxiliary gas (Ar), 10 units; capillary temperature, 320 °C.

m/z 340 and 205 indicated the loss of a methyl group and the cleavage of C_{3-9} bond, respectively. The m/z 205 ion then produced ion at m/z 177, which was attributed to the loss of CO at C-4. Peak 8 was thus identified as 5-hydroxy-7-methoxy-3′,4′-methylenedioxy-6,8-dimethyl homoisoflavanone. Similar fragmentations were observed from MS/MS spectrum of peak 9. Therefore, peak 9 was identified as 5-hydroxy-7,4′-dimethoxy-6,8-dimethyl homoisoflavanone.

Peak 19 (t_R = 70.80 min) and peak 20 (t_R = 72.96 min) displayed $[M-H]^-$ ion at m/z 369 and 355, respectively. The maximum absorption bands of both were at 256 nm. The ion m/z 369 gave the ion at m/z 341, involving the loss of CO. The m/z 341 then yielded ions at m/z 326, 219 and 207, through the loss of a CH_3 group, the cleavage of $C_{9-1'}$ and C_{3-9} bond, respectively. The m/z 207 produced m/z 192 by loss of CH_3 group, suggesting the presence of a methoxyl group in A-ring. The m/z 192 further underwent a loss of CO to yield an ion at m/z 164. By examining the known homoisoflavonoids, whose MW is 370, peak 19 was tentatively characterized as Ophiopogonanone D (8-aldehydo-7-hydroxy-5-methoxy-3′,4′-methylenedioxy-6-methyl homoisoflavanone). Moreover, the MS/MS fragmentations of peak 20 were quite similar to those of peak 19, and thus peak 20 was tentatively identified as 8-aldehydo-7-hydroxy-5,4′-dimethoxy-6-methyl homoisoflavanone.

A preliminary idea of the structural characteristics of homoisoflavonoids could be obtained from their on-line UV spectra. C_{2-3} saturated homoisoflavonoids show a maximum absorption band at 291–296 nm, and C_{2-3} double bond at 265 nm. When a C_6 formyl group is present, the band shifts to 273–274 nm. The results are in agreement with those previously reported [13].

The present results help in unraveling the structural characteristics of homoisoflavonoids in *O. japonicus*. Seventeen of the 24 compounds are C_{2-3} saturated and 7 C_{2-3} double bond. C_{2-3} saturated compounds are the most representative forms. Most C_{2-3} double bond compounds are found in lower concentrations except for peak 15. In addition, the homoisoflavonoids are characterized by 4′-methoxy or 3′,4′-methylenedioxy substitution. Eight compounds are 4′-methoxylated and 10 are 3′,4′-methylenedioxylated. According to the biosynthesis pathway of homoisoflavonoids and the formation of methylenedioxy bridge [17,18], 4′-hydroxylase, 3′-hydroxylase and 4′-O-methyltransferase may possibly appear in the two planting areas.

Nineteen of the 24 homoisoflavonoids were identified in Hang-maidong, 17 in Chuan-maidong and 12 in both. Different cultivating areas are characterized by different combinations and ratios of the components. The peaks 1, 2, 3, 10 and 15 were detected only in Chuan-maidong and the peaks 11, 14, 19 and 20 in Hang-maidong were not detected in Chuan-maidong. Moreover, most of the identified compounds in Hang-maidong are in lower concentrations except for peaks 13, 17, 18, 19 and 20. The major difference is attributed to the concentration and ratio of methylophiopogonanone A and B. In Chuan-maidong, methylophiopogonanone A (peak 17) is the most dominant constituent (ca. 28%), followed by methylophiopogonanone B (peak 18, ca. 21%), and in Hang-maidong, methylophiopogonanone B (peak 18, ca. 44%) followed by methylophiopogonanone A (peak 17, ca. 21%).

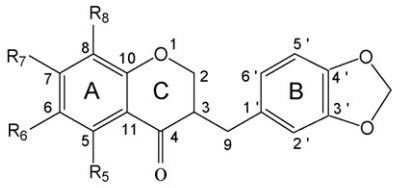
3.2. Homoisoflavonoids, total phenolic contents and antioxidant activity

The results in respect of homoisoflavonoids contents, total phenolic content and the DPPH antioxidant activity in each cultivation region are summarized in Table 2.

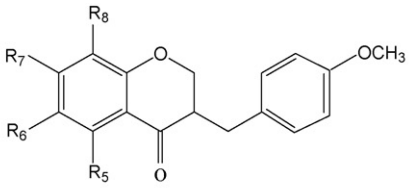
The contents of methylophiopogonanone A and B in Hang-maidong are respectively about 3-fold and 7-fold higher than those in Chuan-maidong. The ratios of methylophiopogonanone A and B in Hang-maidong and Chuan-maidong are about 1.5 and 0.5, respectively. The results are consistent with those of the previous report [19]. Methylophiopogonanone A and B are the major homoisoflavonoids in both cultivating regions. Therefore, Hang-maidong has higher total homoisoflavonoid content than Chuan-maidong. This may be due to the difference between the growth periods of Hang-maidong and Chuan-maidong. Hang-maidong is cultivated for two years, while Chuan-maidong is harvested in the second year. This implies that longer growth period promotes homoisoflavonoid formation.

The total phenolic content varied between 204.62 and 237.54 mg/100 g in Chuan-maidong, and 230.28 and 308.17 mg/100 g in Hang-maidong. There was no significant difference between the total phenolic contents of the two cultivating regions. Other phenolics were also found in *O. japonicus*. The total homoisoflavonoid content of Hang-maidong is higher than that of Chuan-maidong, and therefore Chuan-maidong possesses lower homoisoflavonoid to total phenolic ratio.

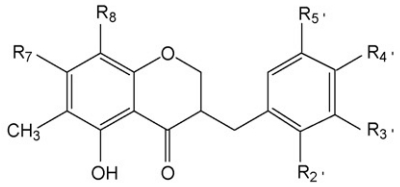
The antioxidant activity, expressed as EC_{50}, varied between 5.52 and 5.86 mg/ml in Chuan-maidong, and 3.64 and 4.78 mg/ml in Hang-maidong. Hang-maidong showed higher antioxidant effects than those of Chuan-maidong. The value of r^2 between total phenolic content and the antioxidant activity is 0.7401. The authors' previous work documented that methylophiopogonanone A and B showed no scavenging effect on superoxide anion, hydroxyl radical and hydrogen peroxide [7]. This suggests that the antioxidant activity is greatly influenced by the composition of phenolics. The



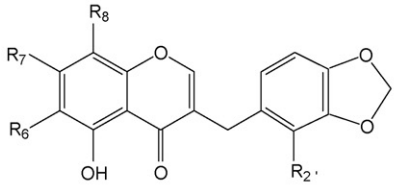
Peak No.	R_5	R_6	R_7	R_8
6	OH	CH_3	OH	OCH_3
8	OH	CH_3	OCH_3	CH_3
12	OH	CH_3	OH	H
17	OH	CH_3	OH	CH_3
19	OCH_3	CH_3	OH	CHO
22	OH	CHO	OH	CH_3



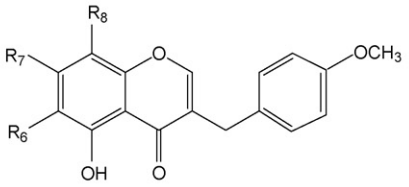
Peak No.	R_5	R_6	R_7	R_8
7	OH	CH_3	OH	OCH_3
9	OH	CH_3	OCH_3	CH_3
13	OH	CH_3	OH	H
18	OH	CH_3	OH	CH_3
20	OCH_3	CH_3	OH	CHO



Peak No.	R_7	R_8	$R_{2'}$	$R_{3'}$	$R_{4'}$	$R_{5'}$
1	OCH_3	OCH_3	OH	—	OCH_3	—
4	OH	CH_3	OH	—	OCH_3	—
5	OH	OCH_3	OH	—	OCH_3	—
11	OCH_3	CH_3	OH	—	OCH_3	—
3	OH	CH_3	—	OCH_3	OH	OCH_3
14	OH	CH_3	—	OCH_3	OH	—



Peak No.	R_6	R_7	R_8	$R_{2'}$
10	CH_3	OH	CH_3	OH
15	CH_3	OH	CH_3	H
21	CHO	OH	CH_3	H
23	CH_3	OH	CHO	H



Peak No.	R_6	R_7	R_8
2	CH_3	OCH_3	CH_3
16	CH_3	OH	CH_3
24	CHO	OH	CH_3

Fig. 2. Chemical structures of homoisoflavonoids isolated from *Ophiopogon japonicus*.

Table 2
Main homoisoflavonoids and total phenolic (TPC) contents and DPPH radical-scavenging activity (EC_{50}) of different cultivating regions.

Materials	Homoisoflavonoids (mg/100 g)		TPC (gallic ac. mg/100 g)	EC_{50} (mg/ml)
	MOA	MOB		
Chuan-maidong				
Mianyang, Sichuan	3.35 ± 0.15	2.13 ± 0.18	204.62 ± 11.10	5.52 ± 0.17
Mianyang, Sichuan	2.67 ± 0.18	1.57 ± 0.11	230.23 ± 7.32	5.66 ± 0.24
Mianyang, Sichuan	5.41 ± 0.23	3.66 ± 0.27	237.54 ± 5.82	5.86 ± 0.26
Hang-maidong				
Cixi, Zhejiang	10.08 ± 0.21	20.71 ± 0.66	308.17 ± 6.06	3.64 ± 0.11
Cixi, Zhejiang	9.76 ± 0.72	20.19 ± 0.81	278.82 ± 5.98	3.80 ± 0.18
Xiaoshan, Zhejiang	8.45 ± 0.64	15.62 ± 0.53	230.28 ± 9.05	4.78 ± 0.32

Values are expressed as means ± S.D. ($n = 3$). MOA: methylophiopogonanone A; MOB: methylophiopogonanone B.

phenolics of Hang-maidong show more active antioxidant effects than those of Chuan-maidong.

4. Conclusion

In this study, LC–DAD–MS/MS method was used for comprehensive identification and characterization of homoisoflavonoids in *O. japonicus* from two cultivating regions. It is found that the two regions contain similar types of homoisoflavonoids, but their composition and concentration are different. Hang-maidong has higher total homoisoflavonoid content and antioxidant activity than Chuan-maidong. The antioxidant activity is related more to the phenolics than to the homoisoflavonoid concentration. The differences in homoisoflavonoid profiles allow differentiation of products from two different regions, but the influence of these differences on the pharmacological action requires further investigation.

Acknowledgments

This study was financially supported by the National Natural Science Foundation of China (Grant No. 30672603) and National Key Technology R&D Program (Grant No. 2006 BAI09B05-6).

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