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Full Length Research Paper

Distribution of arctigenin in different organs of *Saussurea medusa* Maxim. from Qinghai-Tibetan plateau

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Saussurea medusa* Maxim (*S. medusa*) is a rare materia medica used by Tibetans in Chinese phytotherapy. The contents of arctigenin in different organs of *S. medusa* were examined. The samples were analyzed by high-performance liquid chromatography (HPLC). It was found that all samples contained arctigenin at concentrations ranging between 0.016±0.011 mg g^{-1} (Stem) and 7.329±0.360 mg g^{-1} (Seed), respectively. The content of arctigenin in seed was significantly higher than in other organs of *S. medusa*. Compared to distribution of arctigenin in different organs of *S. medusa*, arctigenin mainly exists in reproductive organs and in vegetative organs with trace of *S. medusa.

Key words: Liquid chromatography, arctigenin, organs, *Saussurea medusa* Maxim.

INTRODUCTION

Saussurea medusa Maxim (*S. medusa*) is a rare materia medica used by Tibetans in Chinese phytotherapy. The whole plant of *S. medusa* is widely used for the treatment of rheumatic arthritis, stroke, anthrax, febricity, detumescence and gynopathy (Fan and Yue, 2003; CPMH, 1995). The composition of *S. medusa* has been studied. The constituents are mainly composed of flavonoids, flavonoid glycosides, lignans, coumarins, essential oil, polysaccharides and other substances (Jia et al., 1986, 1989, 1984; DaWa et al., 2007; Li et al., 2002; Fan et al., 2003; Li and Cai, 1998; Midori et al., 2000; Hirano and Oka, 1994; Wang et al., 2008). Arctigenin is one of the major bioactive components in *S. medusa*. It has been reported to exhibit antioxidant, antitumor and anti-fatigue activity (Awale et al., 2006; Cho et al., 2004; Matsumoto et al., 2006; Takasakia et al., 2000; Zhao et al., 2009) and pharmacological properties such as effect on the induction of apoptosis and the putative pathways of its action in HL-60 and K562 cells (Wang et al., 2008). But the distribution of arctigenin in different organs of *S. medusa* has never been researched so far. It is important to study the distribution of arctigenin in *S. medusa* because we can learn about the distribution of lignans and it plays a very important role in the farming of medicinal plant, for example, if we know the distribution of target compounds, we can infer from harvest time and harvest parts from medicinal plant. Therefore, we can save time and money to get the target compounds in the industry. We now report an analytical comparison of arctigenin levels in different organs of *S. medusa* using high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Solvents, chemicals and standard

Methanol used for high performance liquid chromatography (HPLC) was of chromatographic grade (Yuwang Chemical Factory,

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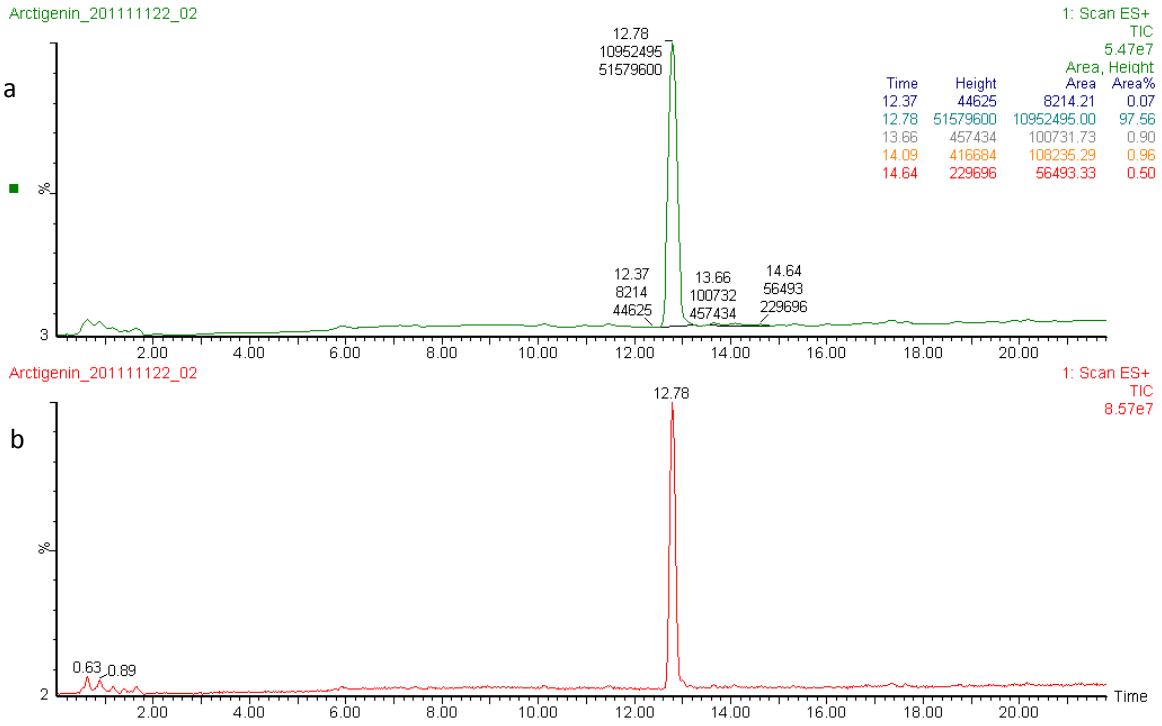

Figure 1. (a) The LC chromatogram of arctigenin (b) LC-MS chromatogram of arctigenin. The sample was accomplished with XterraMS column (150 × 4.6 mm i.d., 5 µm) with a column temperature of 25℃. Mobile phas e A was 0.1% v/v formic acid aqueous solution and mobile phase B 0.1% v/v formic acid acetonitrile solution. Gradient (% B): 0 to 30 min, 15 to 95%, 35 to 40 min, 95 to 95% and flow rate was1.0 ml min^{-1}.

Shandong, China), and the water was ultrapure water (18.25 MΩ/cm^2). All organic solvents used for preparation of crude extract were of analytical grade (Baishi Chemical Factory, Tianjin, China). Standard of arctigenin was obtained from chromatographic separation (Yu et al., 2011). HPLC-MS chromagram showed the purity of arctigenin more than 97% (Figure 1).

Plant materials

The whole plant of *S. medusa* was collected from Yeniugou of Qilian Mountains in Qinghai province of China in August, 2009. The different organs of whole plant from *S. medusa* were dried constantly at 60℃ and then pulverized to powder (abo ut 20-mesh) with a disintegrator.

Apparatus

The analytical system was a Waters 2695 HPLC system. The analytical column was XTerra MS C18 column (150 × 4.6 mm i.d., 5 µm, Waters, USA). The purity analysis, UV and MS analysis were performed on a Waters HPLC/Q-TOF system by Masslynx 4.1.

Sample preparation

All analyses were repeated at least three times, using the optimal method reported by our laboratory (Yu et al., 2010). Dynamic microwave extraction (DME) was performed on microwave apparatus (MG08S-2B microwave instrument, Nanjing Huiyan Microwave System Engineering Co., Ltd. Nanjing, PR China) using reflux system with ice water. Dry pulverized samples (2.0 g) in different organs of *S. medusa* were added methanol 100 ml and extracted for once 20 min in a 500 ml flat bottomed flask with a magnetic stirrer by microwave extraction. The pooled extracts were filtered in a Buchner funnel and transferred to be evaporated to dryness using rotary evaporator (N-1000D rotary evaporation, Shanghai Huixi Precision Instrument Co., Ltd. Shanghai, PR China) at 50℃ and dissolved in 50 ml volumetric flask wit h methanol, and then filtered with 0.45 µm membrane filter for LC analysis and 10 µl of the solution were injected to LC in triplicate.

Calibration

Arctigenin standard 16.8 mg were dissolved in a 50 ml volumetric flask with HPLC grade methanol. The concentration of arctigenin reached 0.336 mg ml^{-1} and filtered with 0.45 µm membrane filter for LC analysis. The injected volumes were 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µl. Every injection was auto-injected in triplicate at least. The linear regression analyses of peak areas at the various injection volume of arctigenin showed the calibration equation and correlation coefficient for the calibration curve.

Recovery and precision

Recovery experiments were performed by standard solutions at three volume levels (1, 2 and 3 ml, respectively) into 1.00 g of the pulverized seed of *S. medusa*. The fortified samples were then extracted and analyzed as the same methods. The recovery values

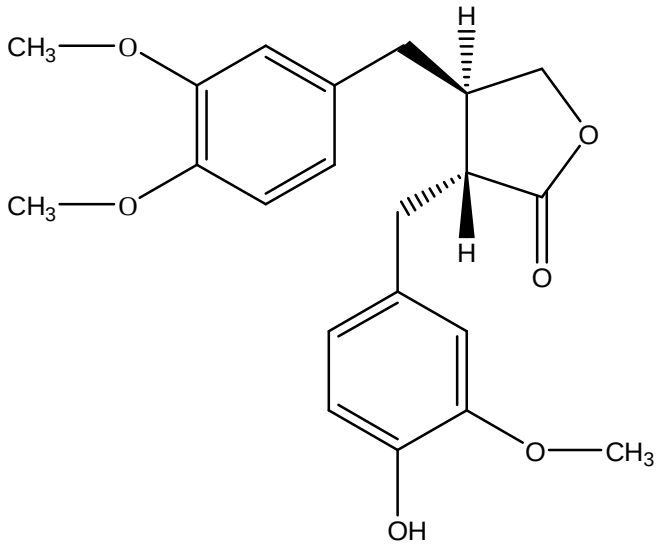

Figure 2. The chemical structure of arctigenin.

Table 1. Analytical results of the average recovery in seeds of *S. medusa.*

S/N	Known arctigenin content (mg g^{-1})	Determination of arctigenin in samples (mg g^{-1})	The average recovery (%)		RSD (%)
1	7.329	7.640	92.56		
2	7.329	7.637	91.67	93.25	2.17
3	7.329	7.650	95.54		
4	7.329	7.990	98.36		
5	7.329	7.970	95.39	97.42	1.81
6	7.329	7.991	98.51		
7	7.329	8.313	97.62		
8	7.329	8.278	94.15	96.96	2.63
9	7.329	8.328	99.11		

were obtained by comparing the results from samples and fortified samples. The precision of the chromatographic system was validated by injecting 10 µl standard solution 9 times in 18 h.

LC analysis

Agilent 1200 system consisted of four G1311A pumps; G1316A column temperature box; G1329A auto-sampler; G1315A detector. Agilent1200 technologies chemstation software was used. The samples *S. medusa* and standard of arctigenin were analyzed by LC. Analysis was accomplished with Phenomenex Fusion-Rp column (250 × 4.6 mm i.d., 4 µm) with a column temperature of 25℃. The mobile phase was methanol-0.2% phosphonic acid-water solution (50:50, v/v) and eluted at a constant flow rate of 1.0 ml min^{-1}. The effluents were monitored at 210 nm by a photodiode array detector. All samples were injected at least in triplicate into LC for analyses. Arctigenin standard was gained from chromatographic separation and structure identified by single x-ray diffraction (Yu et al., 2011). Area normalization method and external standard method was adopted to determine the purity of arctigenin. The result revealed that the purity of arctigenin is more than 97%. Figure 1a and b showed the LC-MS chromatogram of arctigenin. The chemical structure of arctigenin is shown in Figure 2.

RESULTS AND DISCUSSION

Peak identification was based on retention time and ultraviolet absorption spectra. For quantitative analysis, peak areas were used to calculate the amount of arctigenin present in different organs of *S. medusa*. The linear regression equation and correlation coefficient (r) was as follows:

$y = 4330.134x+179.310$ ($r = 0.9992$)

Analytical results of the average recovery (Table 1) in seeds of *S. medusa* showed that the average recovery of

Table 2. Analytical results of precision.

S/N	Area	RSD (%)	Retention time (min)	RSD (%)
1	1928596		12.76	
2	1927446		12.72	
3	1927794		12.75	
4	1917850	0.34	12.77	0.19
5	1924976		12.78	
6	1930123		12.79	
7	1931168		12.78	
8	1941588		12.79	
9	1928596		12.76	

Table 3. Content of acrtigenin in different organs in *S. medusa.*

Different organs	Content (mg g^{-1})
Root	0.029±0.013
Stem	0.016±0.011
Leaf	0.031±0.015
Bract	0.182±0.170
Calyces	0.218±0.130
Seed	7.329±0.360

added standard solutions at three volume levels (1, 2, 3 ml, respectively) was 93.25, 97.42 and 96.96%, and the relative standard deviation (RSD) was 2.17, 1.81 and 2.63%, respectively. Precision was evaluated by the relative standard deviation (RSD) value of retention times and peak areas.

The result showed a high precision (Table 2) of LC system with RSD 0.34% and the value of RSD of retention time was 0.19%. Obvious differences in contents of arctigenin were found in different organs (Table 3) of *S. medusa*. The results showed that all samples contained arctigenin between 0.016±0.011 mg g^{-1} (Stem) and 7.329±0.360 mg g^{-1} (Seed), respectively. The content of arctigenin exists in root, stem and leaf of *S. medusa* with trace. At the same time, the content of arctigenin gradually increased in bract, calyces, sharp rise and reached 7.329±0.360 mg g^{-1} in seed of *S. medusa*.

Conclusion

HPLC was successfully used to analyze the content of arctigenin in different organs from *S. medusa*. During the analysis process, recovery and precision were researched and results showed well. Compared to content of arctigenin in different organs of *S. medusa*, results showed that content of arctigenin increased from root to seed, arctigenin mainly exist in reproductive organs and in vegetative organs with trace of *S. medusa.*

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