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Full Length Research Paper

Variation in icariin and flavonoid contents of barrenwort species

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Flavonoid and icariin contents of five barrenwort species native to Sichuan, China, of the genus *Epimedium* were determined by means of HPLC and UV. The icariin and flavonoid contents of the root, stem and leaf differed significantly among five barrenwort species, and showed the same trend in the five barrenwort species and significantly differed from each other, that's leaf > root> stem, except icariin contents of the *E. davidii* and *E. chlorandrum*. Although only the leaf of five barrenwort species meet the standards, the root and stem still can be utilized for herbalist and manufacturing medicines. The icariin content extracted from long-term storage barrenwort was little available, but not flavonoid contents.

Key words: Barrenwort, icariin, flavonoid, Sichuan.

INTRODUCTION

Barrenwort is a perennial plant of the genus *Epimedium* L. (Berberidaceae) and is a popular botanical supplement used to improve menopausal symptoms and bone health, amongst other indications (Shen et al., 2008). Internationally more than 60 species are known as barrenwort, whereas about 50 are known in China (CCP, 2005).

These occur mostly in the southwestern China. Sichuan is one of the most important centers of diversity of the genus *Epimedium*.

The plant is effective in strengthening kidneys and curing rheumatism, is widely used in the treatment of osteoporosis, hypertension, coronary heart disease (Guo, 1996), and to strengthen immunity, prevent caducity, and cure cancer (Xue et al., 2005; Zhang et al., 2002). It is already known that the most prominent medicinal compounds in the genus are flavonoids including icariin and are used to monitor quality of barrenwort as defined by the Committee of China Pharmacopoeia (CCP, 2005). Icariin and flavone were reported to enhance the osteogenic differentiation of rat primary bone marrow stromal cells (Chen et al., 2005), increase osteoblastic proliferation (Meng et al., 2005), reduced osteoclastic bone resorption (Yu et al., 1999) and to increase mineral content, and prevent osteoporosis in ovariectomized rats (Zhang et al., 2006; Shen et al., 2008).

Many barrenwort species are used in Chinese medicine (Zhang et al., 1995; Xie and Sun, 2006). They can vary in their icariin and flavonoid contents and because raw and processed materials can be mixed from different species, the quality of medicinal preparations is not assured (Sheng et al., 2008). So far, most of the research on *Epimedium* has focused on extracting and identifying novel compounds. Li and Liu (1988) described the isolation and chemical characterization of novel flavonoid compounds from *E. davidii.* Chen et al. (2007) studied Simultaneous determination of 15 flavonoids in *E. brevicormu*, *E. sagittatum* and *E. davidii* using pressurized liquid extraction and high-performance liquid chromatography. And Wu et al. (2008) reported simultaneous determination of seven flavonoids in *E. brevicormu*, *E. sagittatum* and *E. davidii* by liquid chromatography–tandem mass spectrometry method. Zhang et al. (2008) studied simultaneous extraction of epimedin A, B, C and icariin from *E. brevicormu*, *E. sagittatum, E. flavum* by ultrasonic technique. However, there are few reports on the variation in flavonoid and icariin contents of many *Epimedium* species native to

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Table 1. Five species of barrenwort used in the study.

Species	Native to	Plant number	Sample	Symbol
			Root	BRr
E. brevicormu	Nanchong, Sichuan	10	Stem	BRs
			leaf	BRl
			Root	SAr
E. sagittatum	Nanchong, Sichuan	7	Stem	SAs
			leaf	SAl
			Root	DAr
E. davidii	Baoxing, Sichuan	12	Stem	DAs
			leaf	DAl
			Root	CHr
E. chlorandrum	Baoxing, Sichuan	6	Stem	CHs
			leaf	CHl
			Root	FLr
E. flavum	Tianquan, Sichuan	11	Stem	FLs
			leaf	FLl

Sichuan, China. And according to the Chinese Pharmacopoeia (CCP, 2005), many researches used the dried aerial parts (leaf) of *Epimedium* (family Berberidaceae), but underground parts of *Epimedium* were usually collected as medicinal materials by herbalist. So, this paper describes flavonoid and icariin contents of the dried aerial (leaf, stem) and underground parts (root) of five barrenwort species native to Sichuan, China, using high-performance liquid chromatography (HPLC) and ultraviolet (UV). This can provides some clues on the quality of whole-plant, rational use of wild *Epimedium* resource and directing the cultivation.

MATERIALS AND METHODS

We collected five barrenwort species from field, and were divided into root, stem, leaf respectively (Table 1). We obtained 90 samples from barrenwort species.

Determination of icariin and flavonoid contents

Icariin and flavonoid contents were estimated by following the standard methods (CCP, 2005) recommended for studies using HPLC and UV.

All analyses of icariin were performed on an Agilent Series 1100 (Agilent Technologies, USA) LC/MSD Trap system, equipped with a vacuum degasser, a quaternary pump, an autosampler, a column compartment, a diode-array detector and an ion-trap mass spectrometer with electrospray ionization interface, controlled by Agilent LC/MSD Trap Software. A Zorbax SB-C18 column (250 × 4.6 mm I.D., 5 ìm) was used. The mobile phase consisted of acetonitrile and water (70:30). The flow rate was 1.0 ml/min and the injection volume was 10ìl. The column temperature was maintained at 30°C. The analytes were monitored at 270 nm.

Flavonoid are test by UV-2450 (SHIMADZU, Japan), the analytes also were monitored at 270 nm.

Statistical analyses

All data were analyzed using one way ANOVA with Duncan analysis as a posterior test and Nonparametric test with SPSS11.0 software.

RESULTS

Variation in icariin content and flavonoid content among five barrenwort species

Nonparametric-test for pooled data on icariin and flavonoid contents among five barrenwort species (Table 2).

Table 2 shows that the icariin and flavonoid contents of the root, stem and leaf differed significantly among five barrenwort species ($P < 0.05$). Firstly, the icariin content of BRr is much higher than other four, and the CHr have the lowest icariin content. While the flavonoid contents of CHr is much more than other four, the flavonoid contents of DAr is lowest among them. Secondly, DAs contained the greatest amount both in icariin and flavonoid content, whereas SAs and BRs contained less in icariin and flavonoid content respectively. Lastly, DAl recorded the highest icariin and flavonoid content, while the SAl and CHl recorded the lowest icariin and flavonoid content respectively.

Variation in icariin and flavonoid contents about aerial and underground parts of five barrenwort species

In five barrenwort species, one way ANOVA indicated the icariin content are rich in the leaf, significantly differed from the root and stem (Figure 1) ($P < 0.01$), and the stem have much less icariin content, except the *E. davidii*

Table 2. Variation in icariin content and flavonoid content among five barrenwort species.

Sample	N	Icariin content Mean ± S.E (%)	Chi-square	Sig.	Flavonoid content Mean ± S.E (%)	Chi-Square	Sig.
BRr	10	0.243±0.008			4.643±0.077		
SAr	7	0.072±0.002			4.529±0.067		
DAr	12	0.169±0.011	12.833	0.012	4.133±0.199	10.333	0.035
CHr	6	0.047±0.001			4.711±0.003		
FLr	11	0.174±0.018			4.130±0.125		
BRs	10	0.036±0.001			1.014±0.021		
SAs	7	0.013±0.001			1.260±0.070		
DAs	12	0.202±0.030	13.5	0.009	3.028±0.210	13.233	0.01
CHs	6	0.056±0.005			1.538±0.091		
FLs	11	0.091±0.012			2.541±0.081		
BRl	10	0.350±0.004			5.461±0.064		
SAl	7	0.146± 0.005			6.067± 0.414		
DAl	12	0.557±0.002	13.5	0.009	8.639±0.169	13.033	0.011
CHl	6	0.220±0.017			5.372±0.127		
FLl	11	1.030±0.021			9.949±0.443		

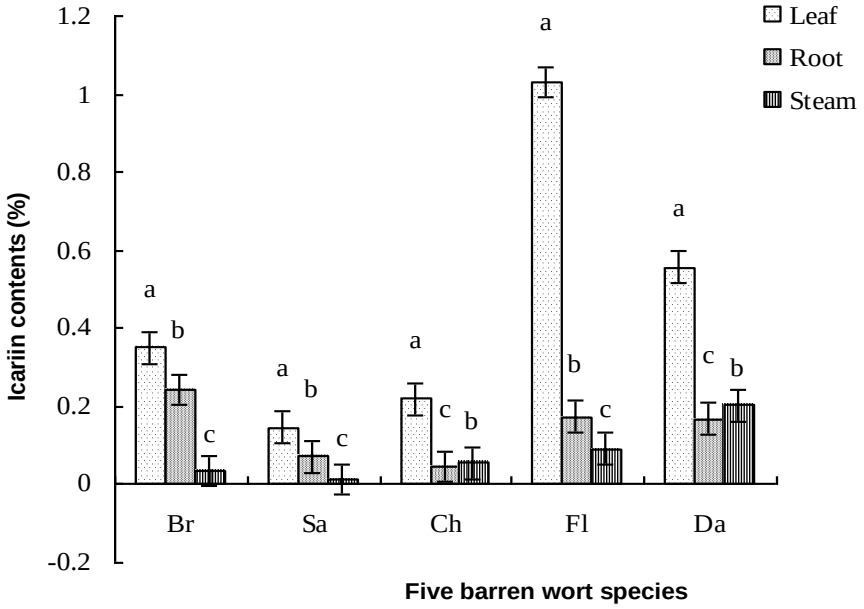

Figure 1. Variation in icariin about aerial and underground parts of five barrenwort species (The bars indicate mean ± SE, and the different letters indicate significant differences according the test at $P < 0.05$).

and *E. chlorandrum.*

The change trends of flavonoid (Figure 2) contents about aerial and underground parts were the same in the five barrenwort species, that's leaf > root> stem, and were remarkably different from each other respectively ($P < 0.01$).

Variation in flavonoid and icariin contents between different deposit time of *E. brevicormu* and *E. sagittatum*

Nonparametric-test for pooled data on icariinand flavonoid (Figure 4) contents between different deposit

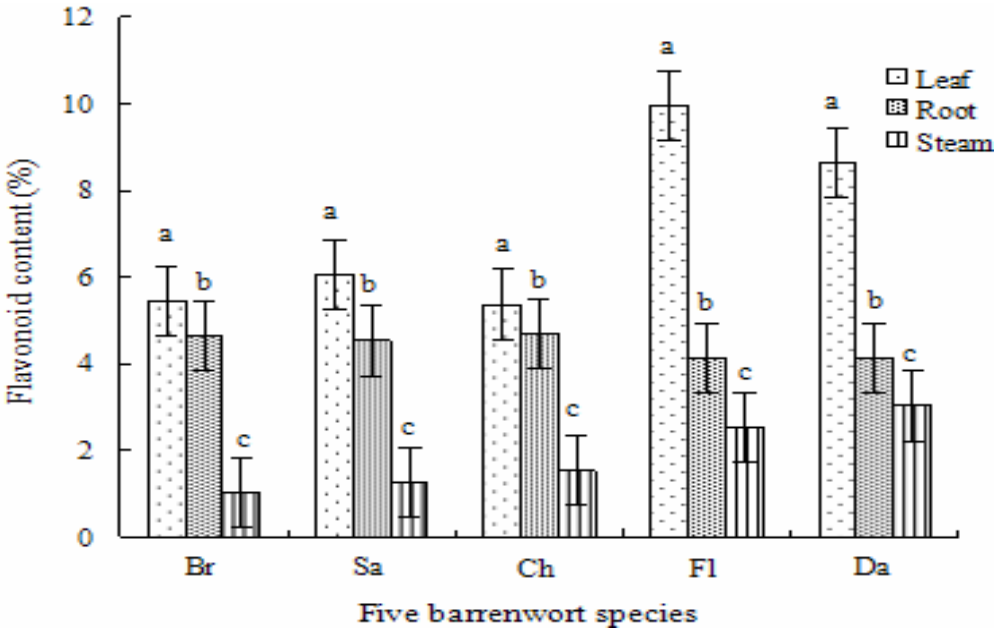

Figure 2. Variation in flavonoid about aerial and underground parts of five barrenwort species.

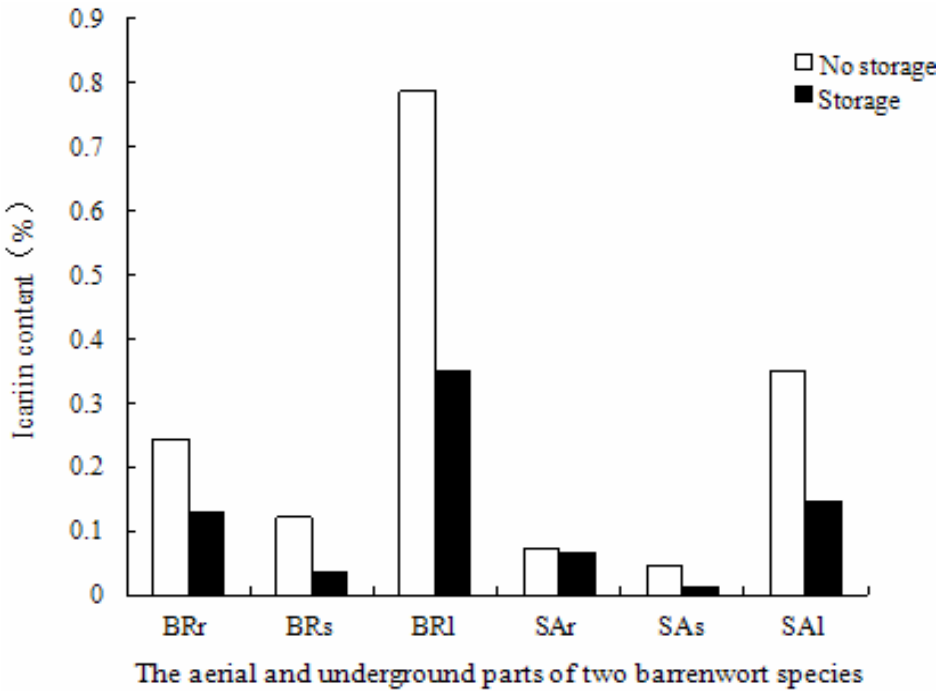

Figure 3. Variation in icariin contents in two treatments of *E. brevicormu* and *E. sagittatum.*

time (freshly and one years later) of *E. brevicormu* and *E. sagittatum*.

It is clear that the fresh *E. brevicormu* and *E. sagittatum* contained the greatest amount icariin contents, and are significantly higher than those stored for a years at room temperature ($P < 0.05$) (Figure 3).

The average flavonoid contents of the aerial and underground parts in *E. brevicormu* and *E. sagittatum* are

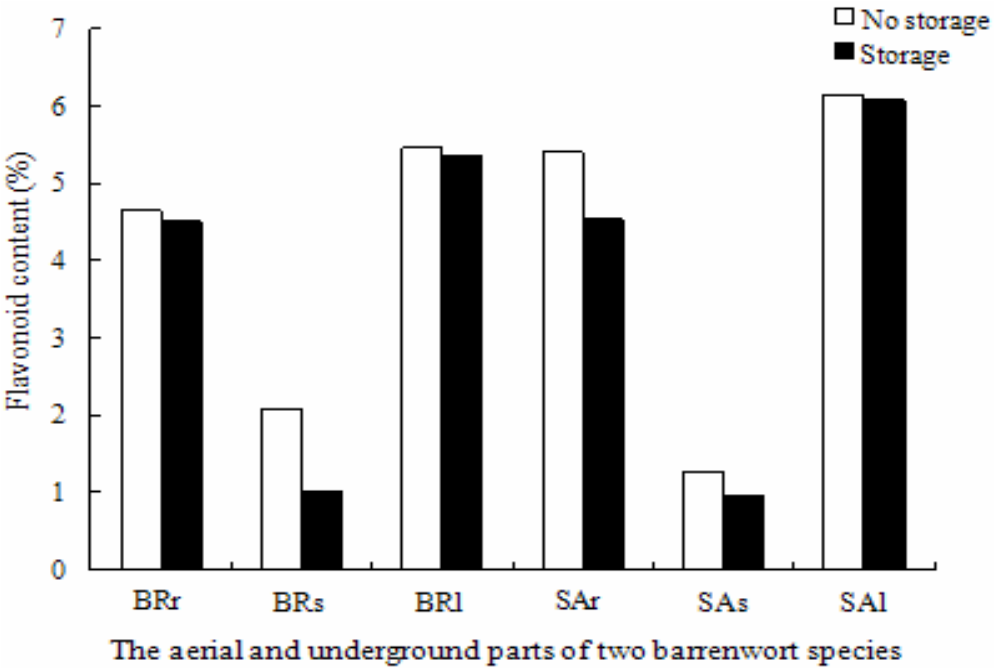

Figure 4. Variation in flavonoid contents in two treatments of *E. brevicormu* and *E. sagittatum.*

remarkably higher than those stored for a years at room temperature ($P < 0.05$), except for BRr, BR and SAl, which showed no significant difference between the no storage and storage ($P > 0.05$) (Figure 4).

DISCUSSION

Different barrenwort species can vary in their icariin and flavonoid contents (Sheng et al., 2008). We studied as many as five barrenwort species native to Sichuan, China, and discovered that the icariin and flavonoid contents of the root, stem and leaf differed significantly among five barrenwort species. The BRr, DAs and DAl contained the greatest amount in icariin content and flavonoid contents were rich in the CHr, DAs and DAl. The icariin and flavonoid contents about aerial and underground parts showed the same trend in the five barrenwort species and significantly differed from each other, that's leaf > root> stem, except icariin contents of the *E. davidii* and *E. chlorandrum*. Based on the standard laid down by the Chinese Pharmacopoeia (2005) requires that flavonoid content as determined by UV is more than 5.0% and icariin content as determined by HPLC is more than 0.5%. We found that only the leaf of five barrenwort species meet the standards, whereas the icariin and flavonoid contents of root and stem do not meet the standards. But both the root and stem, especially the root still can be commercially utilized for herbalist and manufacturing medicines.

Five species of *Epimedium*, namely *E. wushanense*, *E. sagittatu*m, *E. koreanum* Nakai, *E. pubescens* Maxim, and *E. brevicornum* Maxim embody the medicinal species of this genus according to the Chinese Pharmacopoeia. Because of their higher flavonoid and icariin contents, abundance in nature (Takahashi, 1989), and extensive distribution (Wang et al., 2001; Zhang et al., 2002), resulting in great scarcity of naturally occurring Chinese medicinal plants (Lubell and Brand, 2005; Xie and Sun, 2006). Our study indicated *E. davidii* and *E. flavum* are richer in flavonoids and icariin than *E. sagittatu*m and *E. brevicornum* in the leave. So, there exists some high-quality *Epimedium* of the species that can meet the standards even were not recorded by the Chinese Pharmacopoeia. These wild *E. davidii* and *E. flavum* may prove to possess great medicinal value.

Although pure icariin is very stable (Li et al., 1996), we found highly significant differences between the icariin content of freshly prepared samples and of those stored for one year at room temperature, the result was analogous to *E. acuminatum* (Sheng et al., 2008). But the icariinof *E. acuminatum* decomposed was faster than *E. brevicormu* and *E. sagittatum*. This may be caused by the different materials, and the icariin of *E. acuminatum* stored in the solvent, while *E. brevicormu* and *E. sagittatum* were crushed during storage. Flavonoid content of the leave in *E. brevicormu* and *E. sagittatum* showed no significant difference between freshly prepared samples and of those stored. Our study suggested that flavonoid content barrenwort could be stord in a long-term.

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