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Variation in effective composition contents of *E.davidii*

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ABSTRACT

The effective composition content of *E.davidii* were determined in four habitats by High-Pressure Liquid Chromatography (HPLC) and utraviolet-visible (UV), and the different of morpholofical parameters and effective content were analyed by one-way ANOVA statistical analysis. The results showed that both the flavonoid anicariin contents in intensive light were richer than the wide in faint light. So increased light intensity in cultivated *E. davidii* can promote effective components improvement. According to the standard of Chinese Pharmacopoeia, the icariin content of root and stem in *E. davidii* in the wild can not meet the standard of icariin content, but the leaf in habitat 3 can meet the standard of icariin content. In flavonoid content of icariin content, the leaf only can reach the standard of flavonoid content. So, the flavonoid content of *E. davidii* could alternate for the five genuine species to medicine.

KEYWORDS

E.davidii; Effective composition; Icariin.

INTRODUCTION

Epimedium, a genus of Berberidaceae, is a plant group from which a botanical supplement is widely used as a tonic, aphrodisiac and antirheumatic in China, Japan and Korea. *Epimedium* extract can strengthen immunity and has been proven effective against osteoporosis and cardiovascular diseases (Wang & Huang, 2005; Meng, 2005). 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 the natural resources of medicinal *Epimedium* species have been declining dramatically due to over-harvesting and curtailment of habitat over the past several decades, and the plants have become more scarce recently (Ward 2004; Xu et al. 2007). There are urgent to find new *Epimedium* resources to replace the five *Epimedium* species in Chinese Pharmacopoeia. But there are few reports on the other wild *Epimedium*, so we study the flavonoid and icariin contents of *Epimedium davidii* in four habitat, which provides some clues on the quality of cultivated and wild *Epimedium*.

MATERIALS AND METHODS

This study was carried out from April 2009, and collected five plants of wild *E. davidii* respectively in four habitat from a protected area of BaoXing (E 102°53', N 30°36'), northeastern Sichuan, China. All the collected materials used to analyse the effective composition contents. The differences environmental factors were determined in TABLE 1.

TABLE 1 : Environment factors in four habitats

Item	Habitat 1	Habitat 2	Habitat 3	Habitat 4
Elevation (m)	1713	1705	1743	1751
PH value	5.50	7.04	8.03	6.02
Relative light (%)	31.00	32.5	100	38
Relative humidity (%)	90	85	50	79

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

Determination of icariin

All analyses of icariin were performed on an Agilent Series 1100 (AgilentTechnologies, 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 (250mm×4.6mm I.D., 5 μm) 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 10μl. The column temperature was maintained at 30 °C. The analytes were monitored at 270 nm.

Flavonoid contents

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

Statistical analyzes

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

RESULTS

Variation in icariin content and flavonoid content in *E. davidii*

(1) Variation in icariin content in *E. davidii*

One way ANOVA indicated the icariin content are rich in the leaf, significantly differed from the root and stem of *E. davidii* in four habitat (TABLE 2) ($P<0.01$). The icariin content of the root and stem have no significant in *E. davidii* of habitat 1, 2 and 3, but the icariin content of the root significantly differed from stem in *E. davidii* of habitat 4.

TABLE 2 : Variation in icariin content in *E.davidii*

Habitat	Sample	Mean	F	Sig.
	Root	0.18±0.00b	63.98	0.00
1	Stem	0.18±0.01b		
	Leaf	0.45±0.03a		
	Root	0.17±0.01b	561.00	0.00
2	Stem	0.16±0.01b		
	Leaf	0.49±0.01a		
	Root	0.18±0.00b	152.27	0.00
3	Stem	0.20±0.03b		
	Leaf	0.55±0.00a		
	Root	0.17±0.01b	1058.14	0.00
4	Stem	0.10±0.00c		
	Leaf	0.46±0.01a		

(2) Variation in flavonoid content in *E. davidii*

It is clear that the leaf of *E. davidii* contained the greatest amount flavonoid content, and are significantly higher than the root and stem, and the root are remarkably higher than stem in *E. davidii* of four habitats (TABLE 3).

TABLE 3 : Variation in flavonoid content in *E. davidii*

Habitat	Sample	Mean	F	Sig.
	Root	4.71±0.00b	885.13	0.00
1	Stem	2.93±0.02c		
	Leaf	8.27±0.16a		
	Root	4.13±0.13b	805.08	0.00
2	Stem	2.83±0.10c		
	Leaf	8.64±0.10a		
	Root	4.67±0.03b	254.26	0.00
3	Stem	3.64±0.29c		
	Leaf	9.18±.12a		
	Root	4.27±0.05b	1226.79	0.00
4	Stem	2.69±0.09c		
	Leaf	8.41±0.11a		

Variation in icariin content and flavonoid content among four habitats

(1) Variation in icariin content among four habitats

Figure 1 shows that the icariin contents of the root have no difference among the four habitats, the icariin contents of stem in habitat 1, 2and 3 are richer than the habitat 4, and the icariin contents of leaf in habitat 3 differed significantly among four habitats (P <0.05).

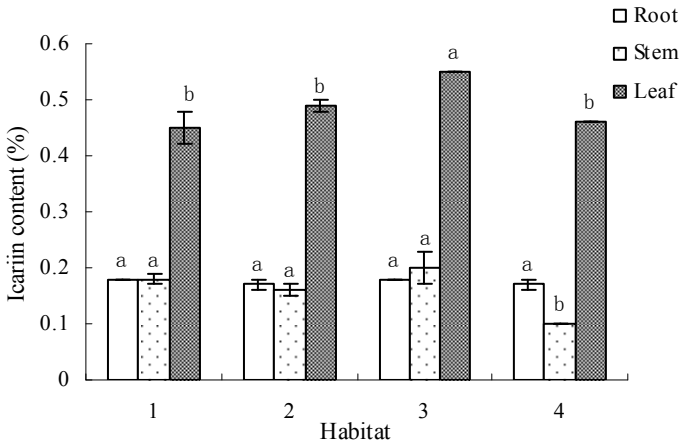

Figure 1 : Variation in icariin among four habitat (The bars indicate mean±SE, and the different letters indicate significant differences according the test at P<0.05)

(2) Variation in flavonoid content among four habitats

Firstly, the flavonoid content of the root in habitat 1 and 3 are significant than habitat 2 and 4. In the stem and leaf, the habitat 3 have much more icariin content than other habitats (Figure 2).

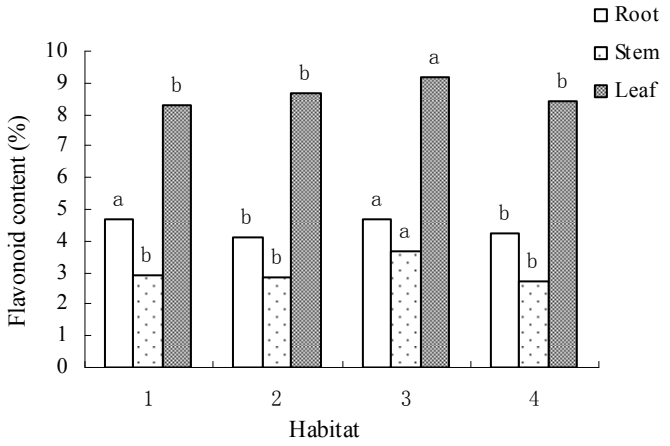

Figure 2 : Variation in flavonoid content among four habitats

DISCUSSION

The light intensity directly influenced medicine quality of the *Epimedium*, the content flavonoid and icariin was higher under intensive light than in faint light (Dong et al., 2003). The flavonoid and icariin content in the Cultivated *E. pubescens* and *E. wushanense* in intensive light are richer than the wild in faint light (Quan et al., 2011). In our study, both the flavonoid and icariin content in intensive light are richer than the wild in faint light. So, increased light intensity in cultivated *E. davidii* can promote effective components improvement.

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%. Our study indicate that the icariin content of root and stem in *E. davidii* in the wild can not meet the standard of icariin content, but the leaf in habitat 3 can meet the standard of icariin content according to the Chinese Pharmacopoeia. But the icariin contents in leaf are lower than in the research of previous study (Quan et al., 2010)

because of difference in harvesting season. In flavonoid content of icariin content, the leaf only can reach the standard of flavonoid content. So, the flavonoid content of *E.davidii* could alternate for the five genuine species to medicine.

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