Journal of Medicinal Plants Research Vol. 5(29), pp. 6523-6527, 9 December, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals
DOI: 10.5897/JMPR11.602

Full Length Research Paper

Comparative analysis of morphological characteristics and effective composition content of wild and cultivated *Epimedium pubescens* and *Epimedium wushanense* (Berberidaceae)

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Accepted 19 July, 2011

The effective composition content were determined in Cultivated and wild of *Epimedium pubescens* and *Epimedium wushanense* by High-Pressure Liquid Chromatography (HPLC) and ultraviolet-visible (UV), and the difference of morphological parameters and effective composition content were analyzed by one-way ANOVA statistical analysis. The results showed that because of the relative light intensity increased, the dwarf phenomenon was occurred in Cultivated *E. pubescens* and *E. wushanense*, but the number of branches and leaf number per plant in cultivated were more than wild, and increased the output of two kinds of *Epimedium.* According to the standard of Chinese Pharmacopoeia, both wild and cultivated leaves the icariin contents of *E. pubescens* can meet the standard. Insignificant difference in flavonoid contents between the cultivated and wild leaves of *E. wushanense*, but both can meet the standard, both wild and cultivated all other parts of *E. wushanense* cannot meet the standard of icariin contents, which may be related to harvesting time.

Key words: *Epimedium pubescens*, *Epimedium wushanense*, wild and cultivated, effective composition, morphological characteristics.

INTRODUCTION

Epimedium, a genus of Berberidaceae, has more than 60 species distributed around the world, whereas about 50 are known in China (CCP, 2005). A plant of the genus is a well-known medicinal plant as well as potential ground cover and ornamental plant (Xu et al., 2007). Five species of *Epimedium*, namely *E. wushanense*, *Epimedium sagittatu*m, *Epimedium koreanum* Nakai, *E. pubescens* Maxim, and *Epimedium 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 many *Epimedium* research about the chemical constituents, extraction methods, domestication cultivation (Chen et al., 2007; Wu et al., 2008; Zhang et al., 2008; Quan et al., 2010). However, there are few reports on cultivated and wild *Epimedium* (Sheng et al., 2008), especially morphological Characteristics. Therefore, this paper study the changes of morphological Characteristics and efficient medicinal components from cultivated and wild *E. pubescens* and *E. wushanense*, which provides some clues on the quality of cultivated and wild *Epimedium* and on breeding *Epimedium*.

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Abbreviations: HPLC, High-pressure liquid chromatography; **UV,** ultraviolet-visible.

Table 1. Habitats of cultivated and wild of *E. pubescens* and *E. wushanense.*

Item	Wild *E. pubescens*	Wild *E. wushanense*	Botanic garden of china west normal university
Locality	106°28′02.6″E, 30°45′49.8″N	106°27′50.1″E, 30°45′56.1′N	106°02′22.3″E, 30°45′36.3′N
Altitude (m)	567	791	265
Slope	30°	15°	0°
PH Value	6.08	7.64	8.46
Relative light intensity (%)	35	57.89	100
Relative humidity (%)	90	85	50

Table 2. The morphological characteristics of wild and cultivated *E. pubescens.*

Item	Wild	Cultivated	F
Leaf area ($cm^{2)}$	24.13±1.95	11.91±0.69	35.62**
Branch height (cm)	22.81±1.62	10.42±0.70	50.67**
The number of branches per plant	6.07±0.52	61.75±8.78	158.49**
Leaf number per plant	14.40±1.29	189.50±19.74	101.26**
Root biomass per plant (g)	8.83±2.61	24.08±2.67	19.42**
Stem biomass per plant (g)	2.64±0.52	5.80±0.66	12.56*
Leaf biomass per plant (g)	7.53±1.61	13.58±1.41	7.91*

MATERIALS AND METHODS

This study was carried out from February 2008, and collected twenty plants of wild *E. pubescens* and *E. wushanense,* respectively from a protected area of Jincheng Mountain Forest Park, northeastern Sichuan, China, then simultaneous collected twenty plants of *E. pubescens* and *E. wushanense* cultivated two years in the China West Normal University, respectively, which were transplant from same habitats with the wild *E. pubescens* and *E. wushanense*. All the collected materials partly used to measure morphological characteristics; another was used to analyze the effective composition contents. The differences environmental factors were determined in Table 1.

Morphological characteristics

The leaf numbers per plant, branch height per plant, the number of branches per plant were measured, leaf area was determined by scanning in vivo measurement of leaf area (SHY-150), the root biomass per plant, stem biomass per plant, leaf biomass per plant were recorded after drying to constant weight in calorstat.

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 (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 (250 × 4.6 mm 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 10 µl. The column temperature was maintained at 30℃. The analytes were monitored at 270 nm. 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

The morphological characteristics

The morphological characteristics of wild and cultivated E. pubescens

One way ANOVA analysis indicated that the morphological characteristics are significantly difference between wild and cultivated *E. pubescens* ($P < 0.05$). The leaf area and branch height per plant of wild *E. pubescens* are remarkably higher than the cultivated *E. pubescens*, while the leaf numbers per plant, the number of branches per plant of wild *E. pubescens* are significantly less than cultivated *E. pubescens* (Table 2). The number of branches and leaf number per plant in cultivated *E. pubescens* are 10.17 and 13.15 times more than wild, respectively. The root biomass per plant, stem biomass per plant and leaf biomass per plant in cultivated

Table 3. The morphological characteristics of wild and cultivated *E. wushanense.*

Item	Wild	Cultivated	F
Leaf area(cm^2)	39.45±1.75	14.39±4.82	117.30**
Branch height (cm)	31.97±1.77	12.00±1.06	72.74**
The number of branches per plant	8.95±0.98	60.50±8.47	147.89**
Leaf number per plant	11.94±2.01	265.25±22.37	556.04**
Root biomass per plant (g)	51.03±6.58	69.25±2.32	5.54*
Stem biomass per plant (g)	12.37±1.53	20.77±2.70	16.06**
Leaf biomass per plant (g)	12.64±1.04	23.41±1.52	93.82**

Table 4. Variation in icariin content and flavonoid content between the wild and cultivated *E. pubescens.*

Ingredient	Part	Types	Mean±S.E (%)	F	M.S
Icariin content	Root	Wild	0.243±0.008	34.305**	0.02
		Cultivated	0.127±0.0178		
	Stem	Wild	0.036±0.001	55.331**	0.024
		Cultivated	0.163±0.017		
	Leaf	Wild	0.550±0.004	840.903**	0.358
		Cultivated	0.839±0.016		
Flavonoid content	Root	Wild	4.643±0.078	0.007	0.00
		Cultivated	4.651±0.055		
	Stem	Wild	1.014±0.021	154.865**	6.52
		Cultivated	3.099±0.166		
	Leaf	Wild	5.461±0.064	112.447**	1.428
		Cultivated	6.436±0.065		

E. pubescens are two times higher than the wild.

The morphological characteristics of wild and cultivated E. wushanense

Table 3 shows that the leaf area and branch height per plant of wild *E. wushanense* are 2.74 and 2.66 times higher than the cultivated, respectively, while the leaf numbers per plant, the number of branches per plant of wild *E. wushanense* are significantly less than cultivated. Among them, the number of branches per plant in cultivated are 6.76 times more than the wild, the Leaf number per plant in cultivated are 22.22 times more than the wild, the cultivated root biomass per plant, stem biomass per plant and the leaf biomass per plant are one times higher than the wild.

Variation in icariin content and flavonoid content

The wild and cultivated E. pubescens

Firstly, the icariin of the root, stem and leaf differed significantly between wild and cultivated *E. pubescens* (Table 4). In the stem and leaf, the Cultivated *E. pubescens* have much more icariin content than the wild, but the icariin content of the cultivated root is lower than the wild. Secondly, the stem and leaf in cultivated *E. pubescens* contained the greatest amount in flavonoid content, and are significantly different from the wild, whereas there is no difference flavonoid content between the wild and cultivated root.

The wild and cultivated E. wushanense

Table 5 shows that the icariin contents of the root, stem and leaf differed significantly between the wild and cultivated *E. wushanense*. In the cultivated, all the parts of *E. wushanense* contained the greatest amount icariin contents than the wild. The average flavonoid contents of root and stem in cultivated *E. wushanense* are remarkably higher than the wild, but the leaf, which showed no significant difference between the wild and cultivated.

Table 5. Variation in icariin content and flavonoid content between the wild and cultivated *E. wushanense.*

Ingredient	Part	Types	Mean±S.E (%)	F	M.S
Icariin content	Root	Wild	0.066±0.001	14.126**	0.000
		Cultivated	0.071±0.001		
	Stem	Wild	0.046±0.001	651.593**	0.001
		Cultivated	0.068±0.001		
	Leaf	Wild	0.349±0.002	751.842*	0.046
		Cultivated	0.374±0.006		
Flavonoid content	Root	Wild	4.529±0.067	67.124**	0.989
		Cultivated	5.341±0.080		
	Stem	Wild	1.260±0.070	60.508**	0.797
		Cultivated	1.989±0.055		
	Leaf	Wild	6.067±0.239	0.858	0.084
		Cultivated	6.303±0.089		

DISCUSSION

Change the habitat of *Epimedium* will lead to influence the morphological characteristics of *Epimedium* (Yang et al., 2007). So the morphological characteristics of *E. pubescens* and *E. wushanense* are significantly difference between wild and cultivated. Due to the light intensity increased in cultivation habitat, the phenomenon of dwarf are detected in cultivated *E. pubescens* and *E. wushanense*, in the leaf area and branch height per plant are of remarkably lower than the wild, this is similar with *E. waushanens*, *E. acuminatum* and *E. leptorrhium* (Sun et al., 2004). 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).

In our study, both the flavonoid and icariin content in the cultivated *E. pubescens* and *E. wushanense* in intensive light are richer than the wild in faint light. 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 indicates that the leaf both in the wild and cultivated *E. pubescens* can meet the standard of flavonoid and icariin content, while others (for example, root and stem) cannot meet. But in the *E. wushanense*, the leaf of the wild and cultivated can only reach the standard of flavonoid content, and found no significant difference in flavonoid content between the wild and cultivated. All the parts of the wild and cultivated *E. chlorandrum* cannot reach the standard of icariin content. Although, icariin contents of cultivated *E. wushanense* do not meet the standards laid down by the Chinese Pharmacopoeia, there exists high-quality of flavonoid content that can meet the standards. This also can be commercially cultivated for manufacturing medicines to substitute for wild resources.

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