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ANALYSIS ON THE QUALITY OF *EPIMEDII FOLIUM* ORIGINATING FROM CULTIVATED *EPIMEDIUM PUBESCENS* MAXIM

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Abstract

The contents of main medicinal componentsin cultivated *Epimedii folium*and yield plant originating from*Epimediumpubescens*Maxim were compared to evaluate the quality of cultivated *Epimedii folium.*The concentration of icariin and epimedin C in the extracts came from cultivated *E.pubescens* and yield one were, respectively determined with RP-HPLC. The results showed that the contents of main medicinal componentsin cultivated *E.pubescens* were all lower than that in yield of *E.pubescens.* The quality of cultivated *Epimedii folium* is inferior to that of yield one. The cultivated *E. brevicornu*materials can be used as medicinal materials because there are considerable medicinal chemical components in cultivated *E.pubescens*.

Introduction

*Epimedii folium*is a kind of traditional Chinese medicine with aphrodisiac, anti-rheumatic and tonic effects. It is usually used to cure impotence, emission, osteomalacia, rheumatism, apoplexy and so on (Chinese Pharmacopoeia Committee 2015). *Epimedii folium*is the dry leafof *Epimedium brevicorn* Maxim, *E. pubescens* Maxim, *E. sagittatum* (Sicb. et Zucc.) Maxim or *E. koreanum* Nakai (Chinese Pharmacopoeia Committee 2015). There are many of the medicinal chemical components such as icariin, caohuoside, baohuoside, epimedin A,epimedin B and epimedin C in *Epimedii folium*(Li *et al.* 2005, Meng*et al.* 2010).

Epimedii folium comes from yield resources for a long time. The yield*Epimedii folium*resources are sharply deteriorating and decreasing because of its increased demand and the change of growing environment. The plants in the genus*Epimedium* are perennial herbaceous(Flora of China 1979). The root and the rhizome of these plants can grow for several years although its leaves die in winter. There are certain content of medicinal chemical components in their roots and rhizomes. As a result, some people usually dug out these plants with their roots and rhizomes. This method seriously destroys epimedii foliumresources. Therefore, the plants of *Epimedii folium*should be cultivated to fulfil the demand of patient for *Epimedii folium*and protect its yieldresources.

In general, the quality of cultivated medicinal materials is lower than that of corresponding yield of medicinal materials. But, the quality of cultivated *Epimedii folium*is not clear. In the present study the cultivation of *E.pubescens* on the quality of*Epimedii folium*was done to improve cultivation technique of *E.pubescens* and protect *Epimedii folium* resources.

Materials and Methods

In the present study Agilent 1260 HPLC instrument, Shimadzu (C18 reverse-phase column, 250×4.6 mm, 5 µm), electronic analytic balance (Precision: 0.0001) and ultrasonator were used.

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Ethanol (AR) and acetonitrile (HPLC grade) used as reagent werepurchased from Tianjin Kemiou Chemical Reagent Co. in March, 2018. Standard epimedin C and icariin (99.8%) were purchased from Sichuan Weikeqi Biotechnology Co. Ltd. in China in May, 2018.

The Shimadzu C18 reverse-phase column (5 µm, 250×4.6 mm) was used as HPLC column. The volume of extract, standard epimedin C and icariin injected was 10 µl. The temperature of HPLC column was 35ºC. The gradient mobile phase consists of acetonitrile and water. The content (v/v) of acetonitrile in the gradient mobile phase varied from 22 to 29% in 0 - 12 min, 29 to 29.5% in 12 - 20 min and 29.5 to 30% in 20 - 22 min. The flow rate of mobile phase was 1 ml/min. The recorder was set at 270 nm in wavelength to detect ingredients eluted from the column.

About 120 plants of alive yield *E.pubescens* with root were collected from Lueyang county, Shanxi province of China in October 2018. Total 90 plants of these materials were planted in 9 plots. Each plot was 2 m^2 in area. The roots of *E.pubescens* were planted in 6-8 cm below ground. These plots were thoroughly irrigated and covered with shading net after planting. The transmittance of shading net was 75%. The leaves and stems of these rest 30 plants of *E.pubescens* were taken as yield medicinal materials. The aerial parts of these planted *E.pubescens* were cut and taken as cultivated medicinal materials in June of the next year.

All of the medicinal materials were respectively dried to a constant weight at 42ºC, crushed and then sieved with sieve of 80 meshes. Each material was weighed for 1 g and extracted with 20 ml ethanol solvent (70%) in the ultrasonic bath for 0.5 hr. The mixture was filtered with filter paper. The residue was extracted with the same solvent (20 ml of 70% ethanol) and filtered once again. This filtrate was merged and added to 40 ml. Each kind of materials was extracted respectively three times. The extracts were respectively filtered with 0.22 µm membrane filter.

Standard epimedin C solutions were prepared at 0.0005, 0.001, 0.005, 0.01 and 0.05 mg/ml, respectively. Standard icariin solutions were prepared at 0.00062, 0.0031, 0.0062, 0.031 and 0.31 mg/ml, respectively.

These standard solutions and prepared extracts were respectively analyzed with Agilent 1260 HPLC instrument according to the above HPLC method. Chromatography peak areas of epimedin C and icariinin each chromatogram were respectively recorded. The standard curve relating the peak area of each chemical composition to its contents was drawn. These contents of icariin and epimedin C in extracts were analyzed according to their chromatography peak areas and the standard curves. Data were analyzed with Statistical Product and Service Solutions.

Results and Discussion

The HPLC chromatograms of standard icariin and epimedin C are presented in Fig. 1. These standard curves of icariin andepimedin C were analyzed and drafted according to the peak areas of those standard solutions and their contents (Table 1 and Fig. 2).

Table 1. The peak areas of standards and their contents.

Epimedin C (Retention time 18.415 min)		Icariin (Retention time 19.761 min)	
Peak area	Concentration (mg/ml)	Peak area	Concentration (mg/ml)
0.0005	60.0215	0.00062	4.5858
0.001	100.084	0.0031	21.877
0.005	498.532	0.0062	45.351
0.01	1063.24	0.031	227.971
0.05	5276.14	0.31	2437.29

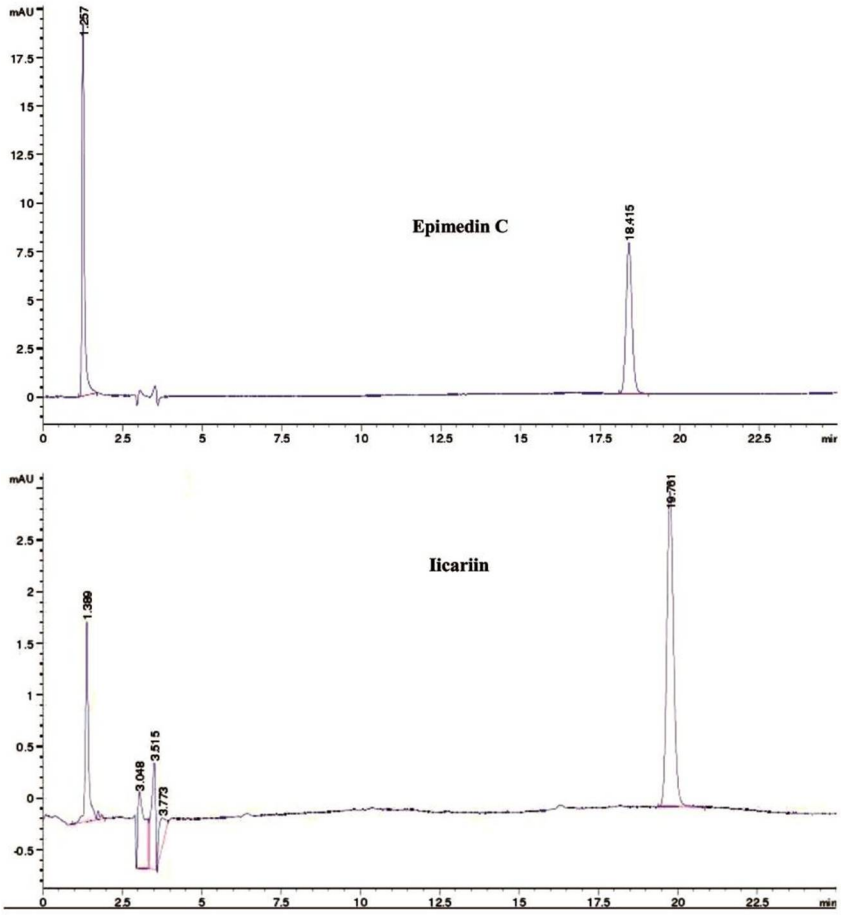

Fig. 1. HPLC chromatograms of standard epimedin C and icariin.

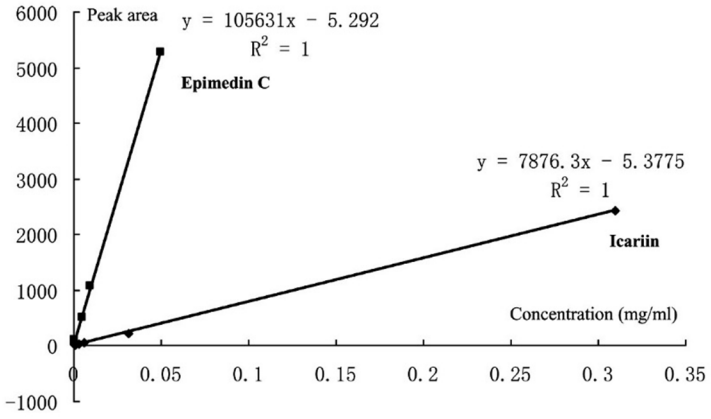

Fig. 2. Standard curves of epimedin C and icariin (x: Concentration, y: Peak area).

The peaks of icariinand epimedin Cin extract chromatograms were identified according to their retention time in HPLC (Fig. 3).

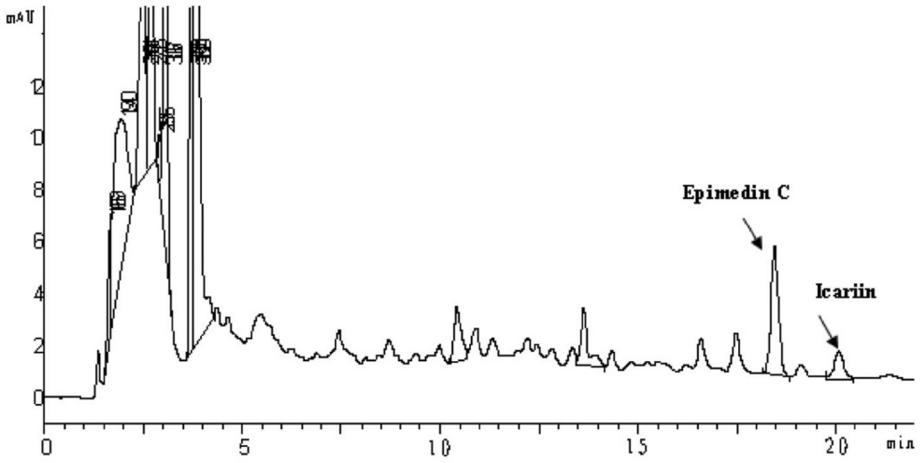

Fig. 3. HPLC chromatograms of cultivated *E. brevicornu* materials.

The concentrations of icariin and epimedin Cin extracts were analyzed according to their peak areas and relative standard curves. Then the contents of icariin and epimedin Cin *E.pubescens* materials were analyzed according the methods of preparation extract (Table 2).

Table 2. Contents of icariin and epimedin C in *E. brevicornu* materials.

Type	Epimedin C			Icariin		
	Peak area	Content (mg/g)	Mean	Peak area	Content (mg/g)	Mean
Yield	626.4	0.239207	0.2423 [a]	223.65263	1.163107	1.1962[a]
E. brevicornu materials	627.4	0.239586		230.44637	1.197609	
	649.7	0.24803		236.42595	1.227975	
Cultivated *E. brevicornu* materials	248.25	0.09601	0.09866 [b]	73.25	0.399303	0.41131[b]
	252.025	0.09744		77.14	0.419058	
	265.5	0.102543		76.452	0.415564	

The mean difference is significant at $p < 0.01$ level. The different letters indicate there is obvious difference between these means, The same letters indicate there is no obvious difference between these means.

The contents of icariin and epimedin Cin cultivated *E. brevicornu*materials were found, respectively lower than these inyield *E. brevicornu*materials. The rate of epimedin C content to icariin content in cultivated *E. brevicornu*materials was consistent with that in yield *E. brevicornu*materials.

The cultivated *E. brevicornu*materials can be used as medicinal materials because there are some medicinal chemical components in it. The content of icariin is obviouslylower than that of epimedin Cin*E. brevicornu*materials. This result is consistent with majority reports (Xie *et al.* 2009, Zhou*et al.* 2013). The contents of some medicinal chemical components in cultivated *E.*

*brevicornu*materials are lower than these inyield *E. brevicornu*materials. The result in the report of Wang Jing *et al.* is same were found to be that in this study (Wang*et al.* 2013). This result maybe related to the cultivation environment of *E. brevicorn*. *E. brevicorn* vigorously grows with adequate water and fertilizer in cultivation environment. Therefore, the contents of some medicinal chemical components as secondary metabolites in cultivated *E. brevicornu*materials are low. The quality of cultivated *Epimedii folium* probably close to that of yield epimedii folium because of improved cultivation environment. Providing similar environment to that of yield *Epimedii folium*for cultivated *Epimedii folium*possibly would increase the its quality.

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