



Original/Otros

Study on the differences of major pharmaceutical ingredients in different parts and processed medicinal material of *Epimedium Brevicornu* Maxim in Taihang mountain

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Abstract

The aim of this study is to evaluate the medicinal values of different parts of *Epimedium brevicornu* Maxim. and the effect of processing on major pharmaceutical ingredients in it. The contents of icariin and epimedin C in different parts and processed medicinal material of *E. brevicornu* in Taihang Mountain were determined with ultrasonic extraction and RP-HPLC. The results indicated that the contents of icariin and epimedin C, respectively 3.4524% and 0.5485%, in the leaf are higher than that in other parts. The contents of icariin and epimedin C, respectively 0.1942% and 0.1342%, in the stem (include petiole) are the lowest. The contents of these ingredients in the root (include rhizome) are close to that in the leaf. The icariin and epimedin C in all parts of *E. brevicornu* reduced after processing. The content of icariin in the processed leaf is about 59.5% of that in unprocessed leaves. The effect of processing on the content of icariin in the stem is unobvious. The content of epimedin C in the processed leaf is about 33.7% of that in unprocessed leaf. The content of epimedin C in the processed stem (include petiole) is about 36.9% of that in unprocessed stem. It is worth to exploit the stem and petiole of *E. brevicornu* because there are certain contents of pharmaceutical ingredients in them. The firepower should be paid attention to and the temperature should not be very high to avoid the damage on pharmaceutical ingredients in *E. brevicornu* when process it.

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Key words: *Epimedium brevicornu*. Medicinal parts. Processing.

ESTUDIO SOBRE LAS DIFERENCIAS DE INGREDIENTES FARMACÉUTICOS MAYOR EN DIFERENTES PARTES Y PROCESADO MEDICINAL MATERIAL DE *EPIMEDIUM BREVICORNU* MAXIM EN TAIHANG MONTAÑA

Resumen

Este estudio pretende evaluar los valores medicinales de diferentes partes de la *Epimedium brevicornu* Maxim y el efecto de su procesamiento sobre sus principales componentes farmacéuticos. El contenido de icariina y epimedin C en diferentes partes y en material medicinal procesado de *Epimedium brevicornu* en la montaña de Taihang fue determinado mediante extracción ultrasónica y RP-HPLC. Los resultados indicaron que el contenido de icariina y epimedin C, respectivamente 3,4524% y 0,5485%, en la hoja son mayores que en otras partes. El contenido de icariina y epimedin C, respectivamente 0,1942% y 0,1342%, en el tallo (peciolo incluido) es más bajo. El contenido de estos componentes en la raíz (rizoma incluido) es similar al de la hoja. El contenido de icariina y epimedin C en todas las partes de *E. brevicornu* se vio reducido después del procesado. El contenido de icariina en la hoja procesada es aproximadamente el 59,5% del de la hoja sin procesar. El efecto del procesado sobre el contenido de icariina en el tallo no es evidente. El contenido de epimedin C en el tallo procesado es de aproximadamente el 33,7% del de la hoja sin procesar. El contenido de epimedin C en el tallo procesado (peciolo incluido) es de aproximadamente el 36,9% de aquel del tallo sin procesar. Vale la pena aprovechar el tallo y peciolo de la *E. brevicornu* porque hay cierto contenido de componentes farmacéuticos en ellos. Hay que controlar la potencia de fuego y la temperatura no debe ser muy alta para evitar dañar los componentes farmacéuticos de la *E. brevicornu*.

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Palabras clave: *Brevicornu epimedium*. Partes medicinales. Tratamiento.

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Introduction

Epimedium brevicornu Maxim is perennial herbaceous plant¹. The leaf of *E. brevicornu* is a kind of traditional Chinese medicine. Many of the medicinal chemical components such as icariin, caohuoside, baohuoside, epimedin A, epimedin B, and epimedin C are flavonoids^{2,3}. As traditional Chinese medicine, the leaf of *E. brevicornu* is named epimedii folium with tonic, anti-rheumatic and aphrodisiac effects. Epimedii folium can be used to cure impotence, emission, osteomalacia, rheumatism, apoplexy and so on⁴.

E. brevicornu widely distribute in China. There is rich resources of *E. brevicornu* in Taihang Mountain. The stem of *E. brevicornu* is herbaceous and its compound leaves consist of nine leaflets. Its petiole branches and form trifoliolate leaf. The leaf is thin and like paper. The total biomass of stem and petiole is close to that of leaf blade. The part of *E. brevicornu* used as Chinese medicine is leaf prescribed in Chinese Pharmacopoeia⁴. Therefore, these stem and petiole are wasted when harvest *E. brevicornu*. We determined the contents of major pharmaceutical ingredients in different parts of *E. brevicornu* distributed in Taihang Mountain to improve rational usage of the resources. In tradition, The processing of epimedii folium is broiling with suet oil to increase its effect. There are differences in the reports on the contents of major pharmaceutical ingredients in the processed epimedii folium. Therefore, the differences between the contents of major pharmaceutical ingredients in the processed epimedii folium and that in unprocessed materials were also studied in our paper to promote the proper usage and rational processing of epimedii folium.

Materials and methods

Reagents and materials

Instruments: HPLC instrument, Shimadzu, MS-2010, Lectronic Analytic Balance (precision: 0.0001), Ultrasonicator and Rotary Evaporator were used in study.

Reagents: methanol (AR), ethanol (AR) and acetonitrile (HPLC grade) were used in experiment. Standard icariin and epimedin C (99.8 %) were purchased from National Institute for the Control of Pharmaceutical and Biological Products in October 2014.

Materials: The *E. brevicornu* materials were collected in Guanshan, Xigou and Jiulianshan of Xinxiang city in Henan province in June 2014. 100 plants were randomly dug in each location. The leaf, stem (include petiole) and root (include rhizome) were separated and drought to constant weight at 45° C.

Methods

Process materials: The leaves of *E. brevicornu* were cut into slices of 5-7 mm in width. The stems (include

petioles) were cut into sections of 12-14 mm in length. 10 g suet oil was melted in pan with small fire and then 50 g *E. brevicornu* materials was put into the pan. The materials was stired and fried in the pan with small fire. The fried materials was taken out when the suet oil completely infiltrated into the materials and these materials appeared with glossy sheen.

Preparation of extract: The *E. brevicornu* materials was crushed and sieved with 80 meshes sieve. 1 g materials was extracted with 20 ml ethanol solvent (70%) in an ultrasonic bath for 1 h. The mixture was filtered and the residue was extracted with same solvent once again. These filtrate was merged and added to 40 ml. The extract was filtered using a 0.22 µm membrane filter. Each kind of materials was extracted respectively three times.

Determine the contents of icariin and epimedin C in extracts: The HPLC column used was a Diamonsil C18 reverse-phase column (5 µm, 250×4.6 mm). The volume of extract injected was 10 µl, and then elution was performed with a gradient mobile phase consisting of acetonitrile and water. The content of acetonitrile in the gradient mobile phase varies as below (v/v): 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 was 1 ml/min and the temperature in HPLC column was 35° C. A variable wavelength recorder set at 270 nm was used to detect ingredients eluted from the column. Standard icariin solutions were prepared at 0.025 mg/mL, 0.15 mg/mL, 0.25 mg/mL, 0.5mg/mL, 2.5 mg/mL and 5 mg/mL respectively. Standard epimedin C solutions were prepared at 0.015 mg/mL, 0.05 mg/mL, 0.15 mg/mL, 0.25 mg/mL, 0.5mg/mL, 2.5 mg/mL and 5 mg/mL respectively. These standard solutions were analyzed according to the above HPLC method. Chromatography peak areas of icariin and epimedin C in standard solutions were respectively recorded to prepare standard curves relating these peak areas to their contents. All of those prepared extracts were analyzed according to the above HPLC method. Chromatography peak areas of icariin and epimedin C in extracts were respectively recorded respectively. These contents of icariin and epimedin C in extracts were analyzed according to their chromatography peak areas and the standard curves. All of the data were analyzed with SPSS (Statistical Product and Service Solutions).

Results and analysis

Standard curves of icariin and epimedin C

The HPLC chromatograms of standard icariin and epimedin C are showed in figure 1. These standard curves of icariin and epimedin C were drafted according to their peak areas and their contents. Standard curve of icariin is $y = 5856528.16x + 141710.54$ (x: content, y: peak area, $R^2 = 0.9998$). The retention time

of icariin is 18.01-18.013 min. Standard curve of epimedin C is $y = 14117474.50x + 181626.91$ (x: content, y: peak area, $R^2 = 0.9998$). The retention time of epimedin C is 16.39-16.41 min.

Differences between these contents of icariin or epimedin C in different parts of E. brevicornu

The peaks of icariin and epimedin C in extract chromatograms were identified according to their retention time in HPLC (Fig. 2). The contents of icari-

in and epimedin C in extracts and unprocessed materials were analyzed according to their peak areas and standard curves also (Table I).

The results shows that there are very significant difference between these contents of icariin or epimedin C in different parts of *E. brevicornu*. These contents of icariin and epimedin C in stem (include petiole) are lowest and that in leaves are highest. The content of epimedin C in root (include rhizome) is about 65.4% of that in leaves. However, the content of icariin in root (include rhizome) is only about 14.9% of that in leaves.

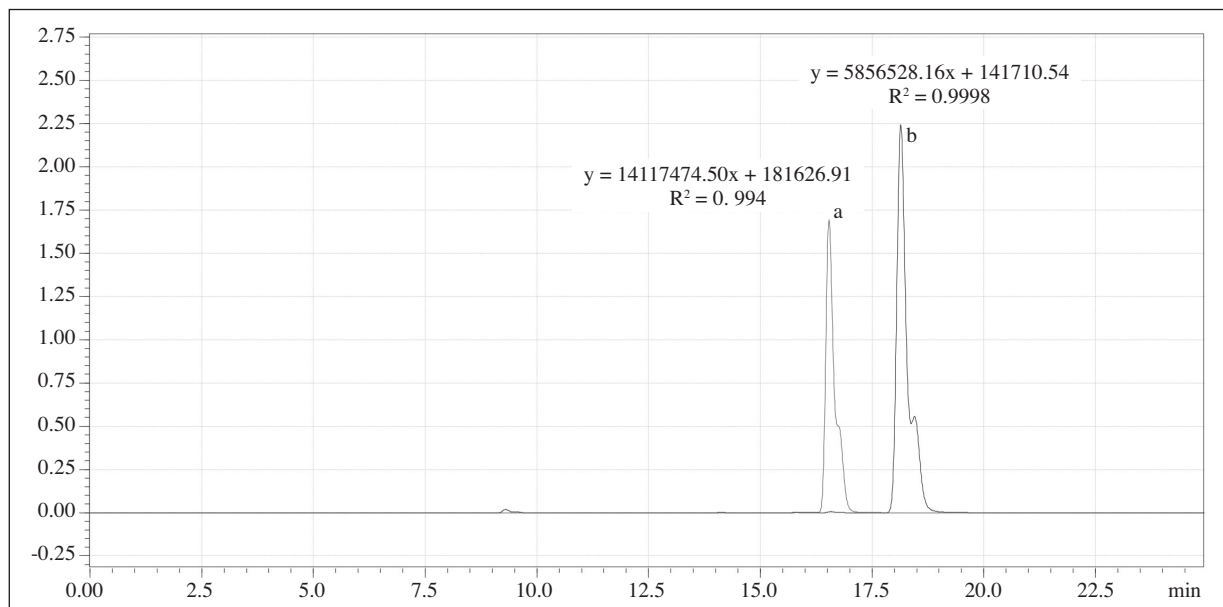


Fig. 1.—HPLC Chromatograms of standard icariin and epimedin C (a: epimedin C, b: icariin).

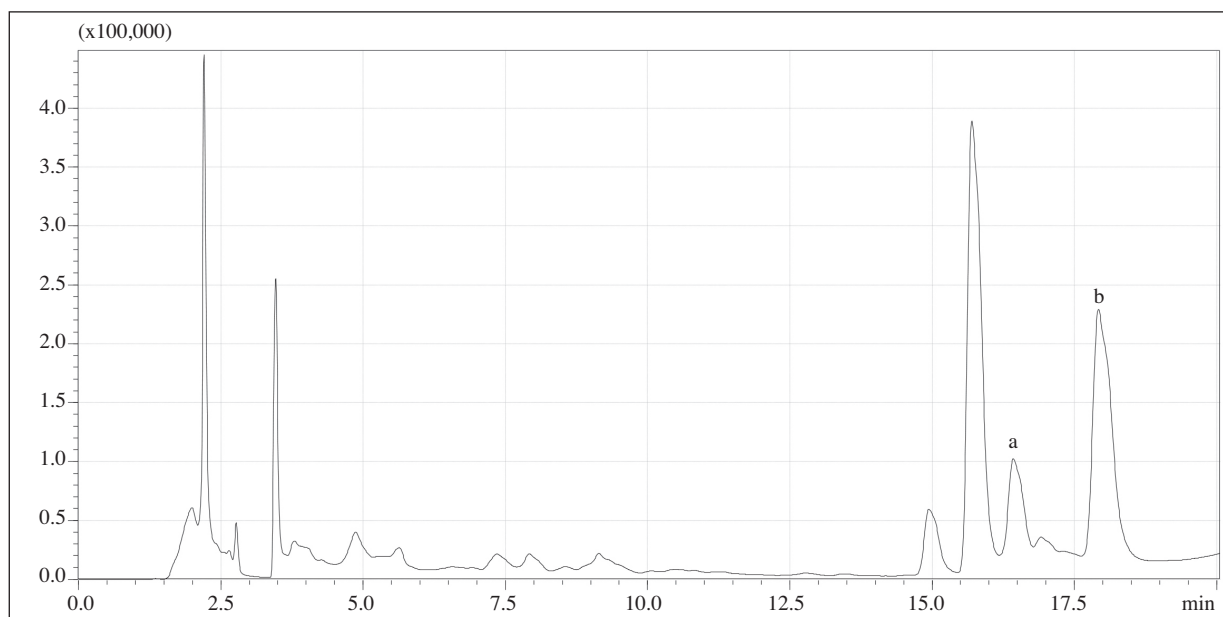


Fig. 2.—HPLC chromatograms of extract from unprocessed *E. brevicornu* leaves (a: epimedin C, b: icariin).

Table I
The contents of icariin and epimedin C in different parts of unprocessed *E. brevicornu* (%)

Parts	Epimedin C (%)		Icariin (%)	
Root (include rhizome) 1	0.351208		0.549924	
Root (include rhizome) 2	0.392668	0.3586 ^a	0.541604	0.5142 ^a
Root (include rhizome) 3	0.3318		0.451088	
Stem (include petiole) 1	0.131984		0.188392	
Stem (include petiole) 2	0.150044	0.1342 ^b	0.173952	0.1942 ^b
Stem (include petiole) 3	0.120684		0.220232	
Leaves 1	0.540148		2.858464	
Leaves 2	0.558748	0.5485 ^c	3.55716	3.4524 ^c
Leaves 3	0.546528		3.941472	
Anova	f=303.413 > f _{0.01} =10.9		f=95.159 > f _{0.05} =10.9	

Note: a, b and c mean the results of multiple comparisons.

Content variances of icariin and epimedin C in materials before and after processing

The representative HPLC chromatogram of extract from processed *E. brevicornu* materials is showed in figure 3. The contents of icariin and epimedin C in these extracts of processed materials were analyzed according to their peak areas and standard curves also (Table II).

The contents of icariin and epimedin C in *E. brevicornu* materials decreased in different degree after processing. The content of icariin in processed leaves, about 59.5% of that in unprocessed leaves, obviously decreased. But there is not obvious difference between

the contents of icariin in processed stem (include petiole) and that in unprocessed stem (include petiole). The contents of epimedin C in *E. brevicornu* materials obviously decreased after processing also. The content of epimedin C in processed leaves is only about 33.7% of that in unprocessed leaves. And the content of epimedin C in processed stem (include petiole) is about 36.9% of that in unprocessed materials.

Discussion

The utilized part of *E. brevicornu* is leaf in traditional Chinese medicine, which possibly is related to

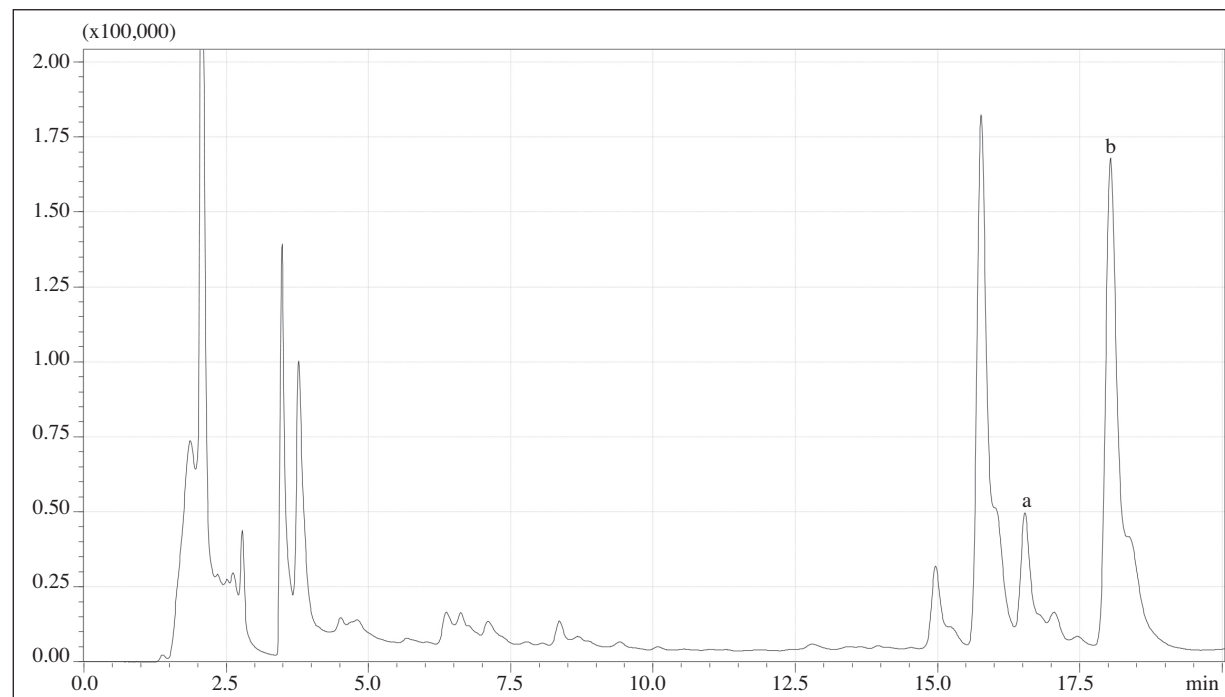


Fig. 3.—Representative HPLC chromatograms of extract from processed *E. brevicornu* materials (a: epimedin C, b: icariin).

Table II
The contents of icariin and epimedin C in processed and unprocessed *E. brevicornu* (%)

Ingredients and processing	Stem (include petiole) 1	Stem (include petiole) 2	Stem (include petiole) 3	Average	Leaves 1	Leaves 2	Leaves 3	Average	
Epimedin C	Unprocessed	0.131984	0.150044	0.120684	0.1342	0.546528	0.558748	0.540148	0.5485
	Processed	0.052316	0.037944	0.044536	0.04493	0.21296	0.17294	0.169648	0.1852
	Anova	f=88.267>f _{0.01} =34.1			f=590.32>f _{0.01} =34.1				
Icariin	Unprocessed	0.220232	0.188392	0.173952	0.1942	2.858464	3.55716	3.941472	3.4524
	Processed	0.221028	0.136612	0.17826	0.1786	1.873396	2.173524	2.114536	2.0538
	Anova	f=0.310<f _{0.05} =10.1			f=17.958>f _{0.05} =10.1				

the high contents of medicinal ingredients and evident efficacy. The result of our investigation shows that the biomass of stem and petiole in *E. brevicornu* is equal to (about 90%) that of leaves. Although the content of icariin in stem (include petiole) is lower than that in leaves, but the contents of epimedin C in them are approximative. Therefore, there is high medicinal value in the stem and petiole of *E. brevicornu*. We can extract medicinal ingredients from the stem and petiole. The stem, petiole and root are utilized as Chinese medicine in some place in China⁵. Although the contents of medicinal ingredients in the root and rhizome of *E. brevicornu* are very high. But excavating the root and rhizome is serious destructive to *E. brevicornu* resources and also harmful to sustainable utilization these resources. Therefore, the root and rhizome of *E. brevicornu* should not be utilized.

Some icariin and epimedin C, specifically epimedin C, can be damaged in structure when *E. brevicornu* materials is processed. The structure of epimedin C is more complicated than that of icariin and more decomposed than icariin in processing^{6,7}. All of these cause the contents of icariin and epimedin C to decrease. The traditional Chinese medicine was decocted in water before it be taken as medicine. The solubility of these medicinal ingredients in water is lower than that in organic solvents. The suet oil is used as accessories in processing *E. brevicornu* materials. There is some suet oil in the *E. brevicornu* materials after processing. The suet oil is also a kind of organic solvent, which increase the solubility of these medicinal ingredients. Owing to these factors, the contents of medicinal ingredients in processed *E. brevicornu* materials are higher than that of unprocessed ones. In addition, there are some medicinal ingredients in suet oil⁸. Therefore, the efficacy of processed *E. brevicornu* materials is evident than that of unprocessed ones. Since processing can damage some medicinal ingredients, The firepower should be paid attention to when *E. brevicornu* materials is processed. The temperature should not be too high to make some medicinal ingredients decompose.

Conclusion

There are obvious differences between these contents of icariin or epimedin C in different parts of *E. brevicornu*. The content of icariin or epimedin C in the leaf is the highest and that in the stem (include petiole) is the lowest. The contents of icariin and epimedin C in all parts of *Epimedium brevicornu* reduced more or less after processing. The decrease of epimedin C is obvious.

Interests Declaration

The authors of this article declare that they have no conflict interests.

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