Bangladesh J. Bot. **48**(2): 367-372, 2019 (June)

COMPARISON OF MAJOR MEDICINAL COMPONENTS IN THE FIBROUS ROOT OF *EPIMEDIUM BREVICORNU* MAXIM

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Keywords: Epimedium brevicornu, Fibrous root, Medical component, RP-HPLC

Abstract

The contents of main medical components in the fibrous root and medicinal materials of *Epimedium brevicornu* Maxim was compared. The contents of epimedin C and icariin in the fibrous root and other parts of *E. brevicornu* were determined with RP-HPLC. The results showed that there were high contents of medical components in the fibrous root of *E. brevicornu*. The content of epimedin C in *E. brevicornu* fibrous root was obviously higher than that of other parts. The content of icariin in *E. brevicornu* fibrous root was close to that in the rhizome and apparently higher than that in the stem and petiole. The contents of epimedin C and icariin in the stem (including petiole) were lowest in all parts of *E. brevicornu*. The fibrous root cut from rhizome when cultivate *E. brevicornu* should be collected and utilized as medicinal materials.

Introduction

Epimedium brevicornu Maxim is a perennial herbaceous plant in Berberidaceae (Flora of China 1979). The leaf of *E. brevicornu* is usually used as a kind of traditional Chinese medicine in China. There are many types of the medicinal chemical components such as icariin, caohuoside, baohuoside, epimedin A, epimedin B and epimedin C (Li *et al*. 2005, Meng *et al*. 2010). The leaf of *E. brevicornu* is named epimedii folium as traditional Chinese medicine with aphrodisiac, anti-rheumatic and tonic effects. Epimedii folium is usually used to cure impotence, emission, osteomalacia, rheumatism, apoplexy and so on (Chinese Pharmacopoeia Committee 2015).

E. brevicornu is widely distributed in China specially in Taihang Mountain. It is regulated in Chinese Pharmacopoeia that only the leaf of *E. brevicornu* can be used as Chinese medicine (Chinese Pharmacopoeia Committee 2015). Medicinal materials growers usually reap the aerial part of *E. brevicornu* when harvest. Then the leaf of *E. brevicornu* is processed. Now, the resources of *E. brevicornu* is insufficient after long time harvest. The cultivation technique of *E. brevicornu* is in study to satisfy the demand of patient on *E. brevicornu.* Medicinal materials growers usually dug out *E. brevicornu* plant, then cut off the fibrous root and withered leaf before ramet cultivation according to the immature cultivation technique. Medicinal materials growers think that cutting off the fibrous root of *E. brevicornu* promote rhizome rooting and germination. It was found that the weight of dry fibrous root is about 1/2 weight of dry aerial part of *E. brevicornu* after investigation. There is certainly some medical components in the fibrous root of *E. brevicornu*. The resources of *E. brevicornu* would be wasted if the fibrous root is given up. Therefore, in the present study the contents of major medical components in the fibrous root and other parts of *E. brevicornu* were determined and compared. This will provide references for fully usage of *E. brevicornu* resources in future.

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Materials and Methods

Agilent 1260 HPLC instrument, Shimadzu (C18 reverse-phase column, 5 µm, 250 × 4.6 mm), Lectronic Analytic Balance (precision: 0.0001), Ultrasonator and Rotary Evaporator were used in the study.

Ethanol (AR) and acetonitrile (HPLC grade) were used as reagent in experiment. Standard epimedin C and icariin (99.8%) were purchased from Sichuan Weikeqi Biotechnology Co. Ltd. in China in May 2018.

More than 30 plants of *E. brevicornu* were randomly dug in Guanshan of Xinxiang city in Henan province China in June 2018. The leaf blade, stem (including petiole), fibrous root and rhizome of these plants were separated and dried to constant weight at 42º C.

The dry leaf blade, stem (including petiole), fibrous root and rhizome of *E. brevicornu* were crushed and sieved with 80 meshes sieve. Each material was weighed for 1 g and extracted with 30 ml ethanol solvent (70%) in the ultrasonic bath for 0.5 hrs. The mixture was filtered with filter paper. The residue was extracted with the same solvent (30 ml of 70% ethanol) and filtered once again. These filtrates were mixed and added to 60 ml. The extract was filtered with 0.22 µm membrane filter. Each kind of materials was extracted three times.

Standard icariin solutions were prepared at 0.00065, 0.0031, 0.0062, 0.0315, 0.31 and 0.5 mg/ml, respectively. Standard epimedin C solutions were prepared at 0.001, 0.005, 0.01, 0.05 and 0.5 mg/ml, respectively.

The Diamonsil C18 reverse-phase column (5 µm, 250 × 4.6 mm) was used as HPLC column. The volume of extract injected was 10 µl. 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 and the temperature in HPLC column was 35ºC. A variable wavelength recorder was set at 270 nm to detect ingredients eluted from the column.

These standard solutions and those prepared extracts were respectively analyzed according to the above HPLC method. Chromatography peak areas of epimedin C and icariin in each chromatogram were respectively, recorded.

All those prepared extracts were analyzed according to the above HPLC method. Chromatography peak areas of icariin and epimedin C in extracts were recorded, respectively. These contents of icariin and epimedin C in extracts were analyzed according to their chromatography peak areas and the standard curves (relating these peak areas to their contents). All data were analyzed with SPSS (Statistical Product and Service Solutions).

Results and Discussion

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

The peaks of epimedin C and icariin in extract chromatograms were identified according to their retention time in HPLC (Fig. 3). The concentrations of epimedin C and icariin in extracts were analyzed according to their peak areas and standard curves also (Table 2). Then the contents of epimedin C and icariin in *E. brevicornu* materials were analyzed according the methods of preparation extract.

It can be seen from the results that there were high contents of medical components in the fibrous root of *E. brevicornu*. The content of epimedin C in the fibrous root was higher than that in other parts including leaf blade. The content of icariin in the fibrous root is close to that in the

rhizome and higher than that in the stem and petiole, although lower than that in the leaf blade (Tables 2, 3). The contents of epimedin C and icariin in the stem (include petiole) are lowest in all parts of *E. brevicornu.*

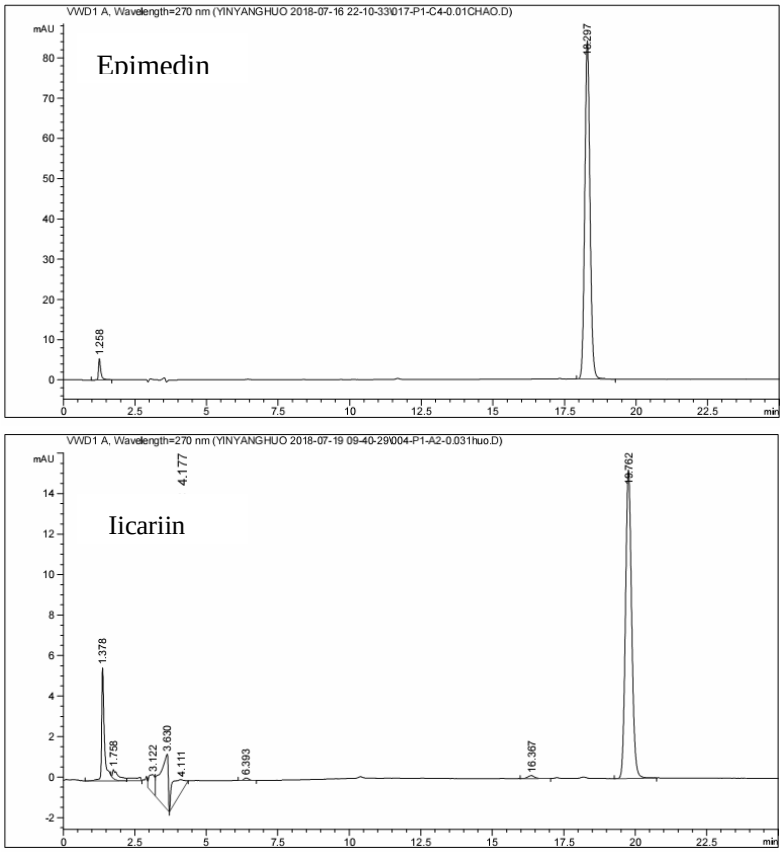

Fig. 1. HPLC chromatograms of standard epimedin C and icariin**.**

Table 1. Analysis results of standard icariin and epimedin C.

Epimedin C (Retention time 18.297 min)		Icariin (Retention time 19.762 min)	
Concentration (mg/ml)	Peak area	Concentration (mg/ml)	Peak area
0.001	100.08	0.00062	4.5858
0.005	498.53	0.0031	21.877
0.01	1063.24	0.0062	45.351
0.05	5276.14	0.031	227.97
0.5	63782.61	0.31	2437.29
		0.5	3930.85

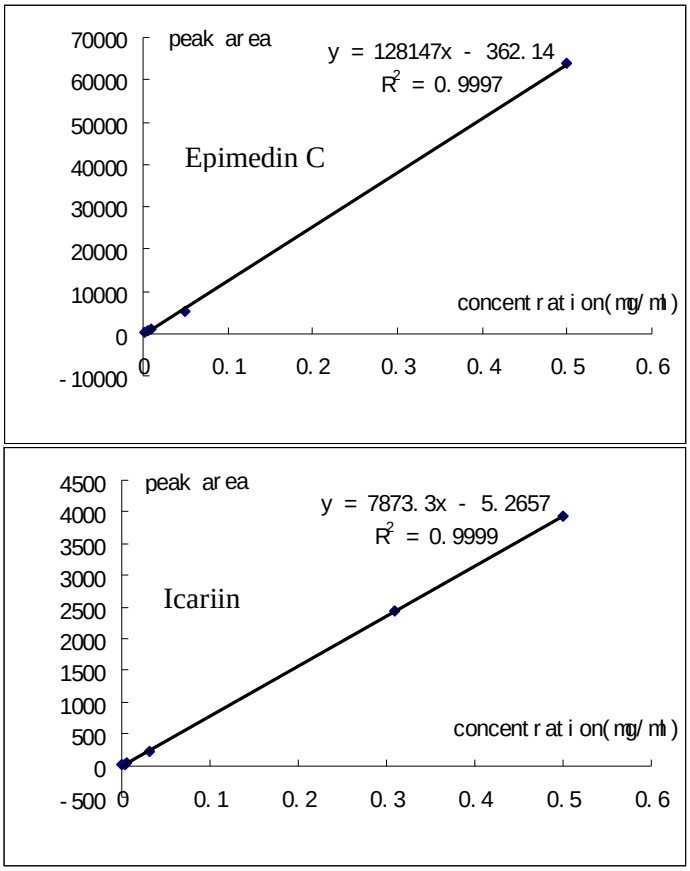

Fig. 2. Standard curves of icariin and epimedin C.

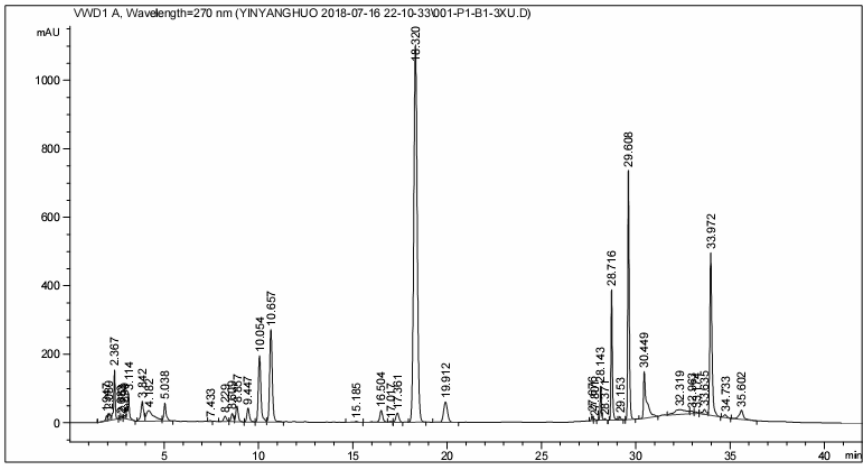

Fig. 3. HPLC chromatograms of *E. brevicornu* materials.

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

Parts	Epimedin C		Icariin	
	Peak area	Content (mg/g)	Peak area	Content (mg/g)
Fibrous root	13064.62	6.287	1079.91	8.270
	13746.01	6.606	1064.21	8.150
	13931.81	6.693	1083.59	8.298
Rhizome	1461.93	0.855	925.69	7.135
	1507.81	0.876	954.24	7.352
	1504.70	0.875	945.53	7.286
Stem and petiole	376.99	0.347	140.33	1.110
	379.09	0.348	143.74	1.136
	392.72	0.354	134.04	1.062
Leaf blade	2625.49	1.399	3051.12	23.292
	2537.07	1.358	2763.01	21.096
	2499.78	1.341	2899.86	22.139

Table 3. Multiple comparisons of medical components contents in different parts.

Epimedin C		Icariin	
Parts	Mean contents comparison* (mg/g)	Parts	Mean contents comparison* (mg/g)
Fibrous root	6.5288[a]	Leaf blade	22.1757[a]
Leaf blade	1.3660[b]	Fibrous root	8.2393[b]
Rhizome	0.8685[c]	Rhizome	7.2577[b]
Stem and petiole	0.3495[d]	Stem and petiole	1.1027[c]

The mean difference is significant at the 0.01 level.*The different letters indicate there is obvious difference between these means, The same letters indicates there is not obvious difference between these means.

There are high contents of medical components in the fibrous root of *E. brevicornu*. The contents of some medical components such as epimedin C are higher than that in medicinal materials from *E. brevicornu*. The fibrous root of *E. brevicornu* is usually cut off the rhizome before ramet cultivation to promote rhizome tooting and germination. The fibrous root is often given up by medicinal materials growers. Thus, the resources of *E. brevicornu* is seriously wasted. It may be suggested that the fibrous root cut from rhizome when cultivate *E. brevicornu* should be collected and utilized as medicinal materials. The fibrous root can also be used to extract medical components to reduce the waste of *E. brevicornu* resources.

It is regulated in Chinese Pharmacopoeia that the part of *E. brevicornu* used as Chinese medicine is leaf (Chinese Pharmacopoeia Committee 2015). But it is not clear that the medicinal materials come from *E. brevicornu* includes blade or not. It was found that the contents of medical components in the stem and petiole of *E. brevicornu* were very low according to the result of the present study. This result is consistent with that of Sun Chao on the whole (Sun *et a.l* 2006, Chu

et a.l 2007). The weight of dry stem (including petiole) is about 1/2 weight of dry leaf of *E. brevicornu*. The stem and petiole of *E. brevicornu* mixed in the medicinal materials reduce its quality. Therefore, epimedii folium should not include stem and petiole.

Acknowledgements

This study was supported by Science and Technology research project of Henan province (NO. 182102310634, NO. 152102210309).

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(Manuscript received on 11 September, 2018; revised on 19 December, 2018)