ANALYSIS ON THE QUALITY OF EPIMEDII FOLIUM ORIGINATING FROM CULTIVATED EPIMEDII PUBESCENTS MAXIM

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Abstract

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

Introduction

Epimedium 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). Epimedium folium is the dry leaf of Epimedium brevicorn Maxim, E. pubescens Maxim, E. sagittatum (Sieb. et Zucc.) Maxim or E. koreanum Nakai (Chinese Pharmacopoeia Committee 2015). There are many of the medicinal chemical components such as icariin, caohuo side, baohuoside, epimedin A, epimedin B and epimedin C in Epimedium folium (Li et al. 2005, Meng et al. 2010).

Epimediium folium comes from yield resources for a long time. The yield Epimediium 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 dig out these plants with their roots and rhizomes. This method seriously destroys epimediium folium resources. Therefore, the plants of Epimedium folium should be cultivated to fulfill the demand of patient for Epimedium folium and protect its yield resources.

In general, the quality of cultivated medicinal materials is lower than that of corresponding yield of medicinal materials. But, the quality of cultivated Epimedium folium is not clear. In the present study the cultivation of E. pubescens on the quality of Epimediium folium was done to improve cultivation technique of E. pubescens and protect Epimediium 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 were purchased from Tianjin Kemiu 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 m2 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 icariin 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 and epimedin 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.**

<table>
<thead>
<tr>
<th>Peak area</th>
<th>Epimedin C (Retention time 18.415 min)</th>
<th>Icariin (Retention time 19.761 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (mg/ml)</td>
<td>Peak area</td>
</tr>
<tr>
<td>0.0005</td>
<td>60.0215</td>
<td>0.00062</td>
</tr>
<tr>
<td>0.001</td>
<td>100.084</td>
<td>0.0031</td>
</tr>
<tr>
<td>0.005</td>
<td>498.532</td>
<td>0.0062</td>
</tr>
<tr>
<td>0.01</td>
<td>1063.24</td>
<td>0.031</td>
</tr>
<tr>
<td>0.05</td>
<td>5276.14</td>
<td>0.31</td>
</tr>
</tbody>
</table>


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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 icariin and epimedin Cin extract chromatograms were identified according to their retention time in HPLC (Fig. 3).

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 materials were analyzed according the methods of preparation extract (Table 2).

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

<table>
<thead>
<tr>
<th>Type</th>
<th>Epimedin C</th>
<th></th>
<th>Icarin</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak area</td>
<td>Content (mg/g)</td>
<td>Mean</td>
<td>Peak area</td>
<td>Content (mg/g)</td>
</tr>
<tr>
<td>Yield E. brevicornu materials</td>
<td>626.4</td>
<td>0.239207</td>
<td>0.2423</td>
<td>223.65263</td>
<td>1.163107</td>
</tr>
<tr>
<td></td>
<td>627.4</td>
<td>0.239586</td>
<td></td>
<td>230.44637</td>
<td>1.197609</td>
</tr>
<tr>
<td></td>
<td>649.7</td>
<td>0.24803</td>
<td></td>
<td>236.42595</td>
<td>1.227975</td>
</tr>
<tr>
<td>Cultivated E. brevicornu materials</td>
<td>248.25</td>
<td>0.09601</td>
<td>0.09866</td>
<td>73.25</td>
<td>0.399303</td>
</tr>
<tr>
<td></td>
<td>252.025</td>
<td>0.09744</td>
<td></td>
<td>77.14</td>
<td>0.419058</td>
</tr>
<tr>
<td></td>
<td>265.5</td>
<td>0.102543</td>
<td></td>
<td>76.452</td>
<td>0.415564</td>
</tr>
</tbody>
</table>

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

References

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