Isolation and purification of esculetin from the seeds of *Euphorbia lathyris* L. using high-speed counter-current chromatography

YU Xia¹,² ZHANG Weiming² SHI Xueping² SUN Lijun²*

1. College of Life Sciences, Nanjing Normal University, Nanjing 210046, China
2. Institute for the Comprehensive Utilization of Wild Plants, Nanjing 210042, China

Abstract A method for the isolation and purification of esculetin from the seeds of *Euphorbia lathyris* L. was developed using high-speed counter-current chromatography (HSCCC). The ethyl acetate extract of the seeds of *Euphorbia lathyris* L. was separated by the HSCCC directly. Different solvent systems were investigated and the results showed that the best solvent system was the two-phase solvent system composed of chloroform-methanol-water (4:3:2 v/v/v). The lower phase was used as the mobile phase and the upper phase was used as the stationary phase. A total of 80 mg esculetin with the purity of 99.04% was successfully obtained from 200 mg crude extract of the seeds of *Euphorbia lathyris* L. The results indicate that optimized HSCCC offers a preferred method for the preparation of esculetin from the seeds of *Euphorbia lathyris* L.

Key words high-speed counter-current chromatography (HSCCC), esculetin, seeds of *Euphorbia lathyris* L.
离技术。它不使用固相载体作固定相，克服了固相载体吸附问题。从续随子种子中提取七叶内酯的关键是溶剂的选择。溶剂的选择直接影响到溶剂萃取的效率以及产物的纯度。

\[ \text{七叶内酯的化学结构式} \]

1. 仪器与试剂
   1.1 TBE-300 1.9 mm 100 mL
   1.2 20 mL TBP-500 TBD-2000 TC-1050 Agilent 1200 RE-2000 Tedia Agilent

1.2 HPLC
   1.2.1 HSCCC

1.2.2

\[ \text{结果与讨论} \]

1.2.3 HSCCC

1.2.4 HPLC

\[ \text{图 1 化学结构式 of esculentin} \]
第 " 期 " 余 " 霞,等:高速逆流色谱法分离纯化续随子种子中的七叶内酯

中的浓度和用来计算分配系数 # (见表)，因此本文选择此溶剂体系进行分离。实验过程中,还对流动相的流速进行了优化,最终选择流动相的流速为。

表 1 分配系数# #的 esculentin in the crude extract from the seeds of Euphorbia lathyris L. in different two-phase solvent systems

<table>
<thead>
<tr>
<th>No.</th>
<th>Solvent system</th>
<th>#</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n-hexane-ethyl acetate-methanol-water</td>
<td>2.47</td>
</tr>
<tr>
<td>2</td>
<td>n-hexane-ethyl acetate-methanol-water</td>
<td>0.37</td>
</tr>
<tr>
<td>3</td>
<td>n-hexane-methanol-water</td>
<td>6.44</td>
</tr>
<tr>
<td>4</td>
<td>light petroleum-ethyl acetate-methanol-water</td>
<td>5.92</td>
</tr>
<tr>
<td>5</td>
<td>chloroform-methanol-water</td>
<td>0.85</td>
</tr>
</tbody>
</table>

2.3 HSCCC

HSCCC 2 HSCCC 5 h

HSCCC 2 HSCCC I ~ V HPLC

Fig. 2 HSCCC chromatogram of the crude extract from the seeds of Euphorbia lathyris L.

HSCCC conditions: solvent system chloroform-methanol-water: 4:3:2 v/v/v the upper phase as stationary phase, the lower phase as mobile phase, flow rate 1.5 mL/min, revolution speed 800 r/min, detection wavelength 254 nm, retention of the stationary phase 70%.

80 mg V 200 mg HPLC 3 99.04%

V HPLC 3

TOF MS [m/z] 201.09 M + Na+ " m/z 177.13 M - H2O 100% 6.16 1H-NMR 500 MHz DMSO d6 δ 6.16 1H d J = 9.5 Hz 3.7 7.4 1H s H5 6.98 1H s H-8 7.87 1H d 4 J = 9.5 Hz H-4 9.40 1H s O H-10 12 H-13 H-17 6.16 1H s H-7-OH 7.48 1H s 1H = 12 13 17 HPLC 1 V 3 HSCCC 5 HPLC b

Fig. 3 HPLC chromatograms of a] the crude extract of the seeds of Euphorbia lathyris L. and b] the prepared esculentin by HSCCC from the crude extract
七叶内酯的方法。该方法具有简便、快速、节省溶剂等特点，具有较好的应用价值，且对于后续的续随子种子的药理研究和综合利用都具有非常重要的参考价值。

参考文献: