Separation and purification of flavonoids from *Nelumbo nucifera* Gaertn. by silica gel chromatography and high-speed counter-current chromatography

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Abstract: Three flavonoids were isolated and purified from *Nelumbo nucifera* Gaertn. by the combination of silica gel chromatography and high-speed counter-current chromatography (HSCCC). The crude extract of *N. nucifera* was separated by silica gel chromatography and the fraction abounding in flavonoids was obtained. Then the fraction was separated by HSCCC with two phase solvent systems composed of ethyl acetate–ethanol–water–acetic acid (4:1:5:0.025) (v/v/v/v). The upper phase was the stationary phase and the lower phase as the mobile phase. Under the conditions of a flow rate of 2.0 mL/min while the apparatus rotated at 800 r/min and the detection wavelength of 254 nm, 6.1 mg of quercetin-3-O-β-D-glucuronide, 14.8 mg of myricetin-3-O-β-D-glucopyranoside and 20.2 mg of astragalin were obtained from 150 mg of the crude sample in one step. The purities determined by high performance liquid chromatography (HPLC) were 97.0%, 95.4% and 96.3%, respectively. The structures of the target compounds were identified by electrospray ionisation mass spectrometry (ESI-MS), 1H-nuclear magnetic resonance (1H-NMR) and 13C-nuclear magnetic resonance (13C-NMR). This method that has practical value not only saves on solvent but also conveniently. It is effective in the separation of flavonoids from *N. nucifera* and provides theoretical foundation for the further development and use of the *N. nucifera* resources.

Key words: silica gel chromatography, high-speed counter-current chromatography, HSCCC.
quercetin-3-O-β-D-glucuronide, myricetin-3-O-β-D-glucopyranoside, astragalin. *Nelumbo nucifera* Gaertn.

**Fig. 1 Chemical structures of the 3 flavone compounds**

### 1  
#### 1.1  
**GS10A**

230 mL β 0.5 ~ 0.8 TBP 5 kg
2 h 3057-11 t PDA Waters 600-996 Varian INOVA-600 Varian Agilent 1100 Series 6320 ion-trap

#### 1.2  

200 ~ 300 5 kg

#### 1.3  

40 g 100: 1 60: 1 40: 1 20: 1 10: 1 5: 1
2.2 HSCCC

HSCCC 2 mg F, 10 mL 2 mL/min 254 nm 20 mL/min 800 r/min 2 mL/min 254 nm 5 mL 2 mL/min 30 min

1.4.1 HSCCC

K_b 2 mg F, 10 mL 2 mL/min 1 min 2 mL/min 5 mL 800 r/min 2 mL/min 254 nm

1.4.2 HSCCC

K_b 2 mg F, 10 mL 2 mL/min 800 r/min 2 mL/min 254 nm

1.5 HPLC

Inertsil ODS-SP 250 mm × 4.6 mm 5 μm 25 °C A 0.19 mL/min 0.05 mL/min 254 nm 0 μL/min 254 nm

1.6 HSCCC

ESI-MS 1H-NMR 13C-NMR DMSO 0.2 mL/min 24.1 Pa 4 kV 250 mm DMSO 0.19 mL/min 0.05 mL/min 0 μL/min 254 nm

Fig. 2 TLC chromatograms of the crude extract of N. nucifera

Developing solvents a. chloroform-methanol b. petroleum ether-ethyl acetate.
徐双双,等:硅胶柱色谱结合高速逆流色谱法分离纯化荷花中黄酮类化合物

| 表 | 不同溶剂体系中黄酮类化合物的分配系数 |
|---|---|---|---|
| I | II | III |
| Petroleum ether-ethyl acetate-methanol-water | 1:1:1 | 1.4 | 1.3 |
| Ethyl acetate-methanol-water | 4:1:5 | 0.025 | 0.01 | |
| Ethyl acetate-ethanol-water | 4:1:5 | 0.025 | 0.025 | |
| Ethyl acetate-ethanol-water-acetic acid | 4:1:5:0.01 | 1.1 | 3.0 | 5.4 |
| Ethyl acetate-ethanol-water-acetic acid | 4:1:5:0.025 | 0.8 | 1.9 | 2.9 |

For compounds I ~ III see Fig. 1.

2.3 HSCCC

<table>
<thead>
<tr>
<th>Solvent system</th>
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<th>II</th>
<th>III</th>
</tr>
</thead>
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<td>8.7</td>
<td>11.2</td>
</tr>
<tr>
<td>Ethyl acetate-methanol-water</td>
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<td>7.1</td>
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化合物的结构鉴定

化合物 F, yellow powder (MeOH); F, compound F, yellow powder (MeOH); F, compound F, yellow powder (MeOH); F, compound F, yellow powder (MeOH); F, compound F, yellow powder (MeOH).

Fig. 3 HSCCC chromatogram of flavone compounds from Nelumbo nucifera Gaertn.

1. quercetin-3-O-β-D-glucuronide; II. myricetin-3-O-β-D-glucopyranoside; III. astragalin.

2.4 HPLC

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For compounds I ~ III see Fig. 1.

Fig. 4 HPLC chromatograms of the component F, quercetin-3-O-β-D-glucuronide, myricetin-3-O-β-D-glucopyranoside, and astragalin.
化合物槲皮素具有较好的实用价值,为进一步开发利用荷花中的黄酮类化合物进行富集,然后采用硅胶柱色谱法分离纯化十分困难。研究证明,采用硅胶柱色谱法对荷花中的黄酮类化合物进行富集,得到化合物槲皮素,结构见图120。以上波谱数据与文献基本一致,因此鉴定其为紫云英苷,葡萄糖醛酸苷、杨梅素(孙印石,刘政波,王建华,等)与文献[3, 4]相似,采用传统的硅胶柱色谱分离纯化十分困难。

HSCCC

-3-O-β-D-葡萄糖醛酸苷

3

参考文献:

2. Lin X X. Food Science.[J]. 2006, 27[10]: 538