Supporting Information

The Efficacy and Mechanism Study of Yiru Tiaojing Granule on Treating Hyperprolactinemia in vitro and in vivo

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**TCL Analysis**

**Qualitative identification of *Paeonia lactiflora***

The three batches of YRTJ granule (20120503, 20121105, 20121107) and negative sample were extracted and dissolved by ethanol. The reference substance of paeoniflorin was dissolved in ethanol and made to a concentration of 1 mg/ml. The samples were analyzed by TLC using precoated sheets with Silica gel GF 254 precoated plates as the adsorbent. The mobile phases were employed: Methylene chloride-ethyl acetate-methanol-formic acid (40:5:10:0.2). After dried in the air, the plate was sprayed with sulfuric vanillin and heated. The chromatograms showed a clearly spotted active principle and noninterference negative sample.

![Fig S1 Thin-layer chromatography of *Paeonia lactiflora.*](image)

**Qualitative identification of *Morinda officinalis***

The three batches of YRTJ granule (20120503, 20121105, 20121107), the reference crude herb of *Morinda officinalis*, and the negative sample were extracted and dissolved by ethanol. Aluminum plates precoated with silica gel GF 254 were used as the stationary phase and a mixture of xylene-ethyl acetate-petroleum
ether-methanol-phosphoric acid-water (2:1:1:0.1:0.5) as the mobile phase. Qualitative identification was carried out by spraying with sulfuric vanillin and then heating. The developed spots were scanned under 365 nm UV light. The chromatograms showed a clearly spotted active principle and noninterference negative sample.

![Chromatogram](image)

**T:** 25°C  
**RH:** 70%

1. YRTJ (20120503)  
2. YRTJ (20121105)  
3. YRTJ (20121107)  
4. Negative sample  
5. Reference crude herb (10 μl)

**Fig S2** Thin-layer chromatography of *Morinda officinalis*.

**Qualitative identification of Glycyrrhiza**

The three batches of YRTJ granule (20120503, 20121105, 20121107), the reference crude herb of Glycyrrhiza, and the negative sample were extracted by diethyl ether. The discarded the ether layer and the water course were extracted by water-saturated butanol. After drying, the residue was dissolved by methyl alcohol. The samples were separated on aluminum plates precoated with silica gel GF254, with ethyl acetate-formic acid-glacial acetic acid-water (15:1:1:2) was used as the mobile phase. The plates were sprayed with sulfuric acid ethanol solution, heated, and analyzed under 365 nm UV light after being dried. A clearly spotted active principle and the noninterference negative sample are shown on the chromatograms.
YRTJ granule was subjected to HPLC analysis. YRTJ was dissolved and solutions were injected into the HPLC system for analysis. The mobile phase of paeoniflorin, liquiritin, and monotropein was a mixture of acetonitrile-0.1% phosphoric acid (18:82), acetonitrile-0.1% potassium dihydrogen phosphate (17:83), and acetonitrile-0.1% phosphoric acid (6:94), respectively. The UV detective wavelength for them was 231 nm. The mobile phase of Orcinol glucoside was a mixture of acetonitrile-0.1% phosphoric acid (5:95). The UV detective wavelength was 216 nm. The flow rate and injection volume were 1.0 ml/min and 20 μl, respectively. The chromatographic standards (98% purity) were purchased from Guangdong Institute For Food and Drug Control.

By comparison with the standard reference compounds, four compounds were
identified: peoniflorin, liquiritin, monotropein, and orcinol glucoside, eluting at approximately 14.505 min, 14.601 min, 9.757 min, and 15.298 min, respectively. The content of peoniflorin, liquiritin, monotropein, and orcinol glucoside was 4.263 ± 0.062 mg · g⁻¹, 1.310 ± 0.029 mg · g⁻¹, 1.310 ± 0.029 mg · g⁻¹, and 0.621 ± 0.033 mg · g⁻¹, respectively (Fig. S4).
Fig. S4 HPLC pattern of YRTJ Granule. 1 (A) Standard reference material (peoniflorin, 14.505 min); (B) blank serum; (C) YRTJ Granule. 2 (A) Standard reference material (liquiritin, 14.601 min); (B) blank serum; (C) YRTJ Granule. 3 (A) Standard reference material (monotropein, 9.757 min); (B) blank serum; (C) YRTJ Granule. 4 (A) Standard reference material (orcinol glucoside, 15.298 min); (B) blank serum; (C) YRTJ Granule.
LC-MS/MS Analysis

To prepare the serum sample for LC-MS/MS analysis, 500 μl methanol was added to 100 μl rat plasma to precipitate proteins. After vortex mixing for 1 min and centrifuging at 10000 rpm for 20 min, the supernatant was collected and dried under nitrogen. Then, the samples and paeoniflorin, orcinol glucoside, and liquiritin internal standards (IS) were eluted with 100 μl of methanol. The samples were injected into the LC-MS/MS system for analysis.

The liquid chromatography was performed on an Agilent 6460 Series liquid chromatography (Agilent Technologies) equipped with a ZORBAN SB-C18 column (3.0 × 100 mm, 1.8 μm, Agilent Corporation). The mobile phase consisted of 0.1% formic acid aqueous solution (A) and acetonitrile (B) with a gradient elution program: 80-20% (v/v), 0.1-0.5 min; 40-60%, 0.5-2.8 min; 10-90%, 2.8-3.5 min; 10-90%, 3.5-4.5 min; 20-80%, 4.5-5 min, at a flow rate of 300 μl/min. The most suitable heated capillary temperature, spray voltage, and collision energy for all target analysts were selected by manual optimization. The analytical conditions of LC-MS/MS for the identification of the three constituents are shown in Table S1.

The LC-MS/MS analysis showed that the YRTJ-medicated serum contained complicated components, with several peaks at various retention times (Fig. S5). The components included prototype components in crude drugs and their metabolites. The retention times of paeoniflorin, orcinol glucoside, and liquiritin were approximately 2.9 min, 3.0 min, and 3.1 min, respectively. The product ion mass spectra of the three components are shown in Fig. S6.
<table>
<thead>
<tr>
<th>Components</th>
<th>RT (min)</th>
<th>Precursor (amu)</th>
<th>Product (amu)</th>
<th>CE (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paeoniflorin</td>
<td>2.9</td>
<td>525.2 [M + CH₃COO]⁻</td>
<td>327.2</td>
<td>-20</td>
</tr>
<tr>
<td>Orcinol glucoside</td>
<td>2.0</td>
<td>330.9 [M + CH₃COO]⁻</td>
<td>122.1</td>
<td>-20</td>
</tr>
<tr>
<td>Liquiritin</td>
<td>3.1</td>
<td>471.1 [M - H]⁻</td>
<td>255.2</td>
<td>-20</td>
</tr>
</tbody>
</table>

1. **Table S1** The analytical conditions of LC-MS/MS for the identification of the three constituents.
**Fig. S6** The product ion mass spectra of the three components. **A** Paeoniflorin; **B** Orcinol glucoside; **C** Liquiritin.

**Conclusion**

The supplement material provided the material basis of YRTJ granules and YRTJ-medicated serum to make the *in vivo/vitro* study more reliable.