

Short communication

## A new lignan from the Jian-er syrup and its content determination by RP-HPLC

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### Abstract

The Jian-er syrup is a commonly used traditional Chinese medicinal preparation refined from two herbs, *Metaplexis japonica* (Thunb.) Makino and *Justicia procumbens* L. A new lignan, named 6'-hydroxy-Justicidin B, was isolated from the ethyl acetate extract of the Jian-er syrup and its structure was established on the basis of spectral analysis. The content of 6'-hydroxy-Justicidin B in the Jian-er syrup was determined by RP-HPLC for the first time. Chromatographic separation was achieved on an Agilent Zorbax SB-C<sub>18</sub> column (150 mm × 4.6 mm i.d., 5 μm) at 25 °C and the mobile phase was a mixture of acetonitrile and water (31:69 v/v) detected at 256 nm at a flow rate of 1.0 ml/min. This method was validated in terms of selectivity, linearity, precision, accuracy, limit of detection, limit of quantitation and solution stability. It can be used to control the quality of this preparation effectively.

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**Keywords:** Lignan; 6'-Hydroxy-Justicidin B; Structural elucidation; Content determination; High performance liquid chromatography; The Jian-er syrup

### 1. Introduction

The quality control plays a very important role for ensuring the curative effect of the traditional Chinese medicine. Nowadays, many published papers have dealt with not only the quality control of these multi-component herbal materials, but also the evaluation and quality control of their related products in the market [1–4]. The Jian-er syrup, a commonly used traditional Chinese medicinal preparation listed in the items of protected traditional Chinese medicines [5], is refined from two herb materials, *Metaplexis japonica* (Chinese herb name: Luomo) and *Justicia procumbens* (Juechuang). Pharmacological studies have showed that it can invigorate the function of the stomach, remove the retention of undigested food and expel the intestinal worms. Previous chemical investigations of the above two herb materials revealed that the pregnane glycosides are the main constituents of *M. japonica* [6], and the ligands and their glycosides are those of *J. procumbens* [7–11]. To date, the standard of quality control demanded for the Jian-er syrup according to the list of protected

traditional Chinese medicines was only the TLC identification of *J. procumbens*. In order to improve its standard of quality control, the characteristics of the chemical constituents of *M. japonica*, *J. procumbens* and the extract of the Jian-er syrup were analyzed by RP-HPLC, respectively. Finally, a marker, a new lignan named 6'-hydroxy-Justicidin B (Fig. 1), was isolated from the ethyl acetate extract of the Jian-er syrup and its structure was established by the spectral analysis. Its content in the Jian-er syrup was also determined by the RP-HPLC for the first time.

### 2. Experimental

#### 2.1. Materials and reagents

*M. japonica* and *J. procumbens* were collected from Jiangxi province and identified by Prof. Hubiao Chen, School of Pharmaceutical Sciences, Peking University. The Jian-er syrup extract (concentrated water extract of *M. japonica* and *J. procumbens* without sugar) and the Jian-er syrup product (containing sugar) were kindly offered by Jiangxi Tianshikang Traditional Chinese Medicine Stock Corporation.

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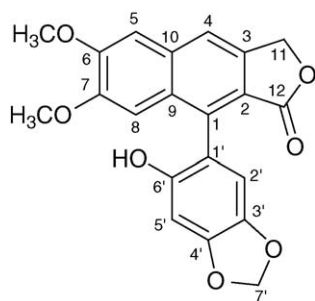


Fig. 1. Structure of 6'-hydroxy-Justicidin B.

6'-Hydroxy-Justicidin B was isolated and its structure was identified in our laboratories. Its purity, confirmed by HPLC with a diode array detector, was over 99%. HPLC grade acetonitrile was purchased from J.T. Baker Company (USA). Other reagents were of analytical grade and the water used was double distilled.

## 2.2. Apparatus and chromatographic conditions

Melting point was measured on a RY-1 melting point apparatus. UV was recorded on a Shimadzu UV-2100s spectrophotometer. IR spectrum was recorded on a Perkin-Elmer spectrophotometer.  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, DEPT, HSQC and HMBC spectra were recorded on a Bruker AV-500 spectrometer (500 MHz for  $^1\text{H}$  NMR and 125 MHz for  $^{13}\text{C}$  NMR) with tetramethylsilane as the internal standard. Mass spectrum was determined on a GCT-MS instrument (Micromass, UK).

All analysis were performed on a Waters HPLC system, equipped with a Waters<sup>TM</sup> 600 pump, a Waters<sup>TM</sup> 600 system controller and a 996 photodiode array detector (Waters, USA). Separation was achieved on a column of Agilent Zorbax SB-C18 (150  $\mu\text{m} \times 4.6 \mu\text{m}$  i.d., 5  $\mu\text{m}$ ) (Agilent Technologies). The chromatographic data were recorded and processed with a waters empower workstation. The flow rate was 1.0 mL min<sup>-1</sup>. The injection volume was 10  $\mu\text{L}$  and the column temperature was 25 °C. When the characteristics of the chemical constituents of *M. japonica*, *J. procumbens* and the extract of the Jian-er syrup were analyzed, respectively, the mobile phase was a mixture of acetonitrile and water in the mode of gradient elution as shown in Table 1 and the column effluents were monitored at 205 and 256 nm simultaneously. On the other hand, when the content of 6'-hydroxy-Justicidin B in the Jian-er syrup was analyzed, the mobile phase was acetonitrile–water (31:69 v/v) and the detected wavelength was 256 nm.

Table 1  
Gradient elution programme using mobile phases A and B

Time (min)	Flow rate (ml/min)	Mobile phase A (%)	Mobile phase B (%)
0	1.0	10	90
50	1.0	60	40
60	1.0	10	90
70	1.0	10	90

A: acetonitrile; B: water.

## 2.3. Extraction and isolation of 6'-hydroxy-Justicidin B

The air-dried whole parts of the plants, *M. japonica* and *J. procumbens* (2 kg, respectively), were chipped and then boiled in water for two times (2 h for the first time and 1.5 h for the second time). The decoctions were combined, filtered and concentrated to yield a dark brown viscous gum (500 g). The resulting gum was partitioned between ethyl acetate and water to afford an ethyl acetate extract (40 g). This extract was chromatographed on a silica gel (100–230 mesh, 1.5 kg) column and eluted successively with a gradient solvent of chloroform–methanol (25:1–12:1). Fractions 10–15 were combined and further purified by preparative RP-HPLC to give 6'-hydroxy-Justicidin B (22 mg).

## 2.4. Preparation of standard solution

A stock solution with 6'-hydroxy-Justicidin B at the concentration of 605  $\mu\text{g/ml}$  was prepared using the solvent of acetonitrile. The stock solution was stored in the refrigerator. The standard solution, containing 6'-hydroxy-Justicidin B at the concentration of 60.5  $\mu\text{g/ml}$ , was prepared by diluting the stock solution with acetonitrile.

## 2.5. Preparation of sample solutions for characteristic study of chemical constituents

### 2.5.1. Preparation of sample solutions for the extract of the Jian-er syrup

**2.5.1.1. Ethyl acetate portion.** Ten millilitres of the Jian-er syrup extract was accurately transferred to a separator and extracted with ethyl acetate for three times (20 ml per time). Then, the ethyl acetate layer was combined and evaporated to dryness. The residue was redissolved with methanol in a 2.0 ml calibrated flask.

**2.5.1.2. *n*-Butanol portion.** Ten millilitres of *n*-butanol (saturated with water) was added to the remainder aqueous layer after extracted with ethyl acetate. Then, the *n*-butanol layer was combined and evaporated to dryness. The residue was redissolved with methanol in a 2.0 ml calibrated flask.

### 2.5.2. Preparation of sample solutions for *M. japonica*, *J. procumbens*

**2.5.2.1. Ethyl acetate portion.** Five grams of crude powder of *M. japonica* and *J. procumbens* was boiled with water for two times, respectively (2 h for the first time and 1.5 h for the second time). The decoctions were combined, filtered and concentrated to the volume of 10 ml on a water bath, respectively. Accurately transferred concentrated solution to a separator and extracted with ethyl acetate for three times (20 ml per time), respectively. The ethyl acetate layer was combined and then evaporated to dryness. The residue was redissolved with methanol in a 2.0 ml calibrated flask, respectively.

**2.5.2.2. *n*-Butanol portion.** Ten millilitres of *n*-butanol (saturated with water) was added to the remainder aqueous layer after extracted with ethyl acetate. Then, the *n*-butanol layer was com-

bined and evaporated to dryness, respectively. The residue was redissolved with methanol in a 2.0 ml calibrated flask, respectively.

### 2.6. Preparation of sample solution for determination of 6'-hydroxy-Justicidin B

Ten millilitres of the Jian-er syrup was accurately transferred to a separator and extracted with ethyl acetate for three times (20 ml per time). The ethyl acetate layer was combined and then evaporated to dryness. The residue was redissolved with methanol in a 2.0 ml calibrated flask.

All solutions were filtered through a 0.45  $\mu\text{m}$  nylon filters (Whatman, UK) before injection into the valve of HPLC.

## 3. Results and discussion

### 3.1. Structural elucidation of 6'-hydroxy-Justicidin B

6'-Hydroxy-Justicidin B: yellow amorphous powder; decomposed point 287 °C; UV,  $\lambda_{\text{max}}$  (CH<sub>3</sub>CN) 256, 305 nm; IR,  $\nu_{\text{max}}$  (film) 3293, 1727, 1617, 1504, 1391, 928  $\text{cm}^{-1}$ ; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): 7.86 (s, 1H, 4-H), 7.43 (s, 1H, 5-H), 6.94 (s, 1H, 8-H), 6.63 (s, 1H, 2'-H), 6.59 (s, 1H, 5'-H), 5.997 (s, 1H, 7'-H<sub>a</sub>), 5.990 (s, 1H, 7'-H<sub>b</sub>), 5.39 (s, 2H, 11-H<sub>2</sub>), 3.92 (s, 3H, 7-OCH<sub>3</sub>), 3.66 (s, 3H, 6-OCH<sub>3</sub>); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): 136.23 (C-1), 118.74 (C-2), 139.90 (C-3), 118.60 (C-4), 106.68 (C-5), 149.68 (C-6), 151.52 (C-7), 105.61 (C-8), 132.94 (C-9), 128.16 (C-10), 68.10 (C-11), 169.68 (C-12), 113.26 (C-1'), 110.43 (C-2'), 139.82 (C-3'), 147.71 (C-4'), 97.80 (C-5'), 149.58 (C-6'), 101.06 (C-7'), 55.85 (7-OCH<sub>3</sub>), 55.32 (6-OCH<sub>3</sub>); DEPT 135 (DMSO-*d*<sub>6</sub>): 118.60 (CH), 110.44 (CH), 106.65 (CH), 105.54 (CH), 97.77 (CH), 55.84 (CH<sub>3</sub>), 55.29 (CH<sub>3</sub>); EIMS, *m/z* 381 [M+H]<sup>+</sup>, 380 [M<sup>+</sup>]; HR-EIMS, *m/z* 380.0899 [M<sup>+</sup>] (calculated for C<sub>21</sub>H<sub>16</sub>O<sub>7</sub> 380.0896). Key HMBC correlations were shown in Fig. 2.

### 3.2. Analysis for the characteristics of chemical constituents of *M. japonica*, *J. procumbens* and the extract of the Jian-er syrup

In order to obtain the characteristics of the chemical constituents of *M. japonica*, *J. procumbens* and the extract of the

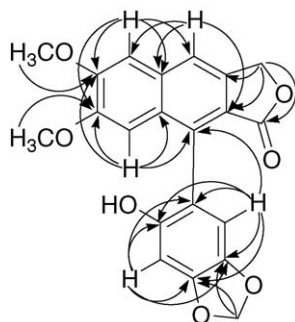


Fig. 2. Key HMBC correlations.

Jian-er syrup, respectively, a mode of gradient elution was used within a reasonable period of time (see Table 1). Photodiode-array detector was applied to get the online UV spectra of all the constituents so that UV spectra of the constituents of *M. japonica* and *J. procumbens* could be compared with those of the related extract of the Jian-er syrup. Some similar peaks could be found from both the chromatograms of ethyl acetate portions of the extract of the Jian-er syrup and those of *J. procumbens*. The sample solutions of *n*-butanol portions were monitored simultaneously at 205, 256 nm. But no similar peak could be found. This was why we isolated the marker from the ethyl acetate portion of the extract of the Jian-er syrup. In order to identify the marker in the Jian-er syrup, the retention times and UV spectra of the sample peaks were compared with those of the marker. The results showed that the excellent agreement between the marker and samples was found in all analyzed samples.

### 3.3. Optimal extraction times

In order to save time and solvent for the sample preparation for the determination of 6'-hydroxy-Justicidin B, the optimal extraction times were studied. According to the above sample preparation procedures, four duplicated samples from the same batch of the Jian-er syrup were extracted with ethyl acetate for 2–5 times (20 ml per time) and analyzed with the established HPLC method, respectively. The contents of 6'-hydroxy-Justicidin B, obtained by different extraction times, were shown in Table 2. It could be seen from Table 2 that the content of 6'-hydroxy-Justicidin B reached the highest value when the sample was extracted for three times. Therefore, the optimal extraction times were selected as three.

### 3.4. Method validation

#### 3.4.1. Linearity

The 2, 4, 6, 8, 10, 12, 14  $\mu\text{l}$  of the standard solution were injected into the valve of the chromatograph three times, respectively. The mean value of peak areas was taken for calibration. The calibrated curve was established by plotting the peak area against the injected quantity of the standard solution using linear regression analysis. It showed that the slope was 6469193.0 with a *y*-intercept  $-37678.1$  and correlation coefficient of 0.9999 and also disclosed an excellent linearity within the range of 0.121–0.847  $\mu\text{g}$ . The above analysis revealed that the external standard method was suitable for the determination of the content of 6'-hydroxyl-Justicidin B in the Jian-er syrup.

Table 2  
Results of extraction time

Extract time	Contents of 6'-hydroxy-Justicidin B ( $\mu\text{g/ml}$ )
2	10.41
3	10.58
4	10.54
5	10.55

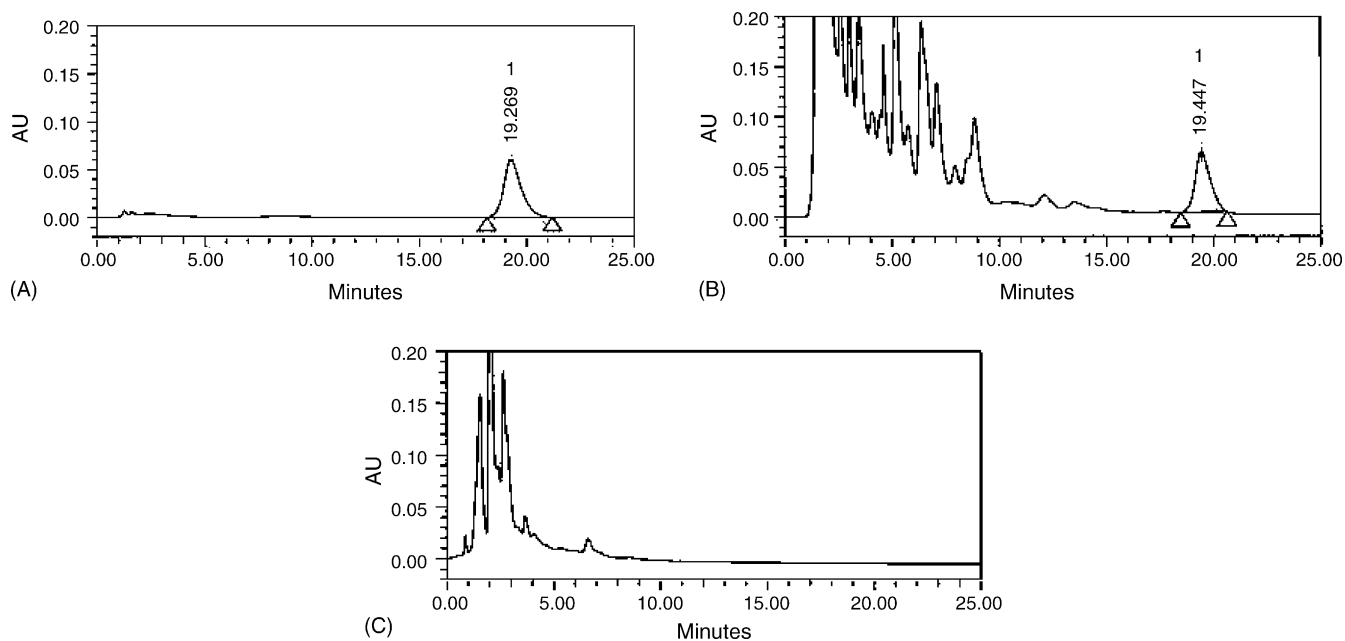


Fig. 3. Chromatograms for the analysis of 6'-hydroxy-Justicidin B. (A) Standard solution, (B) sample solution of Jian-er syrup and (C) blank sample solution of Jian-er syrup. HPLC conditions: Agilent Zorbax SB-C18 (150  $\mu\text{m}$   $\times$  4.6  $\mu\text{m}$  i.d., 5  $\mu\text{m}$ ) column controlled at 25  $^{\circ}\text{C}$ . The mobile phase is acetonitrile–water (31:69 v/v) at a flow rate of 1.0 ml/min. The detective wavelength was 256 nm. (1) 6'-Hydroxy-Justicidin B.

#### 3.4.2. Limit of detection and limit of quantitation

The LOD and LOQ were measured as the concentrations corresponding to signal-to-noise ratio of 3:1 and 10:1, respectively. The LOD and LOQ values of 6'-hydroxy-Justicidin B were found to be 0.1 and 0.3  $\mu\text{g/ml}$ , respectively.

#### 3.4.3. Precision

The system precision was determined by performing six times of replicated analysis of the same standard solution and evaluated by the R.S.D. of the peak area of the analyte. It showed that the obtained R.S.D. value for 6'-hydroxy-Justicidin B was 1.07%.

The precision of the established method was determined by preparing the Jian-er syrup sample of the same batch in six replicated determinations. The R.S.D. value of the assay results was used to evaluate the method precision. The obtained R.S.D. value was 1.45% for the determination of 6'-hydroxy-Justicidin B in the Jian-er syrup.

#### 3.4.4. Accuracy

The accuracy of the method was confirmed by the determination of recovery. Six duplicated samples from the same batch of the Jian-er syrup were spiked with a known amount of 6'-hydroxy-Justicidin B before extraction. The spiked samples were extracted and analyzed under the previously described conditions. The average recovery was 98.68% and R.S.D. was 0.69%. Therefore, The method was regarded as accurate.

#### 3.4.5. Solution stability

In order to examine the stability of standard solution, standard solution was analyzed successively for a period of 6 days. The results showed that the retention time and peak area of 6'-

hydroxy-Justicidin B remained almost unchanged (R.S.D.% less than 1.35%).

#### 3.5. Quantitative results

The newly established method was applied to the determination of 6'-hydroxy-Justicidin B in three batches of the Jian-er syrup. The results of chromatograms for the determination of 6'-hydroxy-Justicidin B were shown in Fig. 3. The retention time of 6'-hydroxy-Justicidin B was about 19 min. Under the proposed analytical conditions, the content determination of 6'-hydroxy-Justicidin B was not subjected to the interference from other components in the chromatograms. The contents of 6'-hydroxy-Justicidin B were within 5.54–11.04  $\mu\text{g/ml}$  (shown in Table 3). R.S.D. values within 1.36–1.65% were found to be satisfactory. For the three batches of the Jian-er syrup, the results of the content of 6'-hydroxy-Justicidin B were quite different from each other. This might be due to the different sources of crude herbs and the processes of preparation production. And this result demonstrated that the need for improving the standard of quality control for the traditional Chinese medicinal preparation was urgent.

Table 3  
Contents of 6'-hydroxy-Justicidin B in batches of Jian-er syrup

Batches	Contents of 6'-hydroxy-Justicidin B ( $\mu\text{g/ml}$ )	R.S.D. (%) ( $n=3$ )
020711	5.54	1.36
030803	11.04	1.65
030919	10.26	1.61

#### 4. Conclusion

This is the first report on the structural elucidation and the content determination of a new lignan from the extract of the Jian-er syrup. The established RP-HPLC method was proved to be simple, precise and accurate. It can be used as a quality control method for the determination of 6'-hydroxy-Justicidin B in the Jian-er syrup, a commonly used traditional Chinese medicinal preparation.

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