HPLC analysis of bioactive flavonoids from the rhizome of *Alpinia officinarum*


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Received 6 May 2005; accepted 17 June 2005

Abstract

*Alpinia officinarum* Hance, a famous traditional Chinese medicine, has been used as an aromatic stomachic, analgesic, and antiemetic in Asia. To evaluate the quality of *A. officinarum* Hance, a sensitive, simple and accurate high-performance liquid chromatographic (HPLC) method was developed for the assessment of two major bioactive flavonoids: galangin and 3-O-methyl galangin. The HPLC system used a Spherisorb octadecylsilyl silica (ODS) column with methanol–water–phosphoric acid (60–38–2) as the mobile phase and detection at 254 nm. Separation of the two compounds was achieved by the HPLC method above and they showed good linear relationships in the range of 0.3–1.4 μg for galangin and 0.02–0.14 μg for 3-O-methyl galangin. The correlation coefficients of the calibration curve for the analytes was higher than 0.999. In addition, the content of these two flavonoids in the rhizome of *A. officinarum* growing at twelve different locations in China was determined to establish the effectiveness of the method.

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Keywords: *Alpinia officinarum*; Galangin; 3-O-methyl galangin; Flavonoid; HPLC

1. Introduction

*Alpinia officinarum* Hance (AO), a traditional herbal remedy, has been used as an aromatic stomachic, analgesic, and antiemetic in Asia (Ching Su New College, 1987). Several constituents of AO have been reported, namely, 1,8-cineole, methyl cinnamate, α-cadinene, galangin, 3-O-methyl galangin, kaempferide, alpinin, galangol, and some diarylheptanoids (Itokawa et al., 1981; Itokawa et al., 1985; Shin et al., 2003). Among them there were two constituents demonstrated to be the major bioactive constituents. One is galangin, a member of the flavonol class of flavonoids, which was present in high concentrations in AO (Heo et al., 2001). Galangin possesses certain biological activities, including anti-clastogenic (Heo et al., 1996), anti-mutagenic (Wall et al., 1988), anti-oxidative and radical scavenging (Cholbi et al., 1991; Imamura et al., 2000), and metabolic enzyme modulating activities (Shih et al., 2000).

The other is 3-O-methyl galangin, which may be effective as a hypolipidemic agent due to its inhibition of pancreatic lipase (Shin et al., 2003). Thus, with the increasing applications of AO in food and the medicinal herb industry, it is essential to establish an analytical method for quality control. The strategy we applied was to determine the two compounds (galangin and 3-O-methyl galangin), which previously were identified as bioactive constituents, for the assessment of the quality of AO. This is the first publication to simultaneously determine galangin and 3-O-methyl galangin in AO by HPLC.

2. Experimental

2.1. Plant material and chemicals

Henan province. The voucher specimens were identified and deposited at the Institute of Biochemistry, East China University of Science and Technology, Shanghai, 200237, China.

Flavonoid standards (galangin and 3-O-

\[
\text{Galangin (C}_{15}\text{H}_{10}\text{O}_{5})
\]

\[
\text{3-O-methyl galangin (C}_{16}\text{H}_{12}\text{O}_{5})
\]

b

Fig. 1. Structures of galangin and galangin-3-methoxy.

Flavonoid standards (galangin and 3-O-methyl galangin, Fig. 1) were isolated and purified in our lab with individual purity not less than 98% (HPLC assay, UV detection).

HPLC-grade methanol was purchased from Caledon Laboratories Ltd., Canada. Redistilled water was prepared in our own lab. Phosphoric acid (≥ 85%) was purchased from Shanghai Chemical Co.

2.2. Sample preparation

An accurately weighed portion of AO was sonicated with methanol at 15–20 °C for 60 min. The methanol extracts were brought to dryness, in vacuo, at 60 °C. The residue was dissolved in methanol, transferred to a 50 ml volumetric flask, and the volume adjusted with methanol. The sample solution was filtered through a 0.2 μm membrane directly into a HPLC sample vial, just prior to HPLC analysis.

2.3. Analytical method

The HPLC system was equipped with an Agilent 1100 serial system, consisting of a quaternary pump, online degasser, auto-sampler, column heater and variable wavelength detector. Separation was achieved on a reversed phase column (ZORBAX, Eclipse SB-C18, 5 μm, 4.6 × 250 mm, Agilent, USA) provided with a C18 guard column and methanol–water–phosphoric acid (60:38:2, v/v/v, isocratically) was employed as the mobile phase. The flow rate was kept constant at 0.8 ml/min and the peaks were identified using UV absorbance at 254 nm. The temperature of the column during analysis was maintained at 40 °C. The injection volume was 10 μl each time.

2.4. Calibration

A mixed stock solution consisting of galangin (1.72 mg·ml⁻¹) and 3-O-methyl galangin (0.17 mg·ml⁻¹) was prepared. 0.4, 0.5, 1.0, 1.3, 1.5 and 2.0 ml of the stock solution was each put into a 25 ml volumetric flask and adjusted with methanol for the standard curves.

3. Results and discussion

3.1. Range of linearity

Typical chromatograms are shown in Fig. 2. The retention time of galangin was 20.4 min and that of 3-O-methyl galangin was 23.9 min. The linearity was investigated for each compound by plotting peak areas against the injected amounts. In the range of 0.3–1.4 μg for galangin and 0.02–
0.14 μg for 3-O-methyl galangin, good correlation of linearity has been achieved. The regression equations and correlation coefficients determined for the references were \[ y = 1213.8 \times +24.576 \] \( R^2 = 0.9997 \) for galangin and \[ y = 2256.1 \times -1.3655 \] \( R^2 = 0.9997 \) for 3-O-methyl galangin.

### 3.2. Reproducibility and repeatability

The study of reproducibility which was performed on three consecutive days \( (n=10) \) indicated a relative standard deviation of 3.65% for galangin and 3.10% for 3-O-methyl galangin and 1.57% and 1.91%, respectively, in the repeatability test.

### 3.3. Precision test

The precision of the analytical method was determined by assaying at least triplicate applications of each sample (Toker et al., 2001). One standard solution was analyzed for six times, consecutively, using the analytical method above. The relative standard deviation of peak areas was 0.44% for galangin and 0.59% for 3-O-methyl galangin.

### 3.4. Recovery test

The recovery test was used to evaluate the accuracy of the method. Five of six parallel solutions were accurately added with certain amount of reference solutions just prior to the extraction. Each solution was injected twice. The average recoveries of galangin and 3-O-methyl galangin were both 99%, and the RSD were 2.54% and 3.12%, respectively. The results are shown in Table 1.

### 3.5. Quantitative determination of AO

Twelve samples were extracted, following the procedure above, and analyzed in the HPLC system. The HPLC/UV profiles are illustrated in Fig. 3. The content of each compound was determined by the corresponding regression equation and was summarized in Fig. 4.

The results indicated that both compounds were detected in all twelve herbal samples and the content of galangin was about ten times more than that of 3-O-methyl galangin in the same sample. In all samples analyzed, the content of galangin ranged from 2.63 mg/g to 11.6 mg/g while that of 3-O-methyl galangin ranged from 0.240 mg/g to 1.13 mg/g.

In our present study, a simple, accurate and rapid HPLC method was developed and this is the first publication of a HPLC determination of 3-O-methyl galangin. The assay is reproducible, sensitive and has been fully validated. Furthermore, it was successfully applied to the determination of the two compounds in different AO samples. The results indicate that herbals from different places show a specific and similar HPLC chromatogram and the evaluation of data might be

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**Table 1**

Recoveries of two compounds \( (n=5) \)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Actual (μg)</th>
<th>Added (μg)</th>
<th>Detected (μg)</th>
<th>*Recovery (%)</th>
<th>Average (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galangin</td>
<td>2.423</td>
<td>1.720</td>
<td>4.14</td>
<td>100</td>
<td>99</td>
<td>2.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.07</td>
<td>96</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>4.17</td>
<td>102</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.16</td>
<td>101</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.11</td>
<td>98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-O-methyl galangin</td>
<td>0.2478</td>
<td>0.1700</td>
<td>0.422</td>
<td>102</td>
<td>99</td>
<td>3.12</td>
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<td>0.409</td>
<td>95</td>
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<td>0.421</td>
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<tr>
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<td></td>
<td></td>
<td>0.415</td>
<td>98</td>
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<td></td>
<td></td>
<td></td>
<td>0.417</td>
<td>100</td>
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</tr>
</tbody>
</table>

* Recovery = [(found−actual)/added] × 100%.
useful in quality assurance as well as for determination of adulteration of the crude drug.

Acknowledgement

This work is supported by the key disciplinary foundation of Shanghai (03DZ19546).

References