Simultaneous Determination by HPLC of Quercetin and Kaempferol in Three *Sedum* Medicinal Plants Harvested in Different Seasons

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A high-performance liquid chromatography method was established for the fast quantification of quercetin and kaempferol in three *Sedum* crude medicines: Sedi Herba (*Sedum sarmentosum* Bunge.), Sedi Linearis Herba (*Sedum lineare* Thunb.) and Sedi Emarginati Herba (*Sedum emarginatum* Migo.). The column used was a YMC-pack ODS-A (250 × 4.6 mm, 5 μm), the mobile phase was a solution of methanol–0.4% phosphoric acid (47:53) with a flow rate of 1.0 mL/min at 35°C and the detection wavelength was 360 nm. The calibration curves for quercetin and kaempferol were linear over the range of 0.01–0.62 μg for quercetin and 0.02–0.78 μg for kaempferol, and the average recoveries were 99.72% [relative standard deviation (RSD): 1.63% and 99.50% (RSD: 1.16%), respectively]. In conclusion, the method established in this paper is accurate and repeatable. It can be used for the determination of quercetin and kaempferol, controlling the quality of the three crude drugs. Furthermore, the experimental data showed that the best harvest season for the three *Sedum* medicinal species should be the full-bloom period between the end of April and the beginning of May.

Introduction

Sedi Herba (*Sedum sarmentosum* Bunge.), Sedi Linearis Herba (*Sedum lineare* Thunb.) and Sedi Emarginati Herba (*Sedum emarginatum* Migo.) are three traditional Chinese crude drugs that have been used in folk medicine to treat hepatitis, dysentery, herpes zoster and swelling. Sedi Herba, which possesses activities against fever and has the function of detoxifying, has been recorded in the Chinese Pharmacopoeia (2010) (1) because of its activities against acute or chronic hepatitis and its effect of reducing glutamic-pyruvic transaminase. In addition, Sedi Linearis Herba and Sedi Emarginati Herba are both used in folk medicine to detoxify, treat fever and stanch bleeding (2, 3).

According to the current state of research, the active pharmaceutical ingredients of Sedi Herba that possess liver-protecting activities and reduce glutamic-pyruvic transaminase primarily exist in aqueous and n-butanol extracts (in which portions the glycosides and the flavonoids principally exist). This suggests that its medicinal properties may result from pharmacologically active bioflavonoids (4). There are a few reports on the determination of quercetin in granules and capsules of Sedi Herba (5, 6), but none on the simultaneous determination of quercetin and kaempferol in the three *Sedum* crude drugs. The more active compounds detected, the better the quality of the drugs can be controlled. Thus, these two compounds of *Sedum* medicinal plants harvested in different seasons were simultaneously determined in this research. Moreover, a credible analytical method was developed to control their quality, the best harvest season for the three *Sedum* medicinal plants was confirmed and their relationship was also inferred.

Experimental

Materials and methods

The standards of quercetin and kaempferol were obtained from the National Institute for the Control of Pharmaceutical and Biological Products in China (batch number: 100081-200406, 110861-200808). The experimental samples were three *Sedum* medicinal species that were harvested in different seasons and from different places in Hubei province: the school campus of South-Central University for Nationalities (SCUEC, Wuhan, Hubei province), Sheshan of Wuhan and Huangmei, as presented in Table I. The three *Sedum* medicinal species were identified as *Sedum sarmentosum* Bunge., *S. lineare* Thunb. and *S. emarginatum* Migo. by professor Dingrong Wan (Pharmaceutical College, SCUEC). Chromatographic grade methanol was purchased from Tedia (Fairfield, OH; batch number: MS1922-001) and water was purified by an Aquapro Hi-End Water Treatment Solution Provider (ASWO-0005-U, Ever Young Enterprises). Phosphoric acid, concentrated hydrochloric acid and all other chemicals were of analytical grade.

Apparatus

The high-performance liquid chromatography (HPLC) analysis was performed on an Agilent 1200 HPLC system composed of a quaternary pump (DE62958975), an autosampler, a column thermostat, temperature-controlled sample trays, an online degasser and an ultraviolet (UV) detector. The analytical column was a YMC-Pack ODS-A column (5 μm, 250 × 4.6 mm).

The full wave scanning of the three *Sedum* medicinal plants was performed on a UV-visible (Vis) spectrophotometer (UV-1800PC, Mapada Co., Shanghai, China).

An Aquapro Hi-End Water Treatment Solution Provider (ASWO-0005-U, Ever Young Enterprises) and an AP-01P Vacuum Pump (Auto Science Company, Tianjin, China) were used to produce and filter the ultrapure water. A rotary evaporator (R-1001N) and a vacuum pump were used for sample preparation.
**Methods**

**Preparation of standard solution**

The stock standard solution of quercetin (31 mg/mL) and kaempferol (39 mg/mL) was prepared by dissolving the dried powders of quercetin and kaempferol (Figure 1) in methanol (1, 7).

**Optimization of the sample extraction**

All samples were extracted via both heated reflux and ultrasonic methods (7, 8). The results showed that refluxing was the better method.

To determine the ideal extraction time, samples were refluxed for 1, 1.5, 2 or 2.5 h (Figure 2). The results showed that the extraction efficiency improved as the reflux time increased. In addition, the extraction efficiency of 2 h was much better than that of the regulation time (1 h) in the 2010 Chinese Pharmacopoeia, but similar to that of 2.5 h. Therefore, 2 h was chosen as the best extraction time.

The crude drugs were crushed, passed through a 20-mesh sieve, extracted only by methanol and analyzed. The chromatograms showed hardly any quercetin or kaempferol. This suggests that both quercetin and kaempferol primarily exist as glycosides in the three drugs and acidic hydrolysis is needed before analyzing. Thus, methanol with different acidity levels was used as the extraction solvent. An increased concentration of HCl significantly improved the extraction efficiency, and methanol–25% HCl (4:1) was found to be the best solvent.

**Sample preparation**

The dried experimental samples (approximately 2.0 g, dried at 60°C) were crushed and passed through a 20-mesh sieve, accurately weighed and refluxed for 2 h in 50 mL of methanol–25% HCl (4:1). The extract was cooled and filtered and the extraction flask was rinsed with methanol. The filtrate was combined with the methanol rinse and diluted to a final volume of 50 mL with methanol. Each sample was filtered through a 0.45 μm membrane into an amber glass HPLC vial before analysis.

**Optimization of chromatographic conditions**

Based on the results of the full wave scanning of the three Sedum medicinal species and the absorbing wavelength of the standard solution of quercetin and kaempferol, 360 nm was chosen as the detection wavelength. To choose the mobile phase, a series of solutions was studied with different ratios of methanol–0.4% phosphoric acid. Ultimately, the ratio of 47:53 was selected for the mobile phase with a flow rate of 1.0 mL/min. The temperature of the column was maintained at 35°C and the injection volume was 20 μL.

The chromatographic conditions above were optimized to separate the primary marker peaks of each Sedum sample with good resolution ($R > 1.5$) and theoretical plate numbers (quercetin and kaempferol: $n > 8,000$). The chromatograms of the standard solution and the three Sedum medicinal plants are shown in Figure 3.

**Results and Discussion**

**Results**

**Method validation**

The reliability of the HPLC method established in this paper was proven by checking its linearity, precision, repeatability, stability and recovery.
To calculate the regression equation, six different concentrations of the standard mixture solution were used in this research; the results are shown in Tables II and III. The regression equations took the form $Y = aX + b$ [the Y-axis was the value of the chromatographic peak area (mAU) and the X-axis was the weight of the marker component]. The regression equations of quercetin and kaempferol and the parameters for linearity are presented in Table IV. The high correlation coefficients of the calibration curves of each marker component indicate good linearity over the range under investigation.

**Precision**

To measure precision, the six standard solution samples were each analyzed thrice to determine the mean. The relative standard deviations (RSDs) of the peak area values of the two standards were 0.02 and 0.08% for quercetin and kaempferol, respectively. The results showed that the method is highly precise.

**Repeatability**

Six *Sedum sarmentosum* Bunge. (May) samples were taken, treated according to the previously described method and analyzed thrice to determine the mean. The average contents of quercetin and kaempferol in *S. sarmentosum* were 1.1644 mg/g (RSD: 0.61%) and 0.1742 mg/g (RSD: 2.79%), respectively, indicating good repeatability.

**Stability**

The solution of one *Sedum sarmentosum* Bunge. (May) sample was analyzed at 0, 2, 4, 8, 12 and 24 h after sample preparation (Table V). The RSDs of quercetin and kaempferol peak area values were 0.83 and 0.55%, respectively. This showed that the two components in these samples were stable for at least 24 h.
Recovery and accuracy
The accuracy was determined by calculating the recovery after a standard addition procedure. In this study, six precisely weighed *Sedum sarmentosum* Bunge. (May) samples were refluxed according to previously described method and analyzed by HPLC after adding 2 mL of quercetin and 2 mL of kaempferol (concentrations: 0.31 and 0.039 mg/mL) to each sample. Each sample solution was injected thrice and the mean and RSD were calculated. The results for quercetin and kaempferol are presented in Tables VI and VII and show the accuracy of the method.

Sample analysis
All *Sedum* samples harvested in different seasons were weighed accurately, prepared according to the previously described method and analyzed by injecting thrice into the HPLC. The amounts of quercetin and kaempferol in these *Sedum* samples are listed in Table VIII, and the variation with respect to the harvest season of the three *Sedum* medicinal plants is shown in Figure 4.

Discussion
The results showed that the amounts of quercetin and kaempferol in both *S. sarmentosum* and *S. lineare* harvested at the beginning of May were higher than in those plants harvested in September and October, whereas only the content of kaempferol in *S. emarginatum* Migo. harvested at the beginning of May was higher than that in October and December. The content of quercetin in *S. emarginatum* Migo. increased in the later harvest months. In addition, comparing the amount of quercetin and kaempferol showed that the quercetin content was much higher than kaempferol in all *S. sarmentosum* samples harvested in different seasons, whereas the content of quercetin was lower

Table VI
Recovery of Quercetin in *Sedum sarmentosum* Bunge. (May) (n = 6)

<table>
<thead>
<tr>
<th>Sample amount (g)</th>
<th>Quercetin in the samples (mg)</th>
<th>Spiked quercetin amount (mg)</th>
<th>Determined quercetin amount (mg)</th>
<th>Recovery (%)</th>
<th>Mean recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5000</td>
<td>0.59</td>
<td>0.62</td>
<td>1.19</td>
<td>98.35</td>
<td>99.72</td>
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<td>0.62</td>
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<td>0.5004</td>
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<td>0.62</td>
<td>1.22</td>
<td>100.83</td>
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<td></td>
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<tr>
<td>0.5000</td>
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<tr>
<td>0.5004</td>
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<td>0.62</td>
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<td>100.83</td>
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Table VII
Recovery of Kaempferol in *Sedum sarmentosum* Bunge. (May) (n = 6)

<table>
<thead>
<tr>
<th>Sample amount (g)</th>
<th>Kaempferol in the samples (mg)</th>
<th>Spiked kaempferol amount (mg)</th>
<th>Determined kaempferol amount (mg)</th>
<th>Recovery (%)</th>
<th>Mean recovery (%)</th>
<th>RSD (%)</th>
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<td>0.5000</td>
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<td>0.165</td>
<td>98.21</td>
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<td>0.5005</td>
<td>0.090</td>
<td>0.078</td>
<td>0.170</td>
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<tr>
<td>0.5004</td>
<td>0.090</td>
<td>0.078</td>
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<tr>
<td>0.5000</td>
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<td>98.81</td>
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<tr>
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<td>0.078</td>
<td>0.166</td>
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<tr>
<td>0.5004</td>
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Table VIII
Amounts of Quercetin and Kaempferol in Three *Sedum* Medicinal Species Harvested in Different Seasons

<table>
<thead>
<tr>
<th>Sample/harvested season</th>
<th>Content of kaempferol (mg/g)</th>
<th>Content of kaempferol (mg/g)</th>
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<tr>
<td>S. sarmentosum Bunge. (May 2)</td>
<td>1.1754</td>
<td>1.1793</td>
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<tr>
<td>S. sarmentosum Bunge. (Jun. 13)</td>
<td>1.0634</td>
<td>0.1414</td>
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<td>S. sarmentosum Bunge. (Sept. 27)</td>
<td>0.4323</td>
<td>0.9553</td>
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<tr>
<td>S. lineare Thunb. (May 2)</td>
<td>0.5095</td>
<td>0.8077</td>
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<tr>
<td>S. lineare Thunb. (Oct. 7)</td>
<td>0.1547</td>
<td>0.0542</td>
</tr>
<tr>
<td>S. emarginatum Migo. (May 3)</td>
<td>0.0275</td>
<td>1.4226</td>
</tr>
<tr>
<td>S. emarginatum Migo. (Oct. 4)</td>
<td>0.0582</td>
<td>0.3027</td>
</tr>
<tr>
<td>S. emarginatum Migo. (Dec. 1)</td>
<td>0.0753</td>
<td>0.3623</td>
</tr>
</tbody>
</table>

Figure 4. Amount variation with respect to season of harvest of three *Sedum* species A. *Sedum sarmentosum* Bunge. B. *S. lineare* Thunb. C. *S. emarginatum* Migo.
than kaempferol in *S. lineare* harvested at the beginning of May, but higher in October. Regarding *S. emarginatum* in different seasons, the quercetin content was always so much lower than kaempferol that quercetin almost needs not be taken into consideration when selecting the best harvest season. In conclusion, the contents of quercetin and kaempferol varied in all *Sedum* medicinal samples harvested in different seasons. Considering the results published earlier on the determination of their total flavonoids (4), it is recommended that the full-bloom period from the end of April to the beginning of May is the best harvest season for the three *Sedum* medicinal plants.

In addition, it was found that the varying amounts of quercetin and kaempferol in *S. sarmentosum* and *S. lineare* were similar according to harvest season, whereas both species differed markedly from *S. emarginatum*. According to the preceding analysis, and considering their morphological and anatomic characteristics (9), it can be concluded that the genetic relationship between *S. sarmentosum* and *S. lineare* is closer than that between either species and *S. emarginatum*.

Acknowledgments
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References
4. Yujie, C., Jing, W., Dingrong, W.; Determination of total flavonoids in three *Sedum* crude drugs by UV–Vis spectrophotometry; *Pharmacognosy Magazine*, (2010); 6(24): 266–270.