

HPLC Determination of Quercetin in Three Plant Drugs from Genus *Sedum* and Conjecture of the Best Harvest Time

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ABSTRACT

To establish a method for quercetin content determination of the three plant drugs from genus *Sedum* and to decide their best harvesting time. Dried herbs of *Sedum sarmentosum* Bunge., *S. lineare* Thunb. and *S. erythrostictum* Migo. are all traditional medicines from Genus *Sedum*. They all have long been used to cure hepatitis, dysentery, swelling poison and so on. It has been reported that flavonoids contained in *S. sarmentosum* Bunge. were one type of the active components to protect liver and reduce serum alanine aminotransferase level. Through the qualitative analysis and TLC assay, we have verified that both *S. lineare* Thunb. and *S. sarmentosum* Bunge. contain flavonoid glycosides whose aglycones include quercetin. In this study, a HPLC methodology was established to determine quercetin which was a common hydrolyzate of the flavonoid glycosides in the three plant medicines. The determination method developed showed good linearity in the range of 41.2-412.0 µg/mL, and had a nice accuracy and repeatability. It indicated that the content of quercetin in *Sedum sarmentosum*, *Sedum lineare* and *Sedum emarginatum* can achieve the quality standard required by Chinese Pharmacopoeia. (No less than 0.01%), The results also revealed preliminarily a relation between collected season and medicine quality. Thus it could be used to control some flavonoids content in the three plant drugs indirectly. By determining the samples of the 3 drugs collected in different seasons, the optimal harvest periods could be preliminarily ascertained.

Key words: Genus *Sedum*, Plant medicines, HPLC determination, Quercetin, best harvest time.

INTRODUCTION

Sedum sarmentosum Bunge., *S. lineare* Thunb. and *S. erythrostictum* Migo., whose dried herbs are traditional herbal medicines, all belong to Genus *Sedum*. As the three plants are closely related with each other, they usually possess similar chemical components. Despite certain differences that existed among their efficacy, all could treat hepatitis, dysentery, swelling poison and so on.¹⁻⁴ It has been proved that sarmentosin, a water-soluble component contained in *S. sarmentosum* Bunge., is one of the active components to protect liver and reduce serum alanine aminotransferase level. Many reports on the methods to determine sarmentosin have appeared, including to the IR, HPLC and GC approaches.⁵⁻⁷ The studies also find that the flavonoids included in *S. sarmentosum* Bunge.⁸ show a better efficacy in liver protection and the enzyme decrease compared with the water-soluble total glycoside,⁹ suggesting that flavonoids should also be main active composition to treat hepatitis. Through the qualitative analysis and TLC assay, we have verified that both *S. lineare* Thunb. and *S. erythrostictum* Migo. also contained flavonoids. And the preliminary HPLC analysis has shown that the three plant drugs all contain the flavonoid glycosides whose aglycones include quer-

cetin. As HPLC has been a routine methodology to determine the active composition in natural plants both at home and abroad,¹⁰⁻¹³ in this study, we have developed a HPLC means to determine the quercetin content of the samples collected in various seasons, trying to find out the variation trends of the some flavonoids content vs. the growing periods, obtain the appropriate harvest seasons and preliminarily control the quality of the 3 medicines finally.

EXPERIMENTAL

Reagents and materials

The quercetin reference substance was purchased from Chinese pharmaceutical and biological product verification station (batch number: 10008-200406); methanol and phosphoric acid employed here were HPLC grade reagents. Deionized (DI) water was used in the experiment. The three plant medicines collected from Jianshi, Huangmei, and Sheshan (Wuhan), Hubei province of China, and were respectively identified as the dried herbs of *S. sarmentosum* Bunge., *S. lineare* Thunb. and *S. erythrostictum* Migo.

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by Professor Wan Ding-rong from college of pharmacy, South-central University for Nationalities.(Table 1)

Chromatographic conditions

High performance liquid chromatography (HPLC) analysis was performed using an Agilent 1200 HPLC system from Agilent (Karlsruhe, Germany), equipped with a quaternary pump, an autosampler, and a VWD UV detector. Quercetin was separated from the sample solutions using a C_{18} column (4.6×250 mm I.D., particle size 5 μ m, Agilent Eclips XDB- C_{18}), with a mobile phase consisting of methanol and 0.40% phosphoric acid(49:51, V/V), at 25°C. The flow rate was 1.0 ml/min, and injections were 20 μ l in volume.

Standard solution preparation

10.3 mg of the quercetin standard substance was accurately weighed into a 100 ml volumetric flask and made up to the volume with methanol. After evenly mixed, 2 ml was accurately transferred to a 10 ml flask and was diluted with methanol to the volume. Thus, quercetin standard solution (20.6 μ g/ml) was obtained.

Sample preparation

About 2.0 g of dried (100°C, to a constant weight) of *Sedum sarmentosum*, *S. lineare* and *S. erythrostictum*. samples powder was taken and weighed accurately and respectively, then 20 ml of methanol was added and refluxed twice(each time for 30 min).The mixture was filtered into a 50ml volumetric flask. The residue was washed with methanol (2×10 ml), and transferred to the same flask, consecutively, and then made up to the volume with methanol, and shaken until evenly mixed. After that, 20 ml was taken, mixed with 5ml of 25% hydrochloric acid solution, refluxed for 30 min, then cooled immediately and transferred to a 50 ml volumetric flask, and made up to the volume with methanol, finally shaken, filtered through a 0.45 μ m millipore filter prior to HPLC analysis.

RESULTS

Determination of the content of quercetin by HPLC

Calibration curve

A calibration curve was established for quercetin by injecting 2, 4, 8, 12, 16, 20 μ l of the standard solutions twice respectively. Linearity was tested by analyzing the average peak area of quercetin of different injection volume. And then the regression equation($y=74.431x-0.9177$) was obtained, and the correlation coefficient (r) was 0.9999. Thus, a good linearity was shown when the quercetin concentration ranged from 41.2 to 412.0 μ g/ml. [Figure 1].

Precision

The precision was assessed by injecting 20 μ l of quercetin standard solution five times respectively. The quercetin content was calculated based on the calibration curve. The content variation (RSD, %) was found to be 1.04%, demonstrating that the instrument used had a high precision.

Stability

The 20 μ l of the same *S. sarmentosum* Bunge. sample (Jianshi, April) solutions were injected into the apparatus in a certain period of time (0,2,4,6,8,12 h) respectively, and quercetin content was calculated based on the calibration curve. As a result, the content variation (RSD, %) was 1.65%, revealing that the sample solution was stable within at least 12 h.

Repeatability

Five same dried samples of the three plant medicines harvested in April were taken respectively to prepare the sample solutions according

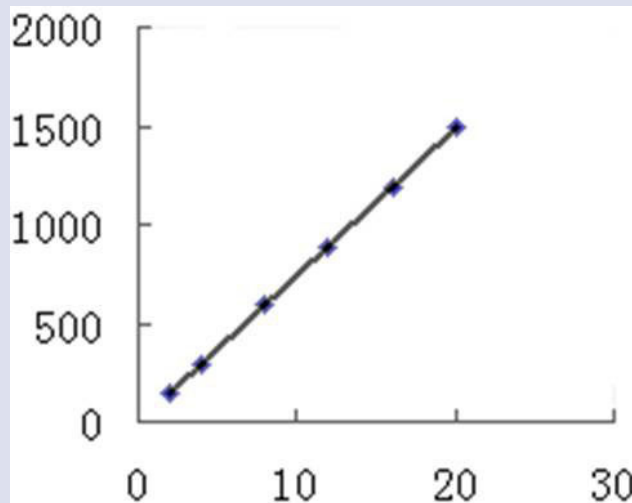


Figure 1: Calibration curve of standard quercetin

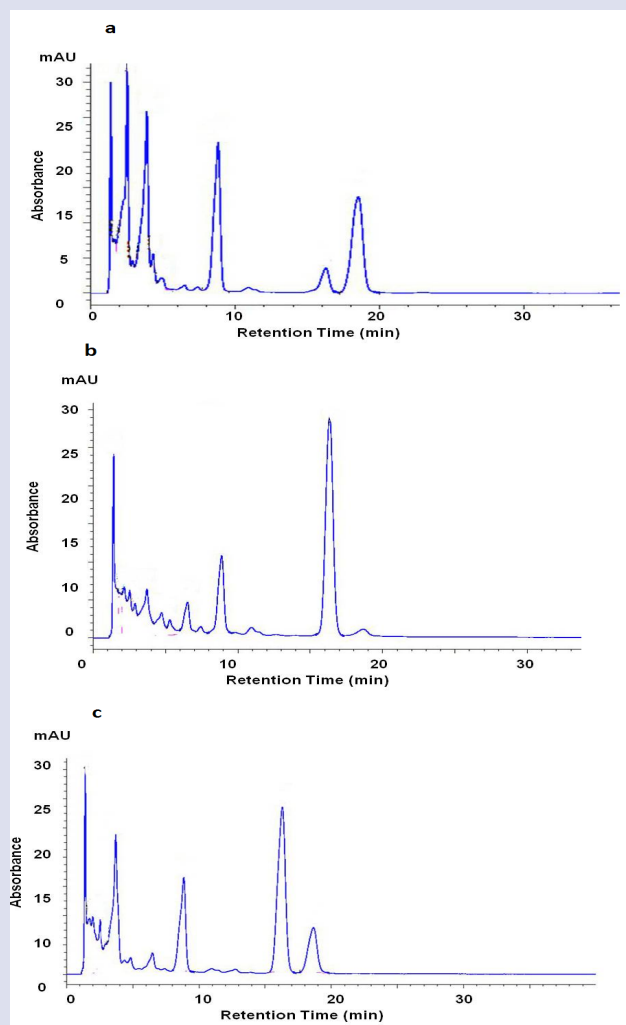


Figure 2: Typical HPLC chromatogram of *S. sarmentosum* collected in April (a: *S. sarmentosum* b: *S. lineare* c: *S. erythrostictum*).

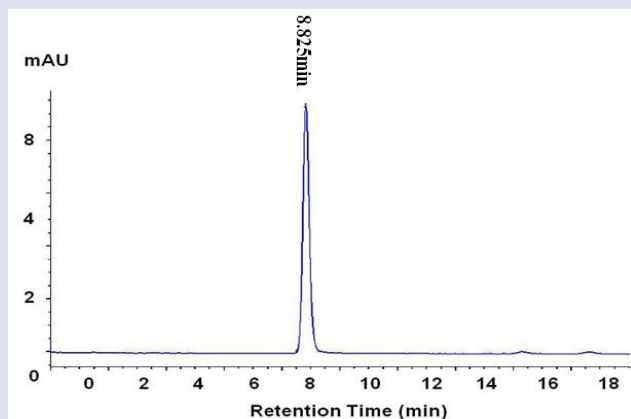


Figure 3: The quercetin standard substance HPLC peak.

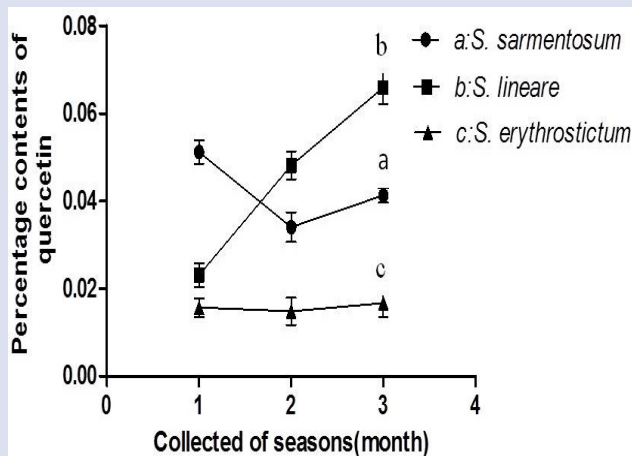


Figure 4: Percentage contents of quercetin contained in the 3 medicines collected in different seasons. (a: *S. sarmentosum* b: *S. lineare* c: *S. erythrostictum*)

Table 2: The quercetin content of the three herbal medicines

Species	Collected seasons	Habitat	Percentage	Specimen number
<i>S. sarmentosum</i>	Apr. 27th	Huangmei, Hubei	0.0512±0.003a	120427
<i>S. sarmentosum</i>	Jun. 28th	Sheshan, Wuhan	0.0340±0.003a	130628
<i>S. sarmentosum</i>	Sep.15th	Sheshan, Wuhan	0.0413±0.002a	130915
<i>S. lineare</i>	Apr. 2nd	Jianshi, Hubei	0.0231±0.003a	130402
<i>S. lineare</i>	Jun. 29th	Sheshan, Wuhan	0.0481±0.003a	150729
<i>S. lineare</i>	Oct. 5th	Sheshan, Wuhan	0.0659±0.004a	141005
<i>S. erythrostictum</i>	Apr. 26th	Huangmei, Hubei	0.0157±0.002a	150426
<i>S. erythrostictum</i>	Jun. 27th	Luotian, Hubei	0.0148±0.003b	140627
<i>S. erythrostictum</i>	Sep. 17th	Huangmei, Hubei	0.0167±0.003b	150917

* Different letters in same column were significantly different at $P < 0.05$

to the method mentioned above prior to the HPLC determination. The consequences showed that the content variation RSD (%) of each sample was less than 2.8%, indicating a good repeatability of this method. [Figure 2, 3, 4]

Recovery Test

In the linear range, 1.0 mg of quercetin which was nearly equal to that contained in 2.004 g of dried *S. lineare* Thunb. Sample collected in June in Huangmei was accurately taken to prepare the solution for recovery test according to the sample preparation method mentioned above. The quercetin content was determined according to the above chromatographic conditions. The recovery test was repeated six times. By comparing obtained quercetin content with that actually injected each time, the average recovery was 100.52%, and RSD (%) was 2.2%, showing that developed determination method had a nice accuracy.

Average retain time of quercetin peak was 8.835 min, and the quercetin standard substance HPLC peak was as follows [Figure 3].

Sample analysis

A total of 9 samples of three plant medicines collected in different periods were determined by the developed analytical method. The results of the quercetin content were given in the following table (Table 2). And the relations of the content change and the collected months were shown as follows [Figure 2].

DISCUSSION

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 in these *Sedum* samples are listed in Table 1, and the variation with respect to the harvest season of the three *Sedum* medicinal plants is shown in Table 2. The results showed that the amounts of quercetin in *Sedum sarmentosum*, *S. lineare* and *S. erythrostictum*, and the content of quercetin in this three drugs can achieve the quality standard required by Chinese Pharmacopoeia. (No less than 0.01%). In conclusion, the contents of quercetin varied in all *Sedum* medicinal samples harvested in different seasons. The content of quercetin was higher in *Sedum sarmentosum* harvested at the beginning of april, but lower in June, and grown a little bit at September, the highest content of quercetin reached 0.0512 percent at april, and then The content of quercetin in *S. lineare* and *S. erythrostictum* increased in the later harvest months, and the highest content of quercetin reached 0.0659 and 0.0167 percent at September respectively.

Table 1: samples and their Sources

Species	Collected seasons	Habitat	Specimen number
<i>S. sarmentosum</i>	Apr. 27th	Huangmei, Hubei	120427
<i>S. sarmentosum</i>	Jun. 28th	Sheshan, Wuhan	130628
<i>S. sarmentosum</i>	Sep.15th	Sheshan, Wuhan	130915
<i>S. lineare</i>	Apr. 2nd	Jianshi, Hubei	130402
<i>S. lineare</i>	Jun. 29th	Sheshan, Wuhan	150729
<i>S. lineare</i>	Oct. 5th	Sheshan, Wuhan	141005
<i>S. erythrostictum</i>	Apr. 26th	Huangmei, Hubei	150426
<i>S. erythrostictum</i>	Jun. 27th	Luotian, Hubei	140627
<i>S. erythrostictum</i>	Sep. 17th	Huangmei, Hubei	150917

CONCLUSION

By the HPLC analysis, we found that *Sedum sarmentosum*, *S. lineare*. and *S. erythrostictum* all contained flavonoid glycosides, whose hydrolysate included quercetin. The results suggested that the common flavonoids existed in the 3 plant medicines should be associated with anti-hepatitis activity.^{14,15}

The results also revealed preliminarily a relation between collected season and medicine quality. In detail, the quercetin content of *S. lineare* Thunb. went up with growing months and reached the climax in October, while both *S. erythrostictum* Migo. and *S. sarmentosum* Bunge. had the relatively high amount in April (flowering period), and were the lowest in June (during this period, the growing state of *S. sarmentosum* Bunge. was poor, and the leaves mostly withered), then increased a little in September, showing preliminarily that the last two would own the best quality if collected in flowering period.

In a word, The HPLC assay of the quercetin in the hydrolysate of the 3 herbal drugs could be used to control the drug quality to a certain extent from the aspect of the related flavonoids content.

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CONFLICT OF INTEREST

All contributing authors declare no conflicts of interest.

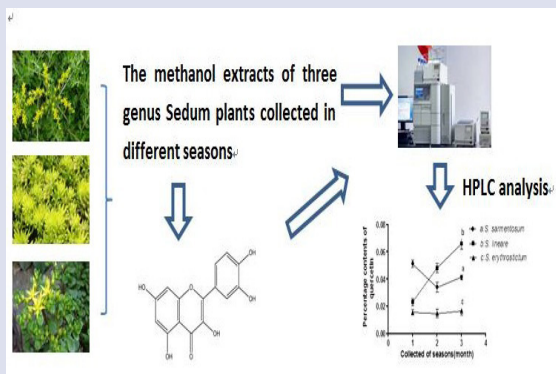
ABBREVIATION USED

TLC: Thin—layer chromatography; **HPLC:** High performance liquid chromatography; **RSD:** Relative standard deviation.

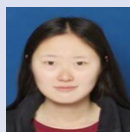
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GRAPHICAL ABSTRACT



ABOUT AUTHORS



Yue-ling Ma: Presently working as a undergraduate student in the School of biological and pharmaceutical engineering, Wuhan Polytechnic University, Wuhan, China. Her area of expertise and interest includes identification and quality evaluation of traditional chinese medicine, research and development of new biologically active substances.

SUMMARY

- To establish a method for quercetin content determination of the three plant drugs from genus *Sedum* and to decide their bestharvesting time. It indicated that the content of quercetine in *Sedum sarmentosum*, *Sedum lineare* and *Sedum emarginatum* can achieve the quality standard required by Chinese Pharmacopoeia. The results also revealed preliminarily a relation between collected season and medicine quality. Thus it could be used to control some flavonoids content in the three plant drugs indirectly.
- The HPLC assay of the quercetin in the hydrolysate of the 3 herbal drugs could be used to control the drug quality to a certain extent from the aspect of the related flavonoids content.

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