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Seasonal, Spatial, and Interspecific Variation in Quercetin in *Apocynum venetum* and *Poacynum hendersonii*, Chinese Traditional Herbal Teas

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Quercetin is of particular importance as it has been found to have functions of suppressing tumors, reducing blood pressure, and scavenging free radicals. It is one of the major flavonoids in *Apocynum venetum* and *Poacynum hendersonii*, whose leaves have long been used as traditional herbal teas in China and Japan. Both species are also cultivated as fiber plants because of their outstanding quality of phloem fiber in stems. To obtain high output of both quercetin and fiber, it is necessary to optimize harvesting time for their leaves. Thus, understanding the developmental patterns of quercetin in leaves and fiber in stems is crucial to achieving this goal. In the present study, temporal and interspecific variations in quercetin in the leaves between *A. venetum* and *P. hendersonii* and spatial variation among *P. hendersonii* populations were studied by HPLC during the period from April to October in 1999. The results show that the content of quercetin in both species reached its highest level in summer and its lowest in autumn. The quercetin content in the leaves of *P. hendersonii* was generally higher than that of *A. venetum* no matter when their leaves were harvested. There was significant difference in quercetin content among three geographical populations of *P. hendersonii*, which might be the result of climatic difference—cooler climate might favor accumulation of quercetin in the leaves of *P. hendersonii.* Furthermore, the developmental patterns of total phenolics in the leaves of the two species were the same as that of the quercetin, that is, summer is an optimal harvesting season for both quercetin and other phenolics. The results obtained here suggest that *P. herdersonii* is a better material for herbal tea or pharmaceutical purposes, and that the best harvest time of its leaves should be summer.

KEYWORDS: Quercetin; *Apocynum venetum*; *Poacynum hendersonii*; total phenolics; Chinese herbal tea

INTRODUCTION

Leaves of *Apocynum venetum* (*A. lancifolium*) and *Poacynum hendersonii* (*A. pictum*) are used as traditional herbal teas in China and Japan (*1*) because both of them are rich in quercetin. Quercetin is one of the important flavonoids in plants (*2*, *3*) and has been found to possess manifold pharmacologic effects, such as scavenging of free radicals (*4*), reducing blood pressure (*5*), and suppressing growth of tumor cells (*6*). Soup made from their leaves is a favorite health-care remedy among the local people (*7*). The two species are also important fiber plants because of their good quality of phloem fiber in their stems (*8*). As heavy defoliation affects the vitality and survival of plants (*9*), to maximize fiber production, their leaves are usually collected in late summer when their stems are well-developed (*10*). In midsummer the plants start to produce fructescences, whereas their height may not increase any more and the number of leaves reaches their maximum. Traditionally, harvesting their leaves in late summer is believed to give highest yield of both leaf and fiber. However, no formal studies have been carried out to examine the developmental patterns of quercetin in leaves of these species, and thus the optimal harvesting time is still unknown. Additionally, although the two species are morphologically distinguishable, they are traditionally called luobuma by local people and, hence, have been used indiscriminately for many years regardless of their potential interspecific difference in quercetin content.

To utilize the biological resources more efficiently, it is necessary to optimize the harvesting time. In doing so, an important step is to characterize the resources. Therefore, our aims of the

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present study were to (1) find the seasonal variation of quercetin content in the leaves of *A. venetum* and *P. hendersonii* and determine the optimal harvesting time of the leaves, (2) compare the difference in quercetin content between the two species to suggest a better material for industrial use, and (3) examine the spatial variation pattern of quercetin content of *P. hendersonii* and the probable ecological factors responsible for the variation to select the suitable cultural conditions for producing commercial quercetin. We also determined the spatial−temporal and interspecific variation in total phenolics of the two species to determine whether the optimal harvesting time for quercetin is also suitable for the other phenolic compounds.

MATERIALS AND METHODS

Study Areas. This study was conducted at the following three sites in Xinjiang, China; each site was positioned via GPS (Garmin, made in Tawan):

1. Shihezi (44° 18′ 37″ N, 86° 3′ 12″ E) is located in the northern part of Xinjiang, and its elevation is 454 m above sea level (asl). The habitat is saline−alkali desert. Annual precipitation is ∼200 mm. Mean annual temperature is 6−6.6 °C. Samples of *A. venetum* and *P. hendersonii* were both collected there.

2. Atushi (39° 47′ 55″ N, 76° 24′ 26″ E) is located in the southern part of Xinjiang. The elevation of the sampling site is 1275 m asl. The habitat is saline−alkali desert. Annual precipitation is ∼70 mm. Mean annual temperature is 11.8 °C. Only *P. hendersonii* was collected, which was the dominant species there.

3. Tashikuergan (37° 47′ 24″ N, 75° 28′ 36″ E) is located in the Pamirs. The elevation of the sampling site is 2996 m asl. The habitat is saline−alkali desert. Annual precipitation is ∼70 mm. Mean annual temperature is 3−4 °C. Only *P. hendersonii* was sampled there.

The three sits are isolated by two mountains, Tienshan and Kunlunshan (*11*).

Sample Collection. For each population nine plants with similar canopy (diameter = 1 m) were randomly selected, which were of similar age. Because both of the species are clonal, the distance between any two selected plants was >30 m to avoid sampling the same clone (*12*). To make our results comparable across different seasons, leaves were sampled at the same stage of development. The leaves were sampled in the mornings of April 28th, July 20th, and October 14th, 1999, respectively. For each species, three leaves were collected, which were ∼20 cm away from the apex on every branch, on each of three plants on each sampling occasion. The leaves from the same plant were combined into a single sample. Each plant was sampled only once; subsequent samples were taken from different plants, so there were 36 samples for the four populations of the two species in total.

Extraction Conditions. Each sample was separately put into a bag, placed in pots filled with ice, and transported to the laboratory immediately. All of the samples were oven-dried to constant weight at 60 °C. The dried leaves were ground to powder with a tissue grinder and passed a 1.2-mm size mesh. One gram of leaf powder was extracted with 100 mL of methanol with added 5.5% (w/v) HCl for 6 h in a Soxhlet extractor in a water bath at 90 °C (*13*). Surplus methanol was evaporated from the extraction to result in a total volume of 50 mL. The extraction was filtered through a 0.45 μm Millipore filter and centrifuged for 10 min in a high-speed centrifuge (8000*g*). The supernatant was saved and retained for analysis with high-performance liquid chromaography (HPLC).

HPLC Apparatus and Chromatographic Conditions. Quercetin contents were determined using an HPLC apparatus consisting of Hewlett-Packard 1100 quaternary pump, an autosampler, and a UV detector set at 370 nm, with a 150 × 4.6 mm Zorbax 5 μm SD-C_{18} column. The column was eluted with methanol/alcohol/water (48:45:7) at a flow rate of 0.8 mL min^{-1}. Authentic quercetin obtained from Sigma (Beijing, China) was used as an external calibration standard. Known contents of quercetin in methanol were injected onto the HPLC for detection and used to obtain a calibration curve equation. Injection volume was 20 μL. Samples of quercetin were detected at 370 nm (*14*) and identified according to its retention time and UV spectra by comparing them with those of the standard. This was done by plotting the area against the content of the sample solution ($r = 0.9999$). We used this equation to calculate the content of quercetin for the samples.

Table 1. Temporal, Spatial, and Interspecific Variations in Quercetin of Leaves of *A. venetum* and *P. hendersonii*[a]

species	population	content of quercetin (mean ± SD) (mg/g of dry weight) April	July	October
A. venetum	Shihezi	0.934 ± 0.052	3.75 ± 0.19	0.085 ± 0.019
P. hendersonii	Shihezi	1.192 ± 0.027	4.661 ± 0.054	0.076 ± 0.017
	Tashikuergan	1.513 ± 0.054	6.44 ± 0.202	0.089 ± 0.015
	Atushi	0.656 ± 0.049	1.497 ± 0.027	0.092 ± 0.009

[a] $n = 3$.

Determination of Total Phenolics. The amount of total phenolics in the extracts was determined according to a modification of the Folin−Ciocalteu method (*15*). A 1.0 mL diluted extract [extract/methanol, 1:50 (v/v), three replicates] was introduced into a test tube and mixed with 1.0 mL of 1 N Folin−Ciocalteu's reagent. The mixture was allowed to stand for 2−5 min, which was followed by the addition of 2.0 mL of 20% Na_2CO_3. After 30 min of incubation at room temperature, the mixture was centrifuged for 10 min (150*g*) and the absorbance of the supernatant was measured at 760 nm on a UNICO 2100 UV−vis spectrophotometer. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram of dry material.

Statistical Analysis. Two-way ANOVA was performed to test seasonal and interspecific differences in quercetin or total phenolics content between *P. hendersonii* and *A. venetum* distributed in Shihezi and to test temporal and spatial variation in quercetin or total phenolics content of the three populations of *P. hendersonii*. Statistical analyses were performed using SPSS 10.0 for Windows.

RESULTS AND DISCUSSION

The quercetin content of the leaves of *A. venetum* and *P. hendersonii* in different seasons and different populations is given in **Table 1**. *A. venetum* and *P. hendersonii* displayed the same patterns of seasonal variation in quercetin content, that is, the quercetin contents of their leaves increased significantly ($p < 0.001$) during vegetative growth of the plants, reached their highest value in summer when the plants came into blossom, and then decreased sharply ($p < 0.001$) and reached their lowest value in autumn when the leaves turned yellow.

Developmental patterns of flavonoids differ among plants. Six patterns of seasonal variation in flavonoids have been observed and are shown in **Figure 1**: V-shaped (**Figure 1a**) (*16*), reverse-V-shaped (**Figure 1b**) (*17*), season-invariant (**Figure 1c**) (*17*), increasing (**Figure 1d**) (*18*), declining (**Figure 1e**) (*19*), and irregular patterns (**Figure 1f**) (*20*). Both *A. venetum* and *P. hendersonii* displayed a reverse-V-shaped developmental pattern of quercetin. Thus, for these species summer would be the best harvesting time for the purpose of extracting quercetin.

As assimilation is often higher than disassimilation during vegetitive growth, the secondary metabolic products of the plants accumulate continually during the course. When plants come into reproductive growth, a considerable amount of photosynthates flows into reproductive organs, so the quercetin content in the leaves decreased significantly after flowering. As temperature and light conditions change in the fall, leaves turn yellow and flavonoids in the leaves decompose or transfer into storage organs, such as root, rhizome, or stem before the leaves fall, which is reflected by the fact that the quercetin contents in the leaves of both *A. venetum* and *P. hendersonii* decreased to trace levels in the fall as observed in other studies (*16*, *21*).

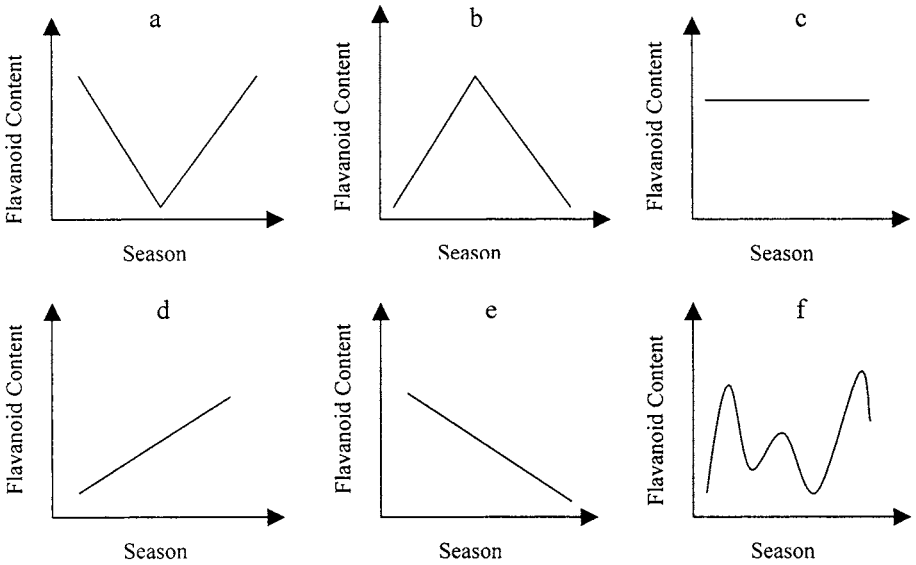

Figure 1. Schematic presentations of patterns of seasonal variation in flavanoids: (a) V-shaped (*16*); (b) reverse-V-shaped (*17*); (c) season-invariant (*17*); (d) increasing (*18*); (e) declining (*19*); (f) irregular pattern (*20*).

Table 2. Temporal, Spatial, and Interspecific Variations in Total Phenolics of Leaves of *A. venetum* and *P. hendersonii*[a]

species	population	content of total phenolics (mean ± SD) (mg of GAE[b]/g dry weight) April	July	October
A. venetum	Shihezi	12.492 ± 0.23	15.451 ± 0.14	9.600 ± 0.37
P. hendersonii	Shihezi	7.542 ± 0.53	9.205 ± 0.36	5.287 ± 0.44
	Tashikuergan	12.245 ± 0.19	14.465 ± 0.15	8.034 ± 0.27
	Atushi	5.364 ± 0.67	7.232 ± 0.71	4.020 ± 0.33

[a] $n = 3$. [b] GAE, gallic acid equivalent.

The quercetin content of *A. venetum* ranged from 0.085 to 3.75 mg g^{-1} of dry weight during its growth period, similar to the finding (1.302 mg g^{-1}) of Cao et al. (*2*). *P. hendersonii* had a much higher content of quercetin in its leaves than did *A. venetum* from spring until fall ($p < 0.001$). The two species in Shihezi were sympatric dominant species and shared the same environmental conditions. Thus, the difference in the quercetin content between the two species might be genetically based. In this sense, we suggest that *P. hendersonii* would be a better material for the purpose of industrial use of quercetin than *A. venetum*.

There was considerable variation in quercetin content of *P. hendersonii* among three populations (**Table 1**), which was statistically significant ($p < 0.001$). There were two possible reasons for the variation. First, the distribution of *P. hendersonii* is not continuous in Xinjiang (*22*), and the three populations are far away from each other and isolated by two mountains, Tienshan and Kunlunshan. These populations might be subject to genetic differentiation in relation to the synthetic potential of quercetin (*23*). Alternatively, the difference might be environmentally induced. The three populations were collected from geographical sites that have different environmental conditions, for example, temperature and light regime, which all affect synthesis and decomposition of quercetin (*24*). Thus, it is not surprising that the three populations had significantly different contents of quercetin.

The content of quercetin in the leaves of *P. hendersonii* had no obvious relationship with annual precipitation among the three populations, whereas it tended to be higher in cooler sites (e.g., Tashikuergan, see **Table 1**). Therefore, cooler environments may favor synthesis of quercetin in the leaves of *P. hendersonii*.

The seasonal, spatial, and interspecific variation patterns of total phenolics content of the two plants were the same as those of quercetin (**Table 2**); there was also a significant increase ($p < 0.001$) in total phenolics from April to July, followed by an obvious decline ($p < 0.001$) after July. This means that the contents of both quercetin and other phenolics reached their highest values in summer, so harvesting leaves for quercetin at this time would not affect the quantities of other phenolics, which supports the conclusion that summer is the optimal harvesting time for leaves of the two species for quercetin production.

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