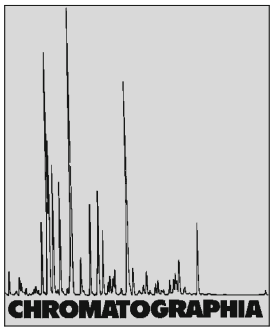
Determination of Salidroside in Medicinal Plants Belonging to the *Rhodiola* L. Genus Originating from the Qinghai–Tibet Plateau



2008, *68*, 299–302

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Received: 2 December 2007 / Revised: 29 April 2008 / Accepted: 29 April 2008
Online publication: 3 July 2008

Abstract

The *Rhodiola* L. genus (Crassulaceae) is one of the most important medicinal plant products used by Tibetans in Chinese phytotherapy. Fourteen species were examined for their content of salidroside. A considerable quantitative variation was observed using high-performance liquid chromatography and this depended on species and regional factors. It was found that all samples contained salidroside at concentrations ranging between 0.02 mg g^{-1} (*R. sinuate*) and 15.95 mg g^{-1} (*R. sacra*), respectively. The content of salidroside in *R. sacra* was significantly higher than in other popular medicinal plants of this genus. This finding indicated that there may be more *Rhodiola* species present in the Qinghai–Tibet Plateau which may be used as a potential source of salidroside.

Keywords

Column liquid chromatography
Salidroside
Crassulaceae
Rhodiola

Introduction

The *Rhodiola* L. genus belongs to the Crassulaceae family [1]. Different Rhodiola species are used in Tibetan traditional folk medicine for centuries in the attempt to maintain body health and to treat various diseases [2, 3]. There are more than 43 species growing between 2,600 and 5,400 m above sea level in the frigid zones on the mountains in the Qinghai–Tibet Plateau. Only 2–4 species are known to be used in the traditional medicine of Tibet [4, 5]. As a traditional herbal remedy *Rhodiola* species have been used for the treatment of a variety of conditions such as clearing heat in the lungs, eliminating toxins from the body, treating various epidemic diseases, edema of limbs, traumatic injuries and burns. *Rhodiola* plants were used by local communities in small quantities but, in recent years, increased commercialization of some species has increased demands and exploitation. Massive harvest, loss of habitat by deforestation and excessive grazing in high altitude pastures in the entire Qinghai–Tibet region now threaten their survival. One possible strategy to address this problem is to encourage the local communities to collect and use other species of *Rhodiola* L. in the Qinghai–Tibet Plateau. A prerequisite for the pursuit of this policy, however, is the evaluation of differences and similarities between the plants with respect to chemical compositions.

Salidroside (*p*-hydroxyphenethyl-β-D-glucoside) is one of the most active ingredients in *Rhodiola* plants. It has been shown to possess a number of medicinal properties including the ability to reduce the effects of anoxia, microwave radiation and fatigue [6–10]. Pharmacological properties have been reported to include anti-aging, antican-

Limited Short Communication
DOI: 10.1365/s10337-008-0705-4
0009-5893/08/08

Table 1. Location and salidroside contents (mg g^{-1}) of the rhizomes of *Rhodiola*

Species	Location, altitude	Voucher	Salidroside (mg g^{-1})
R. sexifolia S. H. Fu	Qushui, Xizang, 3,590 m	Chen2002079	1.01 ± 0.44
	Linzhi, Xizang, 3,130 m	Chen2002123	0.32 ± 0.25
R. chrysanthemifolia (Lévl.) S. H. Fu	Shangri, Xizang, 4,140 m	Chen2002099	3.34 ± 0.50
	Shangri, Xizang, 3,960 m	Chen2002104	2.69 ± 0.21
*R. alsia (Fröd.)*S. H. Fu	Changdu, Xizang, 4,590 m	Chen2002139	1.39 ± 0.21
	Linzhi, Xizang, 3,470 m	Chen2002125	0.53 ± 0.20
R. bupleuroides (Wall. ex Hk. f. et Thoms.) S. H. Fu	Jiacha, Xizang, 3,210 m	Chen2002119	4.51 ± 3.12
	Langkazi, Xizang, 4,480 m	Chen2002095	3.05 ± 2.35
R. macrocarpa (Praeg.) S. H. Fu	Dege, Xizang, 3,620 m	Chen2002147	0.06 ± 0.03
R. sacra (Prain ex Hamet) S. H. Fu	Changdu Xizang, 3,960 m	Chen2002132	15.95 ± 2.31
R. kirilowii (Regel) Maxim	Linzhi, Xizang, 4,220 m	Chen2002127	2.68 ± 0.37
	Changdu, Xizang, 4,620 m	Chen2002138	2.24 ± 0.31
	Qusong, Xizang, 3,980 m	Chen2002112	0.28 ± 0.26
R. sinuate (Royle ex Edgew.) S. H. Fu	Baqing, Xizang, 3,970 m	Chen2002055	0.02 ± 0.01
R. himalensis (D. Don) S. H. Fu	Nangqie, Qinghai, 4,110 m	Chen2002036	3.78 ± 1.52
	Leiwuqi, Xizang, 4,280 m	Chen2002048	1.38 ± 0.31
	Baqing, Xizang, 4,300 m	Chen2002067	0.20 ± 0.05
R. coccinea (Royle) Borrisova	Chenduo, Qinghai, 4,350 m	Liu 971	3.41 ± 2.64
	Changdu, Xizang, 4,810 m	Chen2002140	0.91 ± 0.31
R. crenulata (Hk. f. et Thoms.) H. Ohba	Dege, Xizang, 4,560 m	Chen2002150	4.08 ± 2.08
	Dingqing, Xizang, 4,900 m	Chen2002057	6.34 ± 3.05
R. tieghemii (Hamet) S. H. Fu	Nangqie, Qinghai, 4,490 m	Chen2002039	0.19 ± 0.05
	Nangqie, Qinghai, 3,980 m	Chen2002038	0.22 ± 0.03
R. yunnanensis (Franch.) S. H. Fu	Linzhi, Xizang, 2,460 m	Chen2002128	3.95 ± 0.51
R. fastigiata (Hk. f. et Thoms) S. H. Fu	Maduo, Qinghai, 4,320 m	Chen2002017	0.95 ± 0.35

cer, anti-inflammatory, hepatoprotective and antioxidative effects [11–14]. Salidroside has also been found to be protective against neuronal cell death induced by glutamate and hypoxia/hypoglycemia [15, 16], mitochondria dysfunction induced by sodium azide [17], and against oxidative stress-induced cell apoptosis [18]. Apart from the presence of cinnamoyl derivatives, salidroside is correlated with the activity of Rhodiola extracts which makes it a suitable marker compound. The concentration of salidroside in *Rhodiola* has become one of the quality indicators [1, 19]. We now report an analytical comparison of salidroside levels in 14 *Rhodiola* species using high-performance liquid chromatography (LC).

Materials and Methods

Solvents and Chemicals

All organic solvents were of analytical grade (Xi'an Chemical Factory, Xi'an, China). Methanol was of HPLC grade (Tedia Company, OH, USA). A HPLC grade salidroside standard was purchased from the National Institute for the Control of Pharmaceutical and Biological Products, Ministry of Health, Beijing, China.

Plant Materials

The rhizomes of 14 *Rhodiola* species were collected from the Qinghai–Tibet Plateau in September 2002 (Table 1). The rhizome samples were cut into fragments, dried and stored at room temperature until extraction. All samples were initially identified by their specimens, which were simultaneously collected with their rhizomes. Voucher specimens were deposited at the Herbarium of Northwest Plateau Institute of Biology, the Chinese Academy of Sciences, the People's Republic of China (HNWP).

Sample Preparation

All analyses were repeated at least four times, using the method reported by Linh et al. [20] and Han et al. [1]. Dry, pulverized samples (1 g) of rhizomes of 25 plants (Table 1) were extracted five times in a conical flask with 70% ethanol (20 mL) for 30 min in an ultrasonic bath. The pooled extracts were filtered in a Buchner funnel, transferred to a 100 mL volumetric flask and adjusted to volume with 70% ethanol. Each sample was filtered through a 0.45 µm membrane filter and 10 µL of the solution were subjected to LC in triplicate.

Calibration

Five milligrams of salidroside standard were dissolved in a 10 mL volumetric flask with methanol, ultrasonicated and filled to volume (stock solution). Calibration levels were prepared by diluting the stock solution with methanol. The injected concentrations were 10, 60, 110, 160 and 210 µg mL^{-1}. All calibration levels were injected in triplicate. The linear regression analyses of peak areas at the various concentrations of standard solutions gave the calibration equation and correlation coefficient for the calibration curve.

Recovery and Precision

Recovery was evaluated by spiking a known quantity of the references to 1.00 g of the pulverized samples of *R. kirilowii* (Fig. 1). The fortified sam-

ples were then extracted and analyzed as described above. Three concentration levels were tested. Each sample was analyzed in triplicate. The recovery values were obtained by comparing the results from samples and fortified samples. The precision of the chromatographic system was validated by injecting a mixed reference solution six times during 1 day. The method precision was evaluated by intra-day and inter-day tests. Intra-day experiments were performed by replicate analysis of six aliquots of the sample within 1 day. Inter-day tests were carried out on three consecutive working days with newly prepared mobile phase and samples.

LC Analysis

The LC system consisted of a Waters 600E multisolvent delivery system, a Waters 486 UV–Vis detector and a Rheodyne injector with a 10 µL sample loop. A reversed-phase column (Phenomenex Kromasil C_{18}, 5 µm, 250 × 4.6 mm) was used. The LC protocol published by Lin et al. (2000) was followed. Isocratic elution was employed at room temperature using methanol–water (20:80, *v*/*v*) as the solvent system. Run time was 80 min, flow rate was 1.0 mL min^{-1} and detection wavelength was set to 227 nm. Empower software (Waters) was used to control the instrument and to process the analytical data.

Results and Discussion

Peak identification was based on retention time and ultraviolet absorption spectra. For quantitative analysis, peak areas were used to calculate the amount of salidroside present in different plant materials (Table 1). The linear regression equation and correlation coefficient (*r*) was as follows: $y = 2{,}018{,}844x - 8{,}400$ ($r = 0.9980$). The detection limit for salidroside was found to be 0.01 mg mL^{-1} (RSD < 5%, $n = 5$). The limits of detection (LOD) and quantification (LOQ) were determined to be 0.01 and 0.064 µg mL^{-1} at a signal-to-noise ratio (S/N) of 3 and 10, respectively. Recovery was 96.5% with a RSD < 5% at all tested concentrations. The RSD value of retention times and peak areas were inspected to evaluate precision. The data showed a high precision of the system with a RSD < 5%. The RSD of intra- and inter-day precision was less than 5% for all compounds.

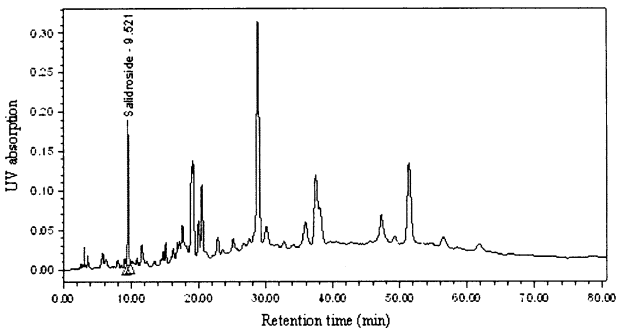

Fig. 1. LC chromatogram of *R. kirilowii* spiked with salidroside. The wavelength was set to 227 nm

Considerable differences were found for the salidroside contents within the 14 species of *Rhodiola* L. (Table 1). All plants contained salidroside but levels ranged between 0.02 mg g^{-1} (*R. sinuate*) and 15.95 mg g^{-1} (*R. sacra*). In *R. kirilowii*, collected from different geographical regions, the highest amount of salidroside was 2.68 mg g^{-1} and the lowest was 0.28 mg g^{-1}. A similar pattern of variation was also observed in other species of *Rhodiola* grown in different geographical regions (Table 1). Wang [21] reported that salidroside levels in six *Rhodiola* species, originating from the Qinghai province of China, ranged between 0.64 and 3.13 mg g^{-1}. Linh et al. [20] found that salidroside contents in *R. rosea*, gathered from various areas in China, ranged between 1.3 and 11.1 mg g^{-1}, respectively. Variation within the plants of *Rhodiola* depended on species and regional distribution [22–27]. In our study, we only analyzed the content of salidroside from 14 species of *Rhodiola* from the Qinghai–Tibet Plateau. The highest level of salidroside (15.95 mg g^{-1}) was found in *R. sacra* collected from the Changdu County of Xizang. *R. sacra* was reported to show anti-radiation effects, could improve learning and memory [28], and possessed antioxidant activities and thus could be a potentially rich source of natural antioxidants [29, 30]. *R. sacra* did also show low levels of toxicity in animal models [31] which indicates that this species may be useful as a potential source of salidroside. The contents of salidroside in *R. sacra* was much higher than that found in *R. crenulata* (4.08–6.34 mg g^{-1}, Table 1) which is a traditional Tibetan medicine. *Rhodiola rosea* also showed lower levels. The latter is known as "golden root" or "roseroot" and is used for centuries in the traditional medicines of Russia, Scandinavia and other countries [32]. Since *R. sacra* is distributed in the Qinghai and Xizang areas of China and Nepal, and also used as an alternative traditional Tibetan medicine, conservation strategies should be undertaken in order to secure future analyses and genetic profiling of that species.

Conclusion

The present study showed that salidroside concentrations were highly variable between 14 species of *Rhodiola* samples which originated from different geographical locations. Therefore, the indiscriminate use of *Rhodiola* derived from various sources as herbal remedies without standardizing the salidroside concentration may ultimately affect their quality and/or efficacy. Further investigation of salidroside variability in other *Rhodiola* species derived from the Qinghai–Tibet Plateau is still needed in order to identify species with high salidroside contents in lesser known species. This approach may help to protect the more commonly used species from extinction and allows for the recovery of threatened *Rhodiola* species.

Acknowledgments

We gratefully acknowledge comments and suggestions made by the reviewers. We would like to thank Ms. Yang Yueqin for the operation of the HPLC system. This study was financed by grants from National Basic Research Program of China (2008CB117013) and International Foundation for Science, IFS D 4258-1 and from the "Western Light" programme of talent cultivation of the Chinese Academy of Sciences (CAS) and the Ministry of Personnel of China.

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