Comparison of different Rhodiola species using NMR- metabolomics, HPTLC and DNA barcoding techniques

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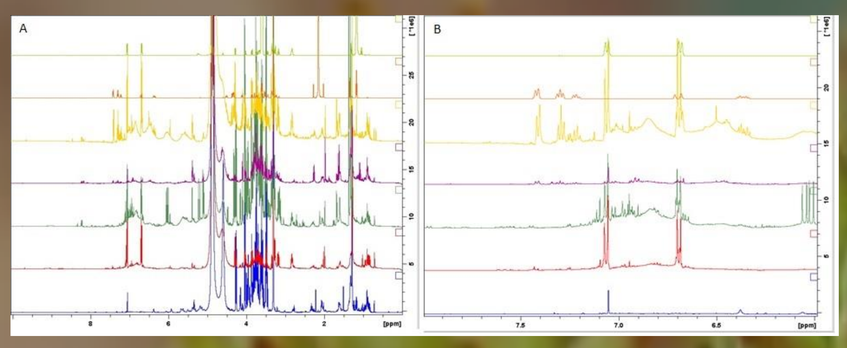
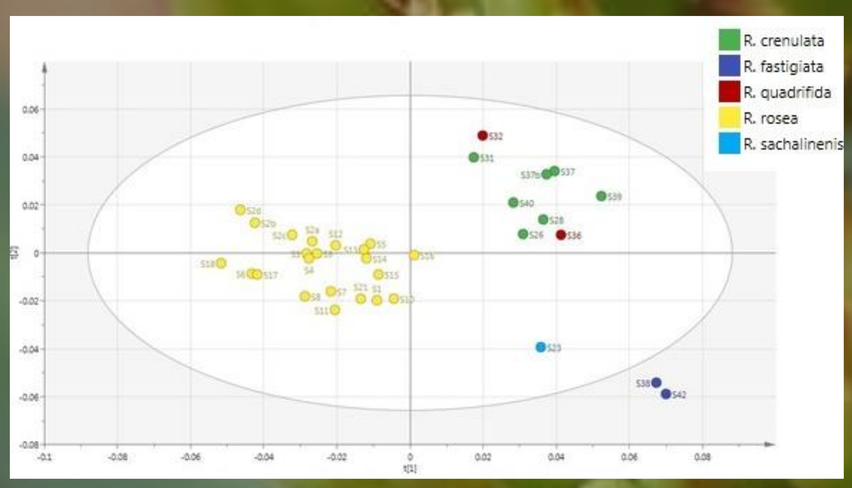
Introduction: Medicinal *Rhodiola* species, including *Rhodiola rosea* L. and *Rhodiola crenulata* (*Hongjingtian*红景天) have been widely used as herbal medicines with numerous claims for their therapeutic effects. These products are registered by a number as pharmaceuticals and throughout China Rhodiola is also taken for wellness and is registered as a self-medicated wellness product for 'blood-boosting and heart-strengthening.' However other species exist that may be found as adulterants in the value chain, these include *Rhodiola quadrifida* (Pall.) Fisch. & C.A.Mey, *Rhodiola sachalinensis* Borris, and *Rhodiola fastigiata* (Hook. f. & Thomson) S.H. Fu.

Faced with resource depletion, environment destruction and higher demand, *R. rosea* and *R. crenulata* are becoming scarce around theworld. This scarcity may add to their economic value, but also increases the risk of adulteration and poor quality (Booker et al. 2015).

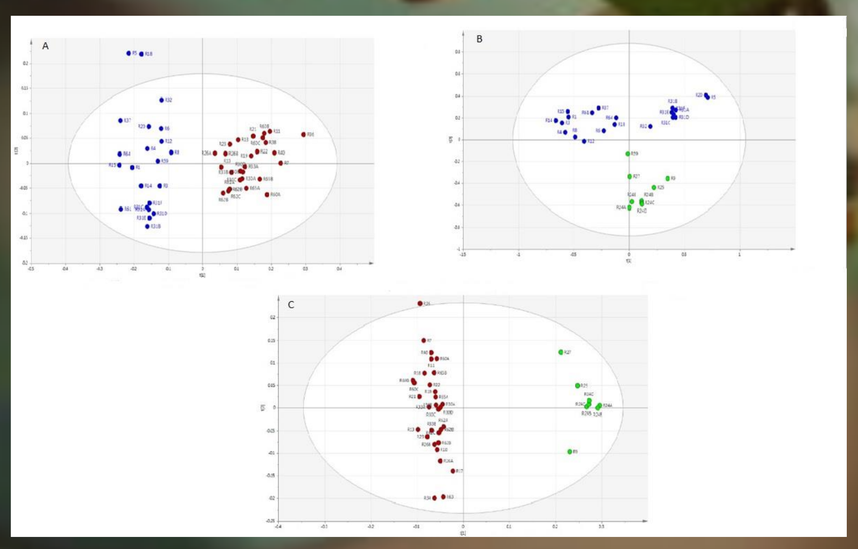
Results:



[1]H-NMR spectra of the reference compounds, salidroside and rosavin, together with the spectra of botanical reference material. 1: R. fastigiata, 2: R. quadrifida, 3: R. crenulata, 4: R. sachalinensis, 5: R. rosea, 6: rosavin and 7: salidroside.(From bottom to top) A: Whole region (0-10ppm); B: aromatic region (6-8ppm)

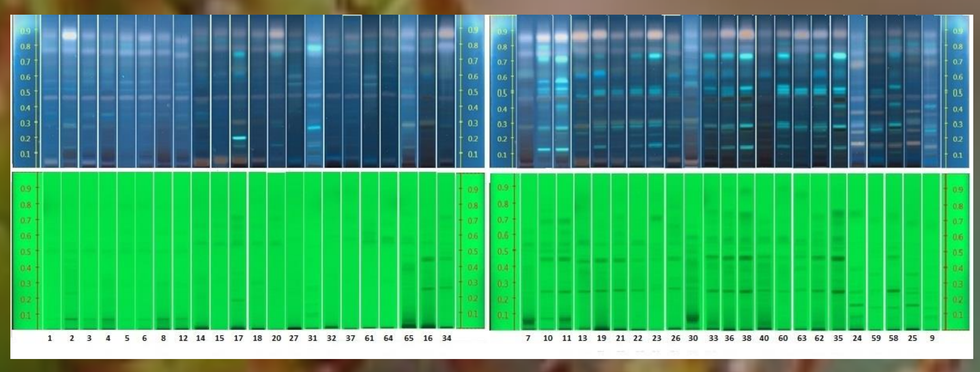


Scores plot of Rhodiola samples using the aromatic [1]H-NMR region shows how principal component analysis can differentiate four of the five species. The scores plot below shows



Score plots of group comparison between Rhodiola species. A: R. crenulata (red) with other Rhodiola spp. (blue); B: R.rosea (green) with other Rhodiola spp. (blue); C: R. crenulata (red) with R. rosea (green).

30% of the *Rhodiola* samples collected from the market were neither *R. rosea* or *R. crenulata*. Some *R. rosea* samples were also being sold as *R. crenulata*. 47.7 % of raw materials samples were not labelled properly and their species information were not clearly illustrated to customers. This highlights the lack of proper local government policies and good quality control strategies. According to our study, different *Rhodiola* species (including *R. rosea* and *R. crenulata)* can be found in the Chinese market. However, they are neither sold separately nor well identified. Therefore, there is a high potential of adulteration and substitution among these species.

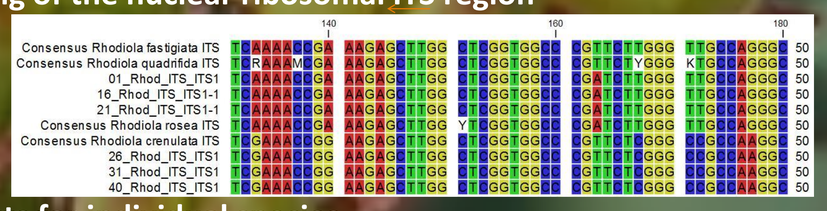


HPTLC results for all Rhodiola market samples, mobile phase (Ethylacetate, methanol, water, formic acid (77:13:10:2)

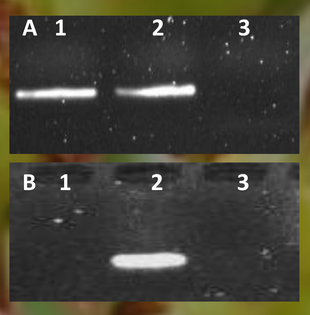
Two types of DNA test were performed to confirm the identity of *Rhodiola* samples:

1. DNA barcode sequencing of the nuclear ribosomal ITS region

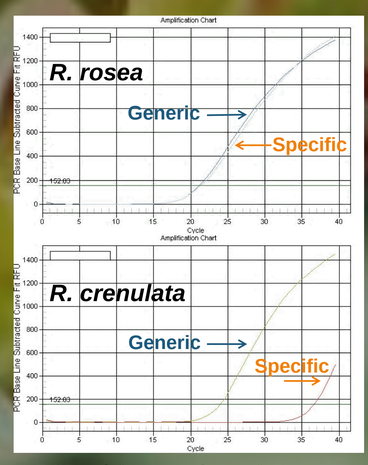
Sequence alignment of a section of the ITS region showing several base differences betwteen R. rosea and R. crenulata



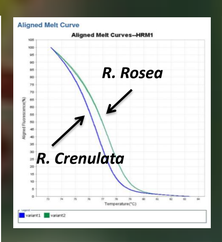
2. Species-specific PCR tests for individual species

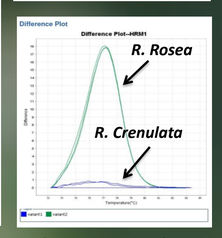


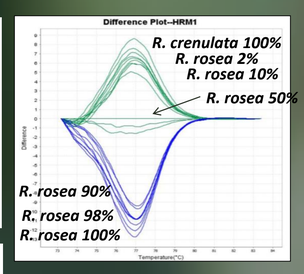
A. *Rhodiola* generic primers
B. Rhodiola rosea-specific primers
   1. *R. crenulata* sample
   2. *R. rosea* sample
   3. Negative control.



qPCR with generic and *R.rosea* specific primers against *R. rosea* and *R. crenulata* templates.







High Resolution Melting Curve for quantify the presence of *R. crenulata* in admixtures with *R. rosea*.

High Resolution Melting Curve using primers designed to discriminate *R.rosea* from non-*R. rosea* samples.

Most of the *R. rosea* samples were correctly labelled, but some *R. crenulata* samples were adulterated, or completely substituted, with *R. rosea* material.

Conclusions: This study provided a method for distinguishing five different species of *Rhodiola*. The metabolomic and phytochemical differences between these different species has been demonstrated through NMR spectroscopy and HPTLC analysis. DNA barcoding could also distinguish these species, and specific PCR tests were able to discriminate individual *Rhodiola* species from potential adulterants. There is a need to study the links between producers and consumers especially when in trans-national trade and re-enforce the hypothesis that poor quality and adulterated products can be products of poorly governed value chains, particularly at the early stages of supply. Moreover, it can be argued that through the establishment of well-controlled and well managed value chains it is possible to better prevent accidental or deliberate contamination and adulteration from occurring

Important parts of this work have been kindly funded through a charitable donation by Dr. Willmar Schwabe GmbH & Co. KG, Germany. Lixiang Zhai was funded through an exchange agreement with Guangdong Pharmaceutical University, Guangzhou, PRC. We thank Lina Du, Yu Liao for collecting samples from Qinghai, Eric Brand and Professor Zhongzhen Zhao (Hong Kong Baptist University) for supplying the samples from Hong Kong, Dr. José Vouillamoz (Agroscope Federal Research Institute, Switzerland) and Professor Alexander Shikov (Saint-Petersburg Institute of Pharmacy, Russia) for providing part of the plant material used in this research. We thank Mr Chen from Sun Ten Co. Ltd, sourcing company, Taiwan and the Yi minority for their help in sourcing plant material on the Tibetan plateau.