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^{1}H nuclear magnetic resonance based-metabolomic characterization of Peucedani Radix and simultaneous determination of praeruptorin A and praeruptorin B

Yue-Lin Song [a], Wang-Hui Jing [a], Yan-Gan Chen [a], Yun-Fei Yuan [b], Ru Yan [a], Yi-Tao Wang [a,*]

[a] State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macao, China
[b] State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, South China Agricultural University, Guangzhou, China

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ABSTRACT

As a widely used traditional herbal medicine, it is crucial to characterize the holistic metabolic profile of Peucedani Radix (Chinese name: Qian-hu). However, it is quite arduous to obtain the whole picture of chemical constituents appropriately with the existing analytical techniques that were based on HPLC–UV or LC–MS/MS system. In present investigation, nuclear magnetic resonance (NMR) spectroscopy coupled with principal components analysis (PCA) was introduced to metabolomic characterization of Qian-hu crude extracts without any chromatographic separation. In addition, the contents of praeruptorin A (PA) and proaeruptorin B (PB) in Qian-hu were simultaneously determined using quantitative ^{1}H NMR (q^{1}H NMR) spectroscopy. Eighteen reference compounds (**1**–**18**), which were purified from this herbal drug extract previously, were recruited for the assignment of the protonic signals in the ^{1}H NMR spectra. Following PCA, 15 batches of Peucedani Radix were divided into two groups (I and II), and angular-type pyranocoumarins, in particular PA and PB, as well as 5-methoxycoumarin were demonstrated as the predominant markers being responsible for the distinguishment of Qian-hu from different districts. The contents of the two analytes (PA & PB) were calculated by the relative ratio of the integral values of the target peak for each compound to the known amount of the internal standard, formononetin (IS). The lower limits of quantitation were determined as 19.5 μg/mL for both PA and PB. The quantitative results indicated that the contents of PA and PB showed quite variable qualities among different extract samples. Above all, ^{1}H NMR spectroscopy, that could not only provide comprehensive profiles of the metabolites but also achieve convenient determination of praeruptorin A and praeruptorin B, is a promising means for evaluating the medicinal samples of Peucedani Radix.

1. Introduction

Peucedani Radix (Qian-hu in Chinese), the dried roots of *Peucedanum praeruptorum* Dunn (Apiaceae) [1], has been widely employed for the treatment of cough with thick sputum and dyspnoea, nonproductive cough and upper respiratory infections [2] for centuries in China. On modern pharmacological models, this herbal drug was highly marked for tracheal and pulmonary arteries relaxant [3], coronary dilatory [2], myocardial protective [4], antitumor-promoting [5] and anti-inflammatory [6] activities, *etc.*

There are several classes of compounds reported in Peucedani Radix, and the major constituents are angular-type pyranocoumarins (APs) that are widely believed to make the main contribution to the pharmacological properties of Qian-hu [7]. This kind of components always comprises a khellactone skeleton with varied acyl substituents at C-3′ and/or C-4′ positions. Many APs were drawing increasing interest due to their strong hypotensive activity typically through acting as a calcium channel blocker and/or a potassium channel opener [3,8]. Recent studies also revealed the prospect of these pyranocoumarins in chemotherapy based on their pharmacological properties of antiproliferation, cytotoxicity and apoptosis-induction [9,10] as well as P-glycoprotein (P-gp) expression suppressing effects [11,12].

As the quality indicators of Qian-hu and related herbal products documented in Chinese Pharmacopoeia (2010 version) [1], praeruptorin A (PA) and praeruptorin B (PB) have been reported diverse biological activities by a great amount of evaluations. PA was intensively proved to be a novel calcium channels blocker [4] and a potassium channel opener [8], and showed the prospects in prevention and therapy of cardiac diseases. It also exhibited antiplatelet aggregative [13], anti-proliferative [13] and anti-inflammatory effects [14,15]. Moreover, the neuroprotective effect of PA was also reported recently [16]. On the other side, PB, as an analogue of PA, has also drawn extensive attention in recent

* Corresponding author. Tel.: +853 8397 4691; fax: +853 2884 1358.
E-mail address: ytwang@umac.mo (Y.-T. Wang).

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years since it was found to possess some other specific pharmacological effects such as inhibition of tumour promoter induced phenomenon *in vitro* [17] and potent anti-inflammatory action in addition to the above-mentioned similar activities of PA [18].

Over the recent years, with the development of much higher magnetic fields with higher sensitivity, one- and two-dimensional NMR spectroscopy has been adopted as routine implements for the analyses of complex matrices [19]. Especially, ^{1}H NMR spectroscopy was proved as a robust and high-throughput analytical method that can provide not only a significant amount of structural information but also a comprehensive profile of the sample in terms of its chemical constituents in just a single measurement [20]. And more fortunately, the availability of various mathematical tools and softwares also provides conveniences for multivariate data analysis, such as principle component analysis (PCA) and partial least squares discriminant analysis (PLS-DA). In previous reports, NMR spectroscopy has been employed for chemotaxonomic evaluation of this traditional Chinese herbal drug [21–24], and the results revealed that ^{1}H NMR spectroscopy is a quick, simple and reliable approach to distinct Qian-hu with other similar herbal drugs, including the species of *P. decursivi, P. medicun, P. rublicanle* and so on, while angular-type pyranocoumarins, in particular PA, were adopted as the chemical markers for recognition. However, neither the assignment of the protonic signals in the ^{1}H NMR spectra nor quantitative analysis of chemical components in Peucedani Radix has been achieved using NMR spectroscopy.

In general, traditional quantitative techniques, HPLC–UV and LC–MS/MS for instance, make use of chromatographic separation of components in the crude extract and determination of the separated components with the adoption of specific detectors, indicating the demands of complicated sample preparation, large amounts of solvents and/or time-consuming procedures. On the contrary, quantitative ^{1}H NMR (q^{1}H NMR) is an ideal strategy to overcome the shortcomings which general quantification techniques usually encounter, thus being regarded as a promising tool for the quality control of herbal drugs. In the past decade, q^{1}H NMR has been widely introduced for the qualitative and/or quantitative analysis of active ingredients in complex plant extracts, such as, quantitative determination of artemisinin in *Artemisia annua* [25], sesquiterpene lactones in *Arnica Montana* [26], ephedrine analogues in *Ephedra species* [27], and hypericin and pseudohypericin in *Hypericum perforatu* [28].

In the case of Peucedani Radix, the quality standard was proposed by Zhang et al. adopting HPLC–UV [25] to monitor PA and PB, without concerning the other components. Thus, a great risk of counterfeit drug is thus, led by the absence of chemical profile.

Therefore, in current investigation, as an important part of our continuous work on Peucedani Radix, we aim to: (1) characterize the metabolic profile of Qian-hu using the detailed signal assignment information based on ^{1}H NMR spectra combining with multivariate statistical analysis; (2) simultaneous determination of praeruptorin A and praeruptorin B using quantitative ^{1}H NMR spectroscopy.

2. Experimental

2.1. Chemicals

Praeruptorin A (PA, **2**), praeruptorin B (PB, **3**) and (+)-praeruptorin E (PE, **4**) (purity >99% for all compounds) were purchased from Shanghai Traditional Chinese Medicine Research Centre (Shanghai, China). Other 10 angular-type pyranocoumarins, including *cis*-khellactone (**1**), *cis*-3′-acetyl-4′-angeloylkhellactone (**5**), *cis*-3′-isovaleryl-4′-acetylkhellactone (**6**), *cis*-3′-angeloyl-4′-senecioylkhellactone (**7**), *cis*-3′,4′-diisovalerylkhellactone (**8**), *trans*-3′-angeloylkhellactone (**9**), 3′-angeloyloxy-4′-oxo-3′,4′-dihydroseselin (**10**), *trans*-4′-angeloylkhellactone (**11**), *trans*-3′-acetyl-4′-isobutyrylkhellactone (**12**), *trans*-3′-acetyl-4′-angeloylkhellactone (**13**) were isolated from Peucedani Radix in our laboratory [29]. 5-Methoxycoumarin (**14**), 5,8-dimethoxycoumarin (**15**), 7-hydroxylcoumarin (**16**), stigmasterol (**17**) and palmitic acid (**18**) were also identified from this herbal medicine. All the chemical structures (Fig. 1) were determined on the basis of NMR spectra (Supplemental figures) and LC–MS/MS spectral data. Moreover, formononetin which was presented by Dr. Qingwen Zhang in our institute, served as internal standard (IS).

$CDCl_3$ (deuterium abundance, 99.96 atom% deuterium) containing 0.03% trimethylsilane (TMS) was purchased from Cambridge Isotope Laboratories Inc. (Miami, FL, USA). Analytical grade $CHCl_3$ was obtained commercially from Kaitong Chemical Co. Ltd. (Tianjin, China).

Table 1
The contents (mg/g) of praeruptorin A and praeruptorin B in Peucedani Radix from different districts (PR1–15).

	Habitats	PA (mg/g)[a]	RSD%[b]	PB (mg/g)[a]	RSD%[b]
PR1	Anguo, Anhui (wild)	23.07	4.7	20.73	3.7
PR2	Anguo, Anhui (cultivate)	16.61	3.9	13.42	5.3
PR3	Taiyuan Shanxi (1)	51.39	5.7	10.53	5.9
PR4	Taiyuan Shanxi (2)	12.15	4.3	7.458	5.5
PR5	Chongqing	12.69	4.1	2.590	5.3
PR6	Chengdu, Sichuan	3.769	5.5	2.172	3.6
PR7	Hangzhou, Zhejiang	5.922	6.0	N.D.	N.A.
PR8	Hongkong	5.327	3.7	2.150	4.5
PR9	Macao	0.01686	5.8	0.2627	5.3
PR10	Nanchang, Jiangxi	15.78	3.8	2.557	4.4
PR11	Wenzhou, Zhejiang (1)	44.90	5.8	5.609	4.8
PR12	Wenzhou, Zhejiang (2)	N.D.	N.A.	N.D.	N.A.
PR13	Guangzhou, Guangdong	37.26	5.6	2.847	4.6
PR14	Shanghai	55.21	4.9	19.51	3.6
PR15	Linfen Shanxi	N.D.	N.A.	N.D.	N.A.

N.D.: not detected. N.A.: not applied.
[a] The mean content of triplicate.
[b] RSD% of triplicate.

2.2. Plant materials

A total of 15 batches of Peucedani Radix (PR1–15) were collected from 15 different locations (Table 1) in China. The botanical origins of the material were authenticated by Professor Pengfei Tu from Department of Natural Medicines, Peking University Health Science Center, Beijing, China, and the voucher specimens were deposited at the Institute of Chinese Medical Sciences, University of Macau, Macao SAR, China. All the crude materials were dried using a universal oven with forced convection (FD115, Tuttlingen, Germany) at 40 °C for 4 days.

2.3. Preparation of samples for NMR spectroscopic analysis

The dried roots were crushed into powder with sample mill (model YF102, Ruian Yongli Pharmacy Machinery, China), following by passing through an 80 mesh sieve. And then, the pulverized crude materials (accurate quantity about 200 mg) were extracted with 5 mL of $CHCl_3$ using ultrasonication for 30 min at 40 °C. Each extracting solution was centrifuged at 10 000 × *g* for 10 min, and the supernatant was filtered through a 0.45 μm nylon membrane filter (Tianjin Jinteng Experiment Equipment Co., Ltd, China). An aliquot (2.5 mL) of the filtrate was transferred into another tube and evaporated under nitrogen flow at 25 °C. Following evaporation, the residues were reconstituted with 600 μL of $CDCl_3$, and quickly transferred into 5-mm tubes (Norell ST500-7) for NMR analysis.

All the reference compounds were also dissolved using 600 μL of $CDCl_3$ in 5-mm tubes (Norell ST500-7) and analyzed parallelly