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Characterization and authentication of *Acori Tatarinowii Rhizoma* and its adulterants by UPLC-Orbitrap-MS/MS chromatographic fingerprints, elements profiles and chemometric methods

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ABSTRACT

UPLC-Orbitrap-MS/MS and ICP-MS were applied to analyze and characterize the chemical composition of *Acori Tatarinowii Rhizoma* (ATR) and its adulterants including *Acori Calami Rhizoma* (ACR) and *Anemones Altaicae Rhizoma* (AAR). Fifty-five, fifty-five and thirty-six organic components were identified in ATR, ACR and AAR by UPLC-Orbitrap-MS/MS, respectively. Forty elements were detected by ICP-MS. AAR is different from ATR and ACR. 19 organic components existed in ATR and ACR were not detected in AAR. And, concentration of β-asarone in AAR was 1000 times lower than ATR and ACR. Chemical components in ATR and ACR are very similar. Partial least squares-discriminant analysis (PLS-DA) was used to distinguish between ATR and ACR. Variable important projection (VIP) was employed to improve the classification ability of PLS-DA. Twenty-one chemical components were identified, and were used to establish the characteristic quantitative fingerprints of ATR and ACR. Our results indicated that the combination of two analytical platforms and chemometrics will provide more information to characterize and distinguish the complex analytical objects, such as similar Chinese herbs.

1. Introduction

The dried rhizome from Acori Tatarinowii Schott is a traditional Chinese medicine (TCM), which is known as *Acori Tatarinowii Rhizoma* (ATR) and is called “Chang Pu”. It belongs to the Araceae family and is used for treatment of nervous diseases [1–10], like epilepsy, nervous disorders [1,11], sedation [4], depression [2], and Alzheimer's disease [3]. It is recorded in the Chinese pharmacopoeia (2015) as the official botanical source of ATR [12]. Another “Chang Pu” is *Acori Calami Rhizoma* (ACR; rhizome derived from the *A. calamus* Linn). It defined as an herbal medicine in national pharmacopoeia in India. ACR is utilized to treat cognitive disorders, epilepsy, asthma, pain and diabetes [13]. In addition, *Anemones Altaicae Rhizoma* (AAR; rhizome from Anemone altaica Fisch. ex Mey), is also called “Chang Pu”. It is a member of the ranunculaceae family. Traditionally, it is used as a substitute for ATR to treat dreaminess, amnesia, rheumatoid arthritis and epilepsy [14]. These three “Chang Pu” have similar bioactivities for nervous diseases [13–15], as well as share several morphological similarities [15,16], making it meaningful but difficult to differentiate.

Tsim et al. had characterized those three species using a combination of macroscopic and microscopic examination, chemical analysis and DNA authentication [15]. However, in this research, only two volatiles, α-asarone and β-asarone, were studied by HPLC in chemical analysis. Zhang et al. analyzed the volatile components in ATR and ACR by GC–MS and screened three volatiles, namely, camphor, longicyclene, and δ-cadinene, to distinguish between ATR and ACR [17]. So far, most researches on ATR focus on the volatile components, especially on asarone [10,18–20], while little attention has been paid to the study on elements and non-volatiles [21]. However, elements and non-volatiles are very important to its medicinal function and quality control. For

Abbreviations: ATR, *Acori Tatarinowii Rhizoma*; ACR, *Acori Calami Rhizoma*; AAR, *Anemones Altaicae Rhizoma*; PCA, principal component analysis; VIP, variable important projection; PLS-DA, partial least squares-discriminant analysis; QC, quality control; TCM, traditional Chinese medicine; SD, standard deviation; LOO, leave-one-out

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example, tatarinan O, one of liginans, have the therapeutic effect on osteoporosis [22]. Diasarone I can inhibit Dengue virus infection [23]. Elements, like Fe and Mn, are related to geo-herbalism of TCM and essential trace elements for human beings [20,24], while toxic elements like Cd, Pb, and As are harmful to metabolism [25]. It is necessary to combine the multiple analytical platforms to characterize their chemical composition comprehensively. UPLC-MS/MS is one of the most promising techniques to obtain massive information of organic components and ICP-MS is every effective to detect almost all elements existed in the earth. This combination of UPLC-MS/MS and ICP-MS will undoubtedly provide us with more information of chemicals. And, chemometrics will help us to transform the information into knowledge. This combination of analytical techniques will be more powerful [17,26–28].

In this study, two analytical platforms, UPLC-Orbitrap-MS/MS and ICP-MS, were combined to characterize the chemical components in ATR, ACR and AAR. With the help of superior separation ability of UPLC, abundant structural information obtained from high-resolution mass spectrometry, the powerful ability of multi-element analysis of ICP-MS, massive chemical information of the three species was obtained. Furthermore, chemometric methods, such as principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA), were employed to distinguish ATR from ACR and AAR.

2. Materials and methods

2.1. Chemicals

Vitexin (purity: 97%, Lot: BBP00886), chlorogenic acid (purity: 98%, Lot: 151022) were purchased from Bio Bio Pha (Kunming, China). Syringic acid (purity: 99.10%, Lot: MUST-16041910), quinic acid (purity: 99.93%, Lot: MUST-16012208), caffeic acid (purity: 99.99%, Lot: MUST-16060613), 4-hydroxycinnamic acid (purity: 99.96%, Lot: MUST-16060613), magnolol (purity: 99.71%, Lot: MUST-16031112) were bought in Chengdu Mansite Bio-Technology Co., Ltd (Chengdu, China). L-tryptophan (purity: GR, Lot: VNOCG-RT), 2-hydroxybenzoic acid (purity: 99.5%, Lot: NPC4S-LF), esculetin (purity: 98%, Lot: RE4QO-MT), 4-(1-propenyl)-1,2-domethoxybenzene (purity: 98%, Lot: H802E-KB), 4-ally-1,2-dimethoxybenzene (purity: 98%, Lot: PH3YH-FS) were gained in TCI (Tokyo, Japan). β-Asarone (purity: 70%, Lot: 03124JRV), α-asarone (purity: 98%, Lot: S18779 V), aspartic acid (purity: 98%, Lot: BCBL8402V), glutamic acid (purity: 98%, Lot: BCBL0977V), L-isoleucine (purity: 98%, Lot: SLBM0249V), phenylalanine (purity: 98%, Lot: BCBL7164V), lysine, leucine (purity: 98%, Lot: BCBL2267V) were acquired in Sigma-Aldrich (St. Louis, MO, USA). L-Tyrosine (purity: 99%, Lot: 20090601-1FG) was acquired by Meryer (Shanghai, China). 3,4-Dihydroxybenzoic acid (purity: 98%, Lot: 0004712-131010000) was purchased from Ark Pharm (Chicago, USA). Acetic acid was of HPLC-MS grade and took out from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile and methanol were of HPLC-MS grade and made available from Merck (Merck, Germany). Distilled water used was recruited from Watsons (Hong Kong, China).

All elemental standard solutions (1000 mg/L) and internal standard solutions (1000 mg/L) were certified and purchased from the National Institute of Metrology, China. Guaranteed reagent grade nitric acid and perchloric acid were purchased from Sinopharm Chemical Reagent, China. Guaranteed reagent grade nitric acid (purity: 65%, product number: 4.110040.2501) was purchased from CNW (Germany).

2.2. Sample collection

In this study, 27 bathes of ATR samples, 13 bathes of ACR samples and 14 bathes of AAR samples were harvested from several provinces in China, shown in Table S1. Those samples were authenticated as ATR, ACR and AAR by Professor Shao Liu (Xiangya Hospital, Central South University) in Changsha. The specimens are preserved in that institute now.

2.3. Analysis of organic components

2.3.1. Standard solutions preparation

22 standard stock solutions including the internal standard solution (magnolol) were prepared at a concentration of 100 mg/L in methanol-water (70:30, v/v) and were stored at −20 °C in refrigerator.

2.3.2. Samples preparation

0.5 g of powdered sample material was accurately weighed and put in a conical flask with 5 mL methanol-water (70:30, v/v). After sonication for 15 min, the mixture was filtered using the filter paper. Adding methanol-water (70:30, v/v) to 10 mL after 1 mL internal standard solution (100 mg/L) was added to the filtered solution. All extracted solutions were stocked at −20 °C in refrigerator. As well as, samples must be filtrated through a 0.22 μm membrane filters prior to UPLC-Orbitrap-MS/MS analysis.

2.3.3. UPLC-Orbitrap-MS/MS analysis

The UPLC-ESI-Q-Orbitrap system consisted of a quaternary Series RS pump, a degasser, an auto-sampler, a column compartment, and a heated electrospray ionization source (ESI) coupled with a high-resolution Q-Exactive focus mass spectrometer (Thermo Fisher Scientific, Germany). XCalibur software (3.0) from Thermo Fisher Scientific (MA, USA) was used to control the instrument and for data processing. Chromatographic separation was achieved on a Shim-pack XR-ODS2 C_{18} column (75 mm × 2.0 mm, 2.3 μm) (Shimadzu, Japan).

Mobile solvent A was 0.1% formic acid in H_2O and mobile solvent B was acetonitrile. The mobile phase was delivered at a flow rate of 200 μL/min with a gradient elution: 0–5 min, 5%–25% B; 5–12 min, 25%–30% B; 12–13 min, 30%–50% B; 13–15 min, 50%–65% B; 15–20 min, 65%–65% B; 20–23 min, 95%–5% B; 23–30 min, 5%–5% B. The total runtime for each injection was 30 min. The injection volume was 2 μL. The column temperature was 35 °C.

The ESI parameters were optimized for accurate mass measurement as follows: sheath gas flow rate, 40 L/min (+ −); auxiliary gas flow rate, 10 L/min (+ −); spray voltage, 3.5 kV (+), 4.0KV (−); capillary temperature, 320 °C (+ −); and S-lens RF level, 50 V (+ −); collision energy, 15, 25, and 35 (+ −); probe heater temperature, 300 (+ −). Nitrogen gas was used for spraying stabilization, high-energy collision dissociation, and damping gas in the C-trap. The mass range in the full scanning experiments was *m/z* 120–1800. The automatic gain control was set at a target value of 1×10^6 for MS acquisition and 5×10^4 for MS/MS acquisition. The effluent from the UPLC column was introduced into the mass spectrometer through a six-port valve, which guided a flow of 0.2 mL/min to the Orbitrap mass spectrometer.

2.3.4. Quality control of UPLC-Orbitrap-MS/MS analysis

The mix-samples and mix-standards were used as quality control (QC) samples to evaluate the variations due to environment and instrument variability. A mixture of the extracted solutions of the fifty-four samples was used as the mix-samples sample (QC1) and a mixture of 22 standard solutions was used as the mix-standards sample (QC2). They were injected thirteen times and as a part of the injection series.

2.4. Analysis of elements

2.4.1. Standard solutions preparation

Calibration solutions were prepared by diluting certified standard solutions with 5% nitric acid in ultrapure deionized water to six concentrations. The concentrations of Li, V, Cr, Ga, As, Rb, Mo, Cd, Sn, Sb, Te, Cs, La, Ce, Pr, Nd, Pm, Eu, Gd, Tb, Dy, Ho, Y, Ni, Co, Er, Tm, Yb, Tl, Pb, and Lu were 0.00, 1.00, 5.00, 10.00, 30.00, and 50.00 μg/L. The concentrations of B, Ti, Cu, Zn, Rh, Ba, and Mn were 0.00, 10.00, 50.00, 100.00, 300.00, and 500.00 μg/L. The concentrations of Sr was 0.00, 20.00, 100.00, 200.00, 600.00, and 1000.00 μg/L. The concentrations of Na, Mg, Al, P, K, Ca, and Fe were 0.00, 100.00, 500.00, 1000.00,