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An expeditious HPLC method to distinguish *Aconitum kusnezoffii* from related species

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ABSTRACT

An optimized HPLC-DAD method aided by similarity and hierarchical clustering analysis (HCA) was applied for identification of four species of the roots of *Aconitum*. The unique properties of this HPLC fingerprints and HCA were validated by analyzing *Aconitum kusnezoffii* Rchb. (AKR) samples and related herbs including *A. karacolicum* Rapcs. (AKP) samples, *A. austroyunnanense* W.T.Wang (AAW) samples and *A. contortum* Finet & Gagnepain (ACF). The results revealed that the chromatographic fingerprint combining similarity measurement and hierarchical cluster analysis could efficiently identify and distinguish AKR samples from its related species.

1. Introduction

Aconitum genus (Ranunculaceae) is a rich source of diterpenoid-alkaloids [1–3], some species under this genus containing the diester alkaloids are highly toxic compounds meanwhile possess anti-rheumatic and pain-relieving efficacy. Among the species, *Aconitum kusnezoffii* Rchb. (AKR) (*Caowu* in Chinese) is included in Chinese Pharmacopoeia as a common-used analgesic and anti-rheumatic herbal medicine [4]. In addition to AKR, the roots of other species of *Aconitum* are also available in some local market as the adulterants of *Caowu*, such as *A. karacolicum* Rapcs. (AKP), *A. austroyunnanense* W.T.Wang (AAW), *A. contortum* Finet & Gagnepain (ACF). The contents and the distribution of the alkaloids among various species are different, consequently their toxicities are diverse. Hence ensuring the species authentication is the most concerned matter. But the reported identification methods limited to macroscopical and microscopical methods [5] as well as non-optimized TLC identification [6]. Those methods do not suffice the precise identification of the species.

Apart from macroscopic and microscopic authentication, chemical identification of TCM materials is an important and useful means as it directly associates with the medicinal functions of TCM materials. Chromatographic fingerprint, a comprehensive and quantifiable identification method, is able to reveal chemical information of herbal medicines with chromatogram, spectrograms and other graphs by analytical and chemical techniques [7–16]. So far, fingerprint of TCM, especially the chromatographic fingerprint, such as high performance liquid chromatography (HPLC) [17–23], gas chromatography [24], thin layer chromatography (TLC) [25], and capillary electrophoresis [26], has attracted much attention and been widely accepted as a feasible means and is a useful method in the identification and authentication of botanical medicines [27–31].

There is so far no application of chromatographic fingerprint to identify differentiation of AKR and its related species reported. In this study, the HPLC-DAD fingerprint combining with similarity measurement and hierarchical clustering analysis were performed aided by the software of

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"similarity evaluation system for chromatographic fingerprint of TCM" and "STATISTICS for windows".

2. Experimental

2.1. Materials

Mesaconitine (MA), aconitine (A) and hypaconitine (HA) were provided by Kunming Institute of Botany Research, Chinese Academy of Sciences, and were used as standard chemicals. Their structures were shown in Fig. 1. Methanol and acetonitrile were of HPLC grade (fisher Inc., USA). Glacial acetic acid, ether, and ammonia were of analytical-reagent grade from Tianjin Chemical Reagent Co. (China). Distilled and deionized water was used for the preparation of all samples and solutions.

2.2. Sample preparation

Twenty-two samples were collected or purchased from different regions of China (Table 1) and dried at 40 °C. All of the samples were identified by Professor Xi-wen Li (Kunming Institute of Botany Research, Chinese Academy of Sciences). Voucher specimen (Nos. 1, 11 and 14) was deposited at Biomedicine Key Laboratory of Shaanxi Province, Northwest University, Xi'an, Shaanxi, P. R. China.

The samples were prepared as follows: 0.4 g of dried powdered sample was precisely weighed and put into a 50 mL amber vial. 0.4 mL of 10% aqueous ammonia then 10 mL of ethyl ether were added to the vial consecutively. The vial was sealed and shaken for 10 min. Sample was extracted by ultrasonication (AS3120A Ultrasonic Cleaner) at room temperature for 30 min, after cooling. The extraction step was repeated and only added ethyl ether twice, and the extracted solution was combined and filtrated through analytical filter paper. The filtrate was evaporated to dryness using a rotary evaporator (BÜCHI, Switzerland) at 40 °C and the dried extract was dissolved in 5.0 mL of acetonitrile. The sample was finally filtrated through a 0.45 μm membrane filter and then injected into the HPLC system.

2.3. Chromatographic conditions

HPLC was performed using a Shimadzu LC-10A (Japan), consisting of a vacuum degasser, binary pump, an auto-sampler and a photodiode array detection (DAD), controlled by Class-up station. The HPLC column was a Kromasil C18 column (250×4.6 mm, 5 μm). The isocratic mobile phase consisted of acetonitrile–0.25% glacial acetic acid (pH value was adjusted to 10.5 by ammonia) (60:40, v/v) was used to elute for 30 min, the flow rate was kept at 1.0 mL/min. Column temperature was kept constant at 30 °C. UV detection: 240 nm. The injection volume was 10 μL.

Table 1
A summary of the tested samples.

No.	Source	Obtained	Year of collection
1	Chengde, Hebei, China	Purchased	2004
2	Bozhou, Anhui, China	Purchased	2004
3	Wuhai, Hubei, China	Purchased	2004
4	Liupanshui, Guizhou, China	Purchased	2004
5	Nanzhao, Henan, China	Collected	2005
6	Qianshan, Liaoning, China	Collected	2005
7	Queshan, Henan, China	Collected	2004
8	Chengdu, Sichuan, China	Collected	2005
9	Shiyan, Hubei, China	Purchased	2005
10	Mianyang, Sichuan, China	Purchased	2005
11	Urumqi, Xinjiang, China	Purchased	2004
12	Urumqi, Xinjiang, China	Purchased	2005
13	Kunming, Yunnan, China	Purchased	2004
14	Gejiu, Yunnan, China	Collected	2004
15	Huize, Yunnan, China	Collected	2004
16	Dali, Yunnan, China	Collected	2004
17	Honghe, Yunnan, China	Collected	2004
18	Qiubei, Yunnan, China	Collected	2004
19	Dali, Yunnan, China	Collected	2004
20	Dali, Yunnan, China	Collected	2005
21	Kunming, Yunnan, China	Purchased	2004
22	Dali, Yunnan, China	Collected	2005

Samples 1 to 10: AKR; Samples 11 to 13: AKP; Samples 14 to 18: AAW; Samples 19 to 22: ACF.

2.4. Calibration, precision, repeatability and stability

Calibration, precision, repeatability and stability of this method were preformed according to our previous work [32].

2.5. Data analysis of chromatogram and chromatographic Fingerprint analysis

The HPLC analysis of all the samples were carried out under the established experimental condition as mentioned in Section 2.3. The data acquired from the representative chromatographic profiles of AKR, AKP, AAW and ACF samples were analyzed and evaluated respectively by using the

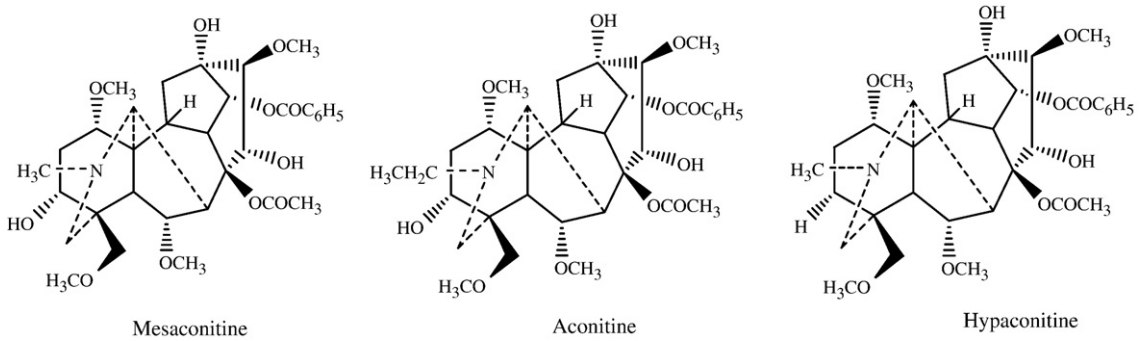

Fig. 1. Structures of mesaconitine (MA), aconitine (A) and hypaconitine (HA).