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Original Article

A comparative study of three tissue-cultured *Dendrobium* species and their wild correspondences by headspace gas chromatography–mass spectrometry combined with chemometric methods

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ABSTRACT

Plant tissue culture technique is widely used in the conservation and utilization of rare and endangered medicinal plants and it is crucial for tissue culture stocks to obtain the ability to produce similar bioactive components as their wild correspondences. In this paper, a headspace gas chromatography–mass spectrometry method combined with chemometric methods was applied to analyze and evaluate the volatile compounds in tissue-cultured and wild *Dendrobium huoshanense* Cheng and Tang, *Dendrobium officinale* Kimura et Migo and *Dendrobium moniliforme* (Linn.) Sw. In total, 63 volatile compounds were separated, with 53 being identified from the three *Dendrobium* spp. samples. Different provenances of *Dendrobiums* had characteristic chemicals and showed remarkable quantity discrepancy of common compositions. The similarity evaluation disclosed that the accumulation of volatile compounds in *Dendrobium* samples might be affected by their provenance. Principal component analysis showed that the first three components explained 85.9% of data variance, demonstrating a good discrimination between samples. Gas chromatography–mass spectrometry techniques, combined with chemometrics, might be an effective strategy for identifying the species and their provenance, especially in the assessment of tissue-cultured *Dendrobium* quality for use in raw herbal medicines.

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1. Introduction

Dendrobium, a precious traditional Chinese medicine, has been used in the preparation of herbal medicines in China for more than 2000 years. Sections of the stems of *Dendrobiums* have long been used to cure throat inflammation, nourish the stomach, promote secretion of saliva or as a tonic to promote the production of body fluid and improve the quality of life [1]. Seventy-four species of *Dendrobium* and two varieties are found in China [2]. The slow growth rate and excessive harvesting had left some of them critically endangered, especially *Dendrobium huoshanense* [3].

Plant tissue culture technique is widely used in the conservation and utilization of rare and endangered medicinal plants due to its remarkable ability of quickly increasing their biomass [4,5]. In the traditional product region, tissue-cultured dendrobiums have already become the major resource of pharmaceutical *Dendrobiums*. It is vital for tissue culture stocks to obtain the ability to produce similar bioactive components as their wild correspondences besides keeping genetic information and morphologies homoplastic between different provenances. Therefore, establishing a fast, quality identification method to evaluate the chemical similarity of the wild and tissue-cultured *Dendrobium* is a critical step for assurance of quality and safety in the traditional Chinese medicine industry.

The volatile components of herbal medicines contain a significant number of compounds and are used as markers for authenticity. The variations of volatile components in plants might be caused by differences in species, habitats, variety, cultivation patterns, or the extraction and analysis methods applied for composition determination [6,7]. Accordingly, it might be practical to establish a gas chromatography–mass spectrometry (GC–MS) fingerprint method based on the analysis of the volatile components to evaluate the similarity between tissue-cultured medicinal plants and their wild correspondences.

In this paper, we aimed to apply the headspace GC–MS technique coupled with a series of chemometric methods to fingerprint the volatile compounds from the stems of tissue-cultured and wild *D. huoshanense*, *Dendrobium officinale*, and *Dendrobium moniliforme*. To our knowledge, no documents have ever mentioned the discrimination and similarity evaluation of the tissue-cultured and wild *Dendrobium* plants by GC–MS method. Therefore, our study might be beneficial for developing a rapid, feasible and economical tool based on GC–MS for the identification and quality evaluation between different provenances of *Dendrobium* species, and provide new insights into the utilization and conservation of rare and endangered medicinal plants by tissue culture techniques.

2. Methods

2.1. In vitro *callus growth protocol, plant materials, and chemicals*

The *in vitro* plantlets of the three tissue-cultured dendrobiums were regenerated via protocorm-like bodies in the laboratories of West Anhui Biotechnology Research Center of Natural Medicine and Traditional Chinese Medicine and were then transplanted in the cultivated base in Huoshan count, Anhui Province, China. The current season's vegetative stems of tissue-cultured and wild *D. huoshanense*, *D. officinale*, and *D. moniliforme* were collected in October 2013, from Huoshan County, Anhui Province, China. All the plant materials were identified by Professor Nai-Fu Chen, Anhui Biotechnology Research Center of Plant Cell Engineering, Anhui Province, China. The voucher specimens were deposited at the Herbarium, College of Biotechnology and Pharmaceutical Engineering, West Anhui University, Anhui Province, China (Table 1).

The authentic chemicals and alkane standard solutions of C8-C20 (mixture no. 115321-01-4PAK) were purchased from ANPEL Laboratory Technologies Inc. (Shanghai, China).

The fresh collected *Dendrobium* samples were washed thoroughly in tap water and then freeze-dried by a Micro-Modulyo lyophilizer (Thermo Fisher Scientific, West Palm Beach, FL, USA), powdered in a blender and then every 2.0 g of dried sample was performed for headspace/GC–MS analysis.

2.2. *Equipment and conditions*

GC–MS analysis was performed with Trace 1300 gas chromatograph coupled to ISQ mass spectrometer (Thermo Fisher Scientific, West Palm Beach, FL, USA) series equipment including a TriPLUS RSH autosampler. The volatile compounds were separated on a TG-5 MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness). Total program time was 39 minutes and the column oven temperature program was: 50°C (maintained for 1 minute) to 60°C at 1°C/min, to 200°C at 5°C/min. The carrier gas was Helium, 1.0 mL/min, split ratio 5:1, injector temperature 250°C. The samples were heated in the agitator oven for 10 minutes with constant incubation mode at 140°C. The injection volume was 2.5μL. The MS transfer line and ion source were at 280°C and 250°C, respectively. The MS mode was electron impact. The mass range scanned was 40–350 atomic mass units.

2.3. *Identification of the separated compounds*

The identification of the separated compounds was carried out by three different methods: (1) retention indices [8] of the compounds to be identified compared the retention index values detected by the same type of capillary column in the National Institute of Technology and Standards mass spectra libraries; (2) retention times of authentic standards in the same equipment and conditions; and (3) mass spectra, with indexes of relative match above 800 (US National Institute of Technology and Standards mass spectra libraries and also authentic chemicals). Compounds were marked as tentatively identified when identification was only based on mass spectral data.

All peaks found in at least two of the three total ion chromatograms (TIC) were taken into account when calculating the total area of peaks (100%) and the relative areas of the volatile compounds.