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Application of pyrolysis-gas chromatography and hierarchical cluster analysis to the discrimination of the Chinese traditional medicine *Dendrobium candidum* Wall. ex Lindl.

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ABSTRACT

The pyrogram fingerprints of *Dendrobium candidum* Wall. ex Lindl. samples from 3 different growing places and 2 other different species were analyzed on the basis of pyrolysis-gas chromatography. An amount of 0.4 mg of sample powder was pyrolyzed in a vertical microfurnace pyrolyzer at 450 °C, and the products were directly introduced into a gas chromatograph equipped with a flame ionization detector or a mass spectrometer. Then, each sample was characterized by the relative peak area of 40 peaks in thus obtained pyrogram. The pyrogram fingerprints of 16 samples from different growing places and species showed good similarity and reproducibility with the relative standard deviations (RSDs) of the retention time less than 0.12% ($n = 5$) and the RSDs of the relative percent of peak areas less than 3.77% ($n = 5$). Furthermore, the discrimination of the samples from different growing places and species was achieved by hierarchical cluster analysis via recognizing the 16×40 data matrix. Thus, the results proved the Py-GC fingerprint combined with chemometric approach is a simple, rapid and selective method which is suitable for the quality control of the raw materials of herbal medicine.

1. Introduction

Herbal medicines (HM) have been used for thousands of years in China and other countries because of their pharmacological activities and low toxicity [1]. *Dendrobium*, known as 'ShiHu', has been used in Chinese medicine therapy because it possesses the functions of clearing heat, eyes-benefiting and immunomodulatory effects [2]. There are 74 species and 2 variations of *Dendrobium* plants found in China and about 30 species of them are used as traditional or folk medicines in China [3]. Among them, the most commonly used and the most expensive species is *Dendrobium candidum* Wall. ex Lindl. because of its excellent immunomodulatory effect. The chemical composition of *Dendrobium* which affects its pharmacological activities often varies to some extent depending on the species and the growing places. However, it is troublesome and difficult to discriminate the *D. candidum* Wall. ex Lindl. materials from different species and identify the fakes of them by traditional methods such as microscopy by a botanist and/or quantitative analysis of several main chemical components with HPLC or TLC. It is likely that the difference of multiple chemical components among sample is not significant enough for their discrimination by the above traditional methods. Therefore, a convenient, precise and holistic approach for quality control is necessary.

Fingerprinting method using chromatographic technique for authentication and quality control of Chinese Medicine Materials has recently been accepted by the World Health Organization (WHO) as a strategy for the assessment of herbal medicines [4]. Furthermore, chromatographic methods are highly recommended for determining the fingerprints of herbal medicine products and their raw materials by the Drug Administration Bureau of China [5]. Several chromatographic fingerprint methods have been developed as useful tools in the authentication and quality assessment of herbal medicines [6–11], and HPLC fingerprinting analysis of the *Dendrobium* species has also been reported [12–14]. However, prior to the final HPLC measurement pretreatments such as solvent extraction and concentration were inevitably utilized, which provided incomplete components for a fingerprint analysis. In a word, a method for quality control requires the properties of speediness and automatism, and the ability to analyze large amount of samples routinely.

Recently, pyrolysis-gas chromatography (/mass spectrometry) (Py-GC(/MS)) were used extensively in the characterization of natural products such as lignin [15,16], food product [17] and botanical medicines [18,19]. This technique yields a pyrogram which consists

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Table 1
A summary of the test samples.

No.	Sample code	Sample species	Source	Time (year, month)
1	ZY-1	*Dendrobium candidum* Wall. ex Lindl.	Yiwu, Zhejiang, China	2007, 1
2	ZY-2	*Dendrobium candidum* Wall. ex Lindl.	Yiwu, Zhejiang, China	2007, 1
3	ZY-3	*Dendrobium candidum* Wall. ex Lindl.	Yiwu, Zhejiang, China	2007, 1
4	ZY-4	*Dendrobium candidum* Wall. ex Lindl.	Yiwu, Zhejiang, China	2007, 1
5	ZT-1	*Dendrobium candidum* Wall. ex Lindl.	Tiantaishan, Zhejiang, China	2007, 1
6	ZT-2	*Dendrobium candidum* Wall. ex Lindl.	Tiantaishan, Zhejiang, China	2007, 1
7	ZT-3	*Dendrobium candidum* Wall. ex Lindl.	Tiantaishan, Zhejiang, China	2007, 1
8	AH-1	*Dendrobium candidum* Wall. ex Lindl.	Huoshan, Anhui, China	2007, 3
9	AH-2	*Dendrobium candidum* Wall. ex Lindl.	Huoshan, Anhui, China	2007, 3
10	AH-3	*Dendrobium candidum* Wall. ex Lindl.	Huoshan, Anhui, China	2007, 3
11	GG-1	*Dendrobium crystallinum* Rchb. f.	Guangzhou, Guangdong, China	2007, 3
12	GG-2	*Dendrobium crystallinum* Rchb. f.	Guangzhou, Guangdong, China	2007, 4
13	GG-3	*Dendrobium crystallinum* Rchb. f.	Guangzhou, Guangdong, China	2007, 5
14	YS-1	*Dendrobium devonianum* Paxt.	Simao, Yunnan, China	2007, 5
15	YS-2	*Dendrobium devonianum* Paxt.	Simao, Yunnan, China	2007, 6
16	YS-3	*Dendrobium devonianum* Paxt.	Simao, Yunnan, China	2007, 7

of the characteristic peaks of the constituents in a given sample without any pretreatment procedures. Based on the peak intensity observed on the pyrogram, not only chemical composition but also discriminative analysis is achieved by further combination with chemometric approaches.

In this study, pyrolysis-gas chromatography using a vertical microfurnace pyrolyzer was applied for construction of Py-GC fingerprint for *D. candidum* Wall. ex Lindl. raw material without any tedious pretreatments. Furthermore, on the basis of Py-GC fingerprint discriminative analyses for *D. candidum* Wall. ex Lindl. samples from 3 different growing places and 2 other different species were performed by chemometric approaches on the basis of principal component analysis (PCA) and hierarchical cluster analysis (HCA).

2. Experimental

2.1. Material

Total 16 samples were used in this study. As shown in Table 1, 10 samples were *D. candidum* Wall. ex Lindl. collected from Zhejiang and Anhui province, 3 samples were *Dendrobium devonianum* Paxt. collected from Yunnan province, and other 3 samples were *Dendrobium crystallinum* Rchb. f. collected from Guangdong province in China.

All samples were dried at 60 °C for 6 h, and then were milled into fine powders (finer than 120 meshes) by an herbal medicine mill (HX-100) prior to Py-GC measurements in order to improve the efficiency of the pyrolysis.

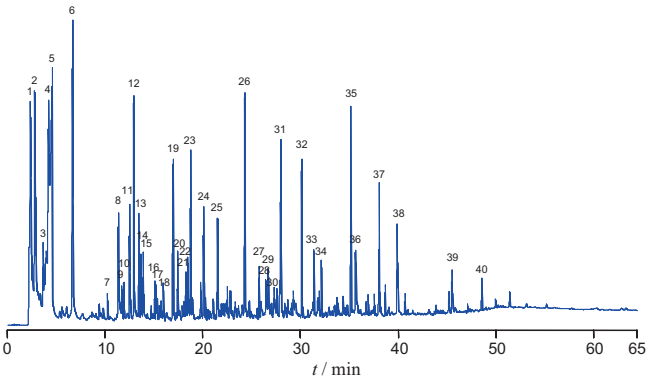

Fig. 1. A typical pyrogram of *Dendrobium candidum* Wall. ex Lindl. sample obtained at pyrolytic temperature of 450 °C.

2.2. Py-GC condition

A vertical microfurnace pyrolyzer (PY2020iD, Frontier Lab Ltd., Fukushima, Japan) was directly attached to a gas chromatograph (CP-3800, Varian, USA) equipped with a flame ionization detector (FID). About 0.4 mg of powdered *Dendrobium* sample taken in a platinum sample cup was first mounted at the waiting position of the pyrolyzer kept at around room temperature, and then it was dropped into the heated center of the pyrolyzer under the flow of nitrogen carrier gas. In order to obtain good peak intensity and appropriate peak number, the optimum pyrolysis temperature of

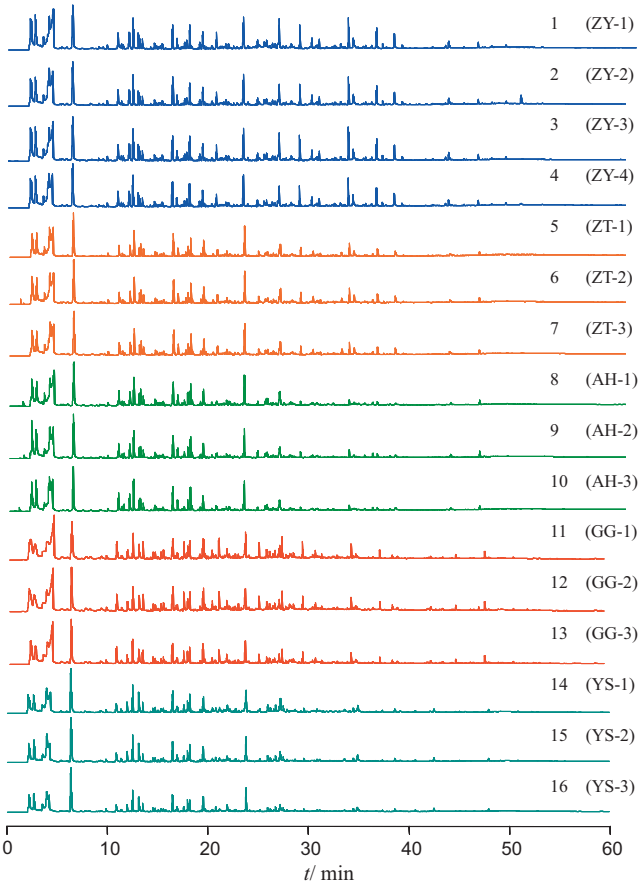

Fig. 2. Typical pyrograms of 16 samples obtained at pyrolytic temperature of 450 °C. (Samples 1–10 are *Dendrobium candidum* Wall. ex Lindl., samples 11–13 are *Dendrobium crystallinum* Rchb. f., samples 14–16 are *Dendrobium devonianum* Paxt.)