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Vibrational microspectroscopic identification of powdered traditional medicines: Chemical micromorphology of *Poria* observed by infrared and Raman microspectroscopy

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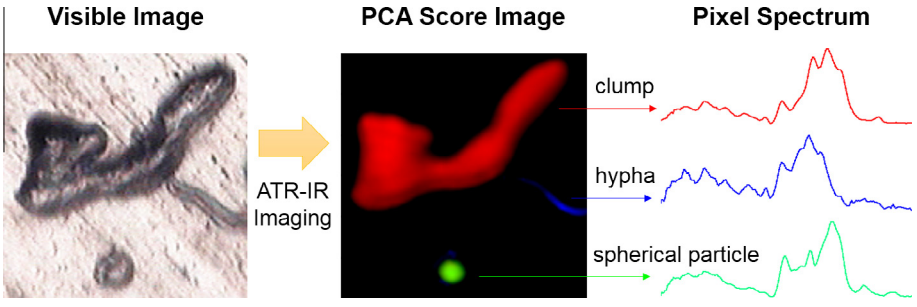
HIGHLIGHTS

- The vibrational microspectroscopic identification method is proposed first.
- Chemical compositions of hyphae and clumps in dry *Poria* powder are characterized.
- Three types of hyphae are classified according to the infrared spectral features.
- A new kind of spherical particles in dry *Poria* powder are found and studied.

GRAPHICAL ABSTRACT



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ABSTRACT

Microscopic identification using optical microscopes is a simple and effective method to identify powdered traditional medicines made from plants, animals and fungi. Sometimes, the criteria based on physical properties of the microscopic characteristics of drug powder may be ambiguous, which makes the microscopic identification method subjective and empirical to some extent. In this research, the vibrational microspectroscopic identification method is proposed for more explicit discrimination of powdered traditional medicines. The chemical micromorphology, i.e., chemical compositions and related physical morphologies, of the drug powder can be profiled objectively and quantitatively by infrared and Raman microspectroscopy, leading to better understanding about the formation mechanisms of microscopic characteristics and more accurate identification criteria. As an example, the powder of *Poria*, which is one of the most used traditional Chinese medicines, is studied in this research. Three types of hyphae are classified according to their infrared spectral features in the region from 1200 to 900 cm^{-1}. Different kinds of polysaccharides indicate that these hyphae may be in different stages of the growth. The granular and branched clumps observed by the optical microscope may be formed from the aggregation of the mature hyphae with β-D-glucan reserves. The newfound spherical particles may originate from the exuded droplets in the fresh *Poria* because they are both composed of α-D-glucan. The results are helpful to understand the development of the hyphae and the formation of active polysaccharides in *Poria* and to establish accurate microspectroscopic identification criteria.

Introduction

Most traditional medicines are made from plants, animals and fungi. Similar names and/or appearances cause the problem of confusion and counterfeit [1]. For example, the dried root of *Stephaniae*

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tetrandrae S. Moore is called "*Fangji*", which is a kind of traditional Chinese medicine free of aristolochic acid. Meanwhile, the root of *Aristolochia fangchi* Y.C. Wu ex L.D. Chou et S.M. Hwang is called "*Mufangji*" and it contains a certain amount of aristolochic acid. The nephropathy may be caused when patients accidentally take in *Mufangji* instead of *Fangji* [2]. Another example is the confusion of "*Chuanmutong*" and "*Guanmutong*". *Chuanmutong* (the dried vine of *Clematis armandii* Franch. or *Clematis montana* Buch. - Ham.) is free of aristolochic acid, but *Guanmutong* (the dried vine of *Aristolochia manshuriensis* Kom.) contains aristolochic acid. The mistake use of *Guanmutong* as *Chuanmutong* may damage the kidney, too [3]. Therefore, the accurate identification of medicinal materials is the first and foremost step of the quality control and quality assurance (QC/QA) of traditional medicines [1].

Discriminating traditional medicinal materials made from plants, animals and fungi by genetic characteristics should be the most confident [4,5]. However, the cost of gene or protein analysis is too expensive when there are too many samples to be determined. At present, macroscopic identification based on the morphological characteristics is still a simple and effective method to discriminate traditional medicinal materials. For example, according to the empirical criteria from the long historical use, botanic materials can be identified by the morphological characteristics of leaves, flowers, seeds, roots, etc. In some cases, even the place of production and the cultivation method can be recognized. If necessary, especially for sliced and powdered materials, the microscopic identification method should be used to check the morphological characteristics of the tissues, cells and ergastic substances by optical microscopes [6,7]. This method has been prescribed by Chinese Pharmacopoeia, United States Pharmacopeia, British Pharmacopoeia, Japanese Pharmacopoeia, etc.

Although the microscopic identification method is simple, quick and effective, some limitations still exist. Obviously, the decisions based on the shape, color and some other physical properties of the tissues, cells and ergastic substances are excessively dependent on the knowledge and experience of the analysts; therefore, the results may be not objective or quantitative enough. Besides, it is very difficult to obtain chemical information about the materials through the microscopic identification. To overcome the abovementioned limits, the combination of the microscopic identification and some chemical microanalysis methods is necessary.

Compared with other chemical microanalysis methods, the vibrational microspectroscopy, including infrared (IR) and Raman microspectroscopy, has greater potential in the microanalysis and identification of traditional medicinal materials. Both macroscopic and microscopic vibrational spectroscopies are direct, nondestructive, label-free and time-saving methods. Fingerprint-like vibrational spectrum can provide comprehensive information about diverse components simultaneously. During the past twenty years, the macroscopic IR and Raman spectroscopy have been successfully applied in the research of traditional medicines including crude materials, preparations and finish products [8,9]. Therefore, the combination of microscopic identification and vibrational microspectroscopy should be a promising approach for the study and quality control of traditional medicinal materials. The vibrational microspectroscopic identification (VMI) method is proposed in this research.

The vibrational spectra and visible images can be obtained together during the IR and Raman microspectroscopy measurements to show the chemical micromorphology, i.e., the chemical compositions and correlated physical morphologies, of traditional medicinal materials. Better understanding about some mechanisms and more explicit identification criteria can be expected because the vibrational spectra make it possible to describe the microscopic characteristics objectively and quantitatively. The identification results based on both physical characteristics and chemical fingerprints should be more accurate than the previous methods using physical and chemical information separately. The morphology–spectroscopy–chemistry method is very significant for the biological and pharmaceutical researches on traditional medicines. The feasibility of the VMI method for crude materials has been demonstrated in a previous study about the chemical micromorphology of *Ginkgo biloba* leaf blades [10]. In this research, the powdered traditional medicinal materials are investigated.

The traditional Chinese medicine *Poria* is the dried sclerotium of *Poria cocos* (Schw.) Wolf [11], which is a kind of fungi parasitizing the roots of pine trees. *Poria* shows multiple medical effects and has been widely used in a lot of TCM formulae. Modern pharmacological researches confirm the functions of *Poria* in antitumor, antibacterial, immunity improvement, diuretic, etc. [12–14] Polysaccharides are the primary active ingredients and compose usually more than 80% of the weight of the dried *Poria*. At least 20 kinds of polysaccharides have been found in *Poria* and most are β-D-glucan [15–18]. According to the criteria prescribed by Chinese Pharmacopoeia [11], the microscopic characteristics of the dry *Poria* powder are granular clumps, branched clumps, colorless and/or colored hyphae. However, there is no explanation about the chemical compositions of clumps and hyphae. In this research, IR and Raman microspectroscopy are used for the first time to determine the chemical compositions of clumps and hyphae and to describe these microscopic characteristics objectively and quantitatively by fingerprint-like spectra. The results could be helpful to draw up explicit and accurate vibrational microspectroscopic identification criteria of *Poria*, as well as to explore the spatial distribution and formation mechanisms of the active polysaccharides.

Material and methods

Materials

Several fresh sclerotia of *Poria cocos* as the one shown in Fig. 1a were obtained at October, 2012, from Luotian, Hubei Province, China. Some fresh sclerotia were cut open (Fig. 1b) and sampled from the inner part to obtain the "fresh *Poria*" samples. The exuded yellow droplet in the fresh sclerotium, as shown in Fig. 1b and c, was collected using a capillary. The other fresh sclerotia were naturally dried in the laboratory for about three weeks. The "dry *Poria*" samples from the inner part of the dried sclerotia were powdered and filtrated through a 60-mesh sieve.

Besides the above samples, there were more *Poria* samples obtained at October, 2012, from Hubei Province and Yunnan Province, China. The inner part of fresh sclerotia of *Poria cocos* were first cut into cubes with the side length of 1–2 cm and then dried naturally. The "*Poria* cube" samples were powdered and filtrated through a 60-mesh sieve. The microscopic characteristics, macroscopic and microscopic IR and Raman spectra of the powder of "dry *Poria*" and "*Poria* cube" samples are identical. The two drying procedures for *Poria* are both accepted by Chinese Pharmacopoeia [11].

Data collection

Optical microscopic observation

A BM2100POL polarizing microscope (Jiangnan Novel Optics, Nanjing, China) was used to observe the microscopic characteristics of the powder of dry *Poria* and *Poria* cube. The powdered sample was loaded on a microscope slide, dispersed by a few drops of deionized water before the cover glass was placed, and then observed in normal transmission mode with non-polarized light. Photographs were taken by a charge coupled device (CCD) camera equipped with the microscope.