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Discrimination of the *Coptis chinensis* geographic origins with surface enhanced Raman scattering spectroscopy

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ABSTRACT

In this paper, we have developed a novel method which can identify the geographic origins of the *Coptis chinensis* using the surface enhanced Raman scattering spectroscopy (SERS) without requiring the strict separation and complex preprocessing. The main characteristic Raman peaks information can be employed to distinguish the *Coptis chinensis* from different origins. The unsupervised exploratory analysis principal component analysis (PCA) is applied to raw SERS spectra as well as the SERS spectra treated with the improved asymmetric least squares (IAsLS) baseline correction method. The supervised DPLS model is employed to validate the discrimination of *Coptis chinensis* origins. The results indicate that the main characteristic Raman peaks information of *Coptis chinensis*, which is consistent with the liquid chromatography analysis results, is different from their geographic origins. Moreover, PCA and DPLS score plots results further depict SERS spectroscopy can be applied to discriminate the origins of *Coptis chinensis*. Therefore, SERS spectroscopy is a suitable method for identifying the origins upon the berberine content in *Coptis chinensis* as well as many other detection applications.

1. Introduction

Traditional Chinese Medicine *Coptis chinensis*, dry rhizome of *Coptis chinensis* Franch, has been used for prevention and treatment of human diseases such as inflammatory and cancer for centuries. The anti-inflammatory and anti-cancer bioactivities of *Coptis chinensis* come from their multi-alkaloid constituents [1,2]. Berberine, the main alkaloid of *Coptis chinensis*, is considered as the most important contributor to therapeutic effects [3]. However, owing to the geographic origins and growth conditions, the berberine contents are different from each other, usually 5%–8% in *Coptis chinensis* [4,5]. Therefore, based on the differences on berberine content, the identification of geographic origins is feasible for quality controlling of herbal materials in modernization and internationalization of Traditional Chinese Medicine *Coptis chinensis* [6–10].

Traditionally, the geographic origins of the *Coptis chinensis* are mainly identified with shape, color and even the smell by the experienced herbalist doctors. In recent years, some modern analytical techniques, such as high performance liquid chromatography, gas chromatography-mass spectrometry and thin layer liquid chromatography, have been applied to analyze the main alkaloids content in *Coptis chinensis* from different producing areas [11–14]. Liu and Chen [11] reported a sensitive and accurate high performance liquid chromatography (HPLC)–electrochemical detection (ECD) method for the determination of isoquinoline alkaloid berberine in *Coptis chinensis* and the LOD (limit of detection) of that was 80 fold lower than LOD obtained by ultraviolet (UV) detection. Thirteen compounds in crude *Coptis chinensis* Franch have been measured by using the ultra-performance liquid chromatography (UPLC) method combined with quadrupole time of flight mass spectrometry (QTOF/MS) by Jiang and coworkers [12]. Meanwhile, the significant difference between the contents of six alkaloids among the *Coptis chinensis* samples from different regions has been obtained using a reversed-phase HPLC by Geng and coworkers [13].

The above methods are laborious and complex on preparation — separation and extraction procedures as well. Therefore, a rapid, simple and direct analytical method is urgently required for the qualitative analysis of *Coptis chinensis* from different origins. Thus, molecular spectroscopic analysis methods were applied to evaluate the main alkaloids contents and to explore the differences in the alkaloids contents of *Coptis chinensis* from different origins [15,16]. Based on quantum chemical calculations, the vibration models of the berberine molecule were assigned by Strekal' and coworkers [17], and the SERS spectra of the

Abbreviations: SERS, surface enhanced Raman scattering spectroscopy; PCA, principal component analysis; IAsLS, improved asymmetric least squares; HPLC, high performance liquid chromatography; ECD, electrochemical detection; LOD, limit of detection; UV, ultraviolet; UPLC, ultra-performance liquid chromatography; QTOF/MS, quadrupole time of flight mass spectrometry; FTIR, Fourier transform infrared; LC, liquid chromatography; TFA, trifluoroacetic acid; DPLS, discriminant partial least squares.

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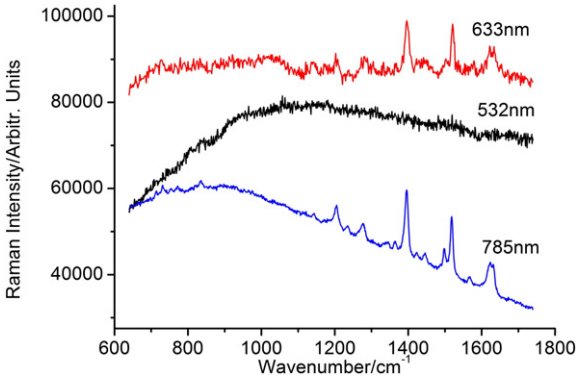

Fig. 1. The normal Raman spectra of solid berberine excited with different wavelength lasers.

berberine were measured with a silver hydrosol and electrode. Moreover, the correlation between experimental and calculated spectra of berberine has been reported by Bashmakova and coworkers at room temperature [18]. Furthermore, the FTIR spectroscopy was also applied to evaluate the infrared spectra of different parts, ages, and heights for *Coptis chinensis* samples rapidly and non-separately by Li and coworkers [19]. Zhao and coworkers [20] proposed a sensitive, rapid, and non-destructive method to analyze the *Coptis chinensis*, *Phellodendron amurense*, and their main active constituent berberine by using silver nanospheres as SERS-active probes.

As a "fingerprint information" spectroscopy, requiring only minimal sample and without complex laborious preparation, the SERS spectroscopy [21] has been applied to evaluate the berberine content in *Coptis chinensis* and to identify the counterfeits. However, the distinction of the *Coptis chinensis* from different producing areas has not been reported by using the SERS spectroscopy on the basis of the berberine contents.

Therefore, the present study is to evaluate the potential of SERS spectroscopy as a screening method for identification of *Coptis chinensis* from different origins. In this paper, the areas of main characteristic Raman peaks will be fitted to explore the differences among the *Coptis chinensis* from different producing areas. Then, an unsupervised exploratory analysis principal component analysis (PCA) [22] and supervised qualitative analysis discriminant partial least squares (DPLS) [23] are employed to evaluate the suitability of SERS spectra for distinguishing the *Coptis chinensis* origins respectively. Moreover, these methods could be potentially applied in many other geographic origins discriminant applications.

2. Experimental

2.1. Sample preparation and Raman spectra measurement

Berberine (J&K, chloride form) was purchased from J&K Scientific. Berberine solution (10^{-4} M) was prepared with deionized water as a standard reference sample. *Coptis chinensis*, was obtained from Chongqing Academy of Chinese Meteria Medica (China), which is derived from three different producing areas (Shizhu Chongqing, Kaixian Chongqing and Wuxi Chongqing). The *Coptis chinensis* was treated by soxhlet extractor with deionized water. Then, the water extraction samples were cooled down and readied for Raman measurement without further processing.

Raman measurements were performed by using a Renishaw (The United Kingdom) inVia micro-Raman spectroscopy system, equipped with three laser excitations (532 nm DPSS laser, 50 mW; 633 nm He-Ne laser, 17 mW; 785 nm diode laser, 250 mW). A Leica microscope with 50× objective was employed to focus the incident laser on the samples for collection of back-scattered Raman signals. Before the Raman spectra were measured, the wavenumber of the Raman band of silicon at 520 cm^{-1} was calibrated, and all data were collected under the same conditions except for the solid standard berberine sample. The spectral range was from 639 cm^{-1} to 1739 cm^{-1}, the acquisition time of each spectrum was fixed at 10 s, and power on the sample was 1% of the laser power. The final Raman spectra dataset is composed of 300 spectra from three *Coptis chinensis* origins.

2.2. LC condition

Liquid chromatography (LC) method was performed by using a Dionex Ultimate 3000 with a Sunfire C18 column (4.6 × 150 mm, 3.5 μm), mobile phase: 23% CAN, 77% TFA (trifluoroacetic acid), sample injection volume was 10 μl, and the retention time was 18 min.

2.3. Programming and software

All programs were written using Matlab R2011b and run under Windows 7 on a personal computer (RAM 4G, CPU 2.83 GHz). The characteristic SERS spectra areas of *Coptis chinensis* water extraction were fitted by the WiRE 3.4 which was built in the Renishaw inVia micro-Raman spectroscopy system.

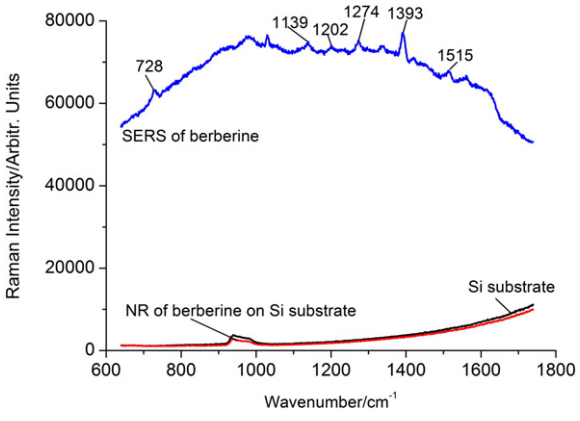

Fig. 2. The normal Raman spectra of the silicon substrate, 10^{-4} M berberine solution on the silicon substrate, and SERS spectrum of 10^{-4} M berberine solution.

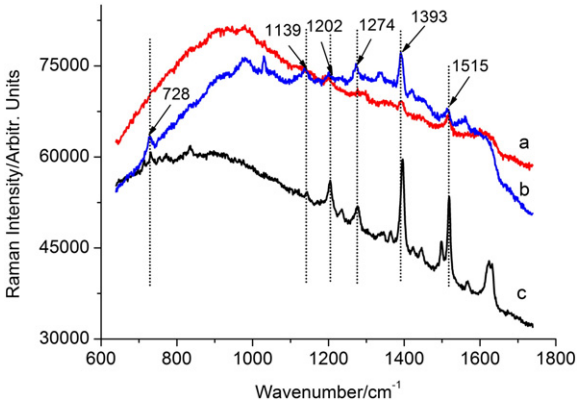

Fig. 3. The raw SERS spectrum of the *Coptis chinensis*: (a) the SERS spectrum of *Coptis chinensis* from Wuxi Chongqing, (b) SERS spectrum of 10^{-4} M berberine solution, and (c) normal Raman spectrum of solid berberine.