Identification of four *Sedum* plant medicines by fourier transform infrared spectra

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ABSTRACT

Background: *Sedum sarmentosum* bunge (*SS*B)., *S. lineare* Thunb. (*SL*T), *S. erythrostictum* migo. (*SE*M), and *S. aizoon* L. (*SA*L) were four widely used Chinese traditional drugs or ethnic drugs, which were easy to be confused with each other. **Objective:** This study aimed at developing a rapid and accurate method to identify the four *Sedum* plant medicines with very similar appearances and close relationships. **Materials and Methods:** The herbal medicines employed here were *SS*B, *SL*T, *SE*M, and *SA*L collected in different places and seasons. Through comparing the infrared (IR) spectra of their 70% ethanol extracts, the results showed that the IR spectra of the four plant medicines possessed not only some common characteristics but also certain notable distinctions, such as shapes, numbers, positions, intensity, and ratios of the absorbing peaks. **Results:** By fourier transform infrared (FT-IR) spectroscopy, the four medicines could be effectively differed, their habitats could be judged preliminarily, and the genetic relationships of the original plants of the four medicines could also be estimated to some extent. **Conclusion:** The application of FT-IR spectroscopy in crude medicine authentication and quality evaluation deserved to be further emphasized.

Key words: Ethanol extracts, fourier transform infrared spectrum identification, *Sedum* plant medicines

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INTRODUCTION

Since IR (infrared) spectrum is a simple, rapid technique with marked characteristics and high reproducibility, it has been widely used in authentication studies involved plants and plant medicines,[1-8] food additives,[9] pollen,[10] as well as crude drug preparations.[11]

It is well known that the quality of a herbal medicine is related to its original plant and habitat. As widely used ethnic drugs by the Tujia Nationality, the whole plants of *Sedum sarmentosum* Bunge. (*SS*B), *S. lineare* Thunb. (*SL*T), *S. erythrostictum* Migo. (*SE*M), and *S. aizoon* L. (*SA*L) could all cure of hemostasis. At the same time, the first three are also applied for hepatitis, dysentery, herpes zoster, and swelling while the last can eliminate stagnant blood and smooth the nerves. Despite some studies reported on identification of the plants from the same genus by the IR spectrum,[2-5] this research employed the technique in authentication of *SS*B, *SL*T, *SE*M, and *SA*L collected in different periods and harvests [Table 1], whose appearances resembled and relationships approached.

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MATERIALS AND METHODS

Materials and reagents

Twenty-one samples of the four drugs were collected in different months from various habitats such as "Tiantangzhai" of Luotian County; "Hongshan", "Sheshan", and "South-Central University for Nationalities (SCUN)" in Wuhan City in East Hubei; "Wuduhe" of Yiling, Yichang City and Jianshi County of Enshi Autonomous Prefecture in West Hubei [Table 1]. They were, respectively, identified as *SS*B, *SL*T, *SE*M, and *SA*L by Professor Wan Dingrong from College of Pharmacy, SCUN. The relevant specimens were deposited in pharmacognosy laboratory of pharmaceutical college of SCUN. The ethanol employed here was analytical reagent.

FT-IR spectroscopy

NEXUS 470 intelligent Fourier transform infrared (FT-IR) spectrometer (American NICOLET) with a resolving powder of 16 cm^{-1}, spectrum range of 4000–400 cm^{-1}, and scanning accumulative limitation of 32 times, was used for the analysis of all the samples in the experiments.

Sample preparation and spectrum measurement

About 1 g of coarse powder of every sample was taken with a certain ratio of stem to leaf, then 20 ml 70% ethanol was added, respectively. Followed with ultrasonic treatment for 40 min and percolation, the filtrate was steamed into thick paste and dried at 60°C to a constant weight. After that, 1.5 mg of the dried powder (100 mesh) was taken and grinded with 300 mg KBr under IR light till evenly mixed. Then the mixture was crushed in a mechanical mold to form a tablet with a diameter of 3 mm and a thickness of 0.6 mm. Finally, the spectrum of an extract could be gained by scanning the sample tablet immediately.

RESULTS

The common peaks shared by the samples

Because the four drugs were all from *Sedum* and the original plants had closed relationships and similar components with each other, the obtained IR spectra entirely exhibited a great consistency in general. There were six common absorbing bands: 3403 ± 25 cm^{-1}, 2930 ± 8 cm^{-1}, 2849 ± 6 cm^{-1}(shoulder peak), 1622 ± 14 cm^{-1}, 1387 ± 4 cm^{-1}, and 613 ± 11 cm^{-1} [Figure 1].

Table 1: *Sedum* plant drug samples and their sources

Origin	Habitat	Collected period	People who gather the plants	Specimen number
*SS*B	Jianshi	Apr, 2006	Wang Congrong	060427
		Jun, 2006	Wang Congrong	060620
		Nov, 2006	Wang Congrong	061119
	Sheshan	Apr, 2006	Wan Dingrong	060403
		Jul, 2006	Wan Dingrong	060720
	SCUN	Mar, 2006	Wan Dingrong	060328
		Jul, 2006	Wan Dingrong	060705
		Dec, 2006	Wan Dingrong	061203
*SL*T	Hongshan	Apr, 2006	Wan Dingrong	060403
		Jul, 2006	Wan Dingrong	060720
		Oct, 2006	Wan Dingrong	061005
		Dec, 2006	Wan Dingrong	061214
*SE*M	Jianshi	Apr, 2006	Wang Congrong	060427
		Jun, 2006	Wang Congrong	060627
		Nov, 2006	Wang Congrong	061125
	Sheshan	Apr, 2006	Wan Dingrong	060403
		Jul, 2006	Wan Dingrong	060720
		Oct, 2006	Wan Dingrong	061004
*SA*L	Yichang	Apr, 2006	Wan Dingrong	060415
	Luotian	Jul, 2006	Wan Dingrong	060715
		Oct, 2006	Wan Dingrong	061004

Differences among species

Although the spectra of the four drug samples were almost alike, each of them still bore its own characters such as different peak shapes, numbers, positions, and intensity.

In detail, the *SA*L samples harvested in different seasons and places possessed common moderate intensive peaks at 1451 ± 2 cm^{-1}, 1224 ± 9 cm^{-1}, 1039 ± 4 cm^{-1} and an obvious weak peak at 873 cm^{-1}, while the other three had none. At 1183 ± 9 cm^{-1}, the four *SL*T samples held their own characteristic absorbing peaks; opposite with *SL*T, shoulder peaks were absent on the left of the peak at 1066 ± 11 cm^{-1} among *SE*M samples. The ratios of $A_{1622 \pm 14}$ to $A_{1385 \pm 2}$ of *SE*M samples collected in various months and habitats were generally lower than 0.78 with

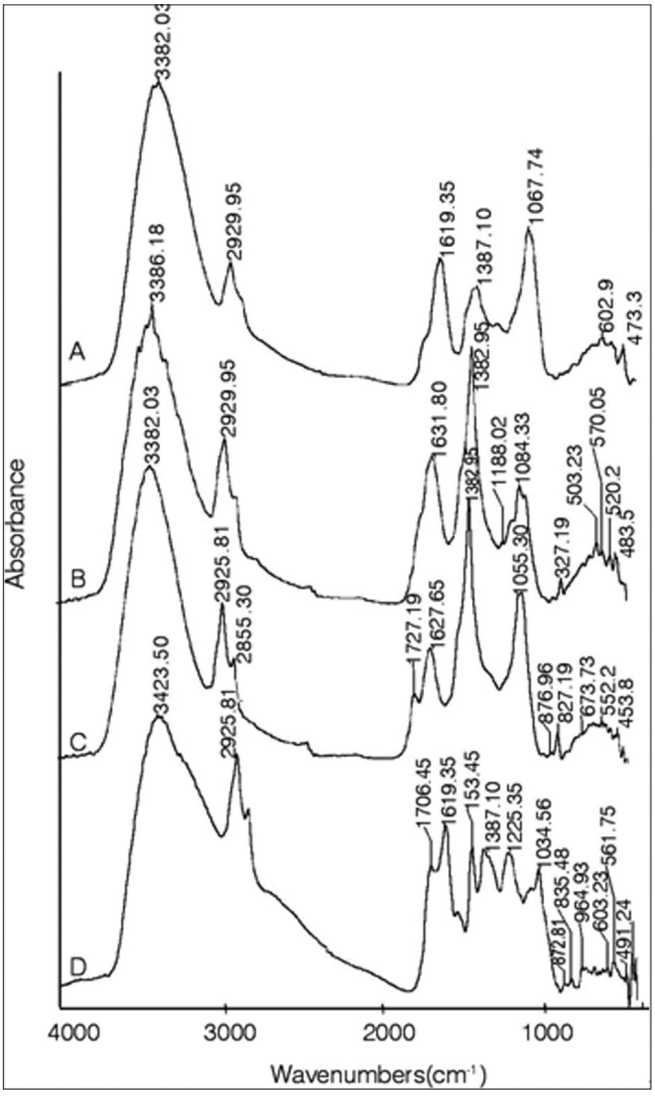

Figure 1: IR spectra of ethanol extracts of four Sedum plant drugs. (A) *SS*B, (B) *SL*T, (C) *SE*M, and (D) *SA*L

an average value of 0.70, while those of *SS*B were mostly above 0.82 with an average value of 1.00. Significant differences of the both were shown after statistics procession ($P < 0.01$). Thus, the ratios could also be used to differ *SE*M and *SS*B [Figure 1].

In the spectra of *SS*B and *SL*T samples, all the peaks in characteristic regions of 4000–1250 cm^{-1} showed great identity in numbers, positions, and relative intensity, plus that common peaks at 830 ± 6 cm^{-1} and 538 ± 18 cm^{-1} of fingerprint regions were almost similar with each other, revealing a close relationship between the two. However, certain stable distinctions could still be found. All the *SS*B samples retained an obvious weak peak at 1261 ± 6 cm^{-1} while *SL*T did not; *SS*B hardly owned a shoulder peak on the left of the peak located at 1070 ± 6 cm^{-1} while *SL*T kept 2 (sometimes 1) on the same side.

Besides, the peak numbers, positions, shapes of *SE*M were more resembling with *SS*B and *SL*T, suggesting a more closed relationship with them compared with *SA*L. This was consisted with the similarity shown on the level of plant morphology, anatomy, and molecular biology.[12-14]

All these supported the traditional clinical utilization of the four herbal drugs from the aspect of preliminary comprehensive information of components.

The spectrum disparities among samples of the same plant drug from different habitats

Certain disparities existed among the samples of the same plant drug (*SS*B or *SE*M) from different places. The IR spectra of *SS*B showed a certain correlated relation between the positions, intensity or ratios of some peaks, and the habitats. As for the positions, a number of samples collected in SCUN and Sheshan in Wuhan City, East Hubei, held absorbing bands at 1628 ± 8 cm^{-1}, which moved to 1613 ± 8 cm^{-1} in the spectra of another three samples gained in Jianshi, West Hubei. After statistics processing, significant differences of the both were shown ($P < 0.05$). As for the ratios, the ratios of $A_{1628 \pm 8}$ to $A_{1387 \pm 4}$ of *SS*B samples harvested in East Hubei were usually lower than 1 with an average value of 0.86, but the ratios of $A_{1613 \pm 8}$ to $A_{1385 \pm 2}$ (corresponding with the ratios mentioned before) of those from the west were all above 1 with an average value of 1.11 [Figure 2]. Significant differences of the both were testified by statistics procession ($P < 0.05$). The same happened to *SE*M too: the three samples harvested in different months in West Hubei bore peaks at 3380 ± 2 cm^{-1}, which changed to 3407 ± 17 cm^{-1} when those were collected in the east [Figure 3].

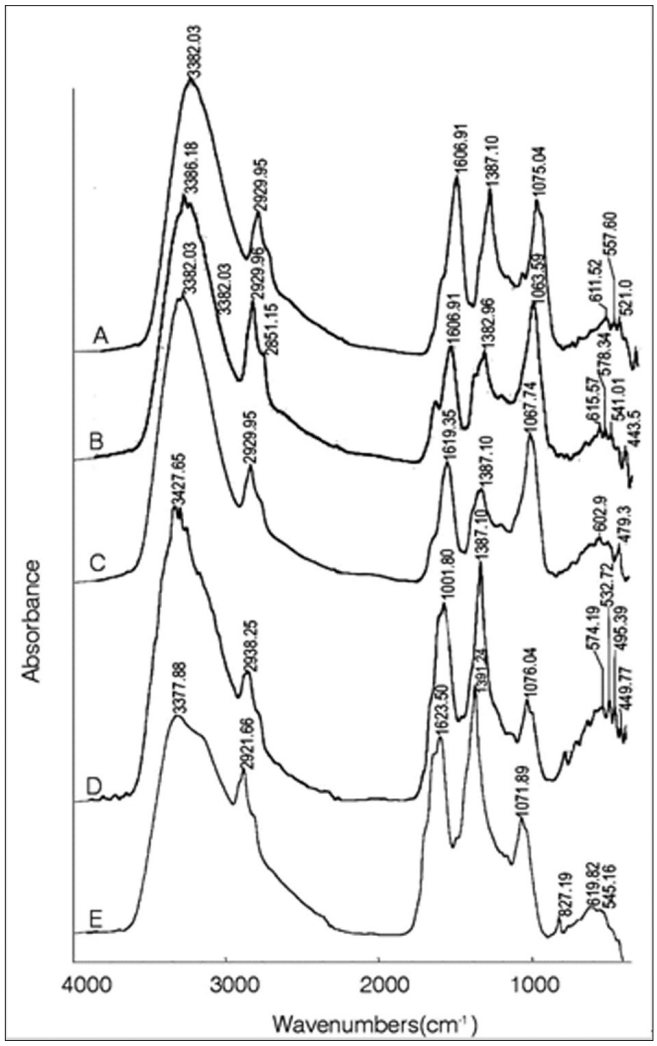

Figure 2: IR spectra of ethanol extracts of SSB from different habitats. (A) Jianshi (060427), (B) Jianshi (060620), (C) Jianshi (061119), (D) SCUN (060328), and (E) Sheshan (060403)

DISCUSSION

Compared with the traditional identification methods, the FT-IR spectroscopy had plenty of advantages. It possessed strong characteristic sense, required low quantity of samples, and was also rapid, simple, and accurate. As the relevant plant drugs from the same family or genus were originally intimate with each other, sometimes they were hardly able to be fast and accurately identified by conventional means. Nevertheless, a rapid and effective identification method for these drugs (including their habitats) could be developed through comparing the peak positions, intensities ratios, and observing whether certain absorbing bands exit or not in their IR spectra.

As components contained in a herbal drug were various and complicated, the peak shape and intensity of IR spectrum were often influenced by interactions among the same or different functional groups. Hence, objective and repeatable IR spectra could be attained as long as the compositions of the plant drugs were relatively stable both in quality and quantity and the samples were all prepared in the same way.

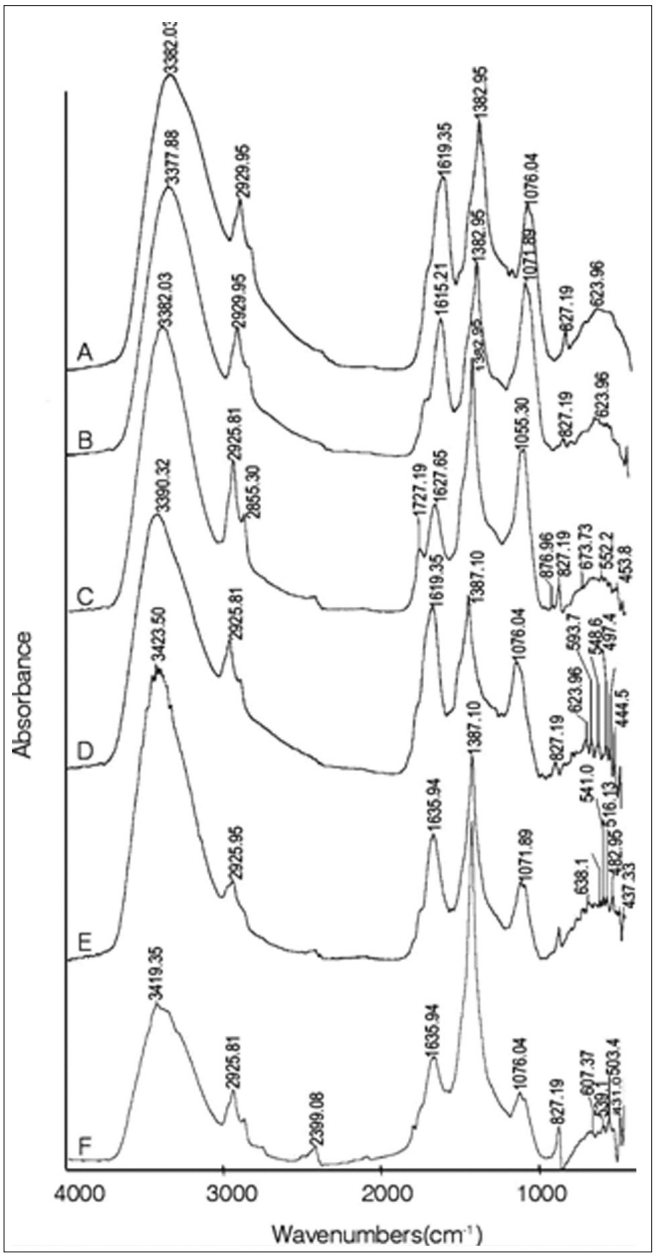

Figure 3: IR spectra of ethanol extracts of SEM from different habitats. (A) Jianshi (060427), (B) Jianshi (060627), (C) Jianshi (061125), (D) Sheshan (060403), (E) Sheshan (060720), and (F) Sheshan (061004).

This study also indicated that the sample extraction technique could effectively settle some difficult problems. Since plenty of ordinary components such as protein and polysaccharide (cellulose) were often contained in various herbal drugs, common absorbing bands generated usually disturbed identification. Nevertheless, the extraction technique could effectively exclude these disturbances. For example, to enrich the unique compositions of each medicine, this study selected 70% ethanol as the solvent to carry out the experiments.

Thus, the overall composition characteristics of the four plant drugs could be attained to a certain extent by the IR spectrum, suggesting this technique was worthy of more attention in identification and quality estimation of plant drugs.

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