Analysis and Assessment of Agrimonia Pilosa Ledeb from Different Sources Using FT-IR Spectroscopy

Bao-qing WANG(王宝庆), Zhe-xiong JIN(金哲雄)
(*College of Pharmacy, Harbin University of Commerce, Harbin* 150076, *China*)

Abstract – To get the IR spectrums of Agrimonia Pilosa Ledeb (APL) from China and Japan areas, and to find out the characters of IR spectrums through the content of different chemical constituents, to provide a fast and effective analysis method monitor the inherent qualities of traditional Chinese medicine-APL. Fourier Transform Infrared Spectroscopy (FT-IR) was applied to detect sample of APL from China and Japan areas. This study showed that the IR spectra of APL from China and Japan areas have their unique IR fingerprint features. The contents of tannin and calcium phosphate in APL from China is different APL from Japan. So FT-IR is a very quick, effective and well repetitive method for monitoring and distinguishing the traditional Chinese medicine.

Key words – *Agrimonia Pilosa Ledeb*; *Fourier Transform Infrared Spectroscopy (FT-IR)*; *chemical constituents*

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1 Introduction

Agrimonia Pilosa Ledeb (APL) is a widely used medicinal plant by asian countries and has been reported to possess various biological activities. In Asia, APL is used in folk medicine as haemostatic, tonic for asthenia, astringent in diarrhoea, and diuretic. For example, APL has been used for blood, cardiovascular, gastrointestinal, genitourinary, inflammatory, liver, respiratory tract, skin and some other conditions.

In addition, APL is also a famous medicinal plant used for cancer therapy in Japan and the antitumor activity of the methanol extract of the roots of this plant has been widely reported. In China, this plant is traditionally used to suppress diarrhoea, to reduce gastric ulcers, to relieve inflammation, to improve eyesight, to calm the liver, to detoxify poison from body. The tannin is the main component of the plant that acts against several rodent tumors and it has been suggested that the immune system adjustment is important in the antitumor effect of tannin[1].

In recent years it is reported that FT-IR is used in traditional Chinese medicine on some aspects, such as identifying counterfeits, controlling qualities, forecasting stability, etc. Although traditional Chinese medicine is very complicated systems, the fingerprint characters in FT-IR spectra will show differences, as long as the categories or contents of their contained chemical components are different. Using these differences of fingerprint characters can identify, compare and control the medicinal materials and their corresponding products quickly, accurately and effectively[2].

Now the mainstream analysis method for monitoring the qualities of chemical constituents in herb medicine is GC-MS method, which can offer much detailed information of chemical components. However, the low reproductivity, expensive instrument and rigorous experiment condition limit it to generalize in some degree. And only a few components selected to test cannot reflect the real qualities of the complicated samples as traditional Chinese Medicine efficiently. Therefore, it is the hot issue to find a fast and effective analysis method for evaluating fully the qualities of such samples. FT-IR spectroscopy presenting the whole features and fingerprint characters of a sample can achieve this goal perfectly. The analysis of FT-IR not only can reveal clearly the main compositions in the sample but also can compare the differences of categories and quantities of chemical constituents in the very similar samples[3].

Here we studied the differences of main constituents of APL using FT-IR fingerprint method. The purpose of this study is to develop a rapid, accurate and feasible analysis method to appraise integrally the inherent qualities of APL and compare sample of chemical constituents APL from China and Japan areas.

2 Experiment

2.1 Apparatus

Spectrometer: Spectrum GX FT-IR system (Perkin-

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Corresponding author: Bao-qing WANG (Baoqingwang2002@yahoo.com.cn)

Elmer), equipped with a DTGS detector was used. Scan range from 400 cm^{-1} to 4 000 cm^{-1} with a resolution of 4 cm^{-1}. the velocity of OPD 0.2 cm/s, Spectra are obtained from the accumulation of a total of 16 scans.

2.2 Samples

The roots of plants were collected from different localities of Harbin district, China and Okayama district, Japan, and authenticated by Professor Zhe-xiong Jin from college of pharmacy, Harbin university of commerce. Voucher specimens were deposited in college of pharmacy of this laboratory.

2.3 Chemicals

Calcium phosphate, Potassium bromide and Tannin were purchased from Sigma-Chemical Company.

2.4 Procedure

The sample was directly frozen and dried into a powder, then, blended with KBr powder, pressed into a tablet. After that, collected the infrared spectra of all samples.

All the second derivative IR spectra were 13-point second derivative IR spectra after 13-point smoothing of the original IR spectra taken at room temperature[4].

3 Results

3.1 IR spectra analysis of Agrimonia Pilosa Ledeb in different areas

Fig. 1 presents the IR spectra of APL in different areas under the room temperature. From the spectra we can see clearly that positions and shapes of the main specific bands are quite similar to each other, except at 1 736 cm^{-1}, 1 438 cm^{-1} and 519 cm^{-1}. Fig. 2 is in the secondary derivative IR spectra that amplify tiny differences of IR spectra at 1 736 cm^{-1} and 1 438 cm^{-1}, 1 438 cm^{-1} (C−H deformation), 1 736 cm^{-1} (C = O stretching), the band of the fingerprint region at about 1 400 cm^{-1} ~1 750 cm^{-1}, So this is a good fingerprint to distinguish APL from China and Japan. 519 cm^{-1} is another special peak.

3.2 IR spectra of Agrimonia Pilosa Ledeb in different ages

Fig. 3 is the IR spectra of APL from China in different ages. Only a slight difference between each peak can be observed, the peaks of 1 736 cm^{-1} strengthens gradually along with the growth age increase. IR spectra results may indicate that the components in APL from china will accumulate with the aging. The analysis results of the peaks at 1 736 cm^{-1} may indicate that the major changed constituents are the compounds of calcium phosphate and tannin.

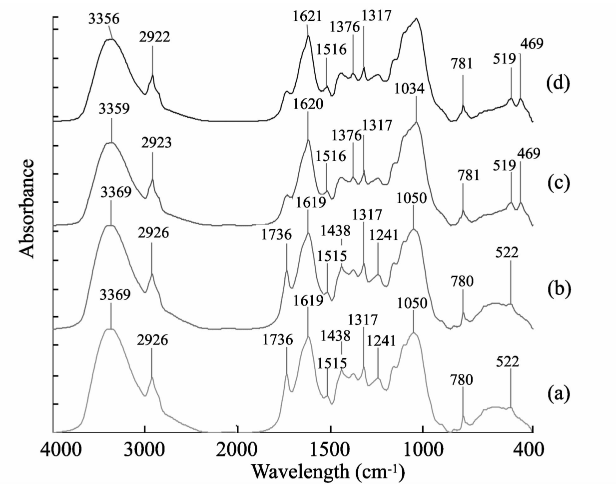


Fig. 1 IR spectra of Agrimonia Pilosa Ledeb from different areas: (a) China1; (b) China2; (c) Japan1; (d) Japan2

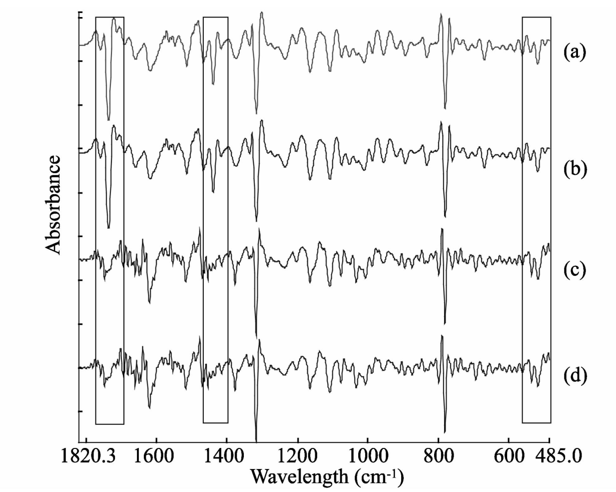


Fig. 2 Secondary derivative IR spectra of Agrimonia Pilosa Ledeb from different areas: (a) China1; (b) China2; (c) Japan1; (d) Japan2

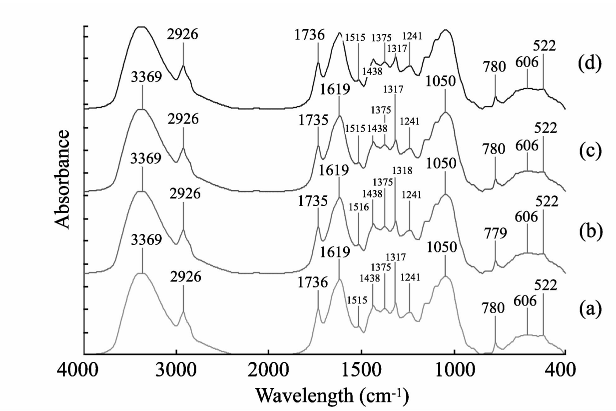


Fig. 3 IR spectra of Agrimonia Pilosa Ledeb from china in different ages: (a) Four years; (b) Three years; (c) Two years; (d) One years

Fig. 4 is the IR spectra of APL from Japan in different ages. Fig. 5 is the secondary derivative IR spectra of the plant from Japan in different ages. The intensity of absorption peak at 1 620 cm^{-1} changes gradually along with the growth age increase. The growth age of influence on the constituents of APL from japan is very small.

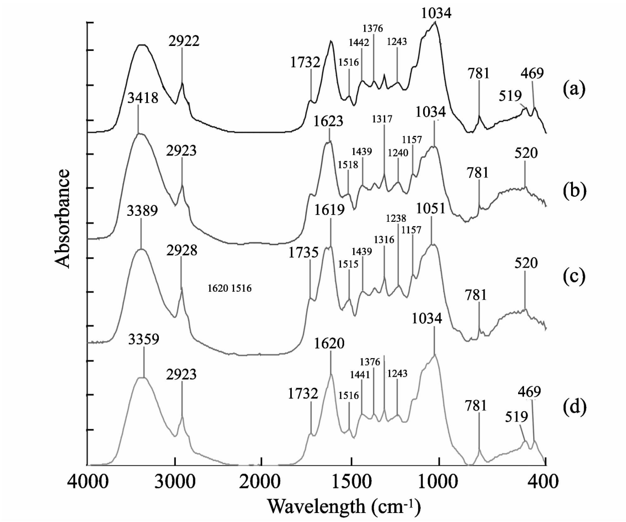


Fig. 4 IR spectra of Agrimonia Pilosa Ledeb from Japan in different ages: (a) Four years; (b) Three years; (c) Two years; (d) One years

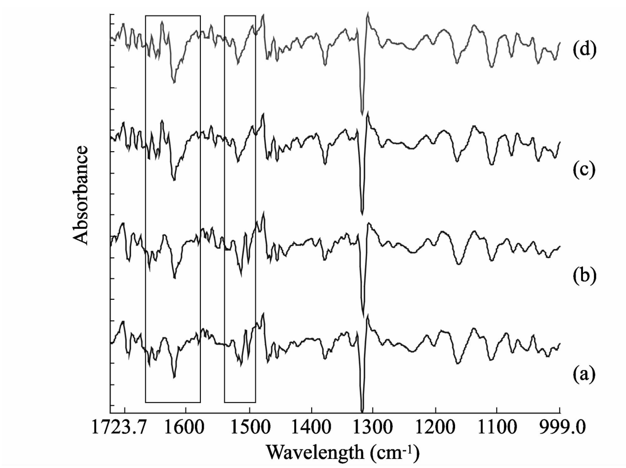


Fig. 5 Secondary derivative IR spectra of Agrimonia Pilosa Ledeb from China in different ages; (a) Four years; (b) Three years; (c) Two years; (d) One years

3.3 IR spectra of Agrimonia Pilosa Ledeb and tannin

According to the literature report, the main medicinal compounds of APL include the tannin. So the tannin has been analysed by FT-IR. As shown in Fig. 6, Comparison of the FT-IR spectrum at 1 736 cm^{-1}, 1 447 cm^{-1}, 1 241 cm^{-1} reveals that the content of tannin in the plant from China is higher than the plant from Japan. This conclusion is consistent with the result of the microscopical identification of the plant . From Fig. 7, we can see clearly that the black fraction (rich tannin) from china plant is obvious higer than Japan plant.

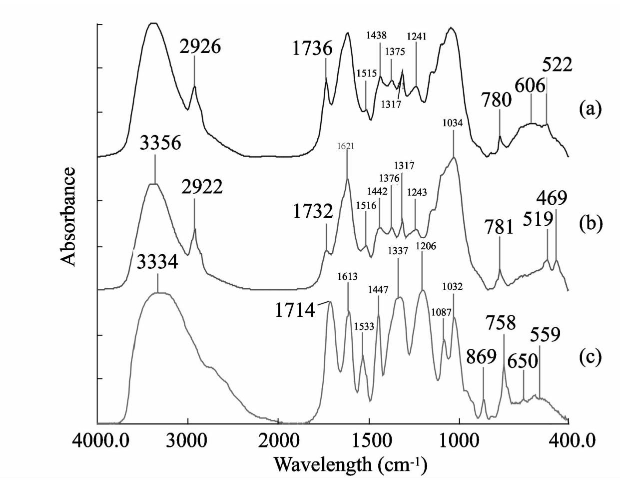


Fig. 6 IR spectra of Agrimonia Pilosa Ledeb and tannin: (a) China; (b) Japan; (c) tannin

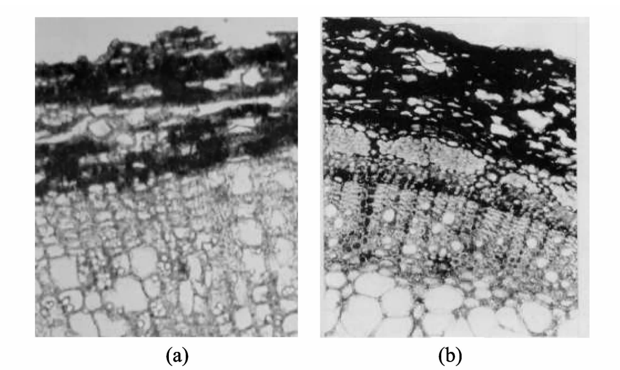


Fig. 7 Microscopical identification of Agrimonia Pilosa Ledeb from china and japan: (a) Japan; (b) China

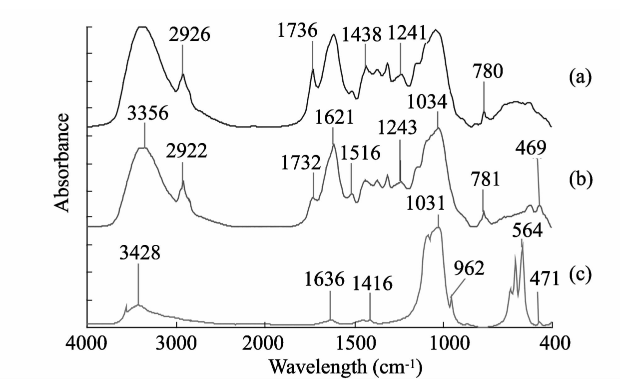
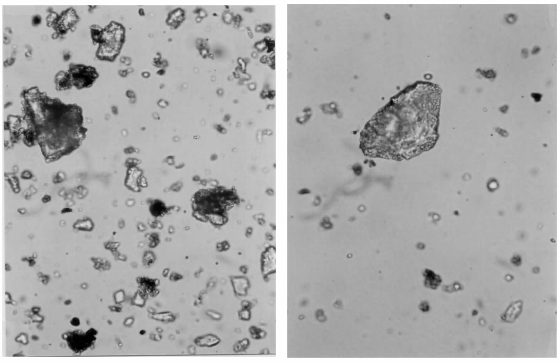


Fig. 8 IR spectra of Agrimonia Pilosa Ledeb and calcium phosphate: (a) China; (b) Japan; (c) calcium phosphate

3.4 IR spectra of Agrimonia Pilosa Ledeb and calcium phosphate

Fig. 8 shows the peak at 1 034 cm^{-1}, 564 cm^{-1}, 469 cm^{-1} belonging to the characteristic absorption of calcium phosphate; and the intensity of peak in Japan plant was lightly stronger than that of China, the relative content of calcium phosphate is higher than that of China. These results are in agreement with that the conclusion of the microscopical identification of APL. The content of cluster crystal of calcium phosphate in Japan plant is slightly higer the content of calcium phosphate of acicular crystal in china plant (Fig. 9). This difference could be due to the climate reason.



(a) (b)

Fig. 9 Microscopical structure of Agrimonia Pilosa Ledeb from China and Japan: (a) Japan; (b) China

4 Discussion

Using correlation analysis technique to treat a series of IR spectra, the identification in Chinese Traditional Medicine can be simplified. These analyses show that the distinctive IR feature in APL from China and Japan had been obtained at 1 736 cm^{-1}, 1 438 cm^{-1} and 519 cm^{-1}. These bands are contribute to the variation of chemical components respectively. The following experiments can provide powerful evidence that the main differences of chemical components are tannin and calcium phosphate. In the IR spectra, the characteristic peaks of tannin and calcium phosphate could be at 1 736 cm^{-1}, 1 447 cm^{-1}, 1 241 cm^{-1}.

The contents of tannin and calcium phosphate contained in the China plant and Japan plant can be identified according to the intensities of the absorption peaks. The content of tannin reveals that APL from China have more medicinal ingredients than the plant from Japan, the high content of calcium phosphate is easier to reduce the content of other medicinal ingredients. Based on these content differences, the results is the same with that from conventional microscopical identification of APL. The microscopical identification of the crude plant or the plant powder is one of the most important techniques to authenticate a crude plant drug. But these bands of IR spectra in the different growth age of the plant are quite similar to each other, which may embody the complexity of the chemical composition, the secondary derivative and two-dimensional IR spectra could be a good choice[5]. Two-dimensional infrared spectroscopy is a nonlinear infrared spectroscopy technique that has the ability to correlate vibrational modes in condensed-phase systems. This technique provides information beyond linear infrared spectra, by spreading the vibrational information along multiple axes, yielding a frequency correlation spectrum.

Generally, the secondary derivative IR spectra can obviously enhance the spectrum resolution and amplify tiny differences of IR spectra, also provide a powerful method for the quality control of APL from China and Japan[6-7].

Currently, the main method for identifying various APL is the microscopical identification. FT-IR spectra a nondestructive, fast and integrity-emphasized method, has important practical utility in identifying and distinguishing the Traditional Chinese Medicine according to geographical origin[8-10]. From this study, We inferred that difference causes in APL from China and Japan are the climate, soil, planting method.

FT-IR technology provided a rapid and effective method for the quality analysis of Agrimonia pilosa Ledeb-Traditional Chinese Medicine from China and Japan.

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