Natural Products Chemistry & Research

Liang et al., Nat Prod Chem Res 2016, 4:1
http://dx.doi.org/10.4172/2329-6836.1000201

Research Article Open Access

Comparative Study on Fluorescence Spectra of Chinese Medicine North and South Isatis Root Granules

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Abstract

Since the spectral imaging technology emerged, it has gained a lot of application achievements in the military field, precision agriculture and biomedical science. When the fluorescence spectrum imaging first applied to the detection of the feature resource of Chinese herbal medicine, the characteristics of holistic and ambiguity made it a new approach to the traditional Chinese medicine testing. In this paper, we applied this method to study the Chinese medicine north and south isatis root granules by comparing their fluorescence spectra. Using cluster analysis, the results showed that the north and south Banlangen can not be divided by ascription. And these indicate that there is a large difference in the quality of Banlangen granules on the market, and fluorescence spectrum imaging method can be used in monitoring the quality of *Radix isatidis* granules.

Keywords: Fluorescence spectrum imaging; Chinese medicine; South isatis root granules; *Radix isatidis* granules

Introduction

Spectral imaging technology emerged in the early 1980s and is a new remote sensing technique which fuses the spectral analysis and the image detection [1,2]. It obtains the spectral image sequences through dividing into light on the continuous spectra of the sample and forming the corresponding image, and gets the spectral curve in the detection band of the sample after processing the spectral image sequences. A sample image at one specific wavelength which is interested in can also be extracted from the image sequences. So it can obtain the image information and spectral properties of sample simultaneously. Spectral information equaling the fingerprint of sample that characterizes the relevant physical and chemical properties can be used as the basis for classification and recognition, which is the application foundation of spectral imaging technology [3]. There are a few different ways to classify the spectral imaging technology. According to the image acquisition mode, the spectral imaging technology can be divided into the scanning imaging, pushbroom imaging and staring imaging [4]. In accordance with the luminescence mechanism, the spectral imaging technology can be divided into absorption spectrum imaging method, reflection spectrum imaging method and fluorescence spectrum imaging method. At present, as a non-destructive test method, the application of spectral imaging technology has been developed from the original military application quickly extended to biomedical, food quality evaluation, etc., and made a lot of achievements [5-7]. Our team applied the technology to the detection of Chinese herbal medicines, and carried out verification, quality classification and genetic relationship identification for some Chinese herbal medicines [8-10].

Radix isatidis is a common Chinese medicinal material, can be divided into north *Radix isatidis* and south *Radix isatidis*. North Banlangen is the dried root of Brassicaceae *Isatis tinctoria*, and south isatis root is the root and rhizome of Acanthaceae plant acanthaceous indigo. Pharmacopoeia of the People's Republic of *China contains them in the name of Radix isatidis* and south isatis root respectively [11]. In life, people also refer to North Banlangen as *Radix isatidis*. Chinese patent drug made of the two kinds of materials are called *Radix isatidis* granules and south isatis root granules. So these two drugs should be clinically used separately for their differences in effective components and content and pharmacological strength. But for a long time in many areas of the south, people often use south isatis root instead of *Radix isatidis*. Even some illegal manufacturers use fake and inferior raw material such as *Clerodendrum cyrtophyllum* to produce *Radix isatidis* granules. In this paper, we used fluorescence spectrum imaging technology to detect the parts of *Radix isatidis* granules and south isatis root granules on the market, then the fluorescence spectrum curves and the quality control of *Radix isatidis* granules were discussed.

Materials and Methods

Spectral imaging system

Staring hyperspectral imaging system mainly includes: UV light source with the center wavelength of 254 nm, the Vari Spec Liquid Crystal Tunable Filter (LCTF) produced by American Cambridge Research and Instrumentation Company, lens, CCD, image acquisition card and computer. The system keeps the image area fixed during the work process, and obtains images of different band through tunable liquid crystal filter. The system block diagram is shown in Figure 1. When testing, the sample is placed on the substrate and emits fluorescence after being excited by UV light. Then fluorescence is separated by LCTF, and the sample images to the CCD receiver target surface to each corresponding wavelength, and finally saves in the computer. The work wavelength range of LCTF is from 400 to 720 nm, and the scanning step is set to 5 nm, so it can obtain a set hyperspectral image block consisted of 65 images for one sample, shown in Figure 2. The exposure time of receiver is 1000 ms.

Sample preparation

All samples used in the experiment include *Radix isatidis* and south *Radix isatidis* standard substance that were purchased from National Institutes for Food and Drug Control, *Radix isatidis* raw material came from Shaanxi, 10 kinds of *Radix isatidis* granules produced by different

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Received January 11, 2016; **Accepted** January 19, 2016; **Published** January 22, 2016

Citation: Liang L, He Q, Chen Z, Pang Q (2016) Comparative Study on Fluorescence Spectra of Chinese Medicine North and South Isatis Root Granules. Nat Prod Chem Res 4: 201. doi:10.4172/2329-6836.1000201

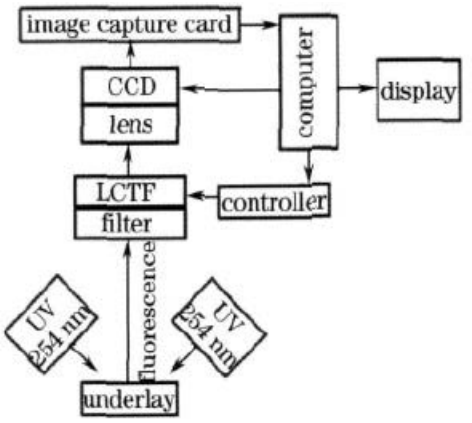

Figure 1: Spectral imaging system block diagram.

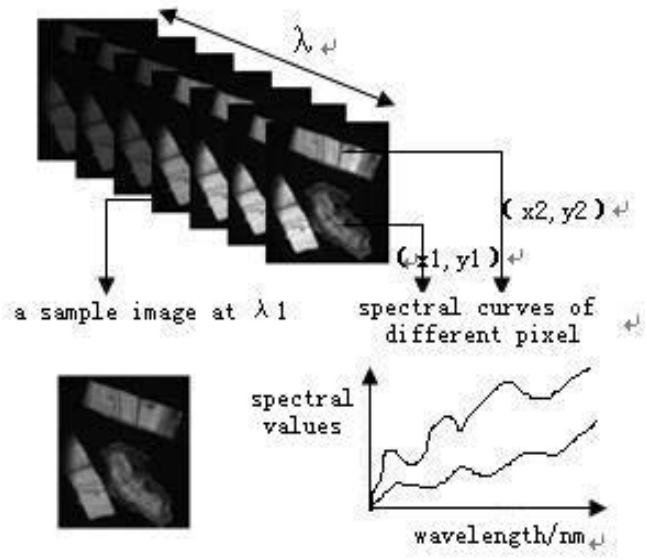

Figure 2: Hyperspectral image block of sample.

manufacturers and 2 kinds of south isatis root granules were bought from the market. A bag of each granules sample was put out, grinded them into powder with a mortar, then filtered the powder through 60 ~ 130 mesh sieves. 6 g of each sample power was tablet evenly in a mold. Finally the images were captured using spectral imaging device.

Image processing

The mean gray value of sample image varies with the change of scanning wavelength, and it represents the corresponding fluorescence intensity of the sample. The background thermal noise in CCD is more stable, so the sample can be regarded as the movement of the target under static background. Frame differential method is used to get the difference image. An appropriate threshold was selected to differentiate the image binary progress, and the white area after image binaryzation is the effective area, which can be used to mask the image one by one to get the effective area of the whole image block. Then the fluorescence spectrum curve of the sample was draw, and the characteristic spectrum curve of the sample was normalized. The whole process bases on MATLBA mathematical software platform.

Results and Discussion

Experiment of *Radix isatidis* granules

Fluorescence spectrum curves of *Radix isatidis*: The fluorescence phenomenon of the material is associated with its molecular structure and component content, therefore, fluorescence spectrum can be used to identify species of material and carry on the quantitative analysis. The fluorescence spectrum curves of granules samples produced by 10 manufacturers are shown in Figure 3. Difference in fluorescence intensity of *Radix isatidis* from some manufacturers is large. The highest is No. 8 sample, and the lowest is No. 5 sample. So it can be used to distinguish different kinds of *Radix isatidis*. The fluorescence intensity of some samples is close and the curve shapes are similar, such as No. 2 and No. 10 sample, indicating that main components for Chinese patent medicine *Radix isatidis* granules are single, and the product quality stability can be guaranteed by controlling the raw material and process technology. Analyzing the 10 samples overall, the differences between them are not only reflected in the fluorescence intensity, but also in the curve shape. Some fluorescence spectrum curves have main peak and sub-peak (No. 8, No. 2, No. 10), while other curve shapes are smooth (such as No. 4, No. 5). The curves reflected that the qualities of different *Radix isatidis* granules vary greatly, and the market for quality monitoring of *Radix isatidis* granules remains to strengthen further.

Normalized characteristic spectrum curves: The meaning of fluorescence spectrum curve and normalized characteristic spectrum curve to identify samples is the same, but the latter can more obviously reflects the difference among samples. So normalization processing of spectrum curves of all *Radix isatidis* granules, standard substances and the raw material is carried out before further discussed, as shown in Figure 4. No. 1 and 2 represent the standard substances and raw material respectively (as reference material), and their characteristic spectrum curves closely overlapped, indicating that the shape of spectrum curves of the same material was basically identical. No. 3 to No. 12 represents 10 kinds of *Radix isatidis* granules. Among them, the trends of curve shape of No. 4, 10 and 12 were consistent, and the most close to the standard substances and raw material. No. 3 and 8 were next, but their sub-peaks at 595 nm wavelength were much higher than the reference material. Comparing No. 5, 9, 11 with No. 3, 8 the main peaks of the former were at 555 nm wave length and the sub-peak at 595 nm, but the latter were just the opposite. No. 7 was the most different from the reference material, the curve trend continues to rise as the

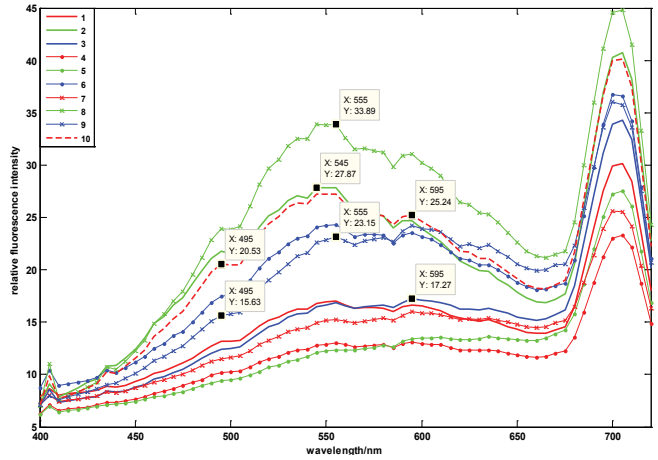

Figure 3: Fluorescence spectrum curves of the *Radix isatidis*.

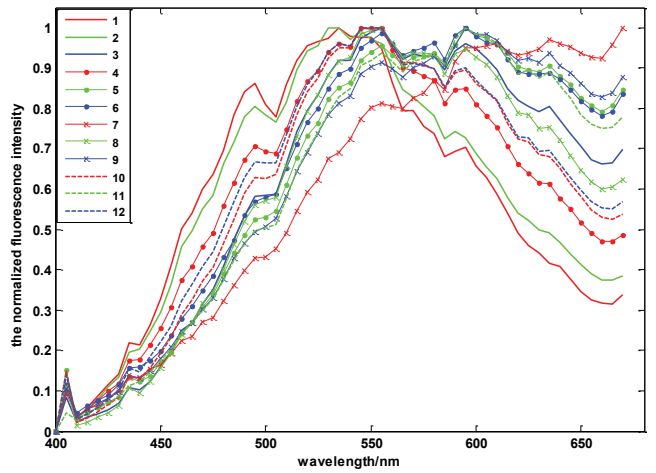

Figure 4: Normalized characteristic spectrum curves.

wavelength changes, and its spectrum feature was completely different from standard substances. These results indicated that the differences of the curves after normalization can reflect the quality of *Radix isatidis* granules better, so we can combine fluorescence spectrum curve with normalized characteristic spectrum curve to establish the quality evaluation methods of *Radix isatidis*, guiding the production of *Radix isatidis* granules and further promoting to the quality management of other Chinese patent drug.

The relationship between granules samples, standard substances and raw material can be directly shown through the cluster analysis results. As shown in Figure 5, L for clustering distance (Table 1), sample 3 and 8 firstly polymerize when L=2.1389; followed sample 5, 9, 11 and 6; then the clustering of sample 4, 10 and 12 is in L=4.8217. The clustering distance of these nine samples is close. Sample 7 and other samples polymerize a class when L=10.4028, suggesting that there is a relatively big gaps in base resource classification for them. After standard substances and raw material polymerizing, all samples lump together in L=13.8156.

Comparison of north and south *Radix isatidis* granules: Firstly we discussed the normalized characteristic spectrum curves of north and south standard substance, shown in Figure 6. The normalization fluorescence intensity of the former is higher than the latter, and envelope area between the curves and horizontal axis at the wavelength range of 400 to 650 nm is also greater than the latter. At the wavelength of 550 to 650 nm, the curve trend of south *Radix isatidis* standard substance is relatively flat, and north *Radix isatidis* standard substance is steep. So these two different families of plants are able to be distinguished.

There is a shortage of raw material for south *Radix isatidis*, so few manufacturers produce south isatis root granules. In Figure 7, No. 1 on behalf of the south banlangen standard material and No. 2 and No. 3 on behalf of two different particle samples, the normalized results show the shapes of characteristic spectrum curves for south isatis root granules and its standard substance are similar, and the curves of two kinds of granules are basically identical. The tiny difference among those is that the wave crest of standard substance at 550 nm is a spike, but the granules samples are gentle. The spectral imaging technology can detect the quality of Chinese patent drug as a whole, rather than a single component. It is an advantage of this technology used in the quality detection of Chinese patent drug.

In contrast, No. 1 to 3 on behalf of the south banlangen standard material and its granules samples and No. 3 to 14 on behalf of the north standard material and its granules samples. From the clustering tree graph, some *Radix isatidis* and south isatis root gathered as a group, indicating that their basic source classification distance is closer, and the rest of *Radix isatidis* samples are divided into a group. Then these two groups aggregated, and gathered a group with *Radix isatidis*

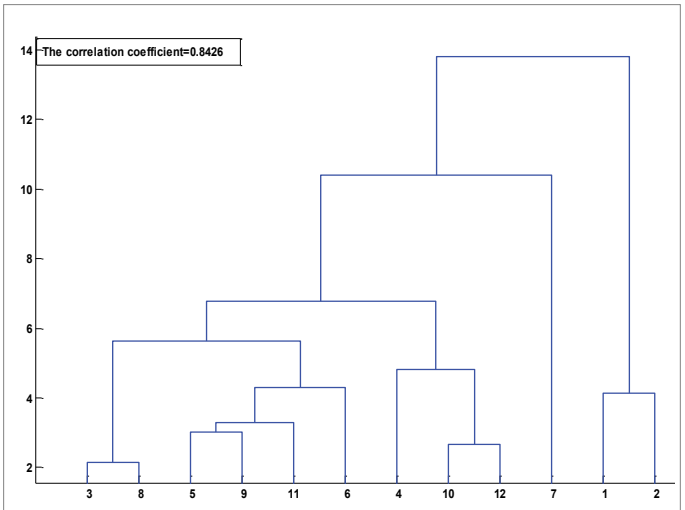

Figure 5: Cluster analysis tree of standard substances etc.

Number of samples	Number of samples	Clustering distance L.
3	8	2.1389
10	12	2.6722
5	9	3.0084
5, 9	11	3.2957
1	2	4.1326
5, 9, 11	6	4.2914
10, 12	4	4.8217
5, 9, 11, 6	3, 8	5.6361
3, 8, 5, 9, 11, 6	4, 10, 12	6.7697
3, 8, 5, 9, 11, 6, 4, 10, 12	7	10.4028
3, 8, 5, 9, 11, 6, 4, 10, 12, 7	1, 2	13.8156

Table 1: The values of L for all samples.

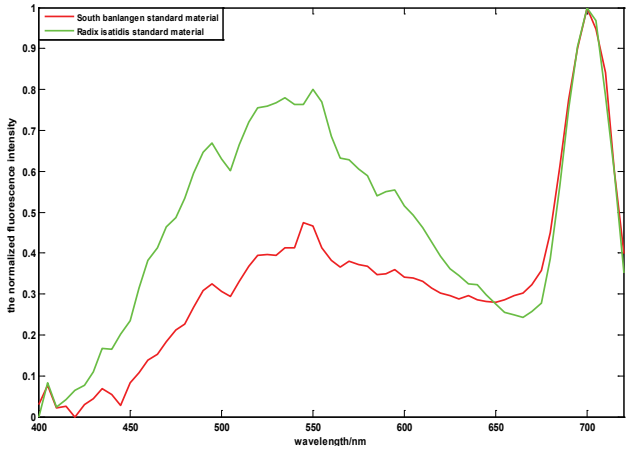

Figure 6: North and south standard substance.

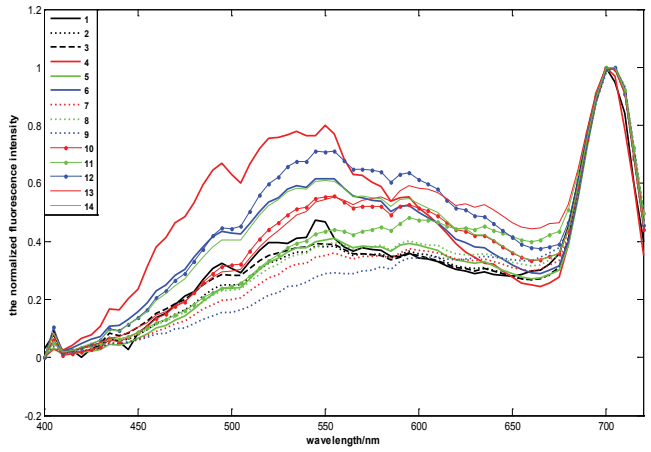

Figure 7: South *Radix isatidis* standard substance and granules.

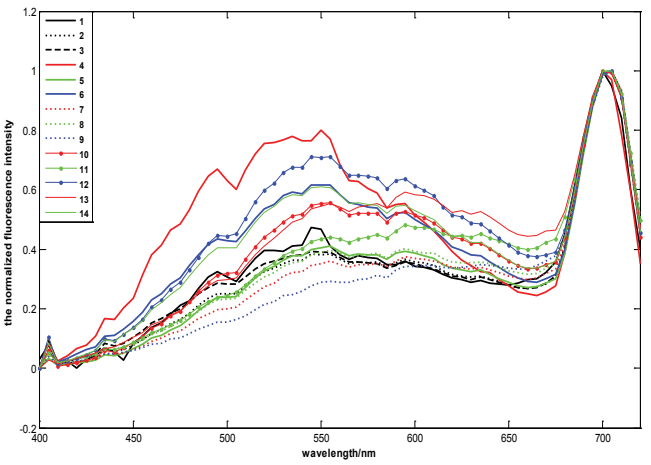

Figure 8: The normalized contrast of north and south.

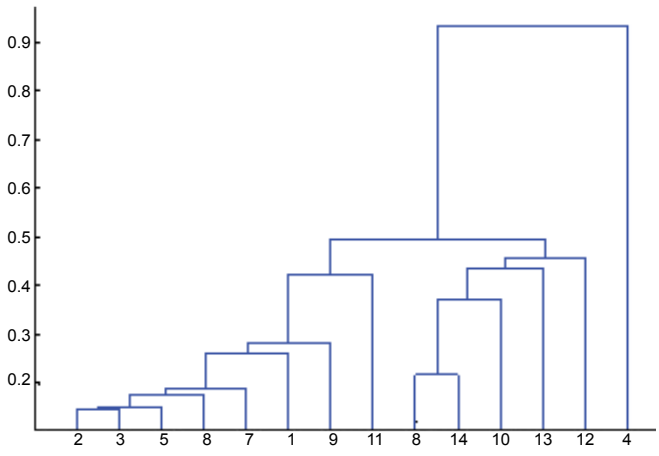

Figure 9: Cluster analysis tree of north and south.

standard substance. These results reveal a common status quo of Chinese patent drug in market, lax on raw material quality control and lack of standardized management, so that there appearing counterfeit and inferior goods on the market, which led to the uneven quality of Chinese patent drug (Figures 8 and 9).

Conclusion

In this paper, we detected some *Radix isatidis* granules on market using spectral imaging technology, and the results show that there is larger difference in quality among samples. Meanwhile comparing them with south isatis root granules, we found that the *Radix isatidis* and south *Radix isatidis* from different families did not be classified based on category. Our study indicates the lack of scientific quality management standard in the use of raw material of Chinese patent drug, and there is still a homonym and adulteration phenomenon, which is the reason for the spotty quality of samples [12]. Fluorescence spectrum imaging method analyses Chinese patent drug on the overall, making up for the disadvantage of other methods that only detect single or two components. So it is expected to become a new method to the quality test for Chinese patent drug which guarantees the stability and reliability of Chinese patent drug.

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