



Short Communication

Preliminary assessment of two non-destructive instrumental techniques for quality evaluation of *Lobelia chinensis* Lour. ☆Hong-Peng Chen^{a,1}, Wen-Jia Pan^{b,1}, Nan Tang^{b,*}, Yuan Zhang^c, Mei-Ling Yu^d, Xing-Da Wu^a^a School of Information Engineering, Guangdong Medical University, Xincheng Road, Dongguan 523808, China^b School of Pharmacy, Guangdong Medical University, Xincheng Road, Dongguan 523808, China^c Department of Pharmacology, Zhongshan School of Medicine, Sun Yat-Sen University, Guangzhou 510080, China^d Department of Pharmacy, the First Affiliated Hospital of Bengbu Medical College, Bengbu 233004, China

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ABSTRACT

Two non-destructive instrumental methods, infrared spectroscopy (IR) and X-ray diffraction (XRD), were studied for quality evaluation of *Lobelia chinensis* Lour. (*L. chinensis*). We obtained the IR spectra and XRD patterns of *L. chinensis* collected from different sources. The similarity of samples was analyzed by calculating the cosine coefficient. The cosine values were in the range of 0.83–0.90, indicating that the main components of *L. chinensis* samples are similar. Sample L1 and L6 showed a slightly lower similarity than that of L2, L3, L4, L5 detected by the two methods, which revealed that IR and XRD methods exhibited analogous detection ability for quality evaluation of *L. chinensis*. The two methods could be highly recommended as simple and rapid detection means for quality evaluation of *L. chinensis*.

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1. Introduction

Lobelia chinensis Lour. (*L. chinensis*), belonging to Campanulaceae family, is a traditional Chinese medicine (TCM). It, included in Chinese Pharmacopoeia 2010 [1], has been used for the treatment of swelling, acute nephritis, detoxification, eczema and furuncle in Chinese folk medicine. Further research has shown that *L. chinensis* exhibits a variety of pharmacological effects, such as anti-tumor [2,3], anti-inflammation [4] and inhibiting the proliferation of vascular smooth muscle cells [5]. These biological activities has been attributed to the various chemical components of *L. chinensis*. Therefore, the quality control of *L. chinensis* is particularly important for its efficacy.

At present, chromatography is the main method used in the quality evaluation of TCM [6–10]. However, chromatography techniques usually require complicated extraction operations and harmful reagents. Furthermore, only components extracted by these reagents can be analyzed. In recent years, “non-destructive” and “overall control” instrumental techniques have received attention in TCM quality evaluation, such as infrared spectroscopy (IR) and X-ray diffraction (XRD) [11–14].

The aim of the study was to conduct the quality analysis of *L.*

chinensis by two non-destructive methods, IR spectroscopy and XRD, and the results showed that they are simple and rapid for the quality control of *L. chinensis*.

2. Experimental

2.1. Materials

The reference herb of *L. chinensis* was purchased from the National Institutes for Food and Drug Control (Beijing, China). Other samples were collected from TCM companies and markets of Guangdong and Guangxi provinces in China. All herbs were identified by pharmacognosy colleagues, Guangdong medical University (Dongguan, China). The number and information of *L. chinensis* samples are shown in Table 1. The herbs were dried at 40 °C for 24 h and ground into powder using a TCM pulverizer (Zhongxiang, Changsha, China), followed by passing through a 100-mesh stainless steel sieve (Shupe, Shanghai, China). The powders were stored in a desiccator and measured by XRD as well as IR methods.

2.2. FT-IR measurement

The sieved powders were mixed with KBr (1:100, m/m). Then the uniformly ground mixture was pressed into tablets for IR measurement. All samples were evaluated by EQUINOX 55 Fourier

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Table 1
Information of *Lobelia chinensis* Lour. samples.

No.	Source	Collection time
L0	Reference	
L1	Nanning, Guangxi (TCM company)	2011.10
L2	Nanning, Guangxi (TCM company)	2013.01
L3	Guangzhou, Guangdong (TCM company)	2012.10
L4	Zhanjiang, Guangdong (TCM company)	2013.01
L5	Qingyuan, Guangdong (TCM company)	2013.05
L6	Guangzhou, Guangdong (TCM market)	2012.10

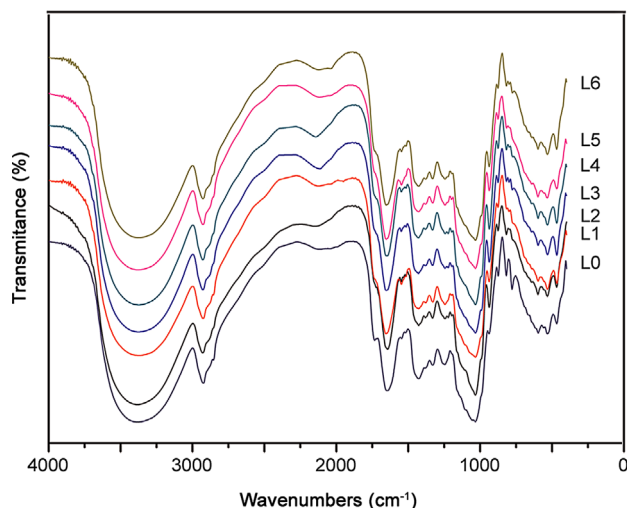


Fig. 1. The IR spectra of *Lobelia chinensis* Lour.

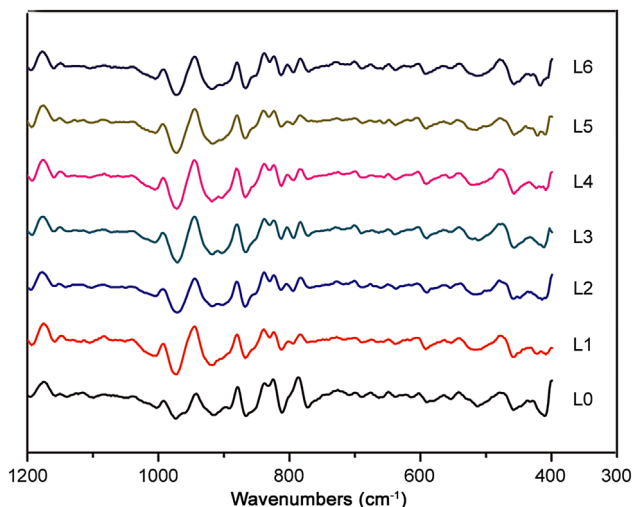


Fig. 2. The first-derivate IR spectra of *Lobelia chinensis* Lour.

transform infrared (FT-IR) spectroscopy (Bruker, Karlsruhe, Germany) with the following typical acquisition parameters: detection range, 4000–400 cm^{-1} ; scanning number, 16; spectral resolution, 4 cm^{-1} .

2.3. XRD measurement

All samples were characterized by D8-advance X-ray diffractometer (Bruker, Karlsruhe, Germany) with a Cu-K α radiation ($\lambda = 1.5406 \text{ \AA}$). The accelerating voltage and the applied current were 40 kV and 40 mA, respectively. The scan range of 2θ was from 10° to 80° with a step of 0.02° . The XRD pattern was analyzed

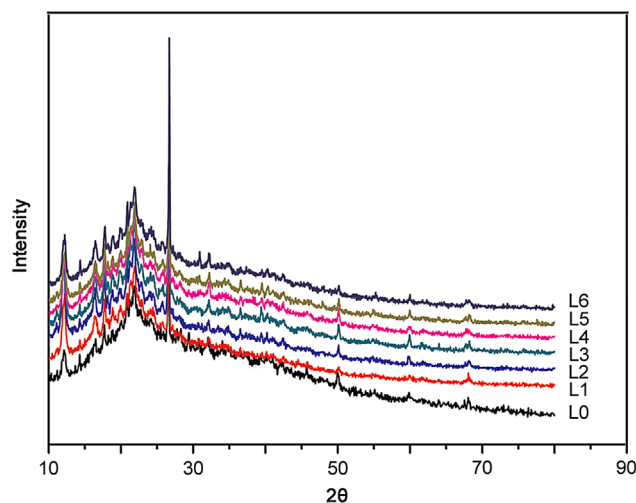


Fig. 3. The XRD patterns of *Lobelia chinensis* Lour.

with MDI Jade 5.0 software.

2.4. The assessment methods

The cosine coefficient was used to estimate the similarity among *L. chinensis* samples. Cosine coefficient is an indicator that measures the cosine value of vectorial angle between two groups' variables. The index is a widely accepted calculation method to evaluate the similarity of TCM. In this study, the data of samples obtained from XRD and IR measurements were first normalized in order to avoid the influence of different sample weights, and then be calculated by using following equation [15]. The cosine value of 0.8 is used as threshold for discriminating *L. chinensis*.

$$\text{Cosine coefficient: } \cos \theta = \frac{\sum_{i=1}^n a_i b_i}{\sqrt{\sum_{i=1}^n a_i^2} \sqrt{\sum_{i=1}^n b_i^2}}$$

where a_i is the wavenumber/ 2θ value of i ($i=1-n$) peak in IR spectrum/XRD pattern of sample; b_i is the wavenumber/ 2θ value of i ($i=1-n$) peak in IR spectrum/XRD pattern of reference.

3. Results and discussion

3.1. IR spectra/XRD patterns of TCM

IR and XRD have additive properties. The crystal, composition and structure of substances determine their IR spectra/XRD patterns. Different substances produce characteristic IR absorption/XRD. The IR spectra/XRD patterns of mixture are the superposition of absorption/diffraction effects generated by each component in the mixture. Therefore, IR spectra/XRD patterns could be used as the fingerprint of the mixture when the components are invariant, which is the theoretical basis of IR/XRD for TCM quality control.

3.2. FT-IR

The IR spectra of *L. chinensis* were obtained by FT-IR spectrometer and are shown in Fig. 1. Similar absorption characteristics of *L. chinensis* samples in IR spectra were observed in the range of 4000–1200 cm^{-1} . However, in the fingerprint region of IR spectra, some differences of absorption characteristics were evident. In order to make the differences more apparent, the first-derivate (FD) spectra of samples in the range of 1200–400 cm^{-1} were established (Fig. 2). The high resolution FD spectra enable to separate the overlapping peaks. In Fig. 2, the differences in number and intensity of peaks among FD spectra appeared in the 800 cm^{-1} . The FD spectra

Table 2The XRD characteristic diffraction peaks of *Lobelia chinensis* Lour. samples.

No.	The characteristic diffraction peaks					
L0	7.2917/81.0	6.1854/12.3	5.3682/51.1	5.0057/34.6	4.4214/24.4	4.2684/19.8
	4.0589/99.5	3.9057/6.5	3.6772/23.4	3.5471/12.9	3.492/23.8	3.3546/100
	3.212/38.9	3.1508/43.3	3.0332/22.8	2.7907/57.2	2.6328/18.0	1.9838/38.3
L1	1.8236/35.8	1.5438/27.0	1.3843/10.5	1.3765/18.7		
	7.2341/100	6.1836/8.9	5.3677/39.1	4.9950/38.0	4.7050/20.3	4.4473/15.0
	4.2601/6.4	4.1513/81.5	4.059/81.0	3.6947/26.8	3.6448/26.6	3.4531/7.5
L2	3.3425/70.9	3.2808/4.0	3.1658/7.5	2.7841/10.2	2.4565/5.0	1.8169/7.2
	1.5393/10.2	1.3759/14.2				
	7.2685/100	6.192/6.3	5.3875/31.3	5.0117/42.0	4.7053/15.9	4.4386/10.0
L3	4.2598/14.6	4.178/98.2	4.0662/73.8	3.896/12.0	3.7127/37.3	3.6389/7.7
	3.4559/12.9	3.3475/72.0	2.7825/11.0	2.4585/5.5	2.2843/7.4	2.2405/5.7
	2.217/8.9	1.8189/9.9	1.5429/10.1	1.3751/9.1	1.396/8.6	
L4	7.257/100	6.1837/6.3	5.3679/40.0	4.9949/40.4	4.7148/17.0	4.4735/15.5
	4.2556/7.2	4.1625/81.7	4.066/78.1	3.8862/9.5	3.689/33.4	3.6276/32.0
	3.4478/11.9	3.3427/91.1	3.2036/8.7	3.1659/15.3	2.7807/16.4	2.5728/9.2
L5	2.4573/4.2	2.2843/8.7	1.8188/13.9	1.5419/8.9	1.3818/14.5	1.3727/13.8
	7.2449/84.0	6.1743/8.0	5.3866/36.5	4.9950/35.4	4.7049/12.3	4.4209/13.9
	4.2476/8.0	4.1477/100	4.0410/80.8	3.8757/5.1	3.6976/22.4	3.4880/12.1
L6	3.34/76.1	3.2784/4.5	3.1637/13.7	2.7791/17.7	1.8176/16.8	1.5433/10.5
	1.3818/13.3	1.3737/13.7				
	7.2112/84.1	6.1745/7.9	5.3806/41.3	5.004/40.4	4.7003/12.3	4.4427/9.5
L6	4.2409/8.0	4.0589/74.5	3.8929/5.5	3.6987/19.1	3.6216/4.8	3.4603/12.0
	3.3376/100	2.7742/13.2	2.6075/18.0	2.4547/9.5	2.2789/10.2	2.2312/16.7
	2.125/17.1	1.817/16.4	1.5414/12.6	1.3825/19.8	1.3712/12.9	
L6	7.2335/61.0	6.165/5.8	5.3746/27.5	4.984/26.7	4.681/10.6	4.4387/5.5
	4.1588/100	4.0588/60.4	3.9020/7.0	3.7217/26.9	3.6333/25.8	3.4712/7.5
	3.3375/85.7	3.2758/5.0	2.8914/7.1	2.7725/13.9	1.8181/8.5	1.6574/5.8
	1.5406/4.2	1.3807/13.5	1.3737/11.4			

(1200–400 cm⁻¹) of *L. chinensis* samples were used for similarities analysis.

The values of cosine coefficient for samples that were calculated with the reference herb (L0) were 0.8622, 0.8934, 0.8802, 0.8772, 0.9001 and 0.8681 from L1 to L6, respectively.

The similarity of six samples relative to L0 (reference herb) was > 85.0%, suggesting that the herbs have similar properties and ingredients. However, the values of L1 and L6 (both approximately 0.86) were slightly less than those of L2, L3, L4, L5 (all more than 0.87). Data were analyzed with the unpaired Student's *t* test and the *p* Value was 0.054, which indicated that L1 and L6 had differences in quality from L2, L3, L4, L5. Table 1 shows that L1, L2, L3, L4, L5 were all collected from TCM companies, while L1 was stored longer than other samples. This indicated that storage time might affect the quality of *L. chinensis*. The chemical components of herbs may decompose or change under the influence of light, air and moisture. L6 was collected from TCM market and showed a slightly lower similarity than that of L2, L3, L4, L5, which might suggest that the quality control in TCM market needs to be strengthened.

Together, these observations indicated that IR has a certain ability to distinguish the quality of *L. chinensis*.

3.3. XRD

The XRD Fourier patterns of *L. chinensis* are shown in Fig. 3. The significant diffraction peaks were exhibited in *L. chinensis* XRD patterns. The geometric topology disciplinarian was similar among sample patterns, and the characteristic diffraction peaks were distributed in the range of 10°–60° (2 θ). In our study, we used Jade 5.0 software to seek diffraction peaks and the results were represented by these diffraction characteristics.

Data of each diffraction peak were expressed in interplanar spacing (\AA , *d*) and the relative diffraction intensity I/I_0 , denoted with $d/I/I_0$. The corresponding characteristic diffraction peaks of samples are shown in Table 2.

The diffraction peaks of samples that were found by Jade

5.0 software were matched with each other using peak position (2 θ) as index, in which the corresponding position without peaks was recorded as “zero”, and the similarity was evaluated using cosine coefficient. The XRD pattern of L0 was taken as reference spectrum.

The cosine coefficients of samples were 0.8344, 0.8720, 0.8802, 0.8933, 0.8882 and 0.8466 for L1–L6, respectively. All values were more than 0.83, indicating that the herbs contained the same main ingredient. Likewise, the values of L1 (0.8344) and L6 (0.8466) were lower than those of samples (all more than 0.87), which were consistent with IR results. The unpaired Student's *t* test was used and the *p* Value was 0.0057, which suggested that L1 and L6 had differences in quality compared with L2, L3, L4, L5. This result indicated that XRD can also exhibit a correlation with *L. chinensis* quality.

4. Conclusion

The IR and XRD methods are non-destructive to the samples. We used them to analyze *L. chinensis*, and the cosine coefficient represented the similarity. It showed that the two kinds of methods exhibited a good correlation with quality evaluation of *L. chinensis*. These observations indicated that IR and XRD could be promising instrumental techniques in quality control of *L. chinensis*.

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