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QUALITATIVE AND SEMIQUANTITATIVE ANALYSIS OF *Cistanche deserticola* BY MID-INFRARED SPECTROSCOPY

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Abstract: In the present study, mid-infrared spectroscopy (MIR) was attempted to apply for quality evaluation of *C. deserticola*. First of all, IR spectra of the herbal sample and its 3 main extracts including PeGs, Man and Polysaccharides were compared. Characteristic absorption peaks within different wave number range were found in different extracts. Then the authors managed to find the relationships between the intensity of the characteristic peaks and the relative content of the PeGs and Man in herbal sample. The IR spectra of the herbal samples were compared with PeGs and Man using Spectrum v5.02 software (Perkin Elmer) for IR Corrdations. It was found that the content of PeGs (y) and its IR Correlation (x) within herbal sample in the range of 1800 ~ 1200cm^{-1} were correlative (R^2 = 0.9927) by the SPSS 11.0 analysis, and the regression equation is $y = 19.846x + 0.0623$. In the same way, the content of Man (y) is correlative with the IR Correlation (x) in the range of 1100 ~ 600cm^{-1} ($R^2 = 0.9705$), and the regression equation is $y = 13.855x - 0.1714$. Therefore, MIR spectroscopy is a fast and effective method in qualitative and semiquantitative analysis of quality evaluation of *C. deserticola*.

Key words: mid-infrared spectroscopy (MIR); *Cistanche deserticola*; qualitative; semiquantitative; quality evaluation
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中红外光谱法对肉苁蓉的定性和半定量分析

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摘要:本文尝试将中红外光谱(MIR)应用于肉苁蓉药材的质量评价和分析中.首先比较肉苁蓉药材粉末样品及其3种主要活性成分提取物,包括本乙醇总苷(PeGs)、甘露醇(Man)和多糖的红外光谱图差异,得出3种提取物的特征红外吸收峰,确定药材样品各特征峰的强弱与PeGs和Man含量高低的关系.然后用Spectrum v5.02软件计算得出不同波段内药材图谱与两种提取物图谱的红外相似系数,通过SPSS11.0软件分析发现,药材PeGs的含量(y)和1800 ~ 1200cm^{-1}范围内的红外相似系数(x)呈极显著相关($R^2 = 0.9927$),回归方程:$y = 19.846x + 0.0623$.同样,药材Man的含量(y)与1100 ~ 600cm^{-1}波数范围的红外相似系数(x)呈极显著相关($R^2 = 0.9705$),回归方程:$y = 13.855x - 0.1714$.因此,MIR可作为肉苁蓉药材质量定性和半定量分析的快速有效方法,直观评价药材质量.

关 键 词:中红外光谱;肉苁蓉;定性;半定量;质量评价

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Introduction

Cistanche deserticola Y. C. Ma, a famous and precious traditional Chinese medicine (TCM), has been indicated for several years as the primary source material of the Chinese herb Cistanche (rou cong rong) in "The Pharmacopoeia of the People's Republic of China" [1]. As many other species of TCM, *C. deserticola* is a complex mixture which contains many kinds of bioactive constituents. Phenylethanoid glycosides (PeGs) from *C. deserticola* are the main bioactive ingredients for improving sexual potency, scavenging free radicals and anti-aging properties [2]. But the purgative function depends on the mannitol content (Man).

Near-Infrared (NIR) spectral methods have successfully been used for qualitative and quantitative analysis in many research fields [3]. However, for lack of integrated interpretation of the spectra, Mid-Infrared (MIR) spectroscopy was seldom used in TCM or other complicated systems before. While, MIR has some incomparable advantages, such as fast, accurate, good reproducibility, need less sample preparations and so on. Since 1998, with MIR spectroscopy, a lot of efforts and accomplishments have been made in TCM[4,5]. In the present study, MIR was attempted to apply as a qualitative and semi-quantitative analysis method in quality evaluation of *C. deserticola*. It appears that, based on our comprehensive literature review, the work described here is the first attempt ever made to develop the MIR technique for qualitative and quantitative analysis of TCM raw material.

1 Experiment

1.1 Sample

76 herbal samples and 3 main extracts had been analyzed. The samples of *C. deserticola* were collected from different habitats and authenticated by Prof. Jun Chen, from the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences. 3 main extracts including PeGs, Man and Polysaccharides were isolated and purified from *C. deserticola* by Prof. Yue Shi, from the same institute.

1.2 Apparatus

Spectrometer (GX Fourier transform infrared spectroscopy (FT-IR), Perkin-Elmer) equipped with a DTGS detector. IR spectra were recorded from the accumulation of 16 scans in 4000 ~ 400cm^{-1} range with a resolution of 4cm^{-1}. Spectra were obtained from the accumulation of a total of 16 scans.

1.3 Procedure

Each sample was ground into powder with over 200 meshes and then blended with KBr powder, grounded again and pressed into a tablet. After that, their IR spectra were collected at room temperature. The IR spectra of the herbal samples were compared with the IR spectra of PeGs and Man using Spectrum v5.02 software (Perkin Elmer) in different ranges without any data processing. The content of PeGs was determined using macroporous resin-UV spectrophotometry methods (RSD = 0.4%)[6]. Content of mannitol was determined by HPLC-ELSD (RSD = 2.4%) [7]. SPSS 11.0 software was used for Correlation and Regression analysis.

2 Results and discussion

2.1 Comparison and assignment of IR spectra of the herbal material and its main extracts

Comparing the IR spectra of the herbal material (a) and its 3 main extracts including PeGs (b), Man (c) and Polysaccharides (d) (Fig. 1), it was found that the strong absorption peaks of different extracts were different. As known, the IR spectra of the herb and some of its extract PeGs (b) and Polysaccharides (d) are all complicated systems consist of the overlapped peaks of all chemical compositions. In Fig. 1, some of the peaks can be seen in the IR spectrum of both herbal samples and the extracts. However, the benzene ring [8] (1604, 1517, 1446cm^{-1}) only existed in the IR spectra of PeGs. As well, the peaks of δ (CH_3) (1374cm^{-1}) and v(C-O-C) (1275cm^{-1}) in the IR spectra of PeGs were different from the other two extracts. It suggested that the peaks should be assigned to ansymmetric stretching vibration of the Ar-O-Me group and should be characteristic absorption bands (ranging from 1800cm^{-1} to 1200cm^{-1}) of PeGs. The peaks of v(C-OH) (1082, 1020cm^{-1}) and sugar ring (929, 882, 700, 631cm^{-1}) in the IR spectra of Man were different from the other two extracts. It also suggested that these peaks (ranging from 1100cm^{-1} to

600cm^{-1}) could be characteristic absorption bands of Man, since the characteristic peaks of PeGs (b), Man (c) and Polysaccharides (d) overlapped in the IR spectra of herbal sample. Furthermore, as the 3 extracts were the high and main content compounds in the herbal sample, all of peaks belonging to the extracts compose of the fundamental shape of the IR spectra of the herbal sample. Then, the relationship between the intensity of the characteristic peaks and the relative content of the main bioactioactrive ingredients in herbal sample were managed to found out.

2.2 Quick quality assessment of *C. deserticola* with intensity of the characteristic peaks

The content of PeGs (PeGs%) and the content of Man (Man%) in *C. deserticola* were determined firstly. Then, from all the herbal samples, we found several samples which with regularly different content of PeGs and Man (Fig. 2 Fig. 3). Figure 2 & 3 showed the IR spectra of different herbal samples with different content of PeGs or Man from high to low (from top to bottom). It was found that higher content of the PeGs or Man could be demonstrated by higher and more similar characteristic peaks. For example, higher content of the PeGs could be demonstrated by higher and more similar characteristic peaks in 1517 ± 1 and 813 ± 3cm^{-1}. The higher of the content of the PeGs in the herbal sample, the higher intensity of the two peaks showed in the spectrum. As well, higher content of the Man could be demonstrated by higher and more similar characteristic peaks in 1022 ± 2 and 630 ± 1cm^{-1}. The higher of the content of the Man in the herbal sample, the higher intensity of the two peaks showed in the spectrum. Fur-

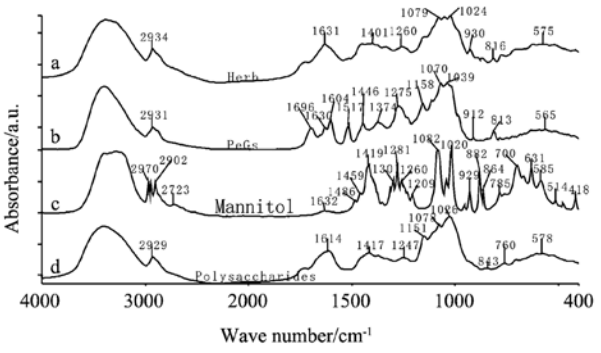

Fig. 1　The IR spectra of Herb (a), PeGs (b), Man (c) and Polysaccharides (d)
图 1　肉苁蓉药材 (a)及其提取物苯乙醇总苷 (b), 甘露醇 (c) 和多糖(d)的红外光谱图

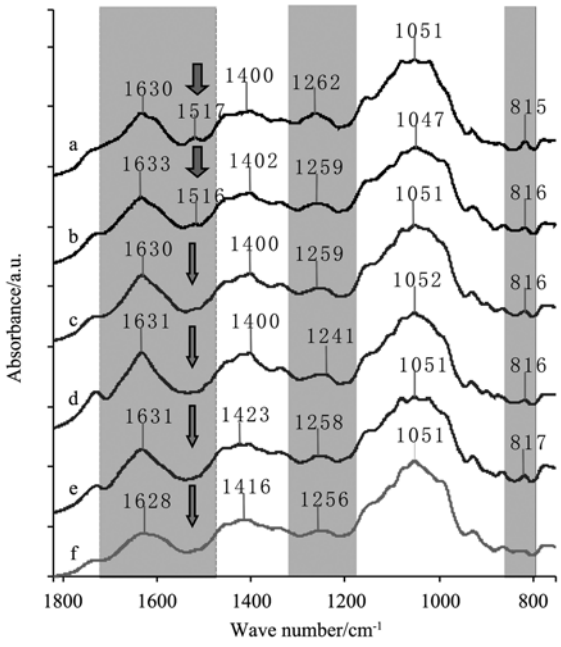

Fig. 2　The IR spectra of different herbal samples with different content of PeGs from high to low (a: 8.41%, b: 5.66%, c: 4.81%, d: 3.87%, e: 2.87%, f: 0.59%)
图 2　苯乙醇总苷含量从高到低的肉苁蓉药材的红外光谱图(a: 8.41%, b: 5.66%, c: 4.81%, d: 3.87%, e: 2.87%, f: 0.59%)

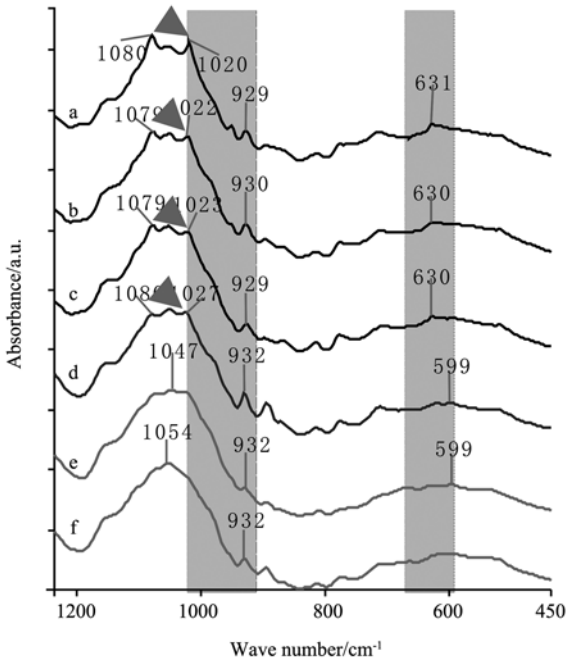

Fig. 3　with different content of Man from high to low (a: 5.15%, b: 3.74%, c: 3.02%, d: 2.53%, e: 2.05%, f: 1.08%)
图 3　甘露醇含量从高到低的肉苁蓉药材的红外光谱图(a: 5.15%, b: 3.74%, c: 3.02%, d: 2.53%, e: 2.05%, f: 1.08%)

thermore, all the procedures including sample preparation, scanning and quality determination need no more than 10 min. It was indicated that FTIR which was non-destructive and needed no sample pre-treatment could be a very promising method for quick qualitative analysis of *C. deserticola*.

2.3 Intensity of the characteristic peak correlated with content of PeGs and Man

The samples which content of the PeGs ranging from 8.41% to 0.59% and their IR Correlations (C1: In the range of 1800 ~ 1200cm^{-1}) which were accounted from the Compare procedure with the IR spectra of PeGs using Spectrum v3.02 software were selected. And in the same way, the herbal samples which content of the Man ranging from 5.15% and 1.08% were used and IR Correlations (C2: In the range of 1100 ~ 600cm^{-1}) had been accounted. Then the PeGs% and C1; Man% and C2 were carried out Correlation and Regression analysis using SPSS 11.0 software (Fig. 4 & 5). The correlations were both significant at the 0.01 level (2-tailed). The Correlation coefficients (R^2) were 0.9927 and 0.9705 respectively. The regression equation for PeGs% (y) to C1 (x) was $y = 19.846x + 0.0623$ and for Man% (y) to C2 (x) was $y = 13.855x - 0.1714$. RSD was 4.3% and 6.6% respectively ($n = 6$). The results showed that IR Correlations (C1, C2) could approximately reflect total content of PeGs and Man. The regression equation of the IR spectra can be used for semiquantitative analysis in quality evaluation of *C. deserticola*. As well, this work could be the basement for developing the MIR technique for quantitative analysis of TCM raw materials by chemometrics method with computer pattern recognition.

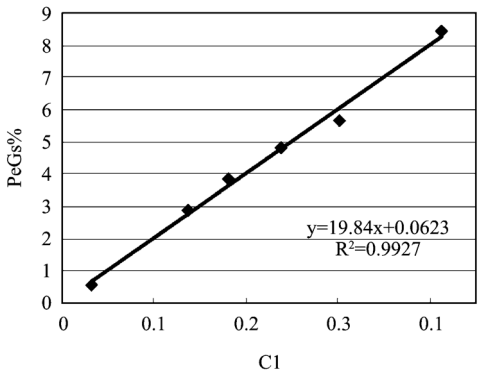

Fig. 4 Linear dependence relation of the content of PeGs (PeGs%) and IR Correlations (C1)
图4 肉苁蓉药材苯乙醇总苷含量测定值与红外相似系数C1的线性相关关系图

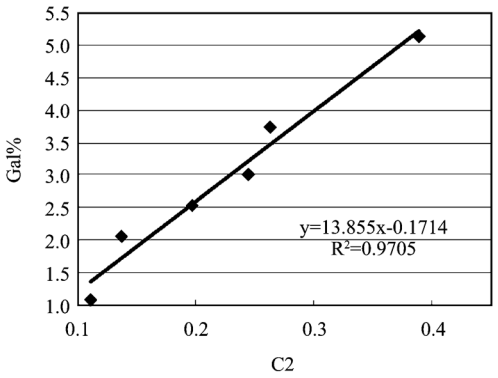

Fig. 5 Linear dependence relation of the content of Man (Man%) and IR Correlations (C2)
图5 肉苁蓉药材甘露醇含量及其红外相似系数C2的线性相关关系图

3 Conclusions

A method based on MIR fingerprinting was established for quick quality evaluation of *C. deserticola*. Compared with the IR spectra of the herbal samples and the extracts, the magnitude of characteristic bands ranging from 1800 ~ 1200cm^{-1} were directly correlated with the content of PeGs significantly ($P < 0.01$). It was also found that higher content of the PeGs could be demonstrated by higher and more similar characteristic peaks in 1517 ± 1 and 813 ± 3 cm^{-1}. As well, in the range of 1100 ~ 600 cm^{-1}, the IR Correlation of herbal sample were correlated with the content of Man ($P < 0.01$). Higher content of the Man could be demonstrated by higher and more similar peaks in 1022 ± 2 and 630 ± 1cm^{-1}. Regression equation of the two contents and the IR Correlations (C1, C2) were $y = 19.846x + 0.0623$ ($R^2 = 0.9927$) and $y = 13.855x - 0.1714$ ($R^2 = 0.9705$) respectively. In summary, the macroscopic quality control method is feasible to illustrate the integrated qualities of *C. deserticola*. It was concluded that MIR is a fast and effective qualitative and semiquantitative analysis method for quality evaluation of *Cistanche deserticola*. As well, it would be a prospective quantitative analysis method for TCM raw materials.

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