Journal of Molecular Structure 1069 (2014) 236–244

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: www.elsevier.com/locate/molstruc

Multi-step infrared macro-fingerprint features of ethanol extracts from different *Cistanche* species in China combined with HPLC fingerprint

Rong Xu [a], Suqin Sun [b], Weicheng Zhu [c], Changhua Xu [b], Yougang Liu [a], Liang Shen [a], Yue Shi [a], Jun Chen [a,*]

[a] The Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100193, China
[b] Department of Chemistry, Tsinghua University, Beijing 100084, China
[c] Harbin University of Science and Technology, Harbin 150080, China

HIGHLIGHTS

- Multi-steps IR show different features on ethanol extracts of four *Cistanche* species.
- Analysis results of IR macro-fingerprint were verified by HPLC fingerprint method.
- Relationships of four species of *Cistanche* were inferred by IR and HPLC fingerprint.
- Main active ingredients of four *Cistanche* were compared and estimated by IR spectra.
- 2D-correlation analysis provided additional further information for differentiation.

ARTICLE INFO

Article history:
Received 31 December 2013
Received in revised form 20 March 2014
Accepted 20 March 2014
Available online 1 April 2014

Keywords:
Fourier transform infrared spectroscopy (FT-IR)
Two-dimensional correlation infrared spectroscopy (2D-IR)
IR correlation coefficients (IRCs)
Cistanche
Ethanol extracts
Discrimination

ABSTRACT

The genus *Cistanche* generally has four species in China, including *C. deserticola* (CD), *C. tubulosa* (CT), *C. salsa* (CS) and *C. sinensis* (CSN), among which CD and CT are official herbal sources of *Cistanche Herba* (CH). To clarify the sources of CH and ensure the clinical efficacy and safety, a multi-step IR macro-fingerprint method was developed to analyze and evaluate the ethanol extracts of the four species. Through this method, the four species were distinctively distinguished, and the main active components phenylethanoid glycosides (PhGs) were estimated rapidly according to the fingerprint features in the original IR spectra, second derivative spectra, correlation coefficients and 2D-IR correlation spectra. The exclusive IR fingerprints in the spectra including the positions, shapes and numbers of peaks indicated that constitutes of CD were the most abundant, and CT had the highest level of PhGs. The results deduced by some macroscopic features in IR fingerprint were in agreement with the HPLC fingerprint of PhGs from the four species, but it should be noted that the IR provided more chemical information than HPLC. In conclusion, with the advantages of high resolution, cost effective and speediness, the macroscopic IR fingerprint method should be a promising analytical technique for discriminating extremely similar herbal medicine, monitoring and tracing the constituents of different extracts and even for quality control of the complex systems such as TCM.

Introduction

Cistanches Herba (named "Rou Cong Rong" in Chinese, CH), one of the most precious traditional Chinese medicine (TCM) recorded by *Shen Nong's Materia Medica* (A.D. 102–200), helps nourish the kidney and strengthen the '*Yang*' and has remarkable curative effects on kidney deficiency, female infertility and senile constipation [1]. Modern pharmacological studies have demonstrated that its extracts exhibit remarkable effects on enhancing the ability to learn and memorize, treating Alzheimer's disease, enhancing immunity, anti-aging, anti-fatigue and promoting bone formation [2–4]. CH has earned the honor of "Ginseng of the desert" and been widely accepted as a health supplement in Japan, Korea and many other countries for its remarkable and reliable medical benefits.

It is generally accepted that there are four species within *Cistanche* (Orobanchaceae) in China, i.e., *Cistanche deserticola* (CD), *Cistanche tubulosa* (CT), *Cistanche salsa* (CS) and *Cistanche sinensis* (CSN) [1], which are often confused used. Among them, only CD has been specified as the original plant of CH by the Chinese Pharmacopoeia since its first edition, and CT has been recorded since the 2005 edition, because CD is becoming extinct

* Corresponding author. Tel.: +86 10 57833180; fax: +86 10 57833352.
E-mail addresses: junzichen63@gmail.com, jchen@implad.ac.cn (J. Chen).

http://dx.doi.org/10.1016/j.molstruc.2014.03.061
0022-2860/

and CT is much more available [5,6]. Although they look similar and derive from the same genera, different therapeutic functions have been observed, maybe due to different constituents and properties. Therefore, it is highly important to develop a reliable method to identify the chemical constituents and properties of different *Cistanche* species for guarantee of the clinical effect.

Jiang and Tu [7] reviewed the progress in the researches on chemical constituents in *Cistanche* species and their corresponding analysis methods. Phytochemical investigation found that 49 kinds of volatile compounds (VCs) mainly including essential oils, 17 phenylethanoid glycosides (PhGs), and 10 iridoids have been identified from CD; 21 VCs, 20 PhGs and 10 iridoids from CT; 38 VCs and 11 PhGs from CS; but 35 VCs and 3 PhGs from CSN that has not been recommended for medical use. Other chemicals such as alkaloids, polysaccharides, sterols and amino acids have only been extracted from CD, but they have not been studied in other species. Among all the constituents, PhGs have been identified as the main active components for curing kidney deficiency, anti-oxidation and neuroprotection. Echinacoside and acteoside (two major constituents of PhGs), have been used as quality markers for CH, which are determined using high performance liquid chromatography (HPLC) [5]. However, those two compounds might not be sufficient to illustrate fully the quality of CH due to its complex bioactive constituents. By now, the constituents of the PhGs of *Cistanche* have been analyzed using several methods such as high-speed counter-current chromatography (HSCCC) [8] and HPLC [9]. Recently, 10 PhGs were identified and further quantified as marker substances of three *Cistanche* species (CD, CT and CSN) using HPLC coupled with diode array detection and high-resolution mass spectrometry [10]. However, most of the chemical analysis methods are not only time-consuming, but also environmentally unfriendly. Nevertheless, the specific markers identified by chromatography cannot fully reflect the whole qualities of complex systems such as the TCMs, which contain hundreds of components and take effect through synergic reaction. Therefore, it is highly desirable to find a rapid and effective method to monitor and capture the constituent features of similar TCMs and extract products.

Multi-step infrared macro-fingerprint method including conventional Fourier transform infrared spectroscopy (FT-IR), second derivative spectroscopy and two-dimensional infrared correlation spectroscopy (2D-IR) was proposed by Suqin Sun and Isao Noda to provide a rapid and effective analysis of complex systems such as TCM [11–13]. It can not only reveal clearly the main constituents in the sample but also distinguish the varieties and contents of chemical constituents in very similar samples. The method has been successfully used to discriminate raw herbal materials of some *Cistanche* species [14,15]. However, the components such as cellulose and lignin have no medicinal values, but exist in the raw herbal materials. The IR absorbance of cellulose and lignin is so strong that it may easily cause overlapping peaks. Therefore, researchers have focused on extracts of the TCMs recently to avoid misjudgment [16,17]. Moreover, CH is usually macerated in alcohol drinks for oral take, but there is no report on the infrared macro-fingerprint of the extracts of all the different species of *Cistanche* in China. Therefore, this paper aims to reveal the main components and holistic variation laws of the chemical constituents in ethanol extracts of different *Cistanche* species by applying multi-step infrared macro-fingerprint method combined with HPLC fingerprint analysis.

Experiment

Samples and materials

All species of the genus *Cistanche* are perennial parasitic herbs, and mainly distributed in arid lands and warm deserts, such as Badan Jara Desert, Tengger Desert, and Gurbantunggut Desert in Junggar Basin and Taklimakan Desert. The plants of each species were gathered from three different sources (Table 1). Two or three plants of each species were sampled from each source and then mixed together. All *Cistanche* samples were identified and confirmed by Prof. Jun Chen. Voucher specimens were deposited in Institute of Medicinal Plant Development (IMPLAD), Chinese Academy of Medical Sciences and Peking Union Medical College (CAMS & PUMC).

C_{18} column (phenomenex, 250 mm × 4.6 mm, 5 μm); acetonitrile was chromatographically pure from Thermo Fisher Scientific, America. Other solvents (ethanol and acetic acid) were analytical reagent (AR) grade, from Beijing Chemical Works, China. Reference substances echinacoside and acteoside were purchased from National Institute of Control of Pharmaceutical and Biological Products. Total PhGs were provided by Prof. Yue Shi (IMPLAD, CAMS & PUMC).

Apparatus and parameters

Spectrometer (GX FT-IR, PerkinElmer), equipped with a DTGS detector was used. Each IR spectrum was recorded from 16 co-added accumulative scans in the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1}. Spectrum was obtained from the 16 accumulative scans. Temperature was controlled by programmable temperature controller (Model 50-886, Love Control Corporation).

Waters 996 high performance liquid chromatography (HPLC) system, including Waters 996 high-pressure pump, Photo-Diode Array (PDA) detector, Empower chromatography workstation (Japan, Shimadzu), and Hamilton automatic sampler was applied for HPLC fingerprint analysis.

Procedure

The dried stems of four species of *Cistanche* were pulverized and then separately extracted with 30 times of ethanol under ultrasonic condition for 20 min. The extract liquids were mixed. Ethanol in extract liquids was then evaporated by using evaporator, yielding the ethanol extracts. Likewise, About 2 mg of obtained powder was blended with 150 mg KBr, then ground again, and pressed into a tablet. After that, the IR spectra of all samples and reference substances were collected at room temperature (20–25 °C). Considering the similarity of the spectral experiments, only one set of the spectra was displayed in Fig. 1. The raw data of FT-IR were processed with on-line spectrum software of PerkinElmer FT-IR spectrometer.

The IR spectra of the herbal samples were compared with each other and with the IR spectra of PhGs and acteoside, and the IR correlation coefficients (IRCs) from different ranges were obtained using Spectrum v3.02 software (PerkinElmer). In addition, all the second derivative IR spectra were obtained after baseline correction, normalization and 13-point smoothing of the original IR spectra by using Savitzky Golay polynomial fitting method.

Table 1
Sample sources of different species of the genus *Cistanche*.

No.	Species Latin name (acronym)	Sample sources location in China (County, Province)
1	*Cistanche deserticola* Y.C. Ma (CD)	Alxa, Inner Mongolia; Jimusaer, Xinjiang; Yongning, Ningxia
2	*Cistanche tubulosa* (CT)	Yutian and Hutian, Xinjiang; Dengkou, Inner Mongolia
3	*Cistanche salsa* (CS)	Yanchi, Ningxia; Guide and Haiyan, Qinghai
4	*Cistanche sinensis* (CSN)	Haiyuan, Ningxia; Alxa, Inner Mongolia; Qitai, Xinjiang