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Fourier-Transform Infrared Spectroscopy for Discrimination of *Cynomorium songaricum* Inflorescence Samples from Different Origins

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Cynomorium songaricum Rupr. has been used as a medicinal tonic in China for thousands of years. The Fourier-transform infrared (FT-IR) spectroscopy and second-derivative infrared (SD-IR) spectroscopy were used to study 200 samples of *C. songaricum* inflorescence collected at 20 sites in four different regions. A resemblance was found among the FT-IR spectra of samples from different origins and developed an IR fingerprint spectrum. The main absorption peaks in the range 1800-1200 cm^{-1} suggests that *C. songaricum* inflorescences may be rich in phenolic compounds, proteins and flavonoids. The SD-IR spectra of samples from different origins revealed an obvious intensity differences for peaks in the range 1800-1200 cm^{-1}, especially for samples from Gansu province of China. An origin discrimination model based on principal component analysis is established. The prediction accuracy of the model is 87.25%. The results indicated that our origin discrimination model can differentiate production areas for *C. songaricum*.

Keywords: *Cynomorium songaricum*, Fourier-transform infrared spectroscopy, Origin discrimination.

INTRODUCTION

Cynomorium songaricum Rupr., a traditional Chinese medicine, is a perennial parasitic herb that lives mainly on the roots of *Nitraria* Linn. and used to treat lumbar weakness, impotence, digestive disorders and diarrhea [1]. *C. songaricum* is abundant in bioactive compounds, including triterpenes, flavanoids, condensed tannins, alkaloids and glycosides [2,3]. It is primarily found in Qinghai and Gansu provinces and in the Ningxia Hui and Inner Mongolia autonomous regions of China, especially in Inner Mongolia. Differences in quality have been observed for *C. songaricum* from different origins. Therefore, it is important to discriminate the sample origin for studies and applications of *C. songaricum*. There have been several reports describing chemical constituents and pharmacologic actions of *C. songaricum* [4-7].

Fourier-transform infrared (FT-IR) spectroscopy can be used for effective, rapid, and non-destructive analysis of complex compounds and has been widely applied in agriculture, food, and chemical fields, among others [8-12]. In Chinese medicines, the origin of multiple materials has been rapidly identified *via* FT-IR spectroscopy coupled with stoichiometry [13-15]. The aim of the present study is to use FT-IR combined with second derivative infrared (SD-IR) spectroscopy and prin-cipal component analysis (PCA) to analyze the characteristics of *Cynomorium songaricum* inflorescence samples from the different origins to develop a method for discriminating the origin of *Cynomorium songaricum*.

EXPERIMENTAL

Collection of samples: The 200 samples of the optimal harvest time of *C. songaricum* were collected at 20 sites in four regions in China *viz*. Qinghai (QH) and Gansu (GS) provinces, and the Ningxia Hui (NX) and Inner Mongolia (NM) autonomous regions (Table-1). Plant material was authenticated by Dr. Xuefeng Lu, Northwest Institute of Plateau Biology, Chinese Academy of Sciences in Xining, P.R. China. Voucher specimens were deposited in the Qinghai-Tibet Plateau Biological Herbarium Department.

TABLE-1
COLLECTION SITES FOR *C. songaricum*

Origins	No.	Collection site	Altitude (m)	Longitude (E)	Latitude (N)	Sample quality
Qinghai province (QH)	1	Nuomuhong	2760	96.35°	36.47°	16
	2	Caka	3073	98.93°	36.77°	14
	3	Delhi city	2817	96.90°	37.32°	11
	4	Dulan County	2867	97.53°	37.20°	9
	5	Wayu	3086	99.32°	36.55°	14
	6	Xiangride	3108	97.92°	36.00°	4
	7	Xiariha	3221	98.21°	36.44°	4
	8	Nachitai	3623	94.62°	35.90°	4
	9	Mohe	3093	98.98°	36.80°	11
Alxa in Inner Mongolia autonomous region (NM)	1	Youqi	1420	101.95°	39.32°	14
	2	Jilantai	1047	105.82°	39.65°	15
	3	Youqi	1437	100.55°	39.72°	13
	4	Zuoqi	1369	105.55°	38.73°	4
	5	Zuoqi	1411	104.05°	40.17°	5
	6	Youqi	1357	100.60°	39.77°	15
	7	Guaishicheng	1362	101.58°	39.60°	4
	8	Youqi	1362	101.60°	39.62°	12
Ningxia Hui autonomous region (NX)	1	Zhongwei	1276	104.99°	37.53°	15
Gansu province (GS)	1	Anxi	1342	95.58°	40.20°	13
	2	Minqin	1329	103.33°	38.93°	3

General procedure: The inflorescence from each dried plant sample was pulverized to a coarse powder and passed through an 800-mesh sieve for IR spectrum. Interference due to H_2O and CO_2 was subtracted during scanning.

Detection method: IR spectra were recorded on an iS50 FT-IR system (Thermo Nicolet, USA) equipped with a DTGS detector. Spectra were obtained over the range 4000-400 cm^{-1}.

RESULTS AND DISCUSSION

IR fingerprint spectrum for *C. songaricum* inflorescence samples: Spectra for 200 samples collected at 20 sites in four regions (Table-1) were averaged using OMNIC v7.0. The IR spectra for *C. songaricum* inflorescence samples from four origins (Fig. 1) is obtained. Comparison of these IR spectra reveals similarity for samples from the four regions, indicating they may have similar chemical constituents. An IR fingerprint spectrum for *C. songaricum* inflorescences (Fig. 2) by averaging IR spectra of all samples, which reflects the overall characteristics of *C. songaricum* inflorescences and can be used to identify the authenticity and investigate the quality of *C. songaricum* material [16]. As shown in Fig. 2, the characteristic peaks in spectra of *C. songaricum* inflorescence samples were observed at 3387, 2928, 1617, 1529, 1444, 1384, 1284, 1237, 1103 and 1056 cm^{-1}.

IR peak assignments: The main IR absorption bands for *C. songaricum* inflorescences were assigned according to literature data. Peak positions and their assignment are listed in Table-2. The peak at 3387 cm^{-1} is due to stretching vibration of O–H groups in phenolic acid compounds and carbohydrates. The peak at 2928 cm^{-1} is attributed to anti-symmetric stretching vibrations of methylene C–H groups. The peak around 1752 cm^{-1} is assigned to stretching vibrations of C=O groups. The peaks at 1653 and 1546 cm^{-1} are due to stretching vibrations of C=O groups of amide-I bands and of C–N bonds, and to bending vibrations of N–H groups of amide-II bands, respectively in proteins [17]. The strong peaks around 1617, 1529, and 1444 cm^{-1} are assigned to stretching vibrations of phenyl groups in aromatic compounds, indicating a high polyphenol

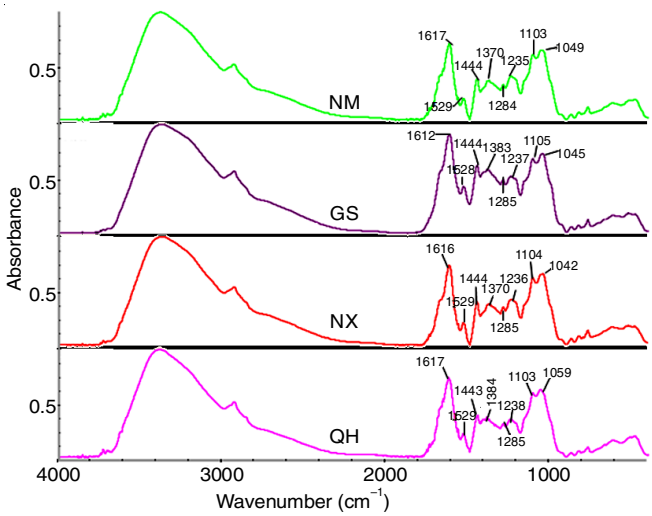

Fig. 1. IR spectra of *C. songaricum* inflorescences from different origins

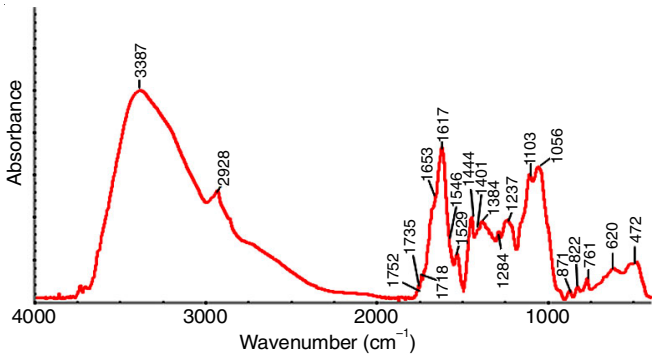

Fig. 2. IR fingerprint spectrum of *C. songaricum* inflorescences

TABLE-2
ASSIGNMENT OF IR SPECTRAL PEAKS
FOR *C. songaricum* INFLORESCENCES

Wavenumber (cm^{-1})	Primary assignment	Main attribution
3387	ν(O–H)	OH
2928	ν_{as}(CH–H)	CH_2
1752	ν(C=O)	
1735,1718	ν(C=O)	Aromatic acid esters
1653	ν(C=O)	Protein
1546	ν(C–N), δ(N–H)	Protein
1617	ν_{as}(C–O)	Polyphenols
1529,1444	ν(Ar: C=C)	Aromatics
1420,1401	δ(=CH)	Aromatics
1384	$\delta_s(CH_2–H)$	CH_3
1370	$\delta_s(CH_2–H)$, δ(C–H)	CH_3, R_3–C–H
1284	ν(C–O), δ(C–C=O), ν(O–H)	Fatty acids and polysaccharide
1237	δ_s(C–C=O)	Glucosides
1103, 1056	ν(C–O)	Polysaccharides, glycosides carbohydrate

content [18]. The peak at 1401 cm^{-1} is assigned to the bending vibrations of C–H groups [19]. The peaks at 1384 and 1370 cm^{-1} are attributed to C–H deformation vibrations of methyl groups [20,21]. The peak at 1284 cm^{-1} is assigned to stretching vibrations of C–O groups in fatty acids and polysaccharides and to stretching vibrations of O–H groups. In addition, the peaks at 1284, 1529, and 1401 cm^{-1} demonstrated that *C. songaricum* inflorescences contain flavonoids [18]. The peak at 1237 cm^{-1} is attributed to C–C=O bending vibrations [22], whereas the peaks at 1103 and 1056 cm^{-1} are caused by C–O stretching vibrations in carbohydrates, glycosides and polysaccharides [23].

Analysis of second derivative infrared (SD-IR) spectra for samples from different origins: The IR spectra of *C. songaricum* samples from four different origins are similar (Fig. 1), except the intensity of the peaks at 1444 and 1384 cm^{-1} is weaker for the QH sample than for the others. Differences among the spectra were amplified by SD-IR and several overlapping peaks in the original spectra were split into two or more peaks, which enhanced the apparent spectra resolution and revealed further chemical information. To further analyze *C. songaricum* from different origins, SD-IR spectra in the range 1800-1200 cm^{-1} were obtained after seven point smoothing of the original spectra (Fig. 3).

Differences not visible in the IR spectra are become clearer in the SD-IR spectra. Fig. 3 shows that the positions of the main peaks in the range 1800-1200 cm^{-1} for *C. songaricum* samples from different origins are similar, indicating they have similar chemical components, but differences in peak intensity are noticeable. The peak intensity is lower for the GS and NX samples than for the NM and QH samples, and the peak intensity in the range 1800-1200 cm^{-1} is lowest for GS samples. This indicates that content of corresponding compounds of GS and NX accumulated lower than that of other origins. Moreover, sharp and strong peaks are evident at 1774, 1752, 1735, 1701, 1685, 1652, 1617, and 1561 cm^{-1}, from which it can be deduced that *C. songaricum* inflorescences are high in ester, acid and

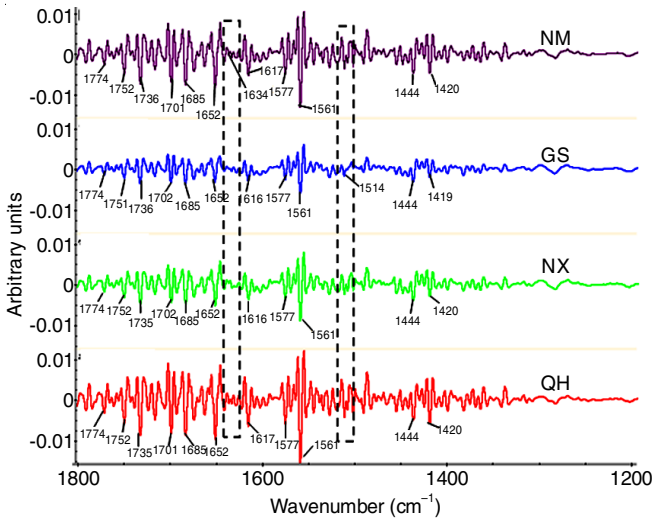

Fig. 3. SD-IR spectra of *C. songaricum* inflorescence samples from different origins

protein content. In addition, GS samples have only one absorption peak around 1514 cm^{-1}, while samples from other origins have two peaks. There is a difference in the shape of the peak around 1634 cm^{-1} for samples from different origins, which indicates that *C. songaricum* inflorescences from the different origins may have different species of aromatic compounds.

Origin discrimination for *C. songaricum* inflorescence samples: To establish an origin discrimination model for *C. songaricum* inflorescence samples from different origins, PCA was used and then estimated its prediction accuracy. The spectral range 1800-1200 cm^{-1} was chosen for PCA because this reflects the characteristics of the samples. For all the spectral data for all the samples, PCA was used. The eigen values and variance proportion for the first four principal components based on all samples are shown in Table-3. The cumulative variance proportion for the first three principal components is 77.35%, which reflects the majority of chemical information for the samples. An origin discrimination model is established for *C. songaricum* inflorescence samples from different origins based on these first three principal components (Fig. 4). For origin discrimination, two or three samples were selected from each site as prediction samples (total of 48 samples), and the remaining 152 samples were used as training samples. As shown in Fig. 4, separation between GS samples and samples from other origins is mainly due to the first principal component (PC1), although the second principal component (PC2) is also a factor in distinguishing GS and QH samples. In addition, *C. songaricum* inflorescence samples from GS, QH, and NM are obviously distinguished, while samples from NX cluster with samples from NM. This result could be explained by the closeness of NX and NM collection sites and habitat similarities. Furthermore, QH samples collected from Caka cluster with GS samples, indicates that the quality of *C. songaricum* in Chaka differs from that of other QH samples but is similar to the quality of GS samples.

To validate the accuracy of present origin discrimination model, we applied the model to 48 prediction samples. The results show a prediction accuracy of 87.25%. This indicates

TABLE-3
EIGEN VALUES AND VARIANCE PROPORTION FOR THE FIRST FOUR PRINCIPAL COMPONENTS IN IR SPECTRA OF *C. songaricum* INFLORESCENCE SAMPLES

Principal component number	Eigen value	Variance proportion (%)	Cumulative variance proportion (%)
1	1105.85	46.44	46.44
2	459.43	18.46	64.90
3	309.95	12.45	77.35
4	156.70	6.30	83.65

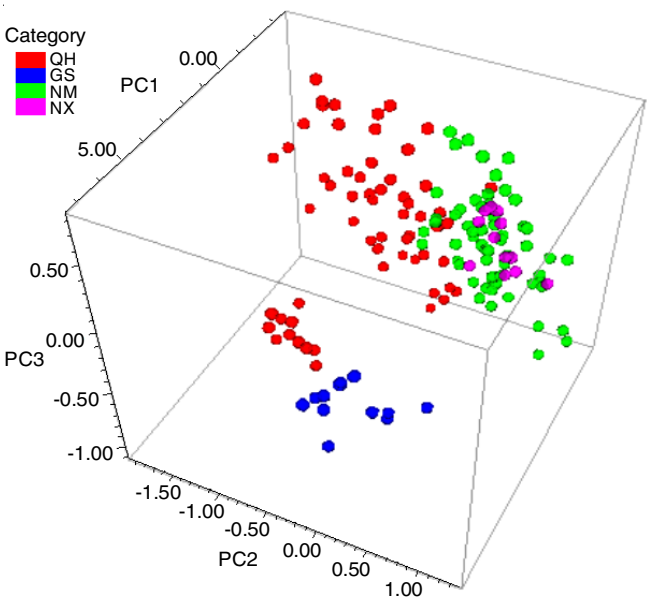

Fig. 4. Origin discrimination model for *C. songaricum* inflorescence samples

that the origin discrimination model based on PCA can be used to determine the origin of *C. songaricum* inflorescence samples.

Conclusion

An IR fingerprint spectrum was established with strong absorption peaks at 3387, 2928, 1617, 1529, 1444, 1384, 1284, 1237, 1103 and 1056 cm^{-1} to distinguish the origin of *Cynomorium songaricum* inflorescence samples. The IR data indicated that *C. songaricum* inflorescences may contain phenolic compounds, proteins, flavonoids, glycosides and fatty acids. An origin discrimination model is established to distinguish *C. songaricum* from different origins on the basis of peaks in the range 1800-1200 cm^{-1}. Validation confirmed that the prediction accuracy of the model is 87.25%. Present results indicated that FT-IR spectroscopy combined with PCA can be used to identify *C. songaricum* from different origins. This method could be applied to distinguish the origin of traditional Chinese medicines rapidly and non-destructively.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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