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Research Article

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Fourier Transform Infrared (FT-IR) Spectroscopy for discrimination of *fenugreek* seeds from different producing areas

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ABSTRACT

Due to fenugreek based on different grown environment has vital differences in quality and physiological efficacy, this study focused on classification and identification of fenugreek seeds from different production areas using Fourier Transform Infrared (FT-IR) Spectroscopy coupled with cluster analysis. Chemometrics fequencies at 1800-400 cm⁻¹ were exploited for the establishment of all chemometric models after systematic noise reduction, 13-point smoothing, and normalization of raw FTIR data. Results showed that the spectral data of fenugreek seeds from different production areas had many similarities and differences mainly because of the different kinds of material compositions. Therefore, infrared spectroscopy coupled with multivariate analysis is an effective, specific, rapid, nondestructive, and nonpolluting identification method of identifying fenugreek seeds from different production areas. The proposed methodology can also reflect the differences among fenugreek seeds material component contents from different producing areas.

Keywords: Fenugreek seeds, Fourier Transform Infrared Spectroscopy, Discrimination, Multivariate statistical analysis, Cluster analysis

INTRODUCTION

Fenugreek (Trigonella Foenum-groecum Linne) is an annual crop belonging to the Fabaceae family [1]. This crop is an important and one of the oldest medicinal plants on record, it is native to an area extending from Iran to northern India, but is now widely cultivated in China, north and east Africa, Ukraine, and Greece [2]. The first recorded use of *fenugreek* is described on an ancient Egyptian papyrus dated to 1500 B.C [3]. There are a huge number of studies that returned positive findings, signifying the efficacy of *fenugreek* seeds as a functional food and as a medicine. In the Middle East and the Balkans, plant is a folk remedy for abdominal cramps associated with both menstrual pain and diarrhea or gastroenteritis [4]. They are also used to ease labour pains. Today, *fenugreek* is used as a folk or traditional remedy for diabetes and loss of appetite, and to stimulate milk production in breastfeeding women [5]. It is also applied to the skin for inflammation. In modern Egypt, *fenugreek* is still used as a supplement in wheat and maize flour for bread making [6, 7]. The plants leaves and seeds are widely consumed in Indo-Pak subcontinent as well as in other oriental countries as a spice in food preparations, and as an ingredient in traditional medicine [3]. In the ancient Indian traditional system of medicine, Ayurveda, *fenugreek* has been suggested as an important medicine to treat a variety of digestive and mucosal conditions [8]. Also has been used traditionally as a carminative,

demulcent, expectorant, laxative and stomachic agent [9]. Traditional Chinese herbalists used it for kidney problems and conditions affecting the male reproductive tract [10], also used as a tonic, as well as a treatment for weakness and edema of the legs [11]. *Fenugreek* is well known for its multiple pharmacological properties including antidiabetic, antifertility, antimicrobial, antioxidative, hypocholesterolemic, antineoplastic, antinociceptive, antiinflammatory, antiulcerogenic, antipyretic, immunomodulatory and antitumor [12-17]. Phytochemical investigations have found that the mature *fenugreek* seed has many active components such as amino acids, fatty acids, vitamins and saponins such as disogenin, gitogenin, neogitogenin, homorientin, saponaretin, neogigogenin and trigogenin, fibers, flavonoids, polysaccharides, fixed oils and some identified alkaloids, that is, trigonelline and choline [9,17]. Given the disparities in the medicinal uses according to the variations in the planting area, significant distinctions may exist in the content of active compounds. Indeed, *fenugreek* may be cultivated in different countries, so its constituents may vary depending on the nature of the seeds under investigation [18].This work presents the results of the application of a chemometric technique to the FTIR (Fourier transform infrared spectroscopy) data obtained from samples of *fenugreek* seeds selected from different regions. Cluster analysis (CA) was applied for the characterization and discrimination of the various seeds samples.

As a method of instrumental analysis, Fourier Transform Infrared (FT-IR) Spectroscopy has been the most extensively used and well-established tool to determine functional groups and polar compounds based on structural vibration analysis for traditional herbal medicine identification. This emergent technique has such obvious advantages as effective, specific, rapid, nondestructive, and nonpolluting [19, 20]. Owing to the fingerprint characters and extensive applicability to the samples, FT-IR has played an important role in pharmaceutical analysis in recent years [21]. The infrared spectrum of any compound is known to express a unique "fingerprint", and this feature combined with the multivariate analytical techniques enable IR spectroscopy to be used in classification of different samples or identification of unknown samples [22].

Multivariate statistical analysis, also referred to as multivariate analysis, was developed from the classical statistical analysis method. This method can analyze statistical data in the case of a plurality of objects and a plurality of indicators associated with each other [23, 24]. Cluster analysis (CA) is one of the main types of the multivariate analysis, also known as group analysis, is a class of statistical techniques, which classifies a set of spectra into two or more groups based on combinations of interval variables [29]. This type of analysis is often very useful at an early stage of an investigation to explore subpopulations in a data set [30]. Clustering is used in many fields, such as mathematics, computer science, statistics, biology, and economics. These technical methods are used to describe data and measure the similarities between the different data sources, as well as the classification of the data source to the type of cluster [31].

Currently, establishing a method, which can promptly and precisely discriminate *fenugreek* seeds from different production areas for assurance of quality control and safe application in medicine is constructive. The aim of this project was to investigate the potential in combining a chemometric technique with the FTIR spectroscopy to discriminate and to study the chemical phylogenetic relationships among different *fenugreek* seeds from different producing areas.

EXPERIMENTAL SECTION

Sample preparation

Nine batches of samples of *fenugreek* seeds were purchased from different provinces in China. And one final sample from Saudi Arabia, which makes a total of 10 samples (table 1). All *fenugreek* seeds were separately pulverized by a disintegrator, screened through a 200-mesh sieve. All samples were stored in a plastic bag for further use.

FTIR spectroscopy analysis:

Apparatus

FTIR spectra were recorded in the region of $4000-400 \text{ cm}^{-1}$ on a TENSOR-27 IR spectrometer (Bruker). The FTIR spectrometer was situated in an air-conditioned room (21 °C, 50% relative humidity).

Analysis conditions

In this experiment, each spectrum was calculated from a total of 32 co-added scans with a resolution of 4 cm⁻¹. Prior to data analysis, the FTIR spectra were baseline corrected and smoothed with their absorbance normalized at 1900 cm⁻¹. Pure potassium bromide (KBr) background spectrum was recorded before analysis of the samples. One

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milligram of *fenugreek* seeds powder was incorporated into 100 mg spectroscopic grade KBr powder (1% w/w) and pressed into a 1.0-mm transparent disk by 8 MPa pressure for transmission infrared spectroscopy. The organic functional groups of the *fenugreek* seeds were analyzed using a TENSOR-27 IR spectrometer (Bruker) in the frequency range of 4000 cm⁻¹ to 400 cm⁻¹. Each sample was scanned with three replicates. The scans of each sample were examined for consistence and the average spectrum of each sample was used for further analyses.

Table 1 The list of *fenugreek* samples

Samples	Source						
S01	Shanxi, China						
S02	Inner Mongolia Autonomous Region, China						
S03	Gansu, China						
S04	Anhui, China						
S05	Henan, China						
S06	Hunan, China						
S07	Shandong, China						
S08	Xinjiang , China						
S09	Xinjiang , China						
S10	Qassim, Saudi Arabia						

Multivariate analysis

Data processing

Data analysis was performed using a multivariate statistical method, cluster analysis (CA) using the SPSS version 19.0 software (USA). Pretreatment includes the removal of absorption intrusions from atmospheric water vapor and CO_2 . Baseline correction is applied to correct the sloping and curved baselines encountered in spectra. Performing FT-IR data preprocessing is often necessary to reduce systematic noise, such as baseline variation, path length differences, and smoothing, and to enhance the influence of the chemical functional groups. The raw FTIR data were obtained after systematic noise reduction, 13-point smoothing, and normalization, and saved as ASC format. The band areas analyzed were in the spectral range of 4000 cm^{-1} to 400 cm^{-1} .

Chemometric model development

The application of spectroscopy coupled with chemometric methods can accentuate the dissimilarities between samples and downgrade the disparity caused by physical consequences in many fields. To report the main components and the holistic variation rules of chemical constituents among different *fenugreek* seeds samples from different origins, data analysis was conducted using a multivariate statistical method in particular CA with the SPSS software (USA). In the current study, taking the characteristic of the spectra of the samples into consideration, the IR fingerprint region of 1800–400 cm⁻¹ was chosen for the establishment of the chemometric model (CA), in which the interferences of H₂O and CO₂ were subtracted.

The aim of (CA) is to launch the best grouping of the multivariate observations. By means of the clustering method of Ward's algorithm using squared Euclidean distance as distance measure method, on the spectral region of $4000-400 \text{ cm}^{-1}$, hierarchical CA was constructed to show the resemblances of spectra graphically in a chemical phylogenetic dendrogram. The spectral distance, also known as the d-value, is a measure of the similarity of the two isolates spectra, and reveals the size of nonoverlapping areas of both spectra. According to the principle of cluster analysis, and in this case, the more similarity between different IR spectra of different *fenugreek* samples, the less relative distance between them on the dendrogram which indicates the more close chemical phylogenetic relationship between them.

RESULTS AND DISCUSSION

Analysis of FTIR spectra of the investigated *fenugreek* seeds

In this study, to investigate the functional groups and polar compounds of the *fenugreek* seeds samples based on structural vibration analysis; the fingerprint technique of FTIR spectroscopy has been applied as a rapid, nondestructive analysis tool, in the spectral region of 4000 cm^{-1} to 400 cm^{-1} . *Fenugreek* seeds have a complex chemical system. As expected, the conventional IR spectrum of *fenugreek* shows its overall chemical composition, allowing the characterization of the herb as a fingerprint. Figure 1 compiles the averaged FTIR spectra of *fenugreek* seeds from different production areas under a room temperature. The general bands assignments for the *fenugreek* seeds FTIR spectrum are shown in Table 2.

No.	Band (cm ⁻¹)	Vibration mode	Samples wavelengths (cm ⁻¹)									
			S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
1	3422	O–H stretching	3422	3421	3398	3421	3421	3354	3421	3444	3394	3421
2	2927	C-H stretching	2927	2926	2927	2927	2927	2927	2927	2926	2926	2927
3	2855	C-H stretching	2855	2856	2855	2856	2855	2856	2856	2856	2855	2856
4	1745	C=O stretching	1745	1744	1745	1745	1745	1745	1745	1743	1745	1745
5	1651	C=O stretching	1651	1649	1651	1651	1651	1653	1650	1649	1652	1650
6	1541	Aromatic skeletal stretching	1541	1541	1541	1540	1541	1541	1541	1541	1540	1541
7	1523	Aromatic skeletal stretching	1523	0	1524	1523	0	0	0	1517	0	0
8	1456	CH ₂ deformation stretching	1456	1457	1456	1456	1456	1455	1457	1457	1456	1457
9	1383	Aliphatic C–H stretching	1383	1384	1316	1384	1384	1384	1384	1384	1384	1384
10	1239	C-C plus C-O plus C=O stretching	1239	1237	1239	1239	1239	1239	1239	0	1239	1239
11	1150	C–O–C stretching, C=O stretching	1150	1154	0	0	0	0	1155	0	0	1155
12	1073	C–O,C=O, C–H stretching	1073	1072	1072	1072	1071	1070	1071	1073	1051	1071

Table 2 Comparison of the main peaks assignment of ten samples of *fenugreek* seeds from different locations

Based on the spectral data, a high similarity was observed between the different *fenugreek* samples. As shown in Figure 1, infrared spectrogram of the 10 different samples of *fenugreek* seeds powder is a typical ladder map and can be generally divided into two parts, namely, 4000 cm^{-1} to 2500 cm^{-1} and 1800 cm^{-1} to 400 cm^{-1} . Detailed averaged peak positions of the samples are marked in the figure. It is obvious that they have distinct fingerprint features particularly in the range of $1300-1700 \text{ cm}^{-1}$ and $1000-1200 \text{ cm}^{-1}$. A broad band was observed at 3700 cm^{-1} to 3000 cm^{-1} , two mid-strong bands were observed at 3000 cm^{-1} to 2800 cm^{-1} , an intense band was observed at 1745 cm^{-1} , and many overlapped bands were detected at the spectral region of 1800 cm^{-1} to 600 cm^{-1} . Through comparative analysis of IR spectra of the ten samples of *fenugreek* seeds from different producing areas, many differences were observed in the position or intensity of the absorption bands, although the IR spectra of the shapes of samples have high similarity.



Fig. 1.Averaged FT-IR spectra of *fenugreek* seeds samples from different producing areas in the range of 4000–400 cm⁻¹

In detail, the strong peak at 3422 cm^{-1} is assigned to the stretching vibration of the O–H groups in phenolic acid compounds and carbohydrates. All the samples have the asymmetric and symmetric stretch vibration of –CH2 group at 2927 and 2855 cm⁻¹ indicating that have fat. The peak at 1745 is due to the stretching vibration of the C=O stretching groups in unconjugated ketone, carbonyl, and aliphatic xylan. The peak at 1651 cm⁻¹ is also assigned to the stretching vibration of the C=O groups of the conjugated and aromatic rings. These data indicate that different type of carbonyl compounds existed in *fenugreek* seeds. The bands centered at 1651, 1541, 1523, and 1456 cm⁻¹ belong to the stretching vibrations of the aromatic ring. The peak at 1383 cm⁻¹ is due to the stretching vibration of the aliphatic C–H stretching groups in methyl and phenol O–H. The peaks at 1239, 1155, and 1073 cm⁻¹ were

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mainly attributed to the stretching vibration of C–O, which showed the characteristic absorptions of polysaccharides and coumarin. The peak at the 820 cm⁻¹ to 500 cm⁻¹ range was mainly attributed to the variable angle vibration and bending vibration of alcohols containing hydroxyl groups and benzene rings. Above all, substantial information on the chemical composition of *fenugreek* seeds can be attained from its IR. Besides the slight differences, the ten samples cannot be effectually identified by the naked eye. Therefore, in order to achieve effective identification, a chemometric method was applied to establish a model for classification and identification.

Multivariate analysis Cluster analysis

Cluster analysis

In this part, FTIR data analysis used a multivariate statistical technique to show spectral information. The technique chosen was CA, which is a perspicuous way to provide intuitive similarity relationships between each sample and the entire data set based on CA of their IR spectra. Nine absorption peaks in the spectral range between 1800 to 400 cm–1 were chosen for classification because they primarily reflect the differences amongst the samples. After that, the paired group algorithm was used without any prior parameterization of the spectral data.

As shown in the dendrogram (Figure 2), the different *fenugreek* seeds samples were grouped into two well-separated clusters with a relative distance of 25. The samples S07, S10, and S02 were the most similar, followed by S05, S09, and S06 that joined to form the first cluster, suggesting the presence of one major cluster in this analysis. Then, the samples S03, S04, and S01 were similar and joined to form a cluster, together with the sample S08, form a major cluster, suggesting the presence of the second major cluster.

The results of the dendrogram grouped the ten samples into 2 clusters, which showed that the S07, S10, S02, S05, S09, and S06 had the most similar chemical compositions. The results reflect clearly the real internal correlation of the different samples and provide a solid foundation for future study.



Fig. 2. Hierarchical cluster analysis with FT-IR spectra of different *fenugreek* seeds samples

CONCLUSION

In this study, FTIR spectroscopy coupled with cluster analysis was applied to discriminate the ten *fenugreek* seeds samples from different productions areas. The general information of the chemical composition and compositional differences of the ten samples was successfully attained, according to the fingerprint technique, including the positions, relative intensities and shapes of the absorption peaks. The present research showed that CA could generate good exploratory and predictive results. Thus, from these results presented in this study, we conclude that the Fourier Transform Infrared (FT-IR) Spectroscopy combined with the chemometric (CA) is an effective, specific,

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rapid, nondestructive, and nonpolluting method and can be can be applied to classify and identify *fenugreek* seeds from different producing areas.

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