Element analysis and characteristic identification of non-fumigated and sulfur-fumigated *Fritillaria thunbergii Miq.* using microwave digestion-inductively coupled plasma atomic emission spectrometry combined with Fourier transform infrared spectrometry

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ABSTRACT

Background: Sulfur-fumigation may induce chemical transformation of traditional Chinese medicines leading to harmful effects following patient ingestion. For quality control, it is urgently needed to develop a reliable and efficient method for sulfur-fumigation identification. **Materials and Methods:** The spectrochemical identification of non-fumigated and sulfur-fumigated *Fritillaria thunbergii Miq.* was carried out to evaluate inorganic elements and organic components. The concentrations of 12 elements, including Zn, Mn, Cu, Fe, Li, Mg, Sr, Pb, As, Cd, Hg, and S of samples were determined by microwave digestion - inductively coupled plasma atomic emission spectrometry (ICP-AES). Meanwhile, Fourier transform infrared spectrometry (FTIR) was used for the study of chemical group characteristic reactions after sulfur-fumigation. **Results:** The concentrations of Fe, Mg, Hg, and S elements showed significant differences between non-fumigated and sulfur-fumigated Fritillaria thunbergii Miq. The characteristic stretching vibrations of some groups in FTIR spectra, such as -OH, -S = O and -S-O, provided the identification basis for the discrimination of non-fumigated and sulfur-fumigated Fritillaria thunbergii Miq. **Conclusion:** The application of microwave digestion - ICP-AES was successfully used in combination with FTIR to authenticate and evaluate the quality of medicinal *Fritillaria thunbergii Miq.* Further applications of this technique should be explored.

Key words: *Fritillaria thunbergii Miq.*, Fourier transform infrared spectrometry, microwave digestion - ICP-AES, sulfur-fumigation

INTRODUCTION

Fritillaria thunbergii Miq. has been widely used as anti-tussive and expectorant drugs in traditional Chinese medicine for more than 2000 years. Fritillary bulb contains a variety of chemical composition, with starch being the major constituent, accounting for 70% of the total biological amount.[1] Steroidal alkaloids, such as peimine and peiminine, make up the remaining bioactive components.[2,3] Currently, most *Fritillaria thunbergii Miq.* are processed by sulfur-fumigation for long-term storage.

Sulfur-fumigation is an effective post-harvest processing treatment that can shorten the drying duration, control pests, and maintain as well as preserve outward appearance and has replaced the traditional post-harvest sun-drying process in the past decades.[4] However, there have been cases where the pursuit of heightened color and luster in Chinese herbal medicines has led to sulfur-fumigation being used excessively, significantly exceeding the standard of SO_2 and heavy metal residues allowed. As sulfur-fumigation

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is a high temperature process, some low-boiling chemical components will be damaged unavoidably.[5,6] However, it is the absorption of sulfur and heavy metals contained in these medicines after patient consumption that is most detrimental to their health.[7]

Inductively coupled plasma atomic emission spectrometry (ICP-AES) has increasingly attracted interest, and is widely used for the analysis of heavy metals and chemical elements in traditional Chinese medicines,[8-10] which offers high precision, high selectivity, and high sensitivity, as well as low limits of detection, and a large linear dynamic range. The method used for sample pre-treatment is an important step to be considered. Microwave digestion has been shown to be a rapid and complete approach for the destruction of organic components, which results in no loss and less pollution than wet digestion and dry digestion.[11] Fourier transform infrared spectrometry (FTIR) method can be used for the study of characteristic reactions, such as dehydroxylation and chemical groups' transformation in the course of sulfur-fumigation.[12] It has been widely applied in TCMs identification,[13] mineral analysis,[14] and food production.[15]

In this study, a combined approach utilizing microwave digestion - ICP-AES and FTIR methods were developed to analyze heavy metals, sulfur, trace elements, and characteristic reactions present in sulfur-fumigated *Fritillaria thunbergii Miq*. to provide a comprehensive, objective, and scientific basis for identification and quality control of non-fumigated and sulfur-fumigated *Fritillaria thunbergii Miq*.

MATERIALS AND METHODS

Microwave digestion - ICP-AES technique

Instrument and operating procedure

An iCAP 6300 ICP Spectrometer (Thermo Electron, England) was used for the measurements. The instrument settings were checked regularly and optimized when necessary. The operating conditions are summarized as follows: RF power: 1150 W, pump rate: 50 r/min, Auxiliary gas flow: 0.5 L/min, nebulizer gas flow: 0.75 L/min, plasma gas flow: 12 L/min, sample washing time: 50 s. Each sample was detected in triplicate, the average and standard deviation values were submitted for data processing. All results were calculated by instrument software.

Chemicals and standards

The stock solutions, S (BW 3180), Cu (GSB 10334), Hg (GSB 09635), Pb (GSB 09647), Cd (GSB 09601), and As (GSB 09521) containing 1000 μg/L of each element and 100 μg/L of each trace multielement (National Research Center for Reference Materials, Beijing, China), were prepared for the reference solution. HNO_3 (GR, Nanjing Chemical Reagent Co., Ltd., Nanjing, China) and 30% H_2O_2 (AR, Nanjing Chemical Reagent Co., Ltd., Nanjing, China) were also purchased.

Mixed trace elements' (Zn, Mn, Cu, Fe, Li, Mg, Sr) calibration standards were prepared by successive dilutions in distilled water with concentrations of 0, 0.5, 1, 2, 5, 10 μg/mL. Heavy metals and sulfur element calibration standards were used in the following ranges: As, Pb, Cd: 0, 0.025, 0.050, 0.100, 0.200, 0.400 μg/mL; Hg: 0, 0.050, 0.100, 0.200, 0.400, 0.800 μg/mL; S: 0, 25, 50, 100, 200, 400 μg/mL.

Sample decomposition

A total of twenty-three *Fritillaria thunbergii Miq*. were acquired from different regions and pharmacies in China. All samples were tested using acid distillation iodine titration method (GB/T 5009.34) for presence of sulfur-fumigation processing. Twelve of them (samples 1-12) were found to be non-fumigated, eleven of them (samples 13-23) were found to be sulfur-fumigated, samples number 24 and 25 were prepared in the laboratory from samples number 1 and 2 by sulfur-fumigation as comparison. All samples were authenticated by an expert in the field.

Grounded *Fritillaria thunbergii Miq*. was precisely weighed (0.5 g) and transferred into a PTFE digester, where 7 mL of HNO_3 and 1 mL of H_2O_2 were added. The digester was sealed tightly and placed in the microwave digestion system (ETHOSONE, Italy, Milestone). The procedure of microwave digestion is summarized as follows: The digester was heated from 25°C to 180°C within 10 min, and then held for 25 min to digest the samples. After complete digestion, the digestion solution was evaporated to 2.5 mL, and transferred to a 50 mL volumetric flask and diluted with de-ionized water. A reagent blank was prepared using the same chemicals and digesting procedure as comparison.

FTIR technique

Instrument and conditions

The same batch of samples were performed on a Nicolet IR100 intelligent FTIR spectrometer (American NICOLET) with a resolving power of 16 cm^{-1}, spectrum range of 4000-400 cm^{-1}, scanning accumulative limitation of 16 times and resolution of 4 cm^{-1}, H_2O and CO_2 interference were deducted when scanning.

Sample preparation and operating procedure

Ethanol extracted samples were prepared as follows: 5 g of powder (45 mesh) of each sample was taken and then 50 mL of 80% ethanol was added. This was followed by ultrasonic treatment for 40 min and percolation. The filtrate was steamed into a thick paste and dried at 65°C to a constant weight. Water extracted samples were prepared following the ethanol extraction method, substituting 50 mL of 80% ethanol with 50 mL of water. 1 mg of the dried powder from ethanol extracted sample or water extracted sample was then taken and ground with 200 mg KBr under IR light till evenly mixed. The mixture was crushed in a mechanical mold to form a tablet. The spectrum of the extract could be obtained by scanning the sample tablet immediately. FTIR spectra and second derivative IR spectra were analyzed by OMNIC 8.0 software.

RESULTS AND DISCUSSION

Analysis of heavy metals, sulfur, and trace elements using microwave digestion - ICP-AES

Validation of the microwave digestion - ICP-AES method

Calibration curves were developed using serially diluted stock element solutions. The detection limit value was defined as the concentration of each element corresponding to three times the standard deviation from the digestion blanks. Analysis parameters are shown in [Table 1]. From Table 1, correlation coefficients (r) of the elements range from 0.9975 to 0.9999, indicating that the applied method has a good linear relationship.

Recovery tests were carried out by spiking known amounts of the reference standard solutions by 0.8-1.2 times to 0.25 g of the testing samples. The resulting recoveries of the method were 88.2-101.7%, RSDs were 0.84-2.77%.

Results of microwave digestion - ICP-AES analysis

In this study, 12 elements were detected, Zn, Mn, Cu, Fe, Li, Mg, Sr, Pb, As, Cd, Hg, and S. A portion of these 12 elements are beneficial to human health, such as Zn, Mn, Cu, Fe, Li, Mg, Sr, whereas others have been shown to be toxic in high concentrations. The results of these elements determined in samples are collectively listed in [Table 2]. T-test was used to evaluate the difference between non-fumigated and sulfur-fumigated samples. Results showed that significant differences exist in Fe, Mg, Hg, and S elements ($P < 0.01$).

Table 1: Analytical conditions and detection limits for determination

Element	Detection wavelength (nm)	Detection limit (ng/mL)	Linear range (μg/g)	Correlation coefficient (r)
Zn	206.2	6.66	0-10	0.9990
Mn	257.6	7.83	0-10	0.9986
Cu	324.7	22.38	0-0.4	0.9992
Fe	259.9	6.57	0-10	0.9995
Li	670.7	19.0	0-10	0.9989
Mg	257.6	15.0	0-10	0.9997
Sr	421.5	0.74	0-10	0.9975
Pb	220.3	3.27	0-0.4	0.9999
As	189.0	3.28	0-0.4	0.9995
Cd	226.5	1.58	0-0.4	0.9963
Hg	184.9	1.34	0-0.8	0.9990
S	180.7	0.21	0-400	0.9991

Fe and Mg elements held higher concentrations in non-fumigated samples. After the sulfur-fumigation process, their concentrations decreased significantly. Decreased Mg and Fe contents will affect the clinical efficacy of medicines. Fe is a critical component for human hemoglobin formation, responsible for oxygen transportation. Decreased Mg can lead to increased excitability of the nervous system, which is disadvantageous to human health.[16]

Hg is a heavy metal element that can cause cumulative poisoning to the human body after substantial absorption. According to the "The Professional Import and Export Stands for Medicinal Herbs and Preparations," the concentration of Hg must be lower than 0.2 mg/kg. In the sulfur-fumigated samples, however, the level of Hg in two samples (number 22 and 23) exceeded the specified range, it is obvious that the samples are unqualified, but they could still be purchased in the market. During the sulfur-fumigation process, industrially inferior sulfur are commonly used which contain heavy metals, such as Hg. In the high treatment temperatures necessary for sulfur-fumigation, these heavy metals will sublimate to the surface of Chinese medicines. This is one of the reasons that sulfur-fumigation cause heavy metals residues in traditional Chinese medicine. In addition, concentration of S element in sulfur-fumigated samples increased fivefold compared to non-fumigated ones as well. Considering how widely *Fritillaria thunbergii Miq*. is used in clinical traditional Chinese medicine decoction and diet, it is self-evident that the increased contents of Hg and S elements will damage human health. Microwave digestion - ICP-AES gives us some available information about inorganic elements analysis in non-fumigated and sulfur-fumigated samples.

FTIR spectra analysis

FTIR spectra and second derivative IR spectra of ethanol extracted samples

Ethanol extracted samples were analyzed by FTIR spectra as well as second derivative IR spectra, the spectral data of these ranges were mean normalized, respectively. The absorption bands of CO_2 and the spectral range of 3696-4000 cm^{-1} contained little useful information for measurement and were excluded from analyses. From [Figure 1a and 1b], non-fumigated *Fritillaria thunbergii Miq*. had fixed absorbances at 1633 cm^{-1}, 1132 cm^{-1}, 1053 cm^{-1}, 996 cm^{-1}, 928 cm^{-1}, and 859 cm^{-1},

Table 2: Average determination results (μg/g) (mean±SD) of heavy metals, sulfur, and trace elements from *Fritillaria thunbergii Miq.* samples

Zn	Mn	Cu	Fe	Li	Mg	Sr	Pb	As	Cd	Hg	S
0.2838±0.0002	0.1743±0.0003	0.0461±0.0002	1.072±0.001	0.0014±0	3.840±0.011	0.0279±0.0001	0.0061±0.0003	0.0002±0.001	0.0024±0	0.0033±0.0001	12.97±0.03
0.3125±0.0002	0.1900±0.0001	0.0395±0.0002	2.253±0.002	0.0024±0	5.642±0.008	0.0257±0	0.0077±0.0005	0.0005±0.0005	0.0021±0.0001	0.0016±0	12.56±0.03
0.4182±0.0002	0.1408±0.0002	0.0488±0.0003	1.355±0.005	0.0017±0	5.237±0.032	0.0250±0	0.0133±0.0004	0.0005±0.0011	0.0033±0.0001	0.0008±0	13.24±0.05
0.3651±0.0007	0.2247±0.0012	0.0395±0.0004	1.816±0.009	0.0021±0	4.806±0.045	0.0247±0.0001	0.0050±0.0002	0.0003±0.0011	0.0047±0	0.0008±0.0001	12.84±0.03
0.2713±0.0002	0.1200±0.0005	0.0295±0.0003	0.8508±0.002	0.0009±0	5.501±0.056	0.0137±0	0.0018±0.0002	0.0002±0.0004	0.0034±0.0001	0.0010±0	12.35±0.02
0.3155±0.0011	0.2249±0.0009	0.0422±0.0002	1.768±0.006	0.0026±0	5.193±0.043	0.0283±0	0.0050±0.0003	0.0003±0.0005	0.0040±0.0001	0.0007±0.0001	11.82±0.03
0.3475±0.0003	0.1945±0.0007	0.0360±0.0002	1.935±0.007	0.0021±0	5.324±0.026	0.0226±0.0001	0.0080±0.0004	0.0001±0.0002	0.0035±0	0.0011±0.0001	11.01±0.03
0.3320±0.0007	0.1334±0.0009	0.0319±0.0003	1.664±0.011	0.0017±0	5.223±0.006	0.0286±0.0002	0.0053±0.0002	0.0004±0.0007	0.0018±0.0001	0.0009±0.0002	10.64±0.03
0.3321±0.0003	0.2208±0.0003	0.0354±0.0001	1.382±0.002	0.0017±0	4.932±0.025	0.0233±0.0001	0.0037±0.0002	0.0005±0.0002	0.0040±0.0001	0.0009±0.0001	11.63±0.01
0.2522±0.0007	0.1416±0.0001	0.0381±0.0003	0.9482±0.06	0.0018±0	5.395±0.035	0.0197±0	0.0030±0.0001	0.0005±0.0003	0.0021±0	0.0006±0.0002	10.81±0.02
0.4302±0.0007	0.1346±0.0006	0.0357±0.0003	3.550±0.012	0.0015±0	4.249±0.021	0.0214±0.0001	0.0047±0.0002	0.0002±0.0003	0.0017±0	0.0008±0.0001	11.91±0.13
0.2138±0.0009	0.1928±0.0011	0.0474±0.0005	4.203±0.022	0.0055±0	5.858±0.02	0.0349±0.0002	0.0106±0.0003	0.0001±0.0003	0.0011±0	0.0007±0.0001	15.07±0.06
0.5424±0.0008	0.2592±0.0006	0.0372±0.0002	1.654±0.003	0.0017±0	3.958±0.01	0.0317±0.0001	0.0058±0.0004	0.0002±0.0002	0.0022±0	0.0630±0.0163	60.78±0.09
0.2823±0.0007	0.1584±0.001	0.0343±0.0004	0.8400±0.0038	0.0010±0	3.748±0.02	0.0302±0.0002	0.0062±0.0004	0.0002±0.0003	0.0022±0	0.0170±0.0008	58.53±0.17
0.2006±0.0006	0.1777±0.0004	0.0333±0.0005	1.016±0.003	0.0010±0	3.068±0.014	0.0237±0.0001	0.0025±0.0005	0.0003±0.0009	0.0023±0.0001	0.0152±0.0013	46.69±0.05
0.2298±0.0005	0.1169±0.0007	0.0354±0.0003	1.197±0.009	0.0013±0	3.972±0.03	0.0321±0.0001	0.0060±0.0005	0.0004±0.0002	0.0021±0	0.0079±0.0002	51.85±0.09
0.2034±0.0008	0.1466±0.0003	0.0357±0.0003	0.9126±0.0015	0.0012±0	3.584±0.018	0.0472±0.0001	0.0052±0.0004	0.0004±0.0004	0.0024±0	0.0077±0.0005	48.16±0.1
0.2420±0.0007	0.1870±0.0005	0.0426±0.0002	1.322±0.004	0.0012±0	3.840±0.011	0.0246±0.0001	0.0036±0.0008	0.0003±0.0003	0.0028±0.0001	0.0055±0.0002	57.71±0.04
0.2307±0.0002	0.1691±0.0009	0.034±0.0003	0.6051±0.0028	0.0032±0	3.633±0.018	0.0221±0.0001	0.0034±0.0003	0.0001±0.0005	0.0021±0.0001	0.0045±0.0003	60.12±0.08
0.2354±0.0008	0.1517±0.0001	0.0371±0.0002	0.7498±0.0022	0.0008±0	3.596±0.01	0.0352±0.0001	0.0058±0.0003	0.0001±0.0004	0.0022±0	0.0034±0.0001	49.96±0.05
0.2336±0.0015	0.1873±0.0001	0.0321±0.0004	0.8181±0.006	0.0010±0	3.568±0.019	0.0273±0	0.0053±0.0004	0.0006±0.0004	0.0024±0	0.0033±0	54.57±0.03
0.1755±0.0002	0.1487±0.0005	0.0303±0.0003	0.9035±0.0036	0.0017±0	3.425±0.012	0.0268±0.0001	0.0058±0.0003	0.0009±0.0008	0.0018±0	0.4751±0.0014	162.7±0.5
0.2372±0.0005	0.1859±0.0002	0.0332±0.0001	0.9086±0.004	0.0010±0	3.335±0.016	0.024±0.0001	0.0053±0.0004	0.0003±0.0005	0.0022±0.0001	0.5268±0.0016	52.56±0.05
0.2160±0.0006	0.1622±0.0004	0.034±0.0001	0.9659±0.0009	0.0010±0	3.356±0.01	0.0291±0.0001	0.0041±0.0001	0.0007±0.0002	0.0020±0	0.0081±0.0005	48.22±0.03
1.205±0.001	0.1445±0.0005	0.0336±0.0002	0.8330±0.0017	0.0009±0	2.970±0.006	0.0199±0	0.0040±0.0004	0.0004±0.0006	0.0017±0.0001	0.0032±0.0001	45.72±0.09

SD: Standard deviation

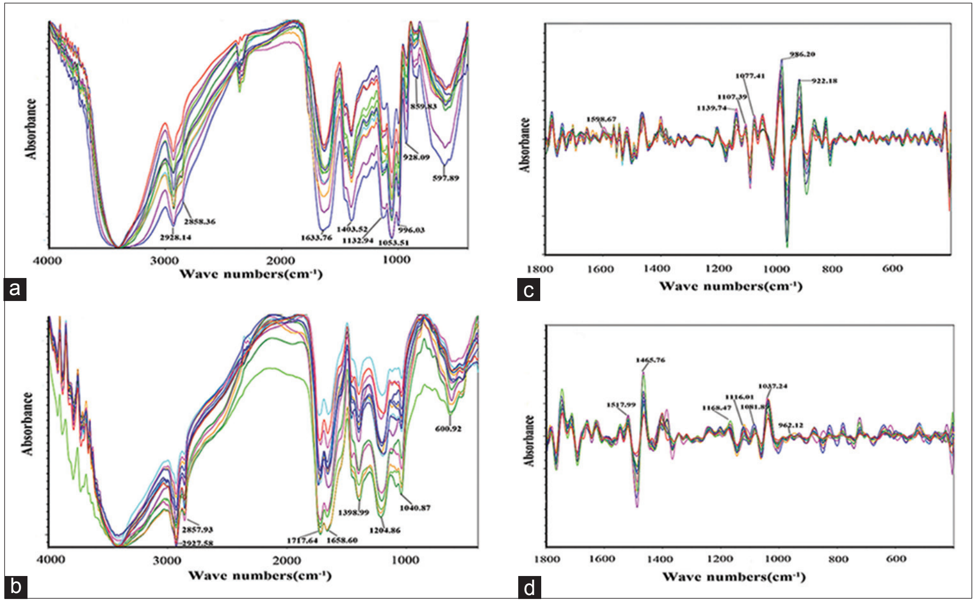

Figure 1: FTIR spectra and second derivative IR spectra of *Fritillaria thunbergii Miq.* extracted by ethanol. (a) FTIR spectra of non-fumigated samples. (b) FTIR spectra of sulfur-fumigated samples. (c) Second derivative IR spectra (1800-400 cm^{-1}) of non-fumigated samples. (d) Second derivative IR spectra (1800-400 cm^{-1}) of sulfur-fumigated samples

while sulfur-fumigated samples had fixed absorbances at 1717 cm^{-1}, 1658 cm^{-1}, 1204 cm^{-1}, and 1040 cm^{-1}. The spectra of these two groups of samples differed markedly in range from 2000 to 600 cm^{-1}. In non-fumigated samples [Figure 1a], the wide bands in 1633 cm^{-1} can be attributed to the stretching of various -C = C. Peaks in 1132 cm^{-1} and 1053 cm^{-1} also involve the contributions of -OH stretching vibrations. Other obvious bands include those found at 996 cm^{-1}, 928 cm^{-1}, and 859 cm^{-1}, which belong to γC-H. As seen from [Figure 1b], at 1717 cm^{-1} and 1658 cm^{-1} absorption, peaks are assigned to -C = O group. The peak at 1204 cm^{-1} is due to vibrations of -S-O, which is absorption of sulfonate. Meanwhile, the peak at 1040 cm^{-1} belongs to -S = O. From the results, the sulfur groups can be clearly identified from infrared spectra after sulfur-fumigation.

From [Figure 1c and 1d], high-resolution second derivative IR spectra were used to evaluate differences, such that it can improve the apparent resolution and provide a more intuitive basis. Sulfur-fumigation derivative absorption peaks following a "sawtooth" shape at 1598 cm^{-1}, 986 cm^{-1}, and 922 cm^{-1} disappeared with respect to non-fumigated samples, and absorbing bands at 1139 cm^{-1}, 1107 cm^{-1}, and 1077 cm^{-1} moved to 1168 cm^{-1}, 1116 cm^{-1}, and 1081 cm^{-1} after sulfur-fumigation in the derivative spectra, which may be the result of increased polarity. The derivative peaks at 1465 cm^{-1} and 1517 cm^{-1} were new appearances and were stronger than other peaks in the spectra of sulfur-fumigated samples.

FTIR spectra and second derivative IR spectra of water extracted samples

The same method was applied to the water extracted samples. From [Figure 2a and 2b], non-fumigated *Fritillaria thunbergii Miq.* showed fixed absorbances at 1052 cm^{-1}, 994 cm^{-1}, and 929 cm^{-1}. Alternatively, sulfur-fumigated samples showed fixed absorbances at 1402 cm^{-1}, 1117 cm^{-1}, and 617 cm^{-1}. In [Figure 2a], the absorption peak was found at 1052 cm^{-1}, belonging to -OH stretching vibrations. Other obvious bands could be identified at 994 cm^{-1} and 929 cm^{-1}, belonging to γC-H, which were similar to the spectra of ethanol extracted samples. For the spectra of sulfur-fumigated samples, the peaks located at 1402 cm^{-1} are $\delta C\text{-}H_3$, and 1117 cm^{-1} and 617 cm^{-1} belong to -C-O-C. The results also provide the evidence of identification.

In [Figure 2c and 2d], derivative absorption peaks at 989 cm^{-1}, 921 cm^{-1}, and 835 cm^{-1} disappeared, whereas a strong derivative absorption peak at 618 cm^{-1} appeared in the spectra of sulfur-fumigated samples.

In summary, after sulfur-fumigation, chemical groups, especially the hydroxyl group, undergo change and new sulfur residue products are also created. Furthermore, FTIR combined with second derivative spectra can provide a simple and reliable basis for analysis of organic groups.

CONCLUSIONS

The study showed that the microwave digestion - ICP-AES technique permits a rapid sequential determination of

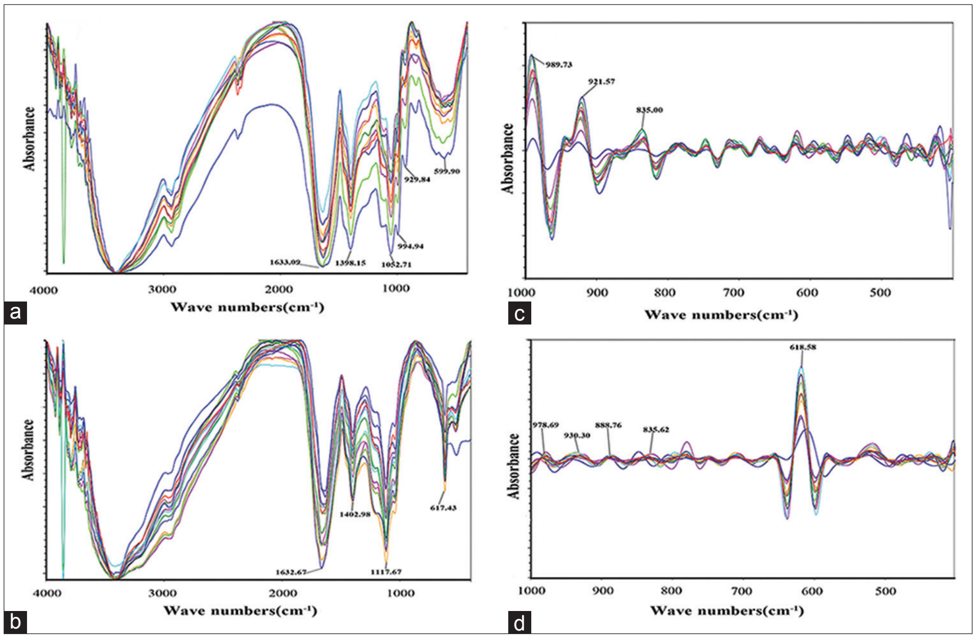

Figure 2: FTIR spectra and second derivative IR spectra of *Fritillaria thunbergii Miq*. extracted by water. (a) FTIR spectra of non-fumigated samples. (b) FTIR spectra of sulfur-fumigated samples. (c) Second derivative IR spectra (1000-400 cm^{-1}) of non-fumigated samples. (d) Second derivative IR spectra (1000-400 cm^{-1}) of sulfur-fumigated samples

different elements in non-fumigated and sulfur-fumigated *Fritillaria thunbergii Miq*. with high accuracy and precision. The contents of Fe, Mg, Hg, and S elements varied significantly in the two processed samples. This method provides an identification profile for inorganic elements analysis.

FTIR, however, is a convenient and effective identification method for herbal medicines (including the relevant plant drugs from the same family or genus), that could be developed through comparing the peak positions, intensity ratios, and observing whether certain absorbing bands exist or not in their IR spectra. It is valuable to assist identification of organic groups. These two complementary approaches could provide more comprehensive and useful idea and information for routine control identification and quality evaluation of non-fumigated and sulfur-fumigated TCMs as well as other processed plant drugs.

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