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Use of X-Ray Diffractometry (XRD) for Identification of *Fritillaria* According to Geographical Origin

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Abstract: *Fritillaria* is a traditional Chinese herbal medicine for anti-tussive and expectorant use in China and some other Asian countries. The *Fritillaria* Chinese medicine material derived from different geographical origins is always difficult to discriminate each other. The objective of the study is to develop a nondestructive and simple method to identify five *Fritillaria* Chinese materia medica such as *Fritillaria thunbergii* Miq., *Fritillaria ussurensis* Maxim., *Fritillaria cirrhosa* D.Don, *Fritillaria pallidifloca* Schrenk and *Fritillaria hupehensis* Hsiao et KC Hsia. X-ray diffraction was utilized to analyze the discrepancy of the *Fritillaria* from different geographical origins. Because *Fritillaria* consists of plenty of starch, which is a very good semicrystalline macromolecule, the X-Ray diffraction spectra of *Fritillaria* powders mainly showed the crystalline properties of starch. The differences in starches crystallinite could be distinguished by X-ray diffraction spectra. X-ray diffraction is proved to be a new and powerful method to discriminate *Fritillaria* from different geographical origins.

Key words: *Fritillaria*, starch, X-ray, crystal type, degree of crystallinity

INTRODUCTION

Fritillaria (Chinese name Beimu), the bulbs of various species of the genus *Fritillaria* (Liliaceae), is a very useful traditional Chinese medicine (TCM) with antitussive and expectorant functions[1-4]. There are many kinds of *Fritillaria* Chinese medicine materials. In China pharmacopoeia (2000 edition), *F. thunbergii* Miq, *F. ussurensis* Maxim, *F. palldifloca* Schrenk, *F. cirrhosa* D.Don and *F. hupehensis* Hsiao et K.C. Hsia are recorded[5]. China has a wide range of *Fritillaria* resources which are distributed extensively. The *Fritillatia* Chinese medicinal materials from different geographical origin are very easy to confuse, in addition, the size of *Fritillaria* bulb is much relevant to the growing conditions[6]. All of these result in the difficulty in original identification of *Fritillaria.*

Up to now, chromatographic method is mainly utilized for the identification of different *Fritillaria* Chinese medicinal materials. Since there are tens of major bioactive components, which are slightly different due to different growing conditions and geographical origins, we can not select only a limited number of specific constituents as essential evaluative criteria. Indeed, there are some contradicting results concerning the contents of some ingredients contained in the *Fritillaria* in the literature[7,8]. In the holistic theory of traditional Chinese medicine, the medicinal materials take effects in curing diseases as a whole. Any method or technique which destroys the wholeness of the traditional Chinese medicine will not be primarily accepted. Recently, there are some methods which keep the integrity of traditional Chinese medicine for discrimination of *Fritillaria* such as Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) and themogravimetric analysis (TGA)[9-10]. FTIR is a nondestructive, fast and integrity-emphasized method. However, FTIR has the poor character of the fingerprint spectrum. If the *Fritillaria* are identified clearly, the two-dimensional correlation infrared spectroscopy under thermal perturbation is always utilized. The DSC and TGA methods are carried out under the heating condition and the chemical constituents in the *Fritillaria* are always destroyed.

X-Ray diffraction has the advantages of strong fingerprint character and nondestruction. The main component in the bulbs of *Fritillaria* species is starch occupying approximately 80% content in the total biomass[11]. Starch is an important polysaccharide reserve in higher plants. It consists of two main components, amylose and amylopectin. Amylose is an α-(1$\rightarrow$4)-D-glucopyranosyl polymer, with linear or lightly branched structures or a mixture of both. The residues in amylopectin are α-(1$\rightarrow$4)-D-glucopyranosse units with α-(1$\rightarrow$6)-linkages at intervals of approximately 20 units, depending on plant sources[12-15]. Starch is a semicrystalline polymer in which amylose forms the crystalline region and amylopectin forms the amorphous region. As for the *Fritillaria* powder, the

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starch crystalline diffraction peaks predominate in the X-ray diffraction spectrum.

In this study, X-Ray diffraction method was utilized to identify the *Fritillaria* from different geographical origins. By analyzing degree of crystallinity of *Fritillaria* powders and starches as well as their crystal type, we could easily discriminate the *Fritillaria* Chinese medicinal materials.

MATERIALS AND METHODS

Sources and Pretreatment of the five Fritillaria samples: *Fritillaria thunbergii* Miq., *Fritillaria ussurensis* Maxim., *Fritillaria pallidifloca* Schrenk, *Fritillaria cirrhosa* D.Don and *F. hupehensis* Hsiao et K.C. Hsiawere provided by Meiwei TCM company (Anguo, Hebei province, China) and were identified by Professor Gao Wenyuan, Tianjin University, China.

The five *Fritillaria* were cleaned, comminuted to powders which were sieved with 160M sifter and then kept in a desiccator. The dried powders were extracted with 85% alcohol in a thermostat at 25°C for 48 h. The solution was filtrated with an anti-acid filter. The residue was washed with 85% alcohol for several times and then desiccated at ambient temperature.

X-ray powder diffraction measurements: Monochromatic Cu-K_α□radiation (wavelength = 1.542 Å) was produced by a BDX3300 X-ray powder diffractometer (Beijing University Equipment Manufacturer, China). The *Fritillaria* and starch powders were packed tightly in a rectangular aluminum cell. The samples were exposed to the X-ray beam from X-ray generator running at 36 KV and 20 mA. The scanning regions of the diffraction angle 2θ were 10-30°, which covered most of the significant diffraction peaks of the starch crystallites. Other operation conditions included: Step interval 0.02, scan rate 2/min, Sollet and divergence slit, 1°, receiving slit, 1° and scattering slit, 0.15°. The same measurements were made at room temperature for three times. Radiation was detected with a proportional detector.

Determination of the degree of crystallinity: The degree of crystallinity of samples was quantitatively estimated following the method of Nara and Komiya[16]. A smooth curve which connected peak baselines was computer-plotted on the diffractograms (Fig. 1). The area above the smooth curve was taken as the crystalline portion and the lower area between smooth curve and the linear baseline which connected the two points of the intensity 2θ of 30° and 10° in the samples was taken as the amorphous section. The upper diffraction peak area and the total diffraction area over the diffraction angle 10-30°, 2θ were integrated using Smadchrom software (Morgan and Kennedy Research, Australia). The ratio of upper area to total diffraction was taken as the degree of crystallinity.

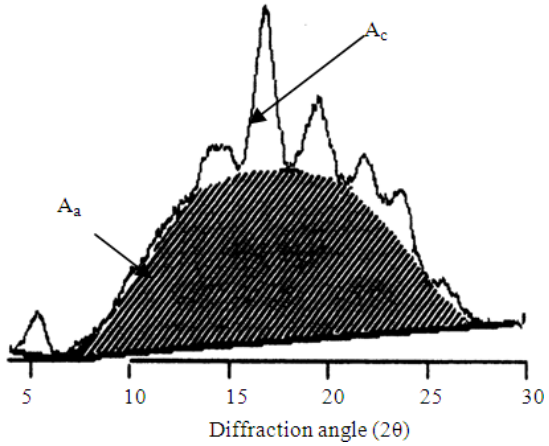

Fig. 1: Calculation of the relative degree of the crystallinity

The equation of the degree of crystallinity is as follows:

$$X_c = A_c/(A_c+A_a)$$

Where:

X_c = refers to the degree of crystallinity
A_c = refers to the crystallized area on the X-ray diffractogram
A_a = refers to the amorphous area on the X-ray diffractogram

RESULTS AND DISCUSSION

X-Ray diffraction analysis of Fritillaria powders: Much of the information about starch granule crystalline properties has been acquired from X-ray powder diffraction studies. Starch can be classified to A, B and C forms[17-19]. In the native granular forms, the A form starch is associated mainly with cereal starches, such as maize starch and wheat starch. The X-ray patterns of these starches give the stronger diffraction peaks at around 15, 17, 18 and 23°. The B form starch is usually obtained from tuber starches, such as potato starch and canna starch. The strongest diffraction peak of the X-ray diffraction pattern appeared at 17° 2θ. And there were also a few small peaks at around 2θ values of 20, 22 and 24°. The C pattern starch is a mixture of both A and B types, such as smooth-seeded pea starch and various bean starches[20].

Because much starch is contained in the *Friitllaria*, the X-Ray diffraction pattern of *Fritillaria* powders mainly showed the crystalline properties of starch. The main crystalline peaks in the X-ray diffraction pattern are attributed to the crystalline peaks of starch.

The X-ray diffractograms of the five *Fritillaria* powders are presented in Fig. 2.

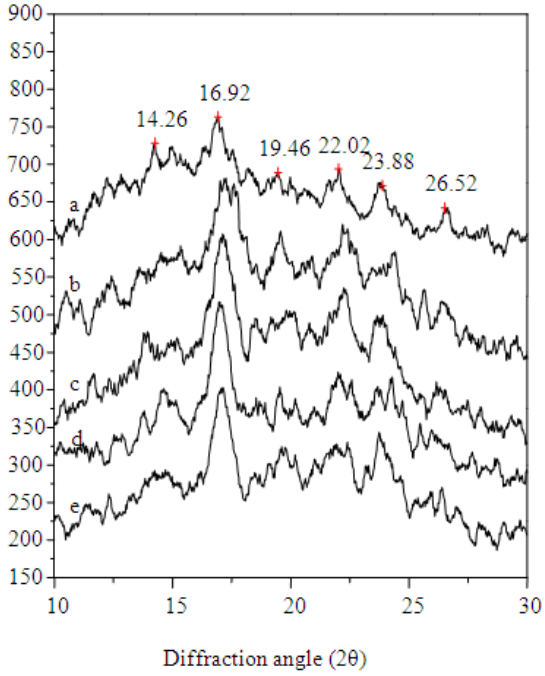

Fig. 2: X-ray diffraction pattern of the five *Fritillaria* powders, a: *F. hupehensis*, b: *F. pallidifloca*, c: *F. cirrhosa*, d: *F. ussurensis*, e: *F. thunbergii*

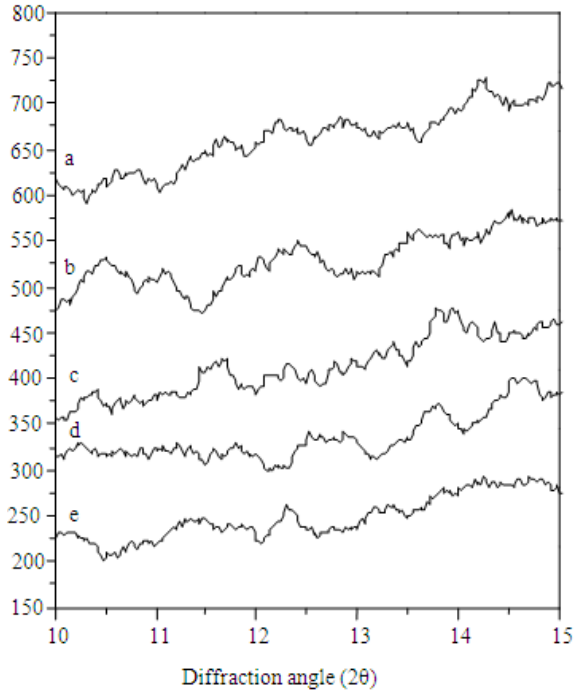

Fig 3: Comparison of the X-ray diffraction pattern of five *Fritillaria* of 10-15°

As can be seen from Fig. 2, *F. thunbergii*, *F. ussurensis* and *F. cirrhosa* powders give the strongest diffraction peak at 17.2° 2θ and a few small peaks at around 2θ values of 15.3, 19.6, 22.2, 24.4 and 26.4°. This result revealed that crystal type of starches contained in the three *Fritillaria* powders is a characteristic B-type. However, the sharp diffraction peak at 17.2° 2θ was converted into two small peaks at 17.2and 17.8° 2θ in the X-ray diffraction pattern of *pallidifloca* powder. This was indicative of A-type, while the other diffraction peaks at 19.6, 22.2, 24.4 and 26.4° are still characteristic of B pattern. In the light of starch granule crystalline properties, we can conclude that the starch contained in the *F. pallidifloca* was classed as C-type, a mixture of both A and B types. As for the *F. hupehensis* powder, there was a strongest diffraction peak at 16.9° 2θ and two symmetrical peaks at 16.3 and 17.5° 2θ based on the peak top. This phenomenon was firstly found in the study of crystalline properties of starch. And now, we temporarily classed this crystal type of starch as 'W' form which was not different from the known types.

In term of the above analysis, the five *Fritillaria* can be sorted into three classes: *F. thunbergii F. ussurensis* and *F. cirrhosa* belong to one class, *F. pallidifloca* belongs to another class, *F. hupehensis* belongs to the third class.

There were also apparent differences in the X-ray diffraction pattern of 10-15 and 25-30° regions, which were enlarged, respectively, in Fig. 3 and 4.

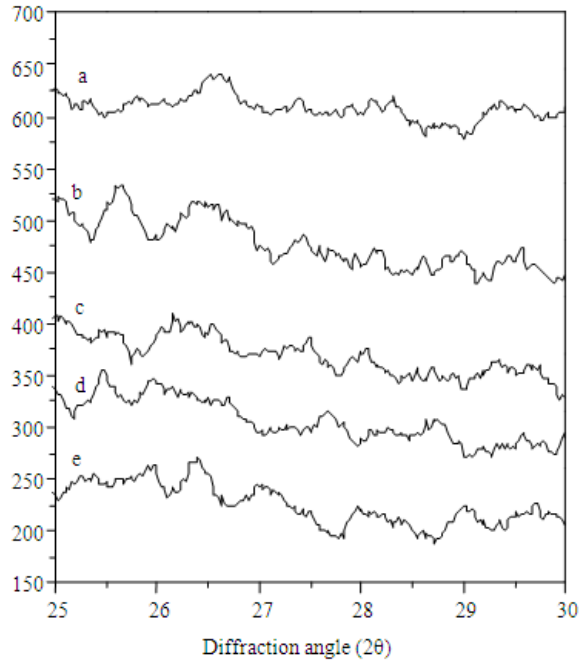

Fig. 4: Comparison of the X- ray diffraction pattern of five *Fritillaria* of 25-30°

The degree of crystallinity of five kinds of *Fritillaria* powders calculated from the Fig. 2 were shown in Table 1. For this evaluation, we utilized the powders which had almost identical moisture contents (~12%) in order to minimize the effect of different moisture contents on crystallinity.

Table1: X-ray diffraction data of the five *Fritillaria* powders

Samples	Degree of crystallinity (%)	Crystal pattern of starch
F. thunbergii	42.1	B
F. ussurensis	43.6	B
F. cirrhosa	35.9	B
F. pallidifloca	37.7	C
F. hupehensis	30.8	W

Table 2: X-ray diffraction data of *Fritillaria* powders after extraction with 85% alcohol

Samples	Degree of crystallinity (%)	Crystal pattern
F. thunbergii	43.2	B
F. ussurensis	40.5	B
F. cirrhosa	41.4	B
F. pallidifloca	44.8	C
F. hupehensis	41.4	W

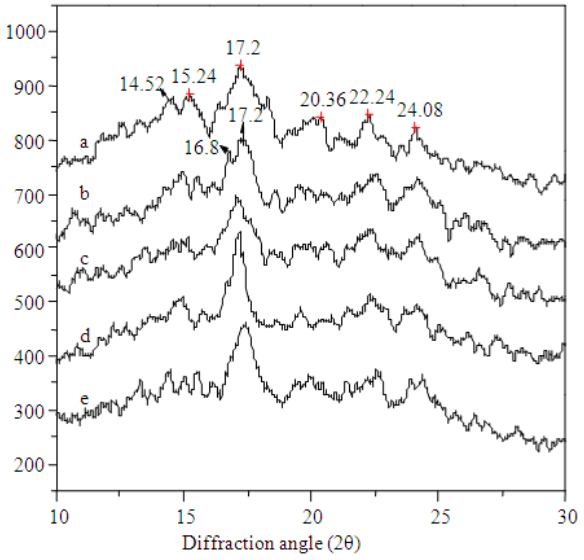

Fig. 5: X-ray diffraction pattern of the five *Fritilaria* powders after extraction with 85% alcohol, a: *F. hupehensis*, b: *F. pallidifloca*, c: *F. cirrhosa*, d: *F. ussurensis*, e: *F. thunbergii*

According to the degree of crystallinity in Table 1, the five *Fritillaria* can also be divided into three classes: *thunbergii F.* and *ussurensis F.* belong to one class, *cirrhosa F.* and *pallidifloca F.* belong to another class, *hupehensis F.* belongs to the third class. If the crystal type of starch contained in *Fritillaria* is considered, the five *Fritillaria* could clearly be separated into four classes: *F. thunbergii* and *F. ussurensis* belong to one class, *F. cirrhosa* belongs to another class, *F. pallidifloca* belongs to the third class and *F. hupehensis* belongs to the fourth class. In addition, the differences of Fig. 3 and 4 could also provide some information to discriminate *F. thunbergii* and *F. ussurensis.*

X-Ray diffraction analysis of *Fritillaria* powders after extraction with 85% alcohol: In Fig. 5 X-ray diffraction pattern of the five *Fritillaria* powders after extraction with 85% alcohol are displayed.

In order to further test the crystal type of starch contained in *Fritillaria*, the *Fritillaria* powder after extraction with 85% alcohol was analyzed by X-ray diffraction. Consistent with the above analysis, the X-ray diffraction pattern of the *F. thunbergii*, *F. ussurensis* and *F. cirrhosa* powders after extraction with 85% alcohol are the characteristic B-type pattern. The peak at 17° 2θ were stronger than the other peaks at 15, 19, 22 and 24° 2θ. For *F. pallidifloca* powders after extraction with 85% alcohol, there were also two small peaks at 16.8º and 17.2° 2θ, which is also an indicative of A-type. The peaks at 15, 19, 22 and 24° 2θ is the characteristic peaks of B-type starch. So, the starch contained in *F. pallidifloca* was classified as C-type. For *F. hupehensis* powder after extraction with 85% alcohol, the strongest peak was also at about 17° 2θ. The two symmetrical peaks centered at 17, 16.3 and 18.2° 2θ, respectively. This result further confirmed that the starch contained in the hupehensis *F.* was 'W' type that was defined for the moment.

The degree of crystallinity of five kinds of *Fritillaria* powders after extraction with 85% alcohol calculated from the Fig. 5 were shown in Table 2. For this evaluation, we utilized the powders which had almost identical moisture contents (~15%) in order to minimize the effect of different moisture contents on crystallinity.

CONCLUSION

The study showed the differences in starch contained in different *Fritillaria* of geographical origins. According to these discrepancies in starch crystal type and crystallinity of *Fritillaria* powder, we could easily separate the five *Fritillaria*. As a new analytical technology, X-ray diffraction method was widely utilized in the study of phase and crystal structure of substance. Diffraction pattern obtained by this method could provide lots of information, strong fingerprint character, stability and reliability. X-ray diffraction could be utilized as identification method of all kinds of traditional Chinese medicine. This experiment not only gave some crystallinite properties on starch contained in *Fritillaria*, but provided some information on the plant taxonomy from macromolecule aspects.

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