



Research Article

Effects of ultrafine grinding on the physicochemical properties and efficacy in relieving cough and resolving phlegm of *Fritillaria taipaiensis* P. Y. Li

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Abstract: *Fritillaria taipaiensis* P. Y. Li (*F. taipaiensis*) is widely utilized for its efficacy in alleviating cough symptoms and reducing mucus production. Its popularity stems from its recognized therapeutic properties and associated health benefits. However, due to the scarcity of its resources and the high market valuation, the traditional application of *Fritillaria taipaiensis* faces the disadvantage of underutilization. To better utilize the resources of *Fritillariae cirrhosae* (*F. cirrhosae*), we prepared an ultrafine powder of *F. taipaiensis* (UPF) and explored the differences in physicochemical properties and pharmacodynamics between it and the traditional powder of *F. taipaiensis* (TPF). Physicochemical properties were characterized by particle size, scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FT-IR). Pharmacodynamic effects were evaluated by cough and phenol red excretion modeling. Relative to TPF, UPF demonstrated superior physicochemical properties, and there was an increase in the dissolution of total alkaloid. Additionally, the effective dose of UPF required to achieve equivalent effects on cough latency prolongation, cough reduction, phenol red excretion enhancement, and the decrease of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α) levels was half that of TPF. The UPF reduced the expression of the neurokinin-1 receptor (NK-1R), transient receptor potential ankyrin1 (TRPA1), surfactant protein A (SP-A) and mucin 5AC (MUC5AC) in the lung tissues. The UPF is superior to TPF in physicochemical properties and cough-relieving and phlegm-reducing effects. The research contributes to the optimization of the daily health consumption of *F. taipaiensis* and the promotion of the adequate utilization of its resources.

Keywords: ultrafine powder; *Fritillaria taipaiensis* P. Y. Li; cough; phlegm

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1 Introduction

The bulbous of *Fritillariae cirrhosae* (*F. cirrhosae*) is a valuable traditional Chinese medicine (TCM) known for its heat-clearing and lung-moistening properties, as well as its ability to resolve phlegm, relieve cough, and eliminate carbuncles^[1]. *Fritillaria taipaiensis* (*F. taipaiensis*), one of the six foundational plants of *F. cirrhosae* herbs, thrives at altitudes of approximately 2,000 m and exhibits advantages in growth and reproduction, thus demonstrating significant developmental potential. These

characteristics have attracted considerable attention from both researchers and investors^[2,3]. The *F. cirrhosae* bulb possesses specific health benefits and serves as a food source^[4]. Its distinct health effects render it widely utilized in TCM and modern formulas as well as in the development of dietary therapies, health products, foods, and daily chemicals^[5]. Medicinal dietary treatments from *F. cirrhosae*, including pear and Lopa Koa cream, Chuanbei almond, Lopa Koa paste, and Chuanbei-stuffed snow pear are extensively employed to treat conditions such as lung heat and dry cough. These treatments play crucial roles in

postoperative health recovery and daily conditioning^[6,7]. Currently, the healthy development of the *F. cirrhosae* industry is constrained by resource scarcity and insufficient basic research on quality control methods and pharmacological mechanisms of the plant^[8]. Additionally, the inability of wild *F. cirrhosae* to meet market demand has led to a supply-demand imbalance for *F. taipaiensis* resources^[9]. Consequently, optimizing the utilization of *F. taipaiensis* in response to these supply-demand disparities is paramount.

F. taipaiensis mainly contains alkaloids, saponins, nucleosides, and other water-soluble compounds. Alkaloids, particularly peimisine, peiminine, and peimine are characteristic and effective constituents of this plant^[10,11]. Alkaloids in *F. taipaiensis* have been found to exhibit diphenyl-1-picrylhydrazyl and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging as well as iron reduction activities^[12]. However, research indicates the low alkaloid content of this plant and poor solubility of its components; hence, the traditional method of administration involving taking the drug in powder form.

Ultrafine grinding technology is an emergent milling technique that is commonly used to produce micron, submicron, and even nano powders, especially in the development of food materials with new functional properties as well as TCM preparations^[13-15]. Compared with samples pulverized by traditional mechanical methods, ultrafine powders have shown better physicochemical properties and better flowability as well as higher bioavailability and bioactivity^[16-18], offering unique advantages and development potential. Literature has reported that when the dosage of ultrafine powder is 1/4–1/2 of the clinical dosage in TCM form, it still has equivalent efficacy^[19,20]. Research on the effect of ultrafine grinding on the efficacy of TCM and food, such as *Pinellia* and *Astragalus*, indicates the use of approximately 1/8–1/4 of the number of herbs to achieve the similar efficacy to the traditional water decoction. *Astragalus* ultrafine powder can enhance the immunity of chickens better than the traditional powder, the coagulation time of *Panax ginseng* ultrafine powder is reduced by 2/3 under the same dose, the time of the pain reaction of mice treated with *Corydalis* ultrafine powder is prolonged by 1/5, and the effect of *Rheum* ultrafine powder on the average propulsion rate of intestinal peristalsis was higher than that of traditional powder by 6%^[21-23]. For food products, ultrafine grinding can not only increase the dissolution of functional components such as bioflavonoids, dietary fibers, and polysaccharides, but also maximizes the retention of various microelements, microorganisms, and other active components, such that their antioxidant and immunity-enhancing effects become more remarkable^[24,25].

In this study, we prepared an ultrafine powder of *F. taipaiensis* (UPF), and systematically analyzed the chemical and physicochemical properties of the ultrafine and traditional powders. Furthermore, we evaluated the efficacy of *F. taipaiensis* in relieving cough and resolving phlegm and conducted a preliminary study on the mechanism.

2 Materials and methods

2.1 Chemicals and materials

F. taipaiensis was obtained from Chongqing Wuxi County Rui Xue Medicinal Herb Plantation Limited Liability Company, China. Other reagents, including ammonia and trichloromethane, were of analytical grade and obtained from Sinopharm Chemical

Reagent Co. (Shanghai, China).

2.2 Preparation of UPF

Referring to the specifications for clinical use of *F. cirrhosae* in the 2020 edition of the Chinese Pharmacopoeia, the traditional powder of *F. taipaiensis* (TPF) was produced using a powdering machine and sieved through a No.6 sieve, whereas the UPF was prepared via ball milling. We used a vertical planetary ball mill to optimize the UPF preparation process using the following parameters powder particle size (as the indicator), one-factor-response surface method experiment, ball milling time, ball material ratio, and rotational speed (as the variable). The results indicated that the particle size of *F. taipaiensis* powder was significantly decreased after ultrafine grinding, and the optimal preparation process was as follows: rotational speed of 300 r/min, time of 75 min, and ball-to-material ratio of 10:1.

2.3 Comparison of physicochemical properties

2.3.1 Particle size

The particle sizes of the UPF and TPF were determined using a laser diffraction particle size analyzer (LS13320/ULM2, Beckman Coulter, Brea, California, USA) through the dry method. The dry powder was subjected to diffraction analysis to establish the particle size distribution and parameters^[26].

2.3.2 Scanning electron microscope (SEM)

The surface morphology of TPF and UPF was examined at a resolution of 3.0 nm in high-vacuum mode using a field emission SEM (QUANTA FEG250, FEI, Beijing, China) with an accelerating voltage of 5 kV.

2.3.3 Fourier transform infrared spectroscopy

The TPF and UPF were analyzed using a Fourier transform infrared spectroscopy (FT-IR) spectrometer (Thermo Scientific™ Nicolet™ iS™ 5 FTIR, Verona, Madison, USA). Scans were performed 16 times with a collection frequency of 4 cm⁻¹ over a range of 4,000 to 400 cm⁻¹. The real-time scanning process accounted for the interference from CO₂ and H₂O. The functional groups in the powders were analyzed.

2.3.4 Determination of total alkaloid content

We weighed approximately 2 g of TPF and UPF, and measured the absorbance at 415 nm using a UV-visible spectrophotometer (Unico Instrument Co., Ltd., UNICO UV-2800A, Shanghai, China) to determine the imperialine content, which represents the total alkaloid content of the samples. A series of solutions of known concentrations (2.0–30 µg/mL) of imperialine were prepared using the same method to construct a standard curve^[27].

2.3.5 Powder properties

A funnel was secured to an iron-frame table with a glass plate positioned perpendicular to the top of the table. The vertical distance (h) from the end of the funnel to the glass plate, and the radius (r) of the cone formed by the powder on the glass plate were recorded. The angle formed between the sloping side of the cone and the horizontal plane was referred to as the angle of repose (AR). The angle of repose was calculated using equation 1 (Eq. 1)^[28].

$$AR = \arctg\left(\frac{r}{h}\right) \quad (1)$$

A sample (m) was taken and placed in a centrifuge tube. The mass of the centrifuge tube and the powder (m_1) were measured. Then, 10 mL of purified water was added, shaken well, left at room temperature for 24 h, centrifuged at 5,000 r/min for 15 min, and the supernatant was carefully discarded. The mass of the centrifuge tube and the powder at this time (m_2) was measured. The water-holding capacity (WHC) was calculated using Eq. 2^[29].

$$WHC(\%) = \frac{(m_2 - m_1)}{m} \times 100\% \quad (2)$$

A sample (m) was placed in a centrifuge tube, and 10 mL of purified water was added. The mixture was vigorously shaken before centrifuging at 5,000 r/min for 15 min in a water bath held at a constant temperature of 60 °C for 30 min. Subsequently, the supernatant was dried at 105 °C until a constant weight was achieved, and the mass of the residue (m_1) was measured. The water solubility (WS) was calculated using Eq. 3^[30].

$$WS(\%) = \frac{m_1}{m} \times 100\% \quad (3)$$

A sample (m) was placed in a measuring cylinder labeled V_1 . Subsequently, 50 mL of purified water was added, and the mixture was stirred thoroughly and left horizontally at room temperature for 24 h. The final volume of the measuring cylinder (V_2) was measured. The swelling capacity (SC) was calculated using Eq. 4^[31].

$$SC(mL.g^{-1}) = \frac{(V_2 - V_1)}{m} \quad (4)$$

Various particle sizes of the sample were placed in a 5 mL measuring cylinder until it reached the 5 mL mark (V). Next, the mass of the sample (m) was accurately measured. The bulk density (BD) was calculated using Eq. 5^[32].

$$BD(g.mL^{-1}) = \frac{m}{V} \quad (5)$$

2.4 Pharmacodynamics study

2.4.1 Animals

Male specific pathogen-free (SPF) grade Kunming (KM) mice weighing 18–22 g were obtained from Xishan Biotechnology Co., Ltd. (Suzhou, China) (license No. SCXK (Su) 2020-0009). Animals were acclimatized for one week before the experiment by keeping them at a constant temperature of 20–25 °C, humidity of 40%–55%, free water, and normal feed. All the animal experimental procedures were approved by the Ethics Committee of Nanjing University of Chinese Medicine (Animal application approval number: 202404A090). Prior to dosing, the mice were fasted for 12 h but given free access to water.

2.4.2 Cough modeling and drug administration

The KM mice were randomly divided into seven groups of 10 each: Control group (Con), Model group (Model), and dextromethorphan group (60 mg/kg) (DH), TPF group was converted from human dose to mouse dose: 1g/kg, low dose group of UPF (0.25 g/kg) (UPFL), medium dose group of UPF (0.5 g/kg) (UPFM), and high dose group of UPF (1.0 g/kg) (UPFH). The drug was administered daily via gavage for seven days. On the eighth day, the mice were placed in a homemade, airtight, plastic transparent container. Subsequently, an ultrasonic nebulizer was used to spray ammonia at a rate of 0.2 mL/min for

20 s. The latency of coughing and the number of coughs within 2 min after drug administration served as the final evaluation indices of drug efficacy.

2.4.3 Evaluation of phlegm resolving effect

The KM mice were randomly divided into seven groups of 10 each: Control (Con), Model, Ambroxol hydrochloride group (70 mg/kg) (AH), TPFH (1 g/kg), UPFL (0.25 g/kg), UPFM (0.5 g/kg), and UPFH (1.0 g/kg). The drug was administered daily via gavage for seven days. On the eighth day, 30 min after drug administration, 0.5% phenol red solution (0.5 mL) was injected intraperitoneally. A tracheal section from below the thyroid cartilage to the tracheal branch was excised, and the segments were placed in a test tube containing 1.5 mL of 5% NaHCO₃ solution. After standing for 1 h to dissolve the tracheal secretion, 200 µL of the supernatant was aspirated, and the optical density (OD) value was measured at 546 nm using an enzyme marker. The phenol red content of the trachea was determined using a phenol red standard curve.

2.4.4 Enzyme-linked immunoassay (Elisa)

The blood samples were incubated at room temperature for 60 min and subsequently centrifuged at 3,000 rpm for 15 min. The resulting supernatant was collected, and the OD of each sample was measured at 450 nm using an enzyme marker according to the instructions of the Elisa kit. The concentrations of IL-6 and TNF-α in the serum were quantified.

2.4.5 Hematoxylin and eosin (H&E) staining

The trachea and lung tissues of the mice were fixed in 4% paraformaldehyde, embedded in paraffin, sectioned, stained with H&E, and examined microscopically to assess the transverse and longitudinal tracheal sections and histopathological alterations in the lungs. The morphological and pathological characteristics of each organ were analyzed.

2.4.6 Western Blot (WB)

Lung tissues from each group were weighed, and RIPA buffer, including phosphatase and protease inhibitors (Tendril, WB0039), was added. Tissue disruption was performed using a tissue crusher (Monad, Absolute 1100), followed by centrifugation at 13,000 rpm for 20 min. This process yielded the supernatant as the total protein extract. Protein concentration was determined using the bicinchoninic acid assay (BCA) method (Amresco, 0332). The proteins from each sample were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and the membranes were incubated using a wet transfer method for 1 h. The membranes were then incubated with a secondary antibody, goat anti-rabbit IgG (H+L) HRP, at a dilution of 1:10,000 (Tandem, S004). The protein bands were visualized using an ECL substrate kit (Millipore, WBKLS0500) and a developer. β-actin served as the internal reference.

2.4.7 Reverse transcription quantitative PCR (RT-qPCR)

Total RNA was extracted from the samples using the Ultra-pure RNA Extraction Kit (Kangwei Century Bioscience and Technology Co., Ltd., CW0581S), and its concentration and purity were assessed using Nano-Drop® ND-2000. Subsequently, total RNA was reverse transcribed into cDNA using the Hi-Script III 1st Strand cDNA Synthesis Kit (+gDNA wiper) (Nanjing Novozymes Bioscience & Technology Co., Ltd., R312-01). RT-qPCR amplification was performed using AceQ Universal SYBR

qPCR Master Mix (Nanjing Novozymes Bioscience & Technology Co., Ltd., Q511-02). The expression level of the internal reference gene β -actin was used as a normalized control. The relative expression was calculated using the $2^{-\Delta\Delta C_t}$ method. The primer sequences are listed in Table 1.

2.5 Statistical analysis

The data were analyzed using GraphPad Prism (Version 8.0). One-way analysis of variance (ANOVA) was performed with a significance level of $P < 0.05$.

3 Results and discussion

3.1 Characterization of UPF and TPF

The particle size range of ultrafine herbal powders is controlled below $75 \mu\text{m}$ [33]. As shown in Fig. 1A, the particle size of UPF showed a single-peak distribution, compared with the traditional powder that showed a double-peak distribution, indicating that the particle size of UPF was more homogenous. The D_{90} (D_{90} indicates that 90% of the powder is below this size.) of the UPF

was reduced from $141.71 \mu\text{m}$ of the TPF to $36.31 \mu\text{m}$ (Fig. 1B), indicating that the particle size of the powder of the *F. taipaiensis* was significantly reduced ($P < 0.01$) after ultrafine grinding.

The results of the powder's chemical and physicochemical properties are shown in Fig. 1C. The results indicate that the AR of UPF increased as the friction between the UPF particles became larger. Additionally, the adhesion and gravitational force between the UPF particles as well as the porosity, increased as the bulk density decreased, thereby facilitating cone formation. Furthermore, the chemical bond force between the powders increased after grinding, enhancing the stability of the cone and resulting in an increased AR [34]. The water solubility results indicate that as the particle size decreased and the specific surface area increased owing to ultrafine grinding, the structure of cellulose was disrupted, leading to increased exposure of hydrophilic groups and enhanced binding ability with water. Consequently, smaller particle sizes resulted in enhanced solubilization.

The SEM images in Fig. 1D show the morphological differences between TPF and UPF particles. TPF exhibits rounded particles, whereas UPF displays irregular edges, cracks, and stick, indicating structural disruption [35, 36].

The FT-IR results in Fig. 1E show that the positions and shapes of the peaks were largely similar before and after ultra-micro milling, with some differences in intensity. This may be attributed to the exposure of some groups in the UPF during ultra-milling or the varying scattering intensities due to differences in powder sizes. Therefore, the functional groups of the ultra-micro powders remained unaffected and the main components remained unchanged. The broad peaks at $3,000\text{--}3,600 \text{ cm}^{-1}$ represent the O-H stretching vibration peaks in the structure of the polysaccharides, whereas the C-H stretching vibration peaks occurred at $2,800\text{--}3,000 \text{ cm}^{-1}$. The absorption peak at $1,634 \text{ cm}^{-1}$ corresponds to C-H or C=O stretching vibration peaks on the benzene ring, and the peak at $1,148 \text{ cm}^{-1}$ represents polysaccharide C-O-C stretching vibrations. Additionally, the absorption peak at 993 cm^{-1} is characteristic of the C-O-C absorption peak in the cellulose sugar ring. In conclusion, the UPF primarily consists of starch polysaccharides, and ultra-micro pulverization does not alter its nutrient composition. Ultra-micro pulverization, with no significant alteration in the structure of the powder, can enhance its performance and facilitate its direct

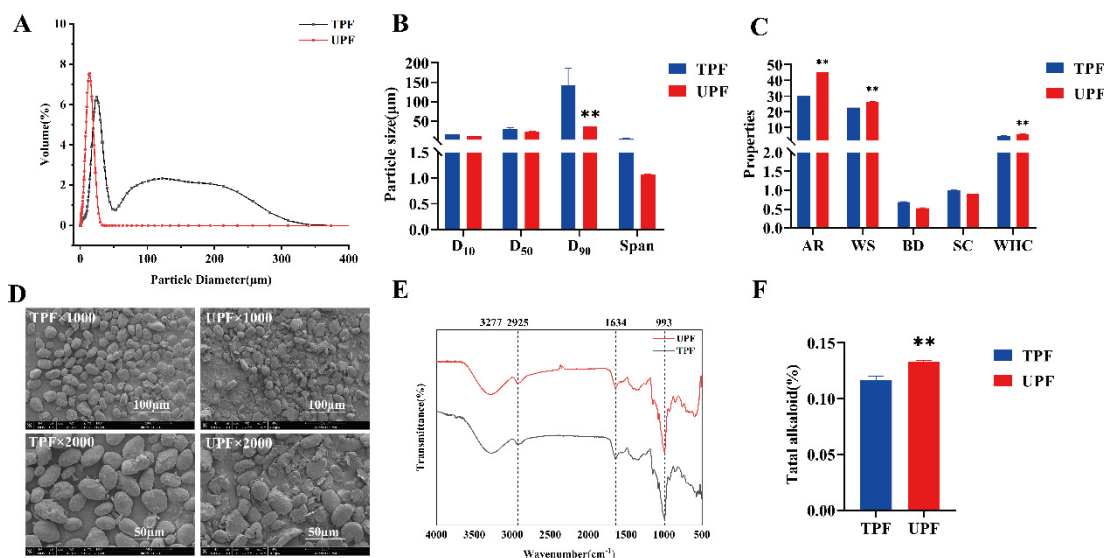


Figure 1 Characterization of TPF and UPF. (A) Particle size distribution (B) Particle size parameters (C) Powder properties (D) SEM images of at 1,000 x and 2,000 x field of view (E) FT-IR images (F) Total alkaloid content.

incorporation into various foods, indicating its potentially broad applications^[37].

The results of Fig. 1F indicate that the total alkaloid content of UPF was significantly higher than TPF ($P < 0.01$). Studies have shown that alkaloidal components are the foundation for the cough-relieving and phlegm-eliminating effects of *F. taipaiensis*^[38].

3.2 UPF is superior to TPF in relieving cough and resolving phlegm

Cough incubation period, frequency of coughing, and phenol red excretion were employed to evaluate the efficacy of cough relief and phlegm elimination. As illustrated in Fig. 2A, compared with the Model group, the cough latency was significantly prolonged ($P < 0.01$) in all treatment groups. Compared with the TPF group, the efficacy of the UPFH was superior ($P < 0.01$). As shown in Fig. 2B, compared with the Model group, the frequency of coughing within 2 min was significantly reduced ($P < 0.05$) in all treatment groups. Compared with the TPF group, the efficacy of the UPFH was superior ($P < 0.05$). The results in Fig. 2C revealed that tracheal phenol red excretion was significantly increased in all treatment groups ($P < 0.01$). Remarkably, there was no significant difference in efficacy between the TPF group and the UPFM group. These results suggest that UPF significantly improves cough relief at the same dosage, while the UPFM group achieves the same level of efficacy as the clinical dose of TPF. Literature reports that when the dosage of ultrafine powder for medication is 1/4 to 1/2 of the clinical dosage in traditional powder, it still has comparable medicinal efficacy^[39-41]. In addition, the enhancement of the efficacy may be associated with a reduction in particle size, which resulted in increased *in vivo* absorption. Gardenia was ultrafinely ground, the content of the active ingredient gardenia glycosides increased by 17.2% compared with the traditional powder^[42]. The adsorption of ultrafine powder was enhanced, and the retention time of powder in the body was prolonged, thereby greatly increasing the degree of drug absorption in the body and achieving the purpose of improving bioavailability^[43,44]. The effect of ultrafine grinding on the *in vitro* dissolution and *in vivo* pharmacokinetics of the active ingredients in seven-flavored Guangzao San was investigated, and

the results indicated that the dissolution of eugenol components in the ultrafine powder was higher than traditional powder in different media, and the AUC value was significantly larger than traditional powder^[45]. In our study, we revealed that the particle size of *F. taipaiensis* ranged from 141.71 μm to 36.31 μm after ultrafine grinding, which was accompanied by particle surface fragmentation. This might contribute to the increase of its active ingredients in the body and enhance the efficacy.

3.3 *F. taipaiensis* exhibited strong anti-inflammatory effects

Pro-inflammatory factors such as IL-6 and TNF- α precipitate neutrophil infiltration, eventually leading to lung tissue damage^[46,47]. TNF- α plays a crucial role in preventing immune responses, including infection. Its secretion induces the release of IL-6, a common inflammatory factor that amplifies the inflammatory cascade and causes inflammatory damage^[48,49]. In this study, the levels of inflammatory factors in mice were assessed (Fig. 3). The results revealed a significant elevation of IL-6 and TNF- α levels in the Model group compared with the Control group ($P < 0.01$), and a variable reduction in the levels of IL-6 and TNF- α in serum in each administration group compared with the Model group. This implies that *F. taipaiensis* has the potential to decrease inflammation in mice. Studies indicated that alkaloids in *F. taipaiensis* could lower the production of NO, TNF- α , and IL-6, and inhibit the mRNA expression of TNF- α and IL-6^[50].

3.4 UPF improved lung and airway histopathology

H&E staining of the lungs and trachea from each group of mice is shown in Fig. 4. In the Model group, prominent pathological damage was observed, including a slight thickening of the alveolar wall and localized widening of the alveolar space in the lung tissues. This was accompanied by a small infiltration of granulocytes (indicated by blue arrows), rare interstitial vascular stasis (indicated by yellow arrows), and fibrin clusters in the lumen of the blood vessels (indicated by red arrows). The tracheal cross-section of the Model group exhibited significant constriction. Tracheal tissue displayed increased mucosal epithelial cell necrosis, detachment, cytosolic solidification, deep staining, fragmentation,

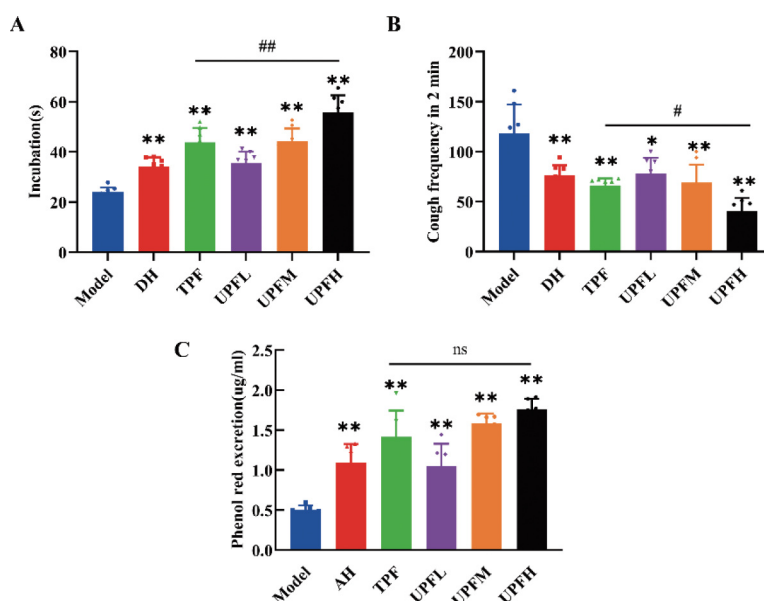


Figure 2 Pharmacodynamic results of cough and phlegm reduction. (A) Cough incubation period, (B) Number of coughs, (C) Phenol red excretion concentration. Data were presented as mean $\pm$ SD. Compared with the model group, * $P < 0.05$, ** $P < 0.01$; compared with the TPF group, # $P < 0.05$, ## $P < 0.01$, $n = 10$.

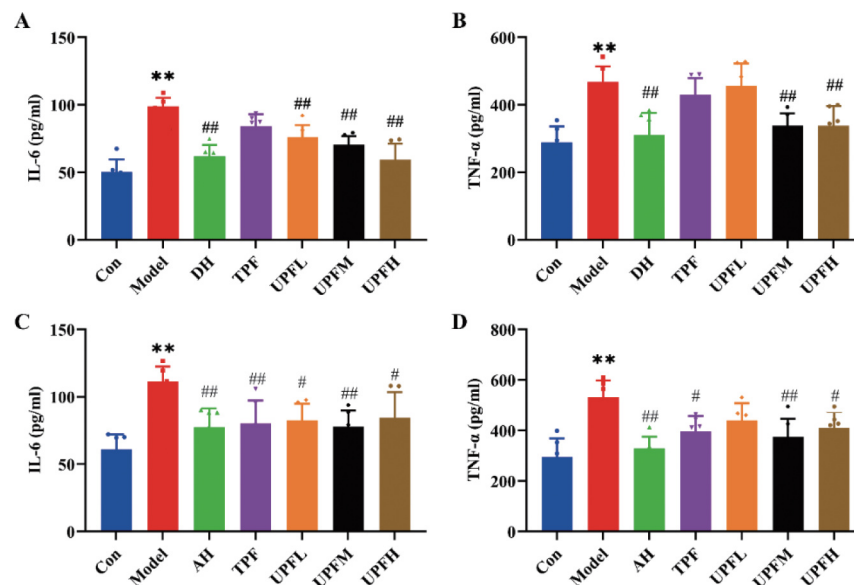


Figure 3 The serum levels of IL-6 and TNF-α. (A) IL-6 in cough suppressant serum, (B) TNF-α in cough suppressant serum, (C) IL-6 in phlegm-reducing serum, (D) TNF-α in phlegm-reducing serum. Data were presented as mean ± SD. Compared with the Con group, ** $P < 0.01$; compared with the Model group, # $P < 0.05$, ## $P < 0.01$, $n = 6$.

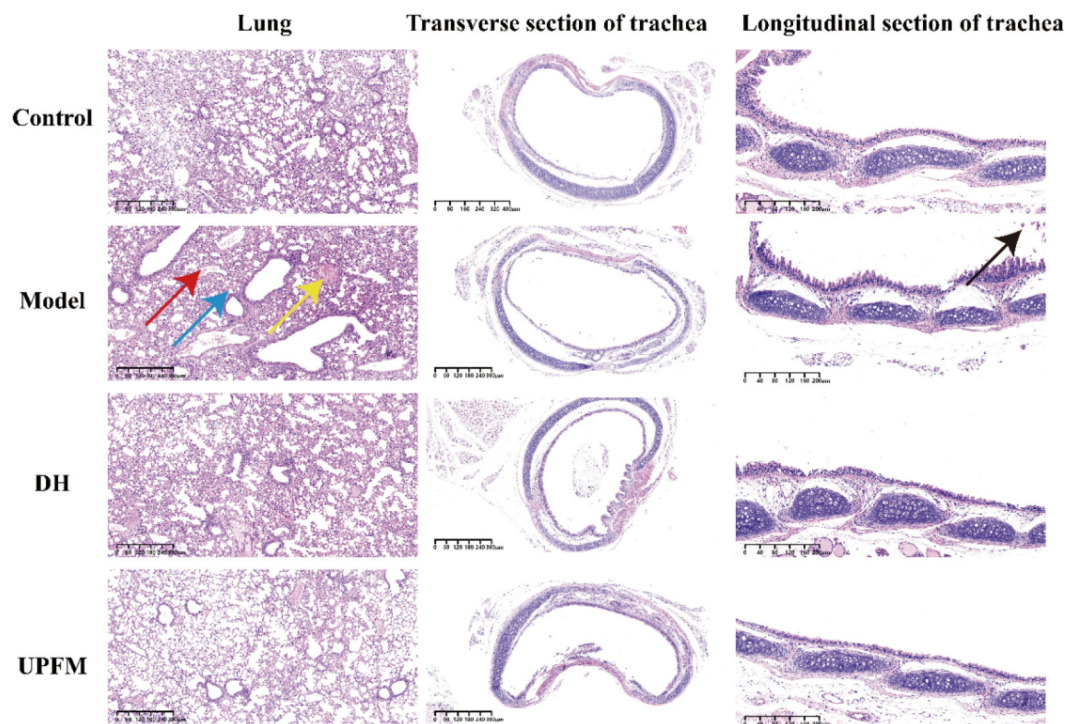


Figure 4 Pathological staining of lung tissue, trachea cross-section and longitudinal section of lung tissue. Representative images of mice lung (5×), transverse section of trachea (10×), longitudinal section of trachea (10×).

dissolution, and disappearance (black arrow). Each administration group demonstrated improvement in inflammatory pathology damage.

3.5 UPF inhibited the expression of cough and mucus-related proteins

To investigate the mechanism by which *F. taipaiensis* alleviates cough and resolves phlegm, we analyzed the expression of proteins associated with these symptoms in lung tissues. The cationic channel TRPA1 can be activated at pH 8 and in an alkaline environment, leading to the release of surfactant protein

(SP) and inflammatory factors due to physicochemical stimuli^[51,52]. Concurrently, SP release mediates the inflammatory response. SP is secreted by both neural and inflammatory cells such as macrophages and functions by binding to the neurokinin-1 receptor (NK-1R). SP has pro-inflammatory effects and has been implicated in inflammatory diseases of the respiratory, gastrointestinal, and musculoskeletal systems^[53]. Fig. 5 depicts the measurements of the transient receptor potential ankyrin1 (TRPA1), NK-1R, Mucin 5AC (MUC5AC), and SP-A in the Con, Model, positive drug, and UPFM groups. The findings indicated a notable increase in these protein expression levels in the Model

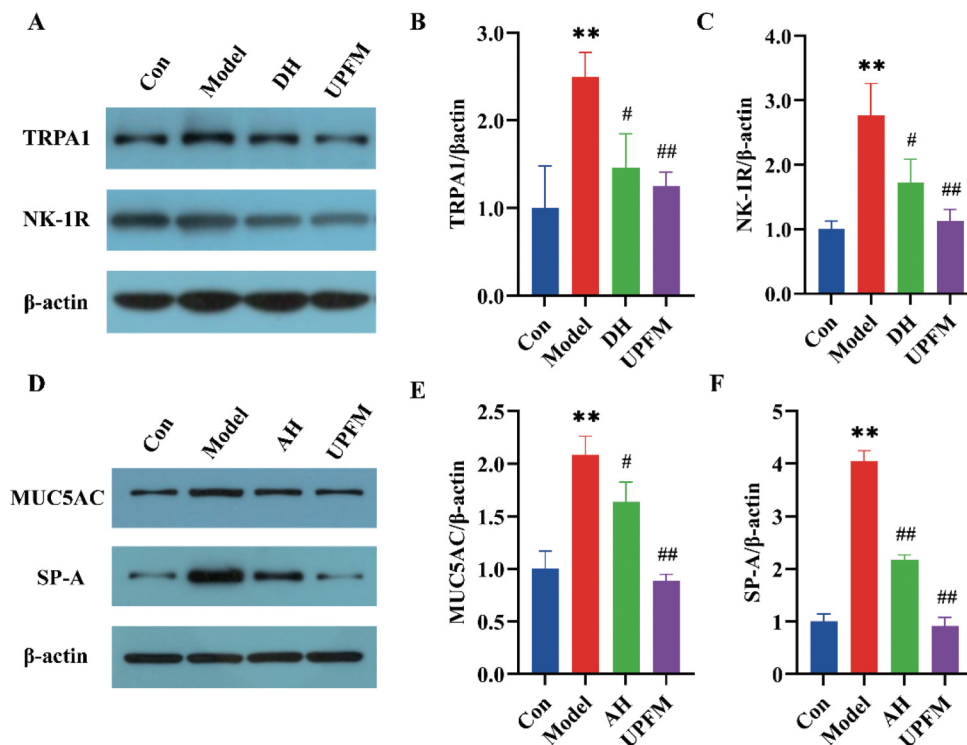


Figure 5 Expression of cough and mucus-related proteins TRPA1, NK-1R, MUC5AC and SP-A. (A) The images of NK-1R, TRPA1 and β-actin. (B) The protein expression of TRPA1. (C) The protein expression of the NK-1R. (D) The images of MUC5AC and SP-A. (E) The protein expression of MUC5AC. (F) The protein expression of SP-A. Data were presented as mean ± SD. Compared with the Con group, * $P < 0.01$; Compared with the Model group, # $P < 0.05$, ## $P < 0.01$, $n = 3$.

group compared to the Control group ($P < 0.01$), whereas drug administration substantially reduced their expressions ($P < 0.05$). This suggests that the cough-relieving effect of *F. taipaiensis* may be associated with the suppression of the expression of these proteins. Verticine, ebeiedine, and suchengbeisine, alkaloidal constituents isolated from the genus *Fritillaria*, inhibit the gene expression and production of MUC5AC in human airway epithelial cells, thereby reducing airway inflammation^[54]. In our study, we noticed that the alkaloid content of UPF was significantly higher than that of TPF, so we speculate that UPF exerted a superior effect associated with an increase in these constituents.

3.6 UPF reduced the expression of cough- and mucus-related genes

The mRNA expression of TRPA1, NK-1R, SP and MUC5AC are shown in Fig. 6. We found that the level of expression of these genes was significantly higher in the Model group than in the Con group ($P < 0.01$). The treated groups exhibited significantly lower expression levels than the model group ($P < 0.05$). The activation of the TRPA1 channel can induce a classical inflammatory response in immune cells through chemical stimulation. Simultaneously, it triggers the release of tachykinin SP from neurogenic sources, which binds to the NK-1R receptor and induces the release of inflammatory factors, known as the neurogenic inflammatory response^[55-58]. The same change was observed in the WB experiment. MUC5AC and MUC5B are the predominant constituents of the airway mucus^[59,60]. The major mucus proteins MUC5B and MUC5AC are primarily expressed in the lungs. SP-A regulates inflammatory cellular responses, including the release of inflammatory cytokines. SP-A belongs to a family of aggregate proteins that protect against allergens, viruses, and bacterial infections and is involved in maintaining normal

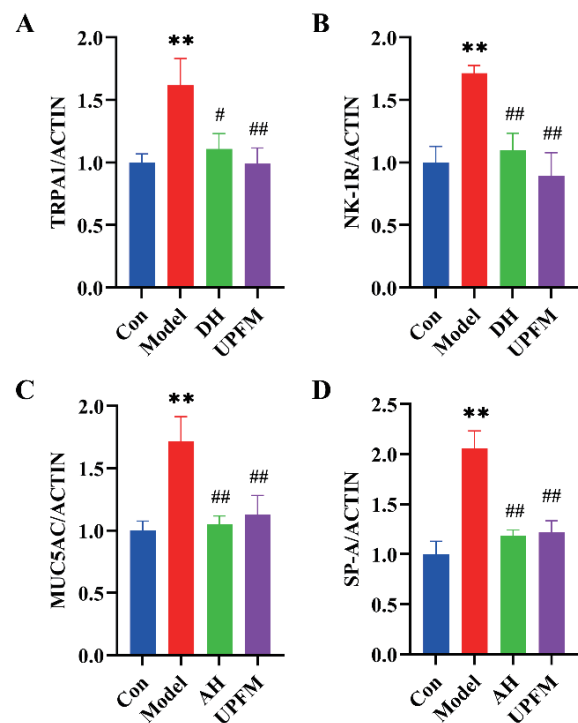


Figure 6 Expression of cough and mucus-related mRNA TRPA1, NK-1R, MUC5AC and SP-A. (A) The relative expression of TRPA1. (B) The relative expression of NK-1R. (C) The relative expression of MUC5AC. (D) The relative expression of SP-A. Data were presented as mean ± SD. Compared with the control group, * $P < 0.01$; Compared with the model group, # $P < 0.05$, ## $P < 0.01$, $n = 3$.

lung structure.^[61] We found that the model group showed stronger inflammatory response and pathological changes by Elisa and HE

research. UPF ameliorated these changes. Therefore, we hypothesized that UPF acts by inhibiting the expression of the above proteins.

4 Conclusion

This study indicated that UPF had superior physicochemical properties relative to TPF. The effective dosage of UPF necessary to achieve equivalent therapeutic effects on cough relief and expectoration was half that required for TPF. The mechanism of the efficacy was related to the inhibition of the expression of the cough-related proteins TRPA1, NK-1R, and the mucus-related proteins MUC5AC and SP-A. The findings of this study provide a reference for the standardized utilization and clinical application of *F. taipaiensis*.

Data availability

Additional data related to this paper may be requested from the corresponding author upon request.

Conflicts of interest

The authors declare that there are no conflicts of interest in this work.

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Author contribution statement

Chun-Mei Mei: Data curation, project administration, writing manuscript, experimental design. **Yu-Wei Zhao:** Data curation, project administration, experimental design. **Fang-Yu Hao:** Formal Analysis, Investigation. **Fu-Gui Chen and Xing-Yu Zhu:** Visualization, investigation. **Xiao-Fang Hu and Mei-Mei Luo:** Visualization, data Curation. **Lin-Yu Zheng:** Formal Analysis, Visualization. **Huan Li and Nong Zhou:** Resources, Writing - Review & Editing. **Wei-Dong Li:** Project Administration, Supervision, Funding Acquisition, Writing - Review & Editing. All the authors have approved the final manuscript.

Informed consent

Not applicable.

Ethics statement

The animal experiments were carried out following the guidelines of the Nanjing University of Chinese Medicine Institutional Committee for the Care and Use of Laboratory Animals and were approved by the Committee on Ethical Use of Animals of Nanjing University of Chinese Medicine (Ethical code: 202404A090).

Use of AI statement

None.

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