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Expectorant, antitussive, anti-inflammatory activities and compositional analysis of *Aster tataricus*

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ABSTRACT

Ethnopharmacological relevance: The root of *Aster tataricus* L. f., recorded in all versions of Chinese Pharmacopoeia, is a traditional Chinese medicine with the function of dispelling phlegm and relieving cough for more than 2000 years. This study was designed to evaluate the expectorant, antitussive, and anti-inflammatory activities of the root of *A. tataricus* and to explore the chemical substances responsible for these activities.

Materials and methods: The 70% ethanol extract of the root of *A. tataricus* (RA-70) was divided into three fractions, Fr-0, Fr-50 and Fr-95. They were all orally administrated to the mice to investigate their potential expectorant activities by a tracheal phenol red secretion method. The most effective fraction, together with shionone, was evaluated the expectorant, antitussive and anti-inflammatory activities by the mouse models of phenol red secretion, ammonia-induced cough, and xylene-induced ear swelling. Furthermore, the chemical components of the effective fraction were analyzed and identified by an HPLC-Q-TOF/MS method.

Results: Treatment with RA-70, Fr-0 and Fr-50 increased the amount of phenol red secretion by 65.3%, 56.5%, and 76.9%, respectively. Fr-50 was chosen for the further investigation and the results showed that Fr-50 at 40, 80 mg/kg significantly enhanced the phenol red secretion of tracheas, increased the latent period and decreased the frequency of cough and inhibited the ear edema in mice. Shionone at 80 mg/kg showed the trend of enhancing sputum secreting, but had no effect on ammonia-induced cough and xylene-induced ear edema. HPLC-Q-TOF/MS analysis indicated that Fr-50 was mainly composed of 12 caffeoylquinic acids (40.8%, in relative peak area), 7 astersaponins (12.0%) and 13 astins/asterinins (pentapeptides, 26.5%).

Conclusions: The root of *A. tataricus* has significant expectorant, antitussive and anti-inflammatory effects. Caffeoylquinic acids, astersaponins, and aster peptides, rather than shionone, may be the main constituents responsible for the expectorant and antitussive activities of *A. tataricus* and act in a synergistic way.

1. Introduction

Cough with copious phlegm is a common symptom of respiratory diseases. Increased sputum may cause irritation of the respiratory mucosa, which leads to coughs. The blocks of bronchioles will not only cause asthma, but also cause secondary infection which results in further damage of the respiratory tract leading to the increased cough, sputum and asthma. In some cases, excessive phlegm may cause respiratory depression or suffocation (Rose et al., 2001; Kishioka et al., 2001). Currently, PM 2.5 is a great trouble of many big cities. Epidemiological studies have shown that air pollution brings about a variety of harmful effects on human health, especially on the respiratory system (Schikowski et al., 2010; Zemp et al., 1999). Cough and mucus were generally caused by respiratory inflammation. High incidences of respiratory diseases make it urgent to research and develop more effective and green therapies. Many of Chinese herbs and Chinese compound preparations have unique advantages in the treatment of such respiratory diseases.

Aster tataricus L. f. (Compositae) is a perennial plant which is widely cultivated in north China. The root of *A. tataricus* (RA) has been used for dispelling phlegm and relieving cough for more than 2000 years in China and has been included in all editions of Chinese Pharmacopoeia. Besides of the effects of expectorant and antitussive, RA possesses more else activities such as antitumor,

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antibacterial, diuretic, antiviral, antioxidant and antiulcer (Zhang et al., 2012).

Thanks to its favorable curative effect for cough, RA is widely used in many Chinese patent medicines and clinical prescriptions, but there were few reports related to the potential effective constituents. Shionone is a specific triterpene of RA and has been used as a marker compound for the quality control of RA in Chinese Pharmacopoeia (Chinese Pharmacopoeia Commission, 2010). Shionone also has been regarded as an effective component of RA because it showed the expectorant (at a dose of 100 mg/kg) and antitussive (at a dose of 300 mg/kg) activities in mouse models (Lu et al., 1999). A research focusing on volatile oil of RA revealed that 1-acetoxy-2-ene(*E*)-4,6-decandiyne increased the mucus secretion of tracheas in mice (Yang et al., 2008). However, it is far from a clear understanding of the active ingredients in RA. The present study was aimed to separate the 70% ethanol extract of RA with macroporous resin into three fractions for screening the active part in terms of the expectorant, antitussive and anti-inflammatory effects. Further, the chemical constituents of the effective part were analyzed and identified by an HPLC-Q-TOF/MS technique.

2. Materials and methods

2.1. Reagents and chemicals

Ammonium chloride was purchased from Xilong Chemicals Co. (Guangdong, China). Pentoxyverine was purchased from Sinopharm Rongsheng Pharmaceutical Co. (Henan, China). Dexamethasone acetate was purchased from Zhejiang Xianju Pharmaceutical Co. (Zhejiang, China). Phenol red was purchased from Nanjing Chemical Reagent Co. (Nanjing, China), and urethane was obtained from Shanghai Qingxi Chemicals Co. (Shanghai, China). Shionone, 3,5- and 3,4-dicaffeoylquinic acids, astins A–C, astersaponins A and C were isolated from RA in our laboratory, and their structures were identified by ^{1}H and ^{13}C NMR data, chlorogenic acid (3-caffeoylquinic acid) was purchased from Zelang Pharmaceutical Technology Co., Ltd. (Nanjing, Jiangsu, China). All above compounds were of the purity > 95%. HPLC-grade acetonitrile and methanol were supplied by Merck (Darmstadt, Germany). Purified water was from Hangzhou Wahaha Company (Zhejiang, China). Other reagents were analytical grade.

2.2. Preparation of plant materials

RA was obtained from Bozhou Medicinal Market (Anhui, China) and identified by Prof. Mian Zhang, one of the authors. Voucher specimens were deposited in our lab of China Pharmaceutical University.

RA was crushed and refluxed with 70% ethanol (v/v) for three times, each for 0.5 h. After concentrated in vacuo, the combined extract (RA-70) was applied on a column of HPD500 macroporous adsorption resin, and eluted with water, 50% and 95% (v/v) ethanol in sequence to give three fractions, Fr-0, Fr-50 and Fr-95.

2.3. Animals and drug administration

ICR male mice (18–22 g) were purchased from Yangzhou University Comparative Medicine Center (Jiangsu, China). All animals were given free access to pellet food and water, housed at a constant temperature (25 ± 2 °C) and relative humidity (50 ± 5%) on a 12-h light/dark cycle. Animal welfare and experimental procedures were carried out in accordance with the Principles of Laboratory Animals and the related ethical regulations of China Pharmaceutical University. All drugs were suspended with 0.5% sodium carboxymethyl cellulose (CMC-Na) solution, and 0.5% CMC-Na solution was taken as control.

2.4. Evaluation on expectorant activity

After 3 days of adaption, the mice were randomly divided into treated and control groups (10/group). Mice in treated groups were orally given indicated drugs and doses once daily for 3 consecutive days, taking ammonium chloride (NH_4Cl, 250 mg/kg) as positive control. After 30 min of the last administration, mice were injected with 2.5% phenolsulphonphthalein. Thirty minutes later, they were sacrificed by intraperitoneally injected with 30% urethane (0.2 ml/10 g), the trachea and bronchi parts were taken out and put into 0.8 ml of 5% $NaHCO_3$ immediately, centrifuged at 2500 rpm for 5 min. The absorbance of the centrifuged supernatant was measured at the wavelength of 546 nm using a microplate reader (Epoch, BioTek, USA) (Han et al., 2010).

2.5. Evaluation on antitussive activity

The mice were exposed to a desiccator sprayed with 15% ammonium hydroxide (0.22 ml) to record the latent period and cough frequency (Zhang et al., 2009; Xu et al., 2001). Mice with latent period less than 1 min and the cough frequency more than three times in 1 min were chosen to be eligible animals for the experiments. After 24 h of recovery, 50 eligible mice were divided into five groups randomly, i.e., control, positive control (pentoxyverine, 17.5 mg/kg), and three treated groups. Mice in treatment groups were orally given Fr-50 fraction at doses of 40 and 80 mg/kg, shionone at 80 mg/kg, respectively, once a day for 3 days. After 1 h of the last administration, the mice were exposed to a 500 ml special glass chamber sprayed with 15% ammonium hydroxide (0.22 ml), and the latent period and the frequency of cough in 2 min were recorded by a skilled observer.

2.6. Evaluation on anti-inflammatory activity

The test was performed as described previously (Akindele and Adeyemi, 2007; Li et al., 2010). Five groups of randomly divided mice (10/group) were separately treated with CMC-Na (control), dexamethasone (10.5 mg/kg, positive control), Fr-50 at doses of 40, 80 mg/kg, and shionone at 80 mg/kg. After oral administration for 30 min, the right ear was evenly applied by 0.02 ml of xylene on the anterior and posterior surfaces, and the left ear was considered as a control. Ears were removed from mice which were sacrificed by cervical dislocation after 30 min treatment, punched into ear disks (6.0 mm in diameter), and weighed. The percentage inhibition of edema was calculated by following formula: % Inhibition = (Wc − Wt)/Wc × 100%, where 'Wc' is the average increase in ear weight in control group, and 'Wt' is that in treatment group.

2.7. HPLC-Q-TOF/MS analysis of Fr-50 fraction

Agilent 1260 HPLC instrument equipped with a quaternary pump (G1311C), an auto sampler (G1329), a column compartment (G1316A), and a diode array detector (G1314B) was connected to a 6520 quadrupole time-of-flight mass spectrometer with a Jet Stream electrospray ionization (ESI) source (Agilent Technologies, USA). HPLC parameters: samples were separated on a Zorbax SB-C18 column (250 mm × 4.6 mm, 5 μm, Agilent) at the temperature of 30 °C; the wavelength for determination was 210 nm; the mobile phase was composed of acetonitrile (A) and 0.01% formic acid (B), eluting in following program (v/v): 0 min, 10% A; 13 min, 18% A; 18 min, 25% A; 30 min, 25% A; 38 min, 28% A; 45 min, 40% A; and 50 min, 80% A. MS parameters: Q-TOF/MS analysis was performed in both negative and positive modes using full scan mode and the mass range was set at 100–1700 Da; the conditions of the ESI source were as follows: drying gas (N_2) flow rate, 8.0 L/min; gas temperature, 32.5 °C; capillary voltage, 3500 V (negative mode) and 4000 V