Determination of Arctiin in Fructus Arctii by Capillary Electrophoresis

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Abstract. This paper investigated the determination of arctiin content in Fructus Arctii by high performance capillary electrophoresis (HPCE) method. The borax solution of 37.5 mmol concentration containing 12.5% methanol was chosen as buffer solution. The experiment was performed at a constant voltage of 16 kV and UV detection wavelength of 277 nm. The content of arctiin in Fructus Arctii was 26.45 mg/g (RSD=3.34%) (n=7). The recovery was in the range of 87.6%-111.8% (n=5). This method is suitable for the detection of the content of arctiin in Fructus Arctii.

1. Introduction
Fructus Arctii is the composite family Arctium Lappa L. dry ripe fruit, pungent, bitter and cold. It is the function of detoxification, detumescence and dispelling wind-heat. It is used treatment of wind-heat type common cold, lung heat cough, sore throat, measles, erysipelas and abscess malignant sore. The Fructus Arctii extractive include lignans, volatile oil, fatty acid, terpenoids, etc. Zhang et al [1] studied the extraction and purification process of total lignans from Arctium lappa L. and to measure the antibacterial activity of total lignans in Arctium lappa L.. Using Arctiin as an indicator, the best extraction process of total Lignan from Arctium lappa L. was investigated by single factor and orthogonal experiments. The purification of the active constituents of Arctium lappa L. was tested by macroporous adsorption resin. The bacteriostasis experiment was performed on the active constituents of the Arctium lappa L. before and after purification by the Oxford Cup method. The best extraction process of Arctium lappa L. was to extract 80 times with 80% ethanol as extraction solvent, and add 12 times of alcohol, 1.5 hours each time. The best purification process was AB-8 type macroporous adsorption resin loading column, 0.05g/mL loading concentration, 6BV distilled water and 5BV 70% ethanol eluting, 70% ethanol eluent eluting part of Arctium lappa L. total wood Lipoprotein. The total lignans of Arctium lappa L. had antibacterial activity against Staphylococcus aureus and Streptococcus pneumoniae. The essential oil from Arctium lappa L. produced in Yunnan was extracted by Hu et al [2] using simultaneous distillation extraction method and its chemical compounds were studied by Gas Chromatograph-mass spectrometry with rate of the carrier gas of 1.0 mL/min. The injection method was split-flow and the ratio of split-flow was 20:1. The temperature of injection port was 280℃ and the sample size was 1 μL. The temperature of the programmed route increased. According to computer retrieval by the spectrum analysis, the structures of 89 components (97.55% of the total contents) were identified. The ultrasound-assisted extraction was used by Wang et al [3] to extract arctiin from Frutus arctii, and the effects of particle size, solvent/sample ratio, extraction time, ultrasound power and
extraction temperature on the concentration of arctiin were studied. According to the experimental investigated and the Fick’s first law, a kinetic model was obtained to describe the ultrasound-assisted extraction process and the calculated values were in good agreement with the experimental data. Xu et al [4] studied extractive of fructus arctii. The fat soluble compounds in the extractive of fructus arctii were removed by ethyl acetate, the aqueous phase drying was isolated by preparative HPLC, and six components were isolated from the extractive of fructus arctii and their structures were identified by physicochemical property analysis, specially by spectral analysis. Their structures were identified as 3,4-dihydroxy cinnamic acid-4-O-β-D-glucoside, 3-caffeoylquinic acid, 3,4-dihydroxy cinnamic acid, 3,5-dicaffeoylquinic acid, 1,5-dicaffeoylquinic acid and 3,4-dicaffeoylquinic acid. Fu et al [5] established a HPLC method for the determining Arctiin, Arctigenin and Chlorogenic acid content in Arctium lappa L. and to make a comparison of content of this three components among different parts as well different time that Arctii Fructus fried. The analysis was performed on Welchrom C18 (5μm, 4.6mm × 250mm) column with mobile phase composed of acetonitrile-0.1% phosphoric and flow rate of 1.0mL/min at gradient elution. The detection wavelength was 280nm and the column temperature was 30℃. The UV and IR spectra of petroleum ether, chloroform and ethanol extracts of Fructus Arctii from different places were measured by Shao et al [6]. It was showed that the UV and IR spectra of the same solvent extract of Fructus Arctii from different places had good similarity and characteristics common peak and the UV and IR spectra of different solvent extracts had significant differences. The ultrasonic-assisted extraction was explored by Zhang et al [7] as an alternative technology for the extraction of arctigenin from the hydrolyzed powder of Fructus Arctii. The experimental conditions were investigated by a central composite design, including extraction temperature, ethanol concentration and solvent to solid ratio. The optimum conditions were obtained that the temperature was 52.8℃ and ethanol concentration was 80% and solvent to solid ratio was 40 mL/g. Qiao et al [8] established HPLC fingerprints of crude and processed Arctii Fructus, analyzed and compared their chemical constituents, and finally revealed the changes of their chemical constituents after processing of Arctii Fructus. The mobile phase was composed of acetonitrile - 0.2% aqueous formic acid with flow rate of 1.0 ml/min at gradient elution. The column temperature was 30 ℃. The detection wavelength was 286 nm. The established chromatographic fingerprints of crude and processed Arctii Fructus were evaluated by similarity analysis and hierarchical cluster analysis. Optimization of the ultrasonic-assisted hot water extraction of polysaccharides from Fructus arctii was investigated by Yu et al [9]. According to single-factor experiments, three independent factors of ratio of water to raw material, extraction time and extraction temperature were tested during extraction. The experiments were arranged by Box-Behnken central composite design experiment. Response surface analysis method was adopted to measure the central composite experiment design. The antioxidant activities of polysaccharides were evaluated by DPPH radical scavenging activity (DPPH·), hydroxyl radical scavenging (·OH), superoxide radical scavenging activity (O2·) and reducing power. In this paper, the Arctiin content in Fructus Arctii was determined by High Performance Capillary Electrophoresis.

2. Experimental section

2.1. Instruments and Reagents

Experimental instruments: CL-1030-type high performance capillary electrophoresis (Beijing Cailu Scientific Instrument Co., Ltd.); HW2000-type chromatography workstation (Nanjing Qianpu Software Ltd.); Capillary (75 μm inner diameter, 52 cm overall length, 44 cm effective length) from Hebei Yongnian Ruifeng Chromatographic Devices Co., Ltd.).

Arctiin (Chinese Drugs and Biological Products); Fructus Arctii (purchase in weifang pharmacy); other reagents used in the experiments were all analytical grade; Double-distilled water was used.
2.2. Experimental Methods
Before the start of the experiment, capillary was successively washed with 1 mol·L$^{-1}$ hydrochloric acid solution, double-distilled water, 1 mol·L$^{-1}$ sodium hydroxide solution, double-distilled water, buffer solution, each for 5 min. After three times running, capillary was cleaned again using the above method. Measurements were carried out at 16 kV voltage and experimental temperature at 21°C. UV detection wavelength was 277 nm. Injection time was 10s (7.5 cm height difference).

2.3. Sample Preparation
Fructus Arctii sample solution: Fructus Arctii was accurately weighed 2.1516 g, added 40 mL water containing 80% methanol, extracted time of 48h at 21°C, filtered, washed and set the volume to 50 mL that was the Fructus Arctii sample solution.

Arctiin standard solution: Arctiin was accurately weighed 0.0046 g, added 2 mL water.

3. Results and Discussion

3.1. Selection electrophoresis conditions
The experiment was carried out at 16 kV voltage. UV detection wavelength was 277 nm.

Based on past experiment experience, 37.5 mmol/L borax solution containing 12.5% methanol was chosen as electrolyte solution.

3.2. Quantitative analysis

3.2.1. Standard curve. First, arctiin standard solution was prepared and its concentrations were 2.3, 1.15, 0.575, 0.2875, 0.1437, 0.0718, 0.0359 mg/mL. Each standard solution was run for three times under the above electrophoresis conditions and the results averaged. The chromatogram of arctiin standard solution was showed in Figure 1. Taking concentration as the abscissa and peak area as the ordinate, the standard curve was drew. Linear regression equation of arctiin (peak area: $y \mu V \cdot s$, density: $x$ mg/mL) and the linear range was as follows: $y = -2653.6 + 84674.5x (r=0.998)$, 0.0359-2.3 mg/mL.

Fig. 1 Electrophorogram of arctiin standard solution 1-arctiin

3.2.2. Precision test. A arctiin standard solution precisely drew and continuously injected for six times under electrophoretic separation conditions, the RSD of arctiin migration time and peak area were 2.25% and 4.91%, indicating good precision.

3.2.3. Determination of sample content. Under selected electrophoresis conditions, Fructus Arctii sample solution was run. Separation chromatogram of the Fructus Arctii sample solution was showed in Figure 2. Measured arctiin content in Fructus Arctii was 26.45mg/g(RSD=3.34%)(n=7).
3.2.4. Recovery. After determination for five times, the recovery of arctiin in Fructus Arctii sample was in the range of 87.6%-111.8% (n=5). The average recovery was 98.9%.

4. Conclusion
This paper investigated the determination of arctiin content in Fructus Arctii by high performance capillary electrophoresis method. Measured arctiin content in Fructus Arctii was 26.45mg/g (RSD=3.34%) (n=7).

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