

Capillary Electrophoresis Method for Determining the Content of Rutin in Oriental arborvitae

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Abstract. The experiment was carried out for the determination of rutin content in Oriental arborvitae by high performance capillary electrophoresis method. The borax solution as buffer solution was chosen, and its concentration was 20 mmol/L at a constant voltage of 20kV and injecting time of 10s. The detection wavelength was 254 nm. Measured rutin content in Oriental arborvitae was 2.3491 mg/g (RSD = 9.12%) (n = 6). The recovery was in the range of 88% - 118% (n=4).

Keywords: Capillary electrophoresis, Oriental arborvitae, Rutin.

1. Introduction

Oriental arborvitae is traditional Chinese medicine. It has the effect of cool blood and hemostasis, clear up the phlegm to stop coughing, hair growth and black hair. It is used for the treatment of vomiting blood, hemoptysis, hematochezia, cough with lung heat, blood fever, hair loss and premature graying hair [1]. It has the component of volatile oil, flavonoids, tannins, inorganic element, etc. It has the antibacterial, anti-inflammatory, hemostasis, preventing cough and dispelling phlegm, antitumor pharmacological action [2]. The inhibition effects of six kinds of plant extracts (including garlic, pomegranate, xanthium and so on) on pathogenic bacteria of some usual vegetable diseases were discussed by Meng [3]. The results showed the inhibition rate of oriental arborvitae on the powdery mildew were better than that on other pathogenic bacteria. For screening the new botanical acaricide, the biological activities of 8 species of plant extracts were determined in laboratory using leaching stem immersion method by Jia et al. [4]. The results indicated that the biological activities of oriental arborvitae, *Euphorbia australis* L., *Rumex japonicas* Houtt and *Punica granatum* Linn. Were high. Luo et al [5] established a method for determining myricetin and quercetin in oriental arborvitae. The separation was carried out on an Inertsil-ODS-3(4.6mm×250mm, 5μm) column, the mobile phase consisted of methanol-0.01 mol/L potassium dihydrogen phosphate solution - glacial acetic acid(37:63:1.5), with a flow rate of 1.0 mL/min and the wavelength of 254 nm. Cheng et al. [6] established an HPLC method for the determining isopimaric acid in the leaves of *platycladus orientalis* and its preparations cebai No. V capsula and isopimaric acid capsula to evaluate the quality of them. The Kromasil C18 (200 mm×4.6 mm, 5μm) column was adopted. The mobile phase was composed of methyl alcohol - water - acetic acid (60:37:3) with flow rate of 1 mL/min and wavelength of 231 nm. Tan et al. [7] established an HPLC method for simultaneously determining the contents of myricetrin, quercitrin, yricetin, quercetin, kaempferol, amentoflavone and hinokiflavone in oriental arborvitae and oriental arborvitae *Carbonisatum*. The experiment were explored on Welch Ultimate LP-C18 column (4.6 mm×250 mm, 5μm), mobile phase was methanol 0.2% phosphoric acid aqueous solution in a gradient elution mode and the wavelength was 330 nm. Meng et al. [8] established an HPLC method for the simultaneous determining myricitrin, quercitrin, amentoflavon and hinokiflavon in order to provide the basis for the quality control and exploitation of *platycladi cacumen*. A Waters ODS C18 column (4.6 mm×150 mm, 5μm) was applied with a gradient elution of methanol-water containing 0.5% acetic acid and the flow rate of 0.8 mL/min and the detection wavelength of 340 nm. Shan et al [9] investigated the flavonoids in oriental arborvitae. The constituents in oriental arborvitae were determined by UPLC-MS. A Waters BEH C18 column (2.1mm×150 mm, 1.7μm) was adopted with a gradient elution of methanol water containing 0.2% formic acid. The mass spectrometer equipped with electrospray ionization source was used as defector and operated in data was collected under the negative ion modes. Eleven constituents were identified.

Gu et al [10] investigated the inhibition of pancreatic lipase and antioxidation of different extracts from oriental arborvitae leaves in vitro. The inhibitory activities of pancreatic lipase of ethanol extract, petroleum aether extract, ethyl acetate extract, n-butyl alcohol extract and water extract were assayed in vitro. The antioxidant activities of these extracts were assessed by two complementary test systems, namely 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and ferric reducing antioxidant power (FRAP) assay. The ethyl acetate extract indicated strong activity in the inhibitory activities of pancreatic lipase. Li et al [11] extracted polysaccharide from oriental arborvitae leaf and analyze its antioxidant activity. With distilled water as the extraction agent, using the method of ultrasonic extraction of Oriental arborvitae polysaccharide, the main factors affecting the extraction yield of polysaccharides by single factor and orthogonal experiments, and the best extraction technology conditions were obtained. The antioxidant activity of polysaccharides from oriental arborvitae leaf was studied by superoxide anion free radical scavenging assays. The optimum ultrasonic extraction conditions were solid - liquid ratio(g:mL) 1:35, temperature 60°C, ultrasonic power 180 W and ultrasonic action time 40 min, under this condition, the oriental arborvitae leaf polysaccharide's extraction quantity was 8.35%. Ding et al [12] established a new determining method for quercitrin from oriental arborvitae leaves by capillary-zone electrophoresis (CZE). The optimized CZE conditions was the buffer composed of 30 mmol/L borax-boric acid (volume 1:3) with the separation voltage of 25 kV and the wavelength of 254 nm.

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.). Rutin (Shanghai Alading Biochemical Technology Co., Ltd.); Oriental arborvitae (Weifang pharmaceutical Co., Ltd.); 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 carded out at 16 kV voltage and experimental temperature at 20°C. UV detection wavelength was 254 nm. Injection time was 10s (7.5 cm height difference).

2.3 Sample Preparation

Oriental arborvitae sample solution: Oriental arborvitae powder was accurately weighed 1.4212 g, added 40 mL water, extracted time of 24h at 20°C, filtered, washed and set the volume to 50 mL that was the Oriental arborvitae sample solution.

Rutin standard solution: Rutin was accurately weighed 50 mg, added 50 mL water with 30% methanol.

3. Results and Discussion

3.1 Selection Electrophoresis Conditions

Based on past experiment experience, we chose 20 mmol/L borax solution as a running buffer solution.

According to the literature, Rutin absorption wavelength was at 254 nm, so we chose the 254 nm detection wavelength.

3.2 Quantitative Analysis

3.2.1 Standard Curve

First, Rutin standard solution that the concentration were 0.2, 0.1, 0.05, 0.025, 0.0125, 0.00625 mg/mL was prepared. Each standard solution was run for three times under the above electrophoresis conditions and the results averaged. Taking concentration as the abscissa and peak area as the ordinate, the standard curve was drew. Linear regression equation of Rutin (peak area: $y \mu V \cdot s$, density: $x \text{ mg/mL}$) and the linear range was as follows: $Y=3137.75+331536.12X$ ($r=0.973$), 0.00625~0.2 mg/mL.

3.2.2 Precision Test

A Rutin standard solution precisely drew and continuously injected for six times under electrophoretic separation conditions, the RSD of Rutin peak area were 8.7%.

3.2.3 Determination of Sample Content

Under selected electrophoresis conditions, Oriental arborvitae sample solution was run. Separation chromatogram of the Oriental arborvitae sample solution was showed in Figure 1. Measured Rutin content in Oriental arborvitae was 2.3491 mg/g (RSD = 9.12%) ($n = 6$).

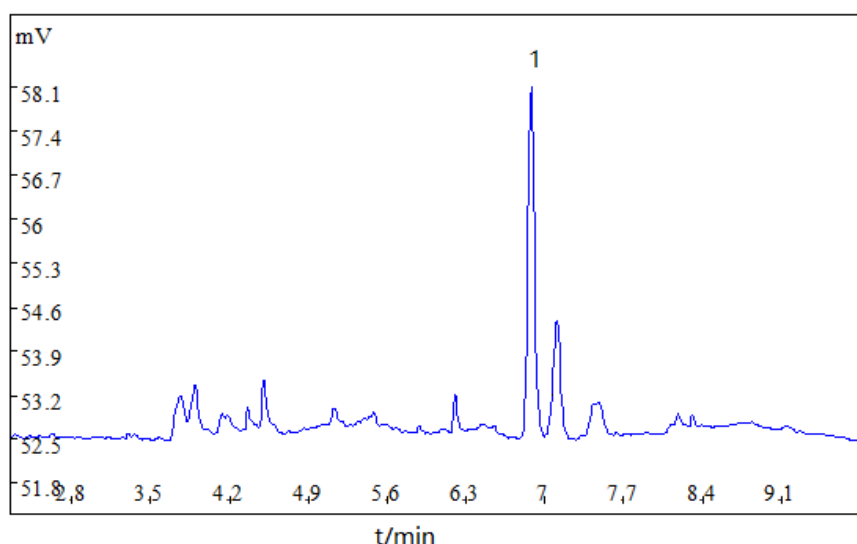


Fig. 1 Electrophorogram of Oriental arborvitae sample solution 1- Rutin

3.2.4 Recovery

After determination for six times, the recovery of Rutin content in Oriental arborvitae sample was in the range of 88% - 118% ($n=4$).

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