

Capillary Electrophoresis Method for Determining the Content of Rutin in Fructus Kochiae

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Abstract. The experiment was Carried out for the determination of rutin content in Fructus Kochiae 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 Fructus Kochiae was 0.115 mg/g (RSD = 10.5%) (n = 6). The recovery was 103.6% (RSD=7.2%, n=4).

Keywords: Capillary electrophoresis, Fructus Kochiae, Rutin.

1. Introduction

Fructus kochiae is traditional Chinese medicine. It has the effect of Clearing heat, removing dampness, expelling wind and stopping itching. It is used for the symptom of painful urination, itchy skin and so on [1]. It has the function of anti-inflammatory and anti-pathogenic microorganism so on. It is chiefly used in the treatment of skin tinea and eczema disease. Its active ingredient was triterpene, saponin and flavonoids [2-4]. Zeng [5] established a RP-HPLC method for the determining 4'-O- β -D-glycosyl-5-Omethylvisamminol in Jingfuzhiyang Granules(Herba Schizonepetae, Fructus kochiae, Radix Saposhnikoviae, Fructus Crataegi, etc.). The sample was performed on Turner Kromasil C18 column (4.6mm \times 200mm, 5 μ m) with mobile phase of methanol-0.05mol/L potassium dihydrogen phosphate-acetic acid-isopropyl alcohol (85:170:4:4, v/v) and detection wavelength of 254nm and the flow rate of 1.0mL/L . The column temperature was 30°C. Luo et al [6] Established a method for measure Momordin Ic in Fructus Kochiae gel, the HPLC analysis was carried out on a Agilent HC-C18 column (160mm \times 4.6mm, 5 μ m) with a mobile phase of methanol-1.3% acetic acid (85:15). The flow rate was 1.0 mL/min and the detection wavelength was 208 nm. Xia et al [7] compared the content of momordin Ic and total saponin in Fructus kochiae from eleven producing areas. The HPLC-ELSD method were adopted for the determining the content of momordin Ic and total saponin, respectively. The content of momordin Ic in Fructus kochiae was related to that of total saponin. Cheng et al [8] investigated the antioxidant activity of the whole herbs of Kochia scoparia and theirs ferulic acid derivatives in vitro through 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) and Fe³⁺ reduction ability experiments. The antioxidant activity of extracts and ferulic acid derivatives were also compared with vitamin C and salicylic acid. The results indicated that the antioxidant activity of extracts from the whole herbs of Kochia scoparia was slightly weaker than that of extracts from the fruits of Kochia scoparia, while the antioxidant activity of N-trans-feruloylmethoxytyramine and N-trans-feruloyltyramine which isolated from Kochia scoparia were slightly weaker than that of vitamin C but better than salicylic acid. They (water, petroleum ether, chlorform, ethylacetate, and methanol) were applied by Wu et al [9] to extract the Fructus kochias, their fungistasis effects on six fungus were investigated by Fusarium gramine, Fusarium oxysporum , Monilia cinerea , Physalos porapiricola , Alternaria artemata and Valsa mali , respectively. The results showed that the water extracts had the strongest fungistasis effects on all six fungus, with antifungal activities all above 74.34%. The five extracts indicated also stronger antifungal activities against Monilia cinerea and Valsa mali. The total flavonoid content and antioxidant activity of several extracts of Kochia scoparia fruits were analyzed by Li et al [10]. The ethanol, acetone and ethyl acetate extracts of Kochia scoparia fruits were established by cold-extraction. The total flavoid content was determined, and the DPPH assay and reducing power assay were utilized to study the antioxidant activity of Kochia scoparia fruits. All extracts of Kochia scoparia fruits possessed potent antioxidant activities, and the

most outstanding one was found to be the ethyl acetate extract. The extracts could be utilized as natural antioxidants. Yu et al [11] determined three common treatment of urticaria Fe, Cu, Mn in traditional Chinese medicine the content of trace elements. The content of elements was obtained by flame atomic absorption spectrometry method with nitric acid and perchloric acid (4+1) decomposition samples. Three kinds of trace elements of the three kinds of traditional Chinese medicine exist obvious difference, ephedra, kochia scoparia, cortex dictamni, element content in the size are as following: Fe>Mn >Cu. Zhao et al [12] investigated the effect of mycelial growth and spore germination about six kinds of aqueous extracts including Kochia scoparia , Bryophyllum pinnatum , Paeonia suffruticosa and so on by mycelial growth and spore germination method. The result indicated that there was a certain inhibition on cucumerinum, and the inhibitory activity on spore germination was better than mycelial growth. The antibacterial activity of aqueous extract of Kochia scoparia and Paeonia suffruticosa was highest.

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.); Fructus Kochiae (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 $\text{mol}\cdot\text{L}^{-1}$ hydrochloric acid solution, double-distilled water, 1 $\text{mol}\cdot\text{L}^{-1}$ 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

Fructus Kochiae sample solution: Fructus Kochiae powder was accurately weighed 2.555 g, added 40 mL water with 10% methanol, extracted time of 2.5h at 60°C, filtered, washed and set the volume to 50 mL that was the Fructus Kochiae 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 $\mu\text{V}\cdot\text{s}$, density: x mg/mL) and the linear range was as follows: $Y=3137.75+331536.12X$ ($r=0.973$), $0.00625\sim 0.2$ 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, Fructus Kochiae sample solution was run. Separation chromatogram of the Fructus Kochiae sample solution was showed in Figure 1. Measured Rutin content in Fructus Kochiae was 0.115 mg/g ($\text{RSD} = 10.5\%$) ($n = 6$).

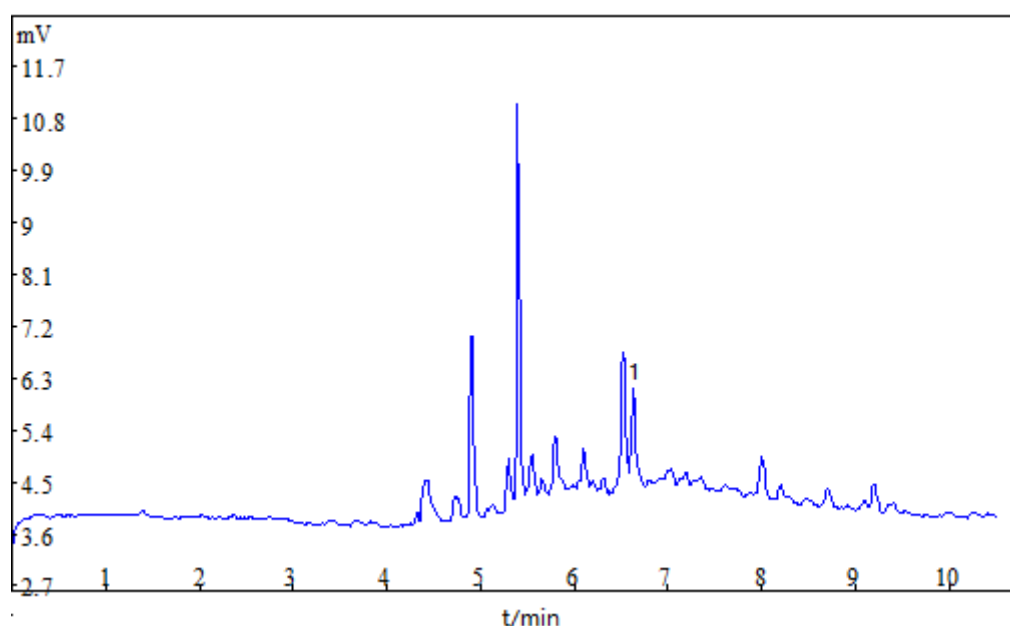


Fig. 1 Electrophorogram of Fructus Kochiae sample solution 1- Rutin

3.2.4 Recovery

After determination for six times, the recovery of Rutin content in Fructus Kochiae sample was 103.6% ($\text{RSD}=7.2\%$, $n=4$).

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