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Preparative isolation and purification of alkaloids from the Chinese medicinal herb *Evodia rutaecarpa* (Juss.) Benth by high-speed counter-current chromatography

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Abstract

High-speed counter-current chromatography (HSCCC) with a two-phase solvent system composed of *n*-hexane–ethyl acetate–methanol–water system (5:5:7:5, v/v) was applied to the isolation and purification of alkaloids from the Chinese medicinal plant *Evodia rutaecarpa* (Juss.) Benth. Five kinds of alkaloids were obtained and yielded 28 mg of evodiamine (**I**), 19 mg of rutaecarpine (**II**), 21 mg of evocarpine (**III**), 16 mg of 1-methy-2-[(6*Z*,9*Z*)]-6,9-pentadecadienyl-4-(1*H*)-quinolone (**IV**), 12 mg of 1-methyl-2-dodecyl-4-(1*H*)-quinolone (**V**) from 180 mg of crude extract in a one-step separation, with the purity of 98.7%, 98.4%, 96.9%, 98.0%, 97.2%, respectively, as determined by high performance liquid chromatography (HPLC). The structures of these compounds were identified by ^{1}H NMR and ^{13}C NMR.

Keywords: *Evodia rutaecarpa* (Juss.) Benth; HSCCC; Evodiamine; Rutaecarpine; Evocarpine; 1-Methy-2-[(6*Z*,9*Z*)]-6,9-pentadecadienyl-4-(1*H*)-quinolone; 1-Methyl-2-dodecyl-4-(1*H*)-quinolone

1. Introduction

Wu-zhu-yu, the dried fruit of *Evodia rutaecarpa* (Juss.) Benth (*E. rutaecarpa*), is a well-known traditional Chinese medicine and officially listed in the Chinese Pharmacopoeia [1], and has been used for a long time in Chinese medical practice. Wu-zhu-yu is used as a remedy for gastrointestinal disorders (abdominal pain, dysentery), headache, amenorrhea, and postpartum hemorrhage [2]. It has also been claimed to have a remarkable central stimulant effect, a transient hypertensive effect [2,3], and positive inotropic and chronotropic effects [4]. In phytochemical studies, a wide variety of compounds including alkaloids were found in the fruits of this plant. Alkaloids, including evodiamine and rutaecarpine, are the major active compounds present in Wu-zhu-yu. Evodiamine and rutaecarpine are often used as standards in the quality control of Wu-zhu-yu products [5]. Here, some of these alkaloids are separated by high-speed counter-current chromatography (HSCCC). The two-phase solvent system composed of *n*-hexane–ethyl acetate–methanol–water (5:5:7:5, v/v) was applied to the separation and purification of alkaloids from the extract of Wu-zhu-yu. Five kinds of major alkaloids including evodiamine (**I**), rutaecarpine (**II**), evocarpine (**III**), 1-methy-2-[(6*Z*,9*Z*)]-6,9-pentadecadienyl-4-(1*H*)-quinolone (**IV**) and 1-methyl-2-dodecyl-4-(1*H*)-quinolone (**V**) were obtained in one-step separation. The chemical structures of these alkaloids are shown in Fig. 1.

2. Experimental

2.1. Apparatus

The HSCCC instrument employed in the present study is TBE-300A high-speed counter-current chromatography

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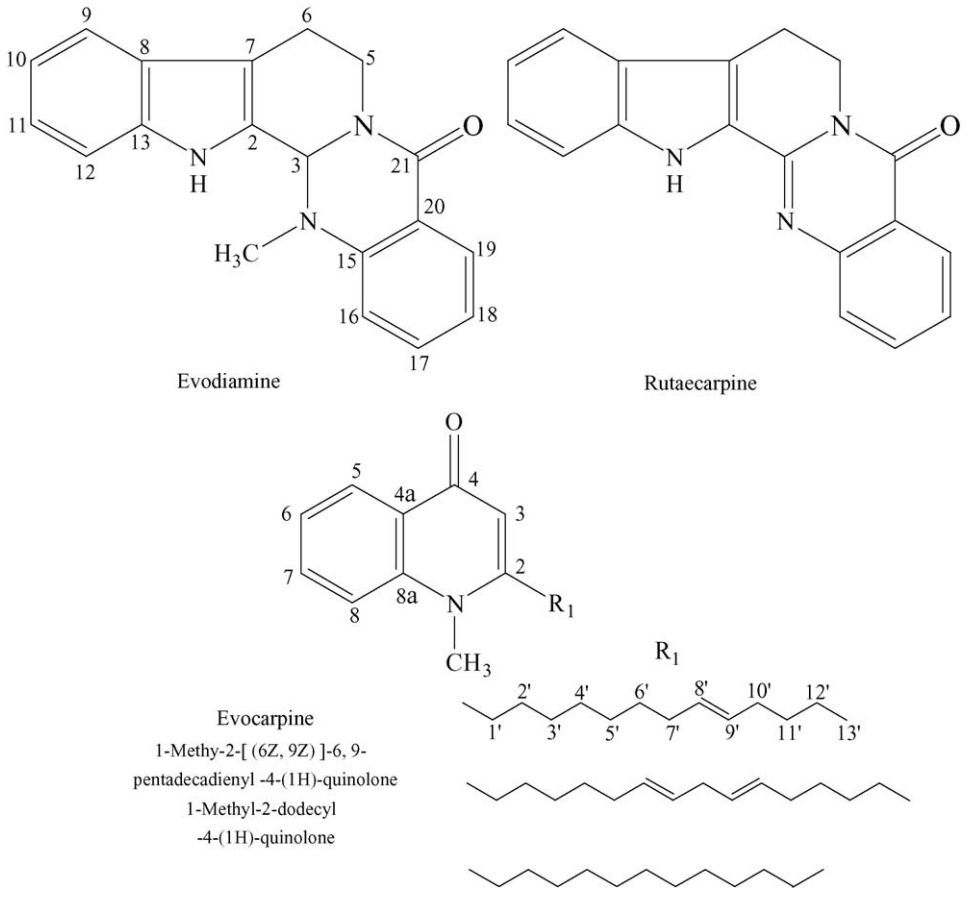

Fig. 1. Chemical structures of alkaloids from *E. rutaecarpa*.

(Tauto Biotechnique Company, Shanghai, China) with three multilayer coil separation column connected in series (I.D. of the tubing = 1.6 mm, total volume = 260 ml) and a 20 ml sample loop. The revolution radius or the distance between the holder axis and central axis of the centrifuge (*R*) was 5 cm, and the β values of the multilayer coil varied from 0.5 at internal terminal to 0.8 at the external terminal ($\beta = r/R$, where r is the distance from the coil to the holder shaft). The revolution speed of the apparatus can be regulated with a speed controller in the range between 0 and 1000 rpm. An HX 1050 constant-temperature circulating implement (Beijing Boyikang Lab Instrument Company, Beijing, China) was used to control the separation temperature. A ÄKTA prime system (Amersham Pharmacia Biotechnique Group, Sweden) was used to pump the two-phase solvent system and perform the UV absorbance measurement. It contains a switch valve and a mixer, which were used for gradient formation. The data were collected with Sepu 3000 chromatography workstation (Hangzhou Puhui Science Apparatus Company, Hangzhou, China).

The HPLC equipment used was Agilent 1100 HPLC system including G1311A QuatPump, G1315B UV–vis photodiode array detector, Rheodyne 7725i injection valve with a 20 μl loop, G1332 degasser and Agilent HPLC workstation.

The nuclear magnetic resonance (NMR) spectrometer used here was a Mercury Plus 400 NMR system (Varian Inc., USA).

A FZ102 plant disintegrator (Taisite Instrument Company, Tianjin, China) was used for disintegration of the sample.

2.2. Reagents and materials

All solvents used for preparation of crude sample and HSCCC separation were of analytical grade (Jinan Reagent Factory, Jinan, China). Methanol used for HPLC was chromatographic grade (Yucheng Chemical Factory, Yucheng, China), and water used was distilled water.

Wu-zhu-yu was purchased from a local drug store and was identified as the dried fruits of *E. rutaecarpa* by Professor Yongqing Zhang (Shandong University of Traditional Chinese Medicine, Jinan, China).

2.3. Preparation of crude sample

Preparation of crude sample was carried out as following. The dried fruits of *E. rutaecarpa* were ground to powder (about 40 mesh) by using the FZ102 plant disintegrator. The powder (100 g) was dipped in 800 ml of ethyl acetate