Fitoterapia 80 (2009) 415–420

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Determination of dehydroevodiamine in *Evodia rutaecarpa* (Juss.) Benth by high performance liquid chromatography and classification of the samples by using hierarchical clustering analysis

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ARTICLE INFO

Article history:
Received 22 February 2009
Accepted in revised form 20 May 2009
Available online 30 May 2009

Keywords:
High performance liquid chromatography
Dehydroevodiamine
Evodia rutaecarpa (Juss.) Benth
Hierarchical clustering analysis

ABSTRACT

A simple, sensitive and accurate liquid chromatographic method with photodiode-array detection was developed for determination of dehydroevodiamine with detection wavelength at 368 nm and column temperature at 30 °C. The separation was carried out on an Agilent Zorbax SB-C_{18} column (250 mm × 4.6 mm, 5 μm) together with a C_{18} guard column. The mobile phase was acetonitrile–water (containing 30 mM sodium acetate trihydrate and 0.15% acetic acid) in the ratio of 30:70 (v/v) delivered at a flow rate of 1 mL/min. Excellent linear behavior was observed over the concentration range investigated, with correlation coefficient (R^2) = 0.9998. This validated method was applied to determine the contents of dehydroevodiamine in 36 samples from different regions of China, and hierarchical clustering analysis was firstly used to classify and differentiate *Evodia rutaecarpa* samples. The analysis is specific and can be successfully applied to analyze *E. rutaecarpa* which is helpful for quality control of the herb.

1. Introduction

The dried unripe fruit from *Evodia rutaecarpa* (Juss.) Benth., popularly known as Wuzhuyu in China, is commonly used as one of the Traditional Chinese medicines (TCMs). The active chemical constituents of Wuzhuyu were divided into fractions of alkaloids, essential oils, limonoids, carboxylic acids and flavonoids [1–5]. Anti-inflammatory [6,7], anti-nociceptive [8,9], anthelmintic [10,11], antidiarrheal [12], anti-anoxic [13,14] and antibacterial effects [15] of the extracts have been reported. It is defined in Chinese Pharmacopoeia [16] that the total content of evodiamine and rutaecarpine in *E. rutaecarpa* should not be less than 0.15%, which indicates that the two alkaloids are the major active compounds in Wuzhuyu. There are many reports about the determination for the contents of evodiamine and rutaecarpine, including thin-layer chromatography (TLC) [17], gas chromatography (GC), gas chromatography/mass spectrometry (GC/MS) [18,19] and high-performance liquid chromatography (HPLC) [20–22]. In the previous paper [23], we developed a direct and rapid HPLC method for simultaneously determining 5 bioactive makers in *E. rutaecarpa*. In the present study, a simple assay method for determining another bioactive compound dehydroevodiamine was developed. It was reported that dehydroevodiamine could suppress the overactivation of GSK-3 and improve the tau hyperphosphorylation and spatial memory deficit of rats [24], inhibit the expression of LPS induced iNOS and COX-2 proteins and suppress their mRNAs from RT-PCR experiment on RAW 264.7 cells [25] and inhibit the IFN-γ/LPS-stimulated NO production in a concentration-dependent manner [26]. But only two literatures [27,28] were found reporting simultaneous determination for alkaloids in *E. rutaecarpa* including dehydroevodiamine from Chinese Taibei. The aim of the

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doi:10.1016/j.fitote.2009.05.017

present study was to establish a simple, sensitive and accurate method for the determination of dehydroevodiamine in *E. rutaecarpa* from various regions in China especially in Guizhou province that was known for the largest output, the best and the most stable quality of *E. rutaecarpa*. Hierarchical clustering analysis (HCA) was performed according to the contents of dehydroevodiamine to classify and differentiate the samples and to evaluate and control its quality better.

2. Experimental

2.1. Chemicals and reagents

HPLC grade acetonitrile was purchased from TEDIA Company, Inc. (Product of Tedia, USA). Ultrapure water was prepared with the Sartorius Arium611UF water purification system (18.2 MΩ, Sartorious Germany). AR grade acetic acid and methanol were obtained from Chongqing Maoye Chemical Company (Chongqing, China). AR grade sodium acetate trihydrate was purchased from Chengdu Kelong Chemical Company (Chengdu, China). Standard substance dehydroevodiamine was isolated and identified in our laboratory whose structure (Fig. 1) was confirmed on the basis of spectroscopic analysis (^{1}H NMR, ^{13}C NMR, ESI-MS, UV). The purity exceeds 98%.

2.2. Plant materials

36 batches of samples were collected from various locations throughout China that were authenticated as *Evodia rutaecarpa* (Juss.) Benth (Table 1).

2.3. Sample preparation

The samples were dried at room temperature and pulverized by a grinder to 60-mesh size before use. A 0.2 g powder was precisely weighed and put into a 50-mL conical flask, then 25 mL chloroform and 5 mL concentrated ammonia were added, which was weighed again and recorded. Then, the conical flask was sealed and extracted under reflux at 70 °C for 2 h, after cooling, chloroform was added to make up to the initial weight. The extracted solution was filtrated through analytical filter paper. 10 mL of the filtered solution was vacuum dried and the dried extract was dissolved in 10 mL methanol. The sample was finally filtrated through a 0.45-μm-membrane filter prior to injection into HPLC.

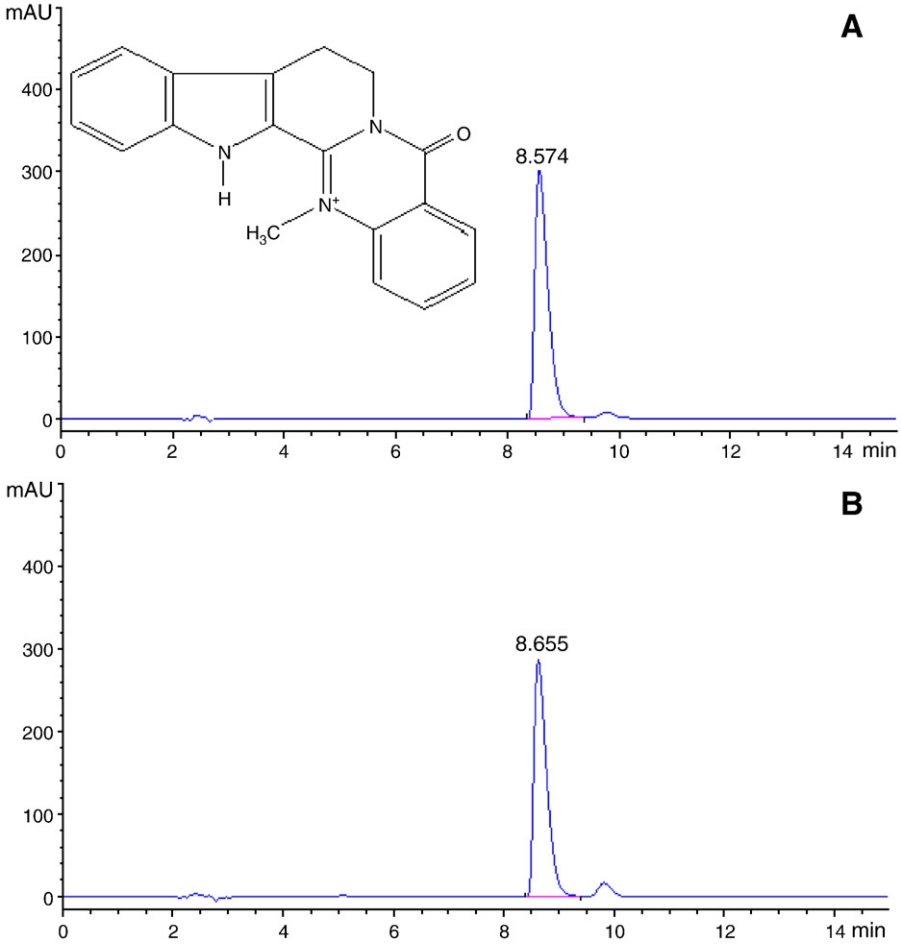

Fig. 1. Representative HPLC chromatograms of the standard (A) and the extract from the fruit of *E. rutaecarpa* B.