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ScienceDirect
Journal of Ethnopharmacology 108 (2006) 257–263
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Chemical and biological comparisons on *Evodia* with two related species of different locations and conditions

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Received 24 June 2005; received in revised form 24 April 2006; accepted 12 May 2006
Available online 26 May 2006

Abstract

Evodia rutaecarpa (ER) and *Tetradium glabrifolium* (TG) are closely related species collected from different locations, with processed versus unprocessed and fresh versus 1-year-old samples. The purpose of this study is to determine the variability of their bioactive constituents; evodiamine, dehydroevodiamine, rutaecarpine and synephrine—as well as their relaxing effects on an isolated rat aortas and uterus using the extracts of the test specimens. The vasorelaxation was greater in ER from Taiwan than from China in spite of lower levels of the relaxing alkaloids evodiamine, dehydroevodiamine and rutaecarpine. On the other hand, the uterine relaxation of ER from China was better than the one from Taiwan, even though constricting synephrine was only contained in Chinese ER. After processing, the relaxation of ER from China in the uterus was increased while the vasorelaxation remained unchanged. Conversely, TG from Wu-ling contained more relaxing alkaloids than that from Lee Mountain. However, the relaxation in both the uterus and the aorta was less in TG from Wu-ling. After 1 year of storage, the vasorelaxation of TG from Lee Mountain was not changed. Taken together, a significant finding in the present study is the lack of correction between chemical composition and relaxing activities. This strongly supports our assumption that biological function evaluations, instead of chemical standardization, is the more adequate way of showing meaningful consistency of natural preparations.

Keywords: *Evodia rutaecarpa*; *Tetradium glabrifolium*; Chemical composition; Relaxing activity; Variability

1. Introduction

Western drugs can be life-saving at times; nevertheless, they may have serious side effects, which often become obvious after months or even years the drug has been introduced to the market. There have been an increasing number of instances where western drugs have been withdrawn from the market due to dangerous and alarming side effects (Preziosi, 2004). There is growing trend of consumers finding a balanced solution with nutraceuticals. Although they come with fewer side effects, they do have other problems. According to recent studies, the consistency of natural products is a major issue. Different brands of a natural substance or different lots of a brand may show a great difference in chemical composition and function. Product standardization or product consistency is a major concern for the industry, as it constitutes serious consumer protection concern. It is generally believed that natural substances are highly variable, especially herbs. Inconsistency of a material's source would lead to inconsistent products. The systematic study to clearly establish chemical and functional variability under different conditions is critical to health food and nutraceuticals,

Abbreviations: ER, *Evodia rutaecarpa*; ERC, *Evodia rutaecarpa* from China; ERCP, *Evodia rutaecarpa* from China processed with liquorices and *Coptis chinensis*; ERT, *Evodia rutaecarpa* from Taiwan; TG, *Tetradium glabrifolium*; TGL, *Tetradium glabrifolium* from Lee Mountain; TGLS, *Tetradium glabrifolium* from Lee Mountain stored 1 year; TGW, *Tetradium glabrifolium* from Wu-ling

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doi:10.1016/j.jep.2006.05.020

which are often single natural substances, traditional Chinese medicine or complex formulations. Due to material variability, the product inconsistency creates a major concern for its effectiveness.

This study is focusing on the chemical and biological comparisons using *Evodia* with two related species of different locations and conditions. Wu-Chu-Yu is the dry unripe fruit of *Evodia rutaecarpa* (Juss.) Benth. (ER) (Rutaceae), recently renamed as *Tetradium ruticarpum* (Juss.) T. Hartley (Huang, 1993). It is commonly used alone or in a combination with other herbs to treat gastrointestinal disorders, postpartum hemorrhages (Chang and But, 1986), amenorrhea, headaches, and hypertension (Tang and Eisenbrand, 1992). Chemistry and relaxing effects on the isolated rat vascular preparations of four main active chemicals; evodiamine (Chiou et al., 1992; Jeng et al., 1995), dehydroevodiamine (Chen et al., 1991; Peng et al., 1993; Chiou et al., 1996), rutaecarpine (Ko et al., 1994; Wang et al., 1996, 1999) and synephrine (Bevan and Osher, 1965; Ledda et al., 1980) have been reported. The uterotonic effects of ER alkaloids are also reviewed (King et al., 1980). In Taiwan, there is a close relative of ER named *Tetradium glabrifolium* (Champ. ex Benth.) T.G. Hartley (TG) (Rutaceae). In the present study, chemical composition and relaxing effects on isolated rat aortic rings and uterine myometrium comparisons were made on ER collected from Taiwan and China, processed and unprocessed, and on TG from two locations in Taiwan, fresh and after storage for 1 year. ER and TG were also compared. Such studies were designed to demonstrate the conditions leading to variability. Also, simultaneous chemicals and functional studies on these specimens may help us understand whether or not chemical standardization can be a meaningful and practical way to show consistency in natural substances.

2. Materials and methods

2.1. Sample specimens

Several specimens of ER and TG, obtained from different localities and conditions, were authenticated by Mr. Muh Tsuen Kao, an experienced botanical consultant of National Research Institute of Chinese Medicine and Institute of Ecology and Evolutionary Biology, National Taiwan University, Taiwan. These voucher specimens were deposited in the National Research Institute of Chinese Medicine for future reference. Several tests and comparisons were made and are described below.

2.1.1. Study 1

Specimens of ER were collected from two widely separated regions, mainland China (ERC) and Taiwan (ERT). The concentrations of the four key chemicals—evodiamine, dehydroevodiamine, rutaecarpine and synephrine—were compared. Their relaxing effects on the contracted rat aorta and uterus were also determined.

2.1.2. Study 2

Specimens of TG were also obtained from two locations, Wu-ling (TGW) and Lee Mountain (TGL), in Taichung County, Taiwan. The concentrations of the four key chemicals—evodiamine, dehydroevodiamine, rutaecarpine and synephrine—were compared. Their relaxing effects on the contracted rat aorta and uterus were also determined.

2.1.3. Study 3

With the results gathered from the above studies 1 and 2, comparisons were made on the four key chemicals—evodiamine, dehydroevodiamine, rutaecarpine and synephrine. Their relaxing effects on the contracted rat aorta and uterus were also determined.

2.1.4. Study 4

A specimen of TGL was tested on two occasions, 1 year apart (TGL and TGLS), for the composition of their four key chemicals—evodiamine, dehydroevodiamine, rutaecarpine and synephrine—and vasorelaxing effects on the contracted rat aorta and uterus were also determined.

2.1.5. Study 5

Two different specimens of ER were obtained from China. One was of the usual dry material (ERC), while the other was processed with other herbs (ERCP), liquorices and *Coptis chinensis* Franch. (Ranunculaceae), which is often done in the preparation of herbal medicine in China. The concentrations of the four key chemicals—evodiamine, dehydroevodiamine, rutaecarpine and synephrine—were compared. Their relaxing effects on the contracted rat aorta and uterus were also determined.

2.2. Phytochemical preparation

2.2.1. Sample processing

Samples of various specimens were pulverized and extracted with methanol (1:100 fruit to methanol) at 40 °C in an ultrasonic bath for 30 min (Zhen et al., 2005). This extraction procedure was repeated and the resulting extracts were combined. They were then dried by rotary evaporation under vacuum at 50 °C. These extracts were used for the chemical composition determinations and biological activity studies.

2.2.2. Chemical analyses

Chemical determinations were made according to our previously published method (Ko et al., 2002, 2003). The sample was re-dissolved in methanol and filtered through a 0.22 μm filter and diluted to 10 ml, once again with methanol. Aliquot of 10 μl sample solution was injected into a reverse phase C18 column (4.6 mm × 250 mm, Nacalai Tesque Inc., Kyoto, Japan). The alkaloids were separated by a gradient using A: 85% acetonitrile, 15% water, 0.085% phosphoric acid and 0.058% sodium dodecyl acetate—and B: 15% acetonitrile, 85% water, 0.085% phosphoric acid and 0.058% sodium dodecyl acetate. Alkaloids were separated by stepwise elution according to the following profile: 0–15 min, 20% A, 80% B; 15–30 min, 35% A, 65% B; 30–45 min, 50%A, 50% B; 45–50 min, 65% A, 35% B; 50–60 min, 100% A. Alkaloids were detected by measuring the