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Full Length Research Paper

Determination of two major xanthone glycosides in rhizome of *Anemarrhena asphodeloides* using high performance capillary electrophoresis

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Anemarrhena rhizome known as "Zhi-Mu" is an important traditional Chinese medicine (TCM). In this study, an accurate, rapid and reproducible high performance capillary electrophoresis (HPCE) method with ultraviolet (UV) detection has been established for the simultaneous determination of two major bioactive xanthone glycosides, mangiferin and neomangiferin in rhizome of *Anemarrhena asphodeloides* (Zhi-Mu). The running buffer was 30 mM borate (pH 9.3) containing 15% methanol. The separation voltage was 30 kV and temperature was 25℃. Gentiopicroside was adopted as an internal standard (IS) and detection wavelength was 254 nm. Within 15 min, the contents of mangiferin and neomangiferin can be simultaneously assayed. The results also indicate that it is important to assay the xanthone content in quality control of "Zhi-Mu" for its great variation among different samples.

Key words: High performance capillary electrophoresis (HPCE), *Anemarrhena asphodeloides*, mangiferin, neomangiferin.

INTRODUCTION

"Zhi-mu" is the rhizome of *Anemarrhena asphodeloides* Bge. (Liliaceae), a medicinal plant distributed widely in mainland China. It has been used as a famous drug in traditional Chinese medicine for more than 2000 years since first recorded in Shennong's Classic of Materia Medica (Editorial board of China Bencao, 1999), and it is known to have functions of clearing heat, draining fire, nourishing yin and moisturizing dryness; for treating exogenous fever, high fever, hyperactivity dry cough, hot flashes, heat diabetes, and intestinal constipation (Committee for the Pharmacopoeia of People's Republic of China, 2010). Pharmacological studies revealed anemarrhena rhizome possesses many biological properties such as antidiabetic (Miura et al., 2001), anticancer (Jeong et al., 2003), antioxidant (Meng et al., 1999), anti-platelet- aggregation (Dong and Han, 1991; Niwa et al., 1988), antifungal (Iida et al., 2000; Park et al., 2003 antiviral (Bae et al., 2007) and antidepressant (Ren et al., 2007), neuroprotective (Oh et al., 2007) properties. Steroidal saponins (Kawasaki and Yamauchi, 1963), polysac- charides (Takahashi et al., 1985), lignan (Kimura et al, 1996; Nikaido et al., 1981; Kim et al., 2009) and xanthones (Hong et al., 1985) were reported as its main chemical constituents in previous phytochemical investigations.

Among them, mangiferin is the major compound which acts as an antioxidant, antiviral, antidiabetic. In addition, further studies confirm that mangiferin also has many other activities such as anti-inflammation (Ojewole, 2005), immunomodulatory, antitumor and anti-HIV effects (Guha et al., 1996). Noemangiferin is another major xanthone in Zhimu (Hong et al., 1997). Having the same gennin with maniferin, its structure is similar to mangiferin. So, it may have similar bioactivities with mangiferin. Nevertheless, the content of neomangiferin is relative high in Anemarrhena rhizome. Based on aforementioned facts, it

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is clear that the assay of mangiferin and neomangiferin is very important in quality evaluation of “Zhi-mu”. Several articles have reported quality analysis of “Zhi-mu” and the quantitative determination of mangiferin and neomangiferin. High performance liquid chromatography (HPLC) and thin layer chromatography (TLC) methods have been widely employed (Chen et al., 2000; Chen et al., 2011; Hong and Han, 1985; Wang et al., 2004). HPLC is the commonly used method for analyzing herbal medicines at present.

Although HPLC is a reliable method, it often requires a large amount of organic reagent consumption for mobile phase and test sample preparation, compared with high-performance capillary electrophoresis method. Alternatively, capillary electrophoresis (CE) has powerful resolving ability and is a simpler, more efficient, and less costly procedure in comparison to HPLC. Meanwhile, multiple modes can be chosen and it is easy to clear up the contaminants in capillary in CE analysis. Therefore, it has been accepted as a good method of separation analysis, and applied in assay of natural medicine including many TCMs (Ding et al., 2005; Jia et al., 2000). However, few published data concerned analysis of “Zhi-mu” by HPCE. In this paper, a HPCE method for rapid separation and determination of two major xanthone glucosides in Anemarrhena rhizome was established. The contents of mangiferin and neomangiferin were assayed in the “Zhi-mu” samples (*A. asphodeloides*) which were collected during our quality survey.

MATERIALS AND METHODS

Plant materials and reagents

The samples of rhizome of *A. asphodeloides* (Zhi-Mu) were collected and bought from different places and marketed in north China. The voucher specimens were deposited at the herbarium of School of Chinese Pharmacy Beijing University of TCM, Beijing. HPLC grade methanol was purchased from Fisher. Super pure water was purified by Milli-Q system (Millipore, Bedford, MA, USA) and filtered through a 0.22 μm filter. Analytical grade methanol, sodium hydroxide, and sodium tetraborate were purchased from Xi’an fine chemicals reagents Co. Ltd. (Xi’an, China). Standard substance of mangiferin was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Batch number: 111607-200301).

Authentic standard of neomangiferin was isolated from the *A. asphodeloides* rhizome in our group. Its structure was unambiguously determined on the basis of spectral data (NMR and MS) and comparing with references as well (Hong et al., 1997) (Figure 1). The purity of the neomaniferin was above 98% determined by HPLC. Internal standard of gentiopicroside was also prepared in our group with the purity of 98%.

Apparatus

The HPCE analysis was carried out on a Beckman MDQ system equipped with a diode array detector, a fluid-cooled column cartridge, an automatic injector and an uncoated fused-silica capillary (Beckman) of 60.2 cm × 75 μm I.D.(effective length 50 cm). A 24 Karat Gold software for system control and data processing was used. A pHs-3C pH meter (Lei-ci Instrumentation Factory, Shanghai, China) was used for pH measurements.

HPCE condition

The applied voltage was 30 kV, using normal polarity. Sample injection was carried out in a hydrodynamic mode for 3 s with a pressure of 20 psi. The capillary temperature was maintained at 25℃ and the detector was set at 254 nm. A measure of 30 mM borate buffer (pH 9.3) containing 15% methanol was used as a running buffer.

When a new capillary was used, the capillary was rinsed with 1 M sodium hydroxide for 15 min, then water for 5 min prior to the first analysis. Between each run, the capillary was rinsed with 0.1 M sodium hydroxide for 3 min, water for 2 min, and buffer for 3 min, sequentially. The pH of the running buffer was adjusted with 0.2 M HCl or 1 M NaOH.

Preparation of standard solution and samples

A stock solution containing the two standards (mangiferin 1105 μg ml^{-1} and neomangiferin 1171 μg ml^{-1}) was prepared in 50% ethanol. The internal standard (gentiopicroside) was dissolved in 50% ethanol with a concentration of 2548 μg ml^{-1}. Accurately piped standard stock 0.1, 0.2, 0.3, 0.5, 1.0 and 2.0 ml to six 5 ml volumetric flasks, respectively and 1 ml internal standard solution was added to each. Adjusted to the volume by adding 50% ethanol, mixed well for constructing calibration plots. The stock and working solutions were stored at 4℃.

The rhizomes of *A. asphodeloides* were ground using a miller. About 300 mg sample (60 mesh) was accurately weighed and extracted with 20 ml of 50% ethanol by ultrasonication (100 W, 40 kHz) for 40 min in a 25 ml volumetric flask. The extract was cooled to room temperature, adjusted to the volume, mixed well, centrifuged at 8000 $rmin^{-1}$ for 5 min. Accurately transfer 2 ml upper clear extraction to a 10 ml volumetric flasks, followed by additional 1 ml internal standard solution (gentiopicroside, 2548 μg ml^{-1}), adjusted to the volume, mixed well, filtered through a 0.45 μm millipore filter membrane.

RESULTS AND DISCUSSION

Method validation

The calibration curves were constructed by injecting the standard solutions across 6 different concentrations (22.1 to 442.0 μg ml^{-1} for mangiferin, 23.42 to 46.84 μg ml^{-1} for neomangiferin). A plot of ratio of the peak area to internal standard peak area versus analyte concentration resulted in calibration equations of Y = 34.949X - 0.1511 (r = 0.9993) for mangiferin and Y = 21.024X + 0.0592 (r = 0.9996) for neomangiferin.

To assess the precision of the method, precision (intra-day and inter-day assay precision) were tested by injected standard solutions. The coefficient of variation of intra- and inter-days studies were both less than 5.0% (n = 5). The precision and accuracy of the assay were satisfactory. The LOD values for mangiferin and neomangiferin were 0.55 and 0.58 μg ml^{-1}, and the LOQ values for mangiferin and neomangiferin were 2.21 and2.34 μg ml^{-1}, respectively. The stability and recovery

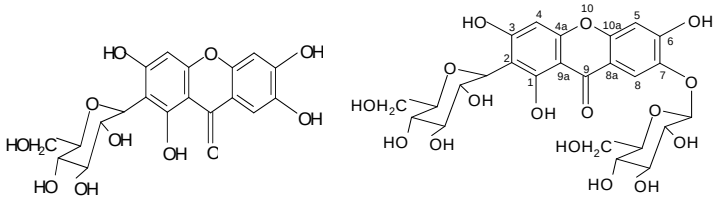

Figure 1. Structure of mangiferin and neomangiferin.

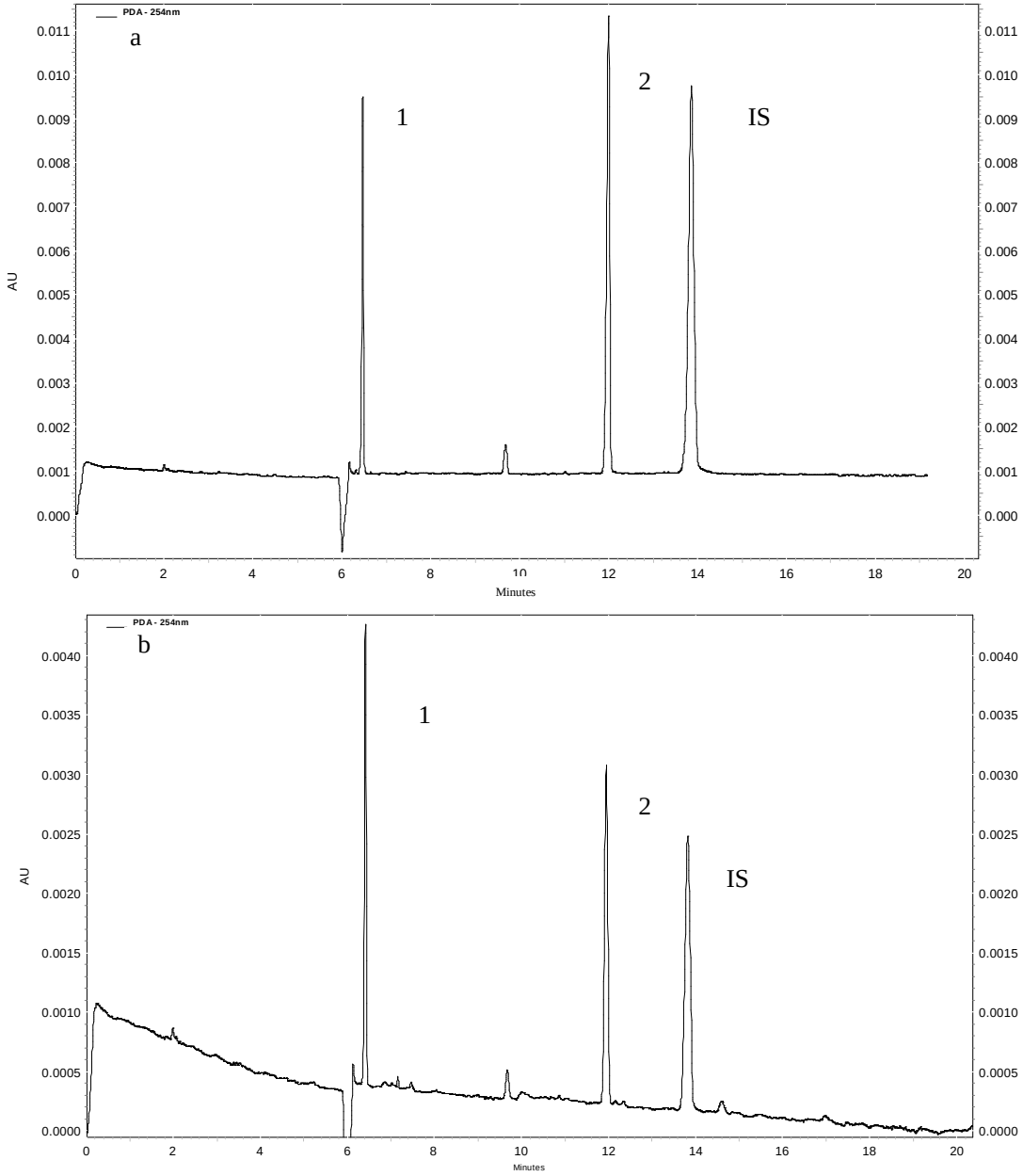

Figure 2. Electropherograms of standard solution of two xanthone glycosides, (a) and extract of "Zhi-mu" (b) CE conditions, capillary 60.2 cm × 75 μm I.D, buffer 30 mM borate buffer (pH 9.3) containing 15% methanol, voltage 30 kV, temperature 25℃, detection wavelengt h 254 nm. 1 = neomangiferin, 2 = mangifein, IS = gentiopicroside.

Table 1. The contents of mangiferin and neomangiferin in rhizome of *Anemarrhena Asphodeloides* (n = 3).

Samples	Location	Content (mg/g dry weight)	
		Mangiferin	Neomangiferin
1	Collected in Pingshan (Hebei Province)	4.5 ± 0.3[a]	18.1 ± 1.3
2	Collected in Wuqi (Shaanxi Province)	9.3 ± 0.3	14.4 ± 0.6
3	Collected in Yixian (Hebei Province)	5.8 ± 0.1	16.3 ± 0.1
4	Collected in Lingchuan (Shanxi Province)	6.8 ± 0.1	21.1 ± 1.0
5	Collected in Shexian (Hebei Province)	6.1 ± 0.0	11.6 ± 0.1
6	Collected in Neiqiu (Hebei Province)	10.6 ± 0.2	15.1 ± 0.3
7	Collected in Yushe (Shanxi Province)	4.4 ± 0.1	19.2 ± 1.0
8	Collected in Lingqiu (Shanxi Province)	3.8 ± 0.1	19.8 ± 0.2
9	Collected in Songshan (Beijing)	12.6 ± 0.1	13.9 ± 0.2

a: Values are expressed as mean ± SD.

were evaluated according to guidelines of Chinese Pharmacopoeia (2010).

Internal standard selection

Several other substances, such as berberine and rutin were tried as internal standard. Finally, gentiopicroside was chosen for its good resolution from all peaks of extracts and its UV absorption character.

Samples analysis

Samples were prepared as described earlier. Using the optimized CE conditions established the amounts of the two xanthone glycosides in samples were determined. The electropherograms of the standards and a sample are shown in Figure 2. Each filtrate was determined in triplicate. The content of each analyte was calculated from the corresponding calibration curve. Contents of two xanthone glycosides in "Zhi-mu" (rhizome of *A. asphodeloides*) are shown in Table 1. The amounts of mangiferin varied from 3.8 to 12.6 mg/g and the amounts of neomangiferin varied from 11.6 to 21.1 mg/g. It is obvious that the contents of the two active constituents in different samples vary greatly. This is in accordance with the results of former researches (Chen et al., 2000; Chen, 2007). So, quality access is a very important and necessary part in selecting and using of this crude drug. Further research on the quality assess of "Zhi-mu" (*A. asphodeloides*) should carried out to determine other active compounds.

Conclusion

A simple capillary electrophoretic system has been developed and evaluated for the rapid, simultaneous determination of mangiferin and neomangiferin. The HPCE method offers high separation efficiency, rapid analysis, and low running cost comparing with HPLC. It is a good method for quality control of rhizome of *A. asphodeloides*.

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