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Full Length Research Paper

Quantification of xanthones in a Mongolian health tea using high-performance liquid chromatography

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***Gentianella acuta*, known as "guixincao", has been used as a health tea with the properties of clearing heat and toxic materials, removing pathogenic heat from blood, and increasing secretion of urine. In this study, high-performance liquid chromatography (HPLC) equipped with ultraviolet (UV) detection was used for quantification of two major bioactive xanthones in the "guixincao" tea samples which were collected during our ethnopharmacological survey. As a result of this study, a simple, specific, precise, accurate, rapid and reproducible HPLC-UV method has been developed to successfully quantify desmethylbellidifolin and bellidifolin in "guixincao" tea. The results also indicate that the samples of "guixincao" tea were rich in bioactive xanthones, which provided a scientific basis for its uses in inner Mongolian.**

Key words: *Gentianella acuta*, xanthones, guixincao tea, high-performance liquid chromatography.

INTRODUCTION

The genus *Gentianella* is mainly distributed in temperate areas, comprises approximately 250 species (Von Hagen and Kadereit, 2001). Many *Gentianella* plants are employed in traditional medicine to stimulate appetite, treat disorders of the gallbladder and treat fever like the other bitter gentians in various regions of the world. In the east of inner Mongolian (China), *Gentianella acuta*, locally known as "guixincao", has been used as a herbal tea with the healthy properties of clearing heat and toxic materials, removing pathogenic heat from blood, and increasing secretion of urine. As such, an ethnopharmacological survey was carried out in the Xilingele and Hulunbeier districts of inner Mongolia from June, 2008 to September, 2009. The results showed that Owenke (one of 55 ethnic minorities in China) in these regions had a habit of drinking "guixincao" tea, which was thought could be anti-ageing and be beneficial to the heart.

Previous chemical investigations revealed that the main secondary metabolites in *Gentianella* were iridoids, xanthones and C-glucoflavonoids (Jensen and Schripsema, 2002; Janković et al., 2005). The medicinal value of the genus *Gentianella* was due to the presence of iridoids and xanthones. Bellidifolin and demethylbellidifolin, two major xanthones isolated from *G. acuta* (Lv and Li, 2009), showed a variety of bioactivities including antioxidant, anti-inflammatory, antibacterial, hypoglycemic, antitumor, cardiovascular protective effects etc (Basnet et al., 1995; Perea and Nagem, 2000; Hirakawa et al., 2005; Jiang et al., 2006; Shi et al., 2009). In addition, some studies reported that bellidifolin and demethylbellidifolin were found to be selective inhibitors of monoamine oxidases A enzymes (Schaufelberger and Hostettmann, 1988; Tovilovic et al., 2005). Furthermore, demethylbellidifolin was reported to significantly reduce the incidence of micronuclei in lymphocytes irradiated *in vitro* with γ-rays, using the micronucleus test (Jankovic et al., 2008).

High-performance liquid chromatography (HPLC) analytical methods have demonstrated excellent selectivity and resolution for xanthones, Some HPLC methods were developed and validated for the determination of naturally occurring bellidifolin and demethylbellidifolin in genus *Gentiana* and *Swertia* (Hostettmann et al., 1984; Xu et al., 2009). However, only a little published data concerned the contents of xanthones in *Gentianella* species (Vinterhalter et al., 2008). In order to provide a scientific basis for its uses in inner Mongolian (China), we

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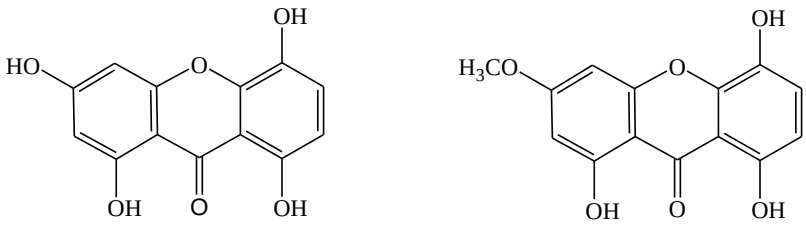

Figure 1. Structure of desmethylbellidifolin and bellidifolin.

conducted HPLC analyses of two bioactive xanthones (bellidifolin and demethylbellidifolin) in the "guixincao" tea samples (*G. acuta*) which were collected during our ethnopharmacological survey from 2008 to 2009.

EXPERIMENTAL

Plant materials and reagents

The plant materials of *G. acuta* were collected from different Xilingele and Hulunbeier districts of inner Mongolia from August, 2008 to September, 2009 by our group and the voucher specimens were deposited at the herbarium of Baotou Medical College, inner Mongolia. The plant materials were naturally dried at 25°C.

HPLC grade acetonitrile and methanol were purchased from Merck (Duren, Germany). Deionized water was purified by Milli-Q system (Millipore, Bedford, MA, USA). Analytical grade methanol, butanol, ethyl acetate and petroleum ether were purchased from Beijing Beihua Fine Chemicals Co. Ltd. (Beijing, China). Two authentic standards of bellidifolin and desmethylbellidifolin were isolated from the Air-dried whole plants of *G. acuta* in our group. Their structures were unambiguously determined on the basis of their spectral data (NMR and MS) and comparing with references as well (Sakamoto et al., 1982; Menkovic et al., 2002) (Figure 1). The purity of the two xanthones was above 98% as determined by HPLC.

High-performance liquid chromatography instrumentation and chromatographic condition

The HPLC system was a SURVEROR series (Thermo Fisher Scientific, USA), and consisted of a quaternary pump (model SRVYR-LPMPP), an auto-sampler (model SRVYR-ASP), and a UV/VIS PLUS detector (model SRVYR-UV5P) coupled with an analytical workstation (Xcalibur 2.0 SR2). The separation was performed on a Phenomenex C_{18} reserved-phase column (5 µm, 250 × 4.6 mm), and isocratic elution was used at a flow rate of 1.0 mL min^{-1} with the solvent system containing methanol and deionized water (70:30). The detection wavelength was set at 350 nm for analysis, and the column temperature was maintained at 25°C (Figure 2).

Preparation of standard solution and samples

A stock solution containing the two standards (bellidifolin 68.5 µg mL^{-1} and desmethylbellidifolin 65.0 µg mL^{-1}) was prepared in methanol and diluted to ten different concentrations for constructing calibration plots. The stock and working solutions were stored at 4°C. The aerial parts of plant were ground using a miller. Around 100 mg sample (60 mesh) was accurately weighed and extracted with 10 mL of methanol by ultrasonication for 30 min. The extract was cooled to room temperature, diluted to 10 mL of methanol, filtered through a 0.45 µm millipore filter membrane, and 5 µL of the filtrate was injected into the HPLC system for analysis.

Quantitative analyses of xanthones in "guixincao" tea (*G. acuta*)

The calibration curves were constructed by injecting the standard solution across 10 different concentrations (2.60 - 65.0 µg mL^{-1} for desmethylbellidifolin, 2.70 - 68.5 µg mL^{-1} for bellidifolin). A plot of the peak area versus analyte concentration resulted in calibration equations of $y = 4\times10^8x + 22964$ ($r = 0.9999$) for desmethylbellidifolin, and $y = 4\times10^8x + 20427$($r = 0.9998$) for bellidifolin.

The specificity, linearity, accuracy, precision (intra-day and inter-day assay precision) and stability were evaluated according to guidelines of Chinese Pharmacopoeia (2010). The LOD values for desmethylbellidifolin and bellidifolin were 0.40 and 0.45 µg mL^{-1}, and the LOQ values for desmethylbellidifolin and bellidifolin were 1.10 and 1.30µg mL^{-1}, respectively. Samples were prepared as described above. A volume of 5 µl of each filtrate was injected into the instrument and determined in triplicate. The content of each analyte was calculated from the corresponding calibration curve.

RESULTS

According to the analytical data in Table 1, the amounts of desmethylbellidifolin varied from 1.46 to 3.29 mg/g and the amounts of bellidifolin varied from 1.21 to 3.46 mg/g. In previous works, quantification of three xanthones (bellidifolin-8-O-glucoside, demethylbellidifolin-8-O-glucoside and demethylbellidifolin) in *Guttulina austriaca* was performed using HPLC, the amounts of desmethylbellidifolin in the shoots of *G. austriaca* from nature is1.67 mg/g, and in the shoots of plants cultured *in vitro*, the amounts of desmethylbellidifolin varied from 0.68 to 1.16 mg/g (Vinterhalter et al., 2008). Six samples collected from different locations in Xilingele and Hulunbeier districts were assayed and the results indicated that "guixincao" tea (*G. acuta*) was a rich source of desmethylbellidifolin and bellidifolin. And these two xanthones also

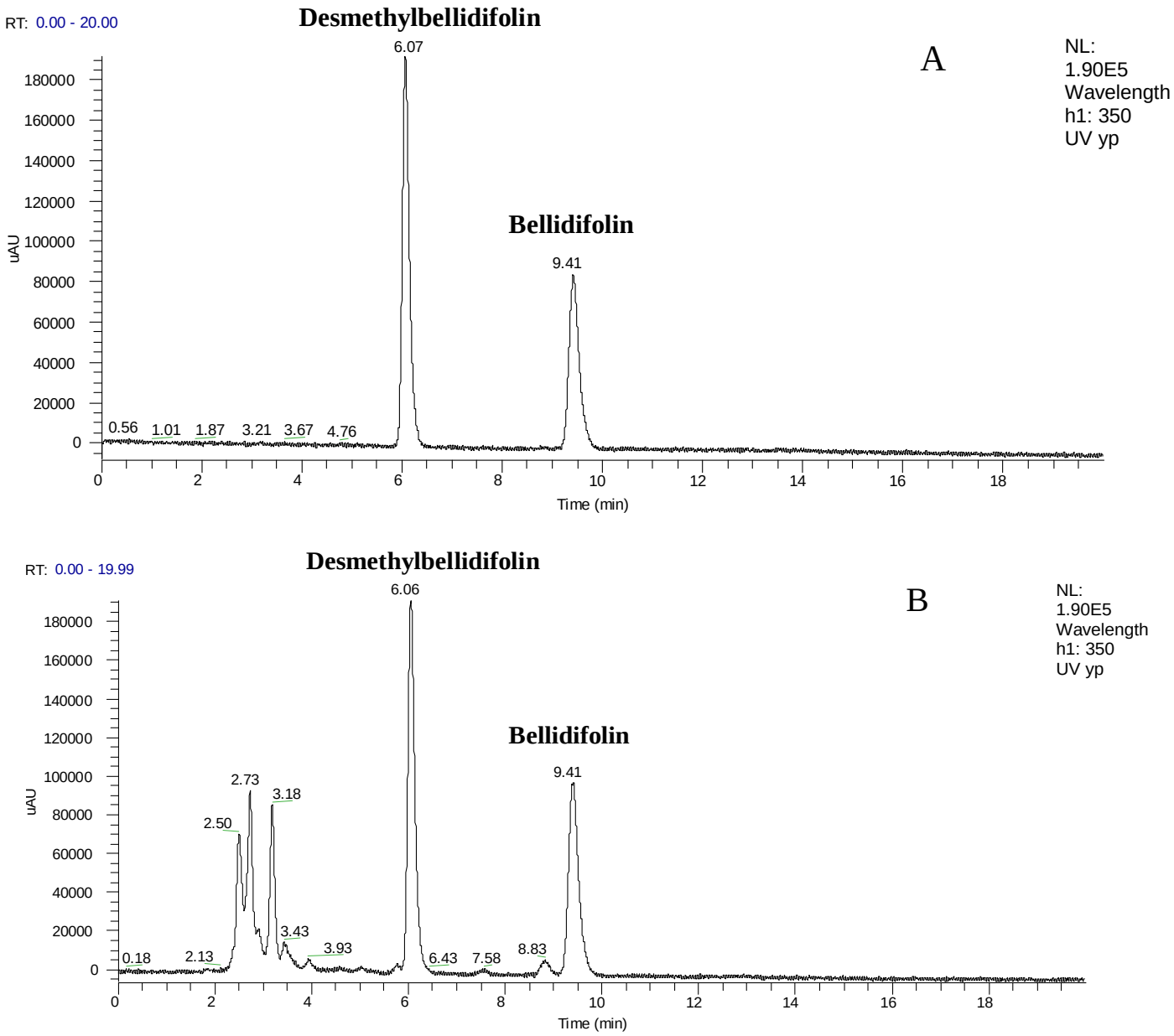

Figure 2. The chromatograms of standard mixture (**A**) and "guixincao" tea (**B**) collected from Mangui (Hulunbeier districts).

Table 1. The contents of desmethylbellidifolin and bellidifolin in "guixincao" tea (*G. acuta*) (n = 3).

Samples	Collecting location and time	Content (mg/g dry weight)	
		Desmethylbellidifolin	Bellidifolin
Sample 1	Mangui (Hulunbeier districts) August 2008	3.02 ± 0.16[a]	3.28 ± 0.13
Sample 2	Genhe (Hulunbeier districts) September 2009	2.42 ± 0.08	2.07 ± 0.11
Sample 3	Derbuer (Hulunbeier districts) September 2008	2.05 ± 0.15	3.46 ± 0.23
Sample 4	Dongwu (Xilingele districts) August 2008	1.77 ± 0.14	2.54 ± 0.19
Sample 5	Xiwu (Xilingele districts) August 2008	3.29 ± 0.16	1.69 ± 0.17
Sample 6	Hariatu (Xilingele districts) September 2009	1.46 ± 0.12	1.21 ± 0.11

a: Values are expressed as mean ± SD.

could inhibit the monoamine oxidases enzyme A by 90.5 and 98.9% at 10^{-5} M respectively, (Urbain et al., 2008). These results might provide strong evidence for the use of “guixincao” as a health tea.

Further research upon the pharmacological effects of the chemical constituents is needed to scientifically assess the efficacy of “guixincao” tea (*G. acuta*).

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