Biochemical Systematics and Ecology 47 (2013) 74–79

Contents lists available at SciVerse ScienceDirect

Biochemical Systematics and Ecology

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

Variation in major flavonoids glycosides and caffeoylquinic acids during florescence of three *Chrysanthemum morifolium* Ramat cv. '*Hangju*' genotypes

Tao Wang, Zaibiao Zhu, Qiaosheng Guo*, Pengfei Mao

Institute of Chinese Medicinal Materials, Nanjing Agricultural University, Nanjing 210095, PR China

ARTICLE INFO

Article history:
Received 18 July 2012
Accepted 10 November 2012
Available online 10 December 2012

Keywords:
Chrysanthemum morifolium Ramat cv. '*Hangju*'
Temporal dynamic
Flavonoids
Caffeoylquinic acids
Luteoloside
Quercitrin
Chlorogenic acid
3,5-*O*-caffeoylquinic acid

1. Introduction

Chrysanthemum morifolium Ramat. (Compositae) is globally the second economically most important floricultural crop following rose, and is one of the most important ornamental species (Teixeira da Silva, 2003; Van Der Ploeg and Heuvelink, 2006). Additionally, the dried capitulum of *C. morifolium*, Chrysanthemi Flos, is an important medicinal material in China, Japan, Korean and other countries (Lai et al., 2007; Jin et al., 2012), which is used for "scattering cold", "'cleaning heat and toxin" and "'brightening eyes" (Chinese Pharmacopoeia Committee, 2010). Modern pharmacological research shows that it has broad pharmacological effects, such as antibacterial, antiviral, anti-inflammatory activities (Ukiyaa et al., 2002; Lee et al., 2003; Kima et al., 2009; Tsuji-Naito et al., 2009; Lii et al., 2010). It is also widely used as a food supplement or herbal tea, and is considered as a healthy food (Lin and Harnly, 2010; Jin et al., 2012).

C. morifolium is rich in flavonoids glycosides and caffeoylquinic acids, which are considered to be the main active components. Quercitrin (Qu) and luteoloside (Lu) were reported to be useful for preventing cardiac, radical scavenging, antioxidation (Park, 2010), and chlorogenic acid (Ch) and 3,5-*O*-caffeoylquinic acid (Cq) possess the functions of antibacterial, antiviral, and antidiabetic activities (Gins et al., 2000). Those pharmacological important components are important indicators for quality control for Chrysanthemi Flos (Chinese Pharmacopoeia Committee, 2010).

* Corresponding author. Tel./fax: +86 25 84395980.
E-mail addresses: gqs@njau.edu.cn, guoqs2@gmail.com (Q. Guo).

http://dx.doi.org/10.1016/j.bse.2012.11.004

C. morifolium has been cultivated in China for over 2000 years. During its long evolution, eight main varieties of *C. morifolium* have been developed, in which '*Hangju*', '*Boju*', '*Chuju*', and '*Gongju*' are the four standard varieties of *C. morifolium* cited in the Chinese Pharmacopeia (Chinese Pharmacopoeia Committee, 2010). Among them, *C. morifolium* cv. '*Hangju*' is widely cultivated in China because of the strong market demand for this cultivar. *C. morifolium* cv. '*Hangju*' originated from Tongxiang county, Zhejiang province, and it had been introduced to several other areas to meet the increased demand. Numerous genotypes have been developed during this distribution of cultivation.

The content of phytochemicals is influenced by numerous factors including harvesting time, genotypes, cultivation techniques, and climatic conditions that occur during the growth period (Liu et al., 2010; Gao et al., 2011). It is important to find the genotypes that are rich in bioactive compounds and to study the effects of seasonal variation on the levels of the bioactive compounds to optimize harvesting protocols. Previous works has demonstrated that the components in *C. morifolium* cv. '*Hangju*' capitulum vary with different stages of flower development, origins or genotypes (Guo et al., 2008; Wang et al., 2009; Shao et al., 2010; Sun et al., 2010). However, in previous studies, materials were often purchased from local markets, or only the contents of total bioactive compounds rather than composition was considered, e.g. total flavonoids. Thus it is difficult to compare the results.

Hence, we collected three widely cultivated genotypes of *C. morifolium* cv. '*Hangju*' from five origins, and harvested the capitulum in five different periods of florescence. The components variation in *C. morifolium* cv. '*Hangju*' capitulum in terms of Lu, Qu, Ch, and Cq were analyzed and evaluated. The main objective of current work was to determine the effect of harvest time on quality of *C. morifolium* cv. '*Hangju*' and to provide useful information about the suitability of some genotypes widely cultivated.

2. Materials and methods

2.1. Plant materials

Three widely cultivated *C. morifolium* cv. '*Hangju*' genotypes were collected from five distribution areas in China (Table 1) in April 2010. All were field-grown in October 2010 in research field of the Institute of Chinese Medicinal Materials, Nanjing Agricultural University, Nanjing, P.R China using conventional commercial cultivation methods under identical conditions. Each plot measured 1.5 × 2 m (3 m^2). The plots were arranged in a completely randomized block design with three replications. Each field plot consisted of 48 plants, spaced 0.25 m apart with a row spacing of 0.25 m. Chrysanthemum capitulum was collected in five flowering stages in October 2011. Five stages of chrysanthemum flower development were determined according to the method of Guo et al. (2008) as follows: tubular-shaped florets and ray florets being not opened yet (A stage), ray florets being opened 30% while tubular flower being not opened (B stage), ray florets being opened 50% and tubular florets being opened 30% (C stage), ray florets being opened 70% and tubular florets being opened 50% (D stage), ray florets and tubular florets being fully opened (E stage). Fresh flowers were treated with 100 °C steam for 3 min to deactivate enzymes, oven-dried at 60 °C for 6 h, and were ground to pass through a 0.5 mm sieve.

2.2. Reagents and solvents

Luteoloside, quercitrin, chlorogenic acid, and 3,5-*O*-caffeoylquinic acid (Chinese pharmacopoeia reference standard) were purchased from China Pharmaceutical Biological Products (Nanjing, China). HPLC grade of methanol and acetonitrile were obtained from Tedia Co., Inc. (Fairfield, OH, USA).

2.3. Sample solution preparation

Powdered *C. morifolium* capitulum 0.25 g was exacted by sonication (300 W; 45 kHz) for 40 min in 25 mL 70% methanol. The solution was filtered through microporous membrane (pore size = 0.45 μm) (Chinese Pharmacopoeia Committee, 2010) and used for the determination of luteoloside, quercitrin, chlorogenic acid, and 3,5-*O*-caffeoylquinic acid.

Table 1
Sampling information of *C. morifolium* Ramat cv. '*Hangju*'.

No.	Samples code	Genotypes	Origins	Location
1	TZ	*Zaoyang*	Tongxiang, Zhejiang province	N30°38′, E120°32′
2	TW	*Wanyang*	Tongxiang, Zhejiang province	N30°38′, E120°32′
3	MH	*Hongxin*	Macheng, Hubei province	N29°10′, E115°00′
5	JZ	*Zaoyang*	Jurong, Jiangsu province	N33°17′, E118°51′
6	JW	*Wangyang*	Jurong, Jiangsu province	N33°17′, E118°51′
7	JH	*Hongxin*	Jurong, Jiangsu province	N33°17′, E118°51′
9	SH	*Hongxin*	Sheyang, Jiangsu province	N33°46′, E120°15′
11	SXZ	*Zaoyang*	Ruicheng, Shanxi province	N34°42′, E110°40′
12	SXW	*Wanyang*	Ruicheng, Shanxi province	N34°42′, E110°40′