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Analytical Methods

Study on ginsenosides in different parts and ages of *Panax quinquefolius* L.

Chenling Qu [a], Yuping Bai [a], Xiangqun Jin [b], Yutang Wang [a], Kun Zhang [a], Jingyan You [a], Hanqi Zhang [a,*]

[a] College of Chemistry, Jilin University, 2699 Qianjin Street, Changchun 130012, PR China
[b] College of Pharmacy, Jilin University, Changchun 130021, PR China

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ABSTRACT

The contents of 12 ginsenosides (Rg_1, Re, F_{11}, Rf, Rg_2, Rh_1, Rb_1, Rc, Rb_2, Rb_3, Rd, Rh_2) in different parts and ages of *Panax quinquefolius* L. (American ginseng) were quantified by high pressure microwave-assisted extraction (HPMAE) high-performance liquid chromatography coupled with evaporative light scattering detection (HPLC-ELSD). The analytical method was established and analytical performances were evaluated. The chemical marker of American ginseng F_{11} was detected, and Rf which is the chemical marker of Asian ginseng was not found. Rare ginsenoside Rh_1, Rg_2 and Rh_2 were also studied in this experiment. The total contents of these 12 ginsenosides in the different parts of 5-year-old American ginseng follow this order: leaf > root-hair > rhizome > root > stem. Therefore, compared with the other parts of American ginseng, the leaf is a better available source of ginsenosides. The contents of ginsenosides in root and leaf also change with age.

1. Introduction

Ginsenosides are the primary bioactive components of Asian ginseng (*Panax ginseng* C.A. Meyer), American ginseng (*Panax quinquefolius* L.) and San qi (*Panax notoginseng*). All these kinds of ginsengs are well known for their use in healthy food and traditional medicine (Zhang, Chen, Wu, & Wang, 2006). Asian ginseng is the most familiar herbal medicine which has been used as a tonic, sedative, anti-fatigue, or anti-gastric ulcer drug, and also has antidiabetic and antitumor activities (Lee, Lee, Kim, Park, & Lee, 1997; Shin, Bae, & Kim, 2006). American ginseng, which is cultivated in United States and Canada, is used to reduce stress, lower high blood sugar and adjust immunity (Vuksan et al., 2001). Modern pharmacological studies have shown that San Qi has anticarcinogenic and hepatoprotective activities, as well as protective effects on cardiovascular and cerebrovascular systems (Konoshima, Takasaki, & Tokuda, 1999). Ginsengs have been not only used as therapeutic agents but also marketed as dietary supplements and raw materials of health food (Shen, Ren, & Chen, 2003; Wang et al., 2008). For example, the root of Asia ginseng has been used as additives to drinks for hundreds of years.

More than 40 ginsenosides have been identified, isolated and characterised till now (Teng et al., 2003). Based on their aglycone moieties, ginsenosides can be mainly divided into two categories: 20(S)-protopapaxdiol (ginsenoside Rb_1, Rb_2, Rb_3, Rc, Rd and Rh_2) and 20(S)-protopapaxtriol (ginsenoside Re, Rf, Rg_1, Rg_2 and Rh_1) (Fig. 1a) (Kim, Ha et al., 2007). Other ginsenosides which do not belong to the two categories are also identified, such as oleanolic acids (Ro, Rhs, R_1, F_4) and pseudoginsenoside F_{11} (Fig. 1b) (Leung, Chan, Bensoussan, & Munroe, 2007; Wang, Wang, Wu, Osinski, & Yuan, 2005). Each kind of ginseng has its own characteristic ginsenoside which does not exist in other kinds of ginsengs. For example, ginsenoside F_{11} is the characteristic component of American ginseng, while ginsenoside Rf is the chemical marker of Asian ginseng. Reverse C18 column was usually employed to separate ginsenosides (Chen, Xie, Fu, Lee, & Wang, 2007; Liu, Han, Duan, Huang, & Wang, 2007; Shangguan et al., 2001; Wan, Lai et al., 2006; Wan, Yang, Li, Wang, & Cui, 2006; Wood, Bernards, Wan, & Charpentier, 2006; Zhang et al., 2006). However, polyvinyl alcohol-bonded stationary phase was also used to separate ginsenosides (Quiming, Denola, Soliev, Saito, & Jinno, 2007).

Only few articles reported the changing trends of contents of ginsenosides in ginsengs along with the age and parts. Wang et al. (2005) investigated the amounts of Rg_1, Re, Rf, Rb_1, Rc, Rb_2 and Rd in the root of 5- and 7-year Illinois-cultivated American ginseng and those cultivated for 3 or 4 years in Wisconsin. Li, Mazza, Cottrell, and Gao, (1996) studied ginsenoside Rg_1, Re, Rb_1, Rc, Rb_2, Rd in the root and leaf of American ginseng cultivated at different sites. While Lim, Mudge, and Vermeylen, (2005) also studied the genotype effects. In a previous study in our laboratory (Shi, Wang, Li, Zhang, & Ding, 2007), seven major ginsenosides Rg_1, Re, Rb_1, Rc, Rb_2, Rb_3 and Rd in different parts and ages of Asian ginseng were studied by HPLC coupled with UV detection.

The conventional methods for extracting Chinese herbal medicine include reflux extraction (RE), Soxhlet extraction (SE), ultrasonic extraction (UE) and supercritical fluid extraction (SFE) etc. These extraction methods confront with some problems, such as

* Corresponding author. Tel.: +86 431 85168399; fax: +86 431 85112335.
E-mail address: analchem@jlu.edu.cn (H. Zhang).

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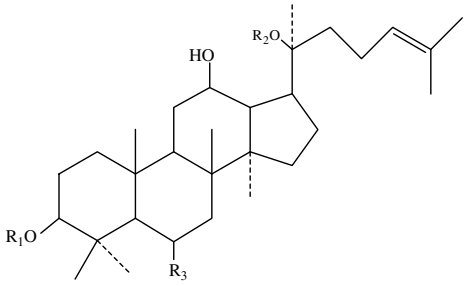
a



Ginsenoside	R_1	R_2	R_3
Rg_1	-H	-glc	-O-glc
Re	-H	-glc	-O-glc(2-1)glc
Rf	-H	-H	-O-glc(2-1)glc
Rg_2	-H	-H	-O -glc(2-1)rha
Rh_1	-H	-H	-O-glc
Rb_1	-glc(2-1)glc	-glc(6-1)glc	-H
Rc	-glc(2-1)glc	-glc(6-1)ara (f)	-H
Rb2	-glc(2-1)glc	-glc(6-1)ara (p)	-H
Rb3	-glc(2-1)glc	-glc(2-1)xyl	-H
Rd	-glc(2-1)glc	-glc	-H
Rh_2	-glc	-H	-H

Glc = glucose, ara (p) = alpha-L arabinopyranose, ara (f) = alpha-L arabinofuranose, rha = rhamnose

b

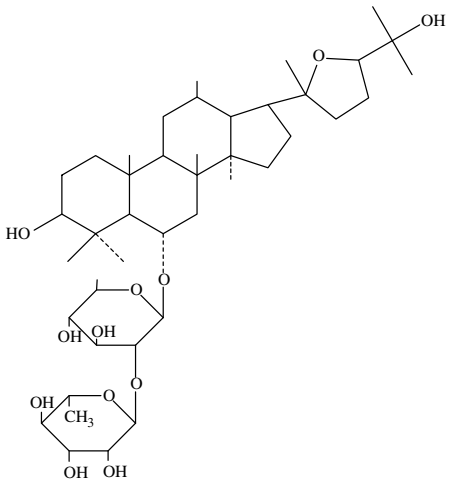


Fig. 1. Structure of Rg_1, Re, Rf, Rg_2, Rh_1, Rb_1, Rc, Rb_2, Rb_3, Rd and Rh_2 (a) and F_{11} (b).

long time and low efficiency. Recently other extraction methods were also employed, e.g. Li's group used pressurised liquid extraction to extract ginsenosides (Wan, Lai et al., 2006; Wan, Yang et al., 2006). And microwave-assisted extraction (MAE) in which the extraction time is dramatically reduced is popular in these years (Kim, Murthy, Hahn, Lee, & Paek, 2007; Kwon, Belanger, Jocelyn Pare, & Yaylayan, 2003; Shi et al., 2007; Shu, Ko, & Chang, 2003; Zhang et al., 2006). It includes atmospheric pressure microwave-assisted extraction (APMAE) and high pressure microwave-assisted extraction (HPMAE).

HPLC-UV method, which uses acetonitrile and water as elution solvents and measures the absorbance at wavelength of 203 nm, is a general method to determine ginsensosides. Acetonitrile used in the gradient elution also has absorption at 203 nm and makes baseline drift. Besides UV detector, evaporative light scattering detector (ELSD) was also used to detect ginsenosides (Kim et al., 2007; Kim et al., 2000; Kwon et al., 2001). Kim's group (Kim et al., 2007) simultaneously quantified 14 ginsenosides in Korean red ginseng by HPLC-ELSD. The working principle of ELSD is to evaporate the elution solvent and remain the non-volatile objects to be detected, so ELSD can solve the baseline drift problem well. Another reason for the use of ELSD in our study was that the chemical marker ginsenoside F_{11} does not have UV absorption at 203 nm. Recently Kwon, Jeong, Lee, and Hong, (2008) used pulsed amperometric detection to determine glycosides which include ginsenoside Rg_1, Re, Rf, Rb_1, Rc, Rb_2 and Rd.

Many scientists usually focused on the root of different kinds of ginsengs. In the present study, besides the root, we also studied the