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# Determination of Authentic Chiisanoside in *Acanthopanax senticosus* by High Performance Liquid Chromatography

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**Abstract** - High performance liquid chromatography (HPLC) was used for the analysis of chiisanoside in each stem and root of *Acanthopanax senticosus* collected from South Korea, North Korea, China and Russia. A reverse-phase system using a gradient of $H_2O$ and acetonitrile as the mobile phase was developed and detection was at 210nm. The analysis was successfully carried out within 30 min. Chiisanoside was measured in the stem and root of *A. senticosus* collected from various countries.

**Key words** - *Acanthopanax senticosus*, Araliaceae, Chiisanoside, HPLC

## Introduction

*Acanthopanax* species grown in the Korean peninsula belong to the family Araliaceae. *Acanthopanax* species has been traditionally used as a tonic and a sedative, as well as in the treatment of rheumatism and diabetes (Perry and Metzger, 1980; Yook, 1990). This plant has been widely used as food products and health supplements in Korea.

Many investigations have shown that *Acanthopanax* species exhibit a variety of pharmacological activities such as anti-bacterial, anti-cancer, anti-inflammatory, anti-hyperglycemic, anti-leishmanicidic, anti-oxidant, anti-pyretic, anti-xanthine oxidase, immunostimulatory, hypocholesterolemic, and radioprotectant effects (Davydov and Krikorian, 2000; Shin and Hong, 2005). Chemical analysis of *Acanthopanax* species has revealed a diverse range of secondary metabolites such as lignans, coumarins, flavonoids and terpenoids (Shin and Lee, 2002).

Among the secondary metabolites isolated from *Acanthopanax* species, chiisanoside which is the main component of *A. chiisanensis* was reported to possess anti-inflammatory (Jung *et al.*, 2005; Won *et al.*, 2005), anti-aggregating (Jin *et al.*, 2004), anti-cancer (Yook *et al.*, 1996), anti-rotaviral (Bae *et al.*, 2001), pancreatic lipase (Jiang *et al.*, 2006; Yoshizumi *et al.*, 2006), anti-diabetic activities (Kim *et al.*, 1980) and an effect of mitogen-induced proliferation of lymphocytes (Kim *et al.*, 1999).

Hence, the presence of important chiisanoside has been increased for the development of new medicinal crops. Therefore, it is necessary to develop more efficient and simple analytical methods for the analysis of chiisanoside in the stem and root of *A. senticosus*. This report describes a simple HPLC method for analyzing chiisanoside in the stem and root of *A. senticosus* collected from South Korea, North Korea, China and Russia.

## Materials and Methods

### Plant materials

The stem and root of *Acanthopanax senticosus* (= *Eleutherococcus senticosus*) were collected from Jeongseon at South Korea, Mt. Baekdu at North Korea, Yanbian at China and Khabarovsk at Russia. They were botanically identified by Prof. Seon Haeng Cho, Gongju National University of Education, Korea.

### Instruments and reagents

HPLC chromatograms were recorded with a Gilson 305 HPLC (USA) system equipped with a Gilson UV 119. Mucleosil 100-5C18 (4.6×250mm, 5㎛) column was used for the stationary phase. Water and acetonitrile used in this research were of HPLC grade and all other reagents were analytical grade.

### Preparation of chiisanoside

Air-dried powder of *A. senticosus* stems was extracted with MeOH under reflux. The MeOH extract was suspended in water, and then fractionated successively with equal volumes of $CH_2Cl_2$, EtOAc and *n*-BuOH. Among them, a portion of the EtOAc fraction

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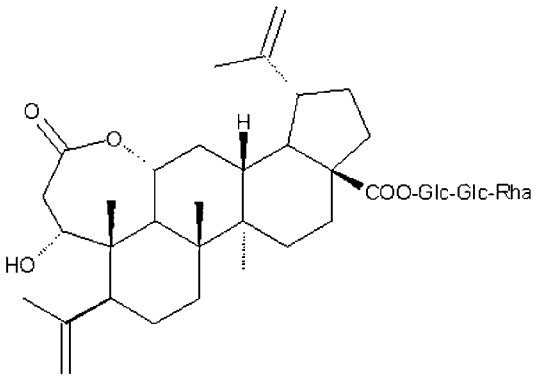
was chromatographed on a silica gel eluting with a gradient of $CHCl_3$-MeOH to afford compound **1** (Hahn *et al.*, 1984; Lee *et al.*, 2003).




### Sample preparation

For the analysis of chiisanoside in *A. senticosus*, each 10g of the stem and root of *A. senticosus* collected from Jeongseon at South Korea, Mt. Baekdu at North Korea, Yanbian at China and Khabarovsk at Russia was extracted with 20mL of 50% MeOH by reflux and evaporated *in vacuo*. The residue was dissolved in 2mL of 50% MeOH and filtered with a 0.45㎛ filter. The resulting solution was used HPLC analysis.

### HPLC condition

For the identification and quantification of chiisanoside *via* HPLC, the stationary phase used was a Mucleosil 100-5C18 (4.6 × 250mm,5㎛) column and a mobile phase program was used, which started at 90 : 10 and then next 30 min to 50 : 50 in a linear gradient solvent system of $H_2O$ : MeCN at flow rate of 1.0 mL/min. The column eluent was monitored at UV 210nm. The injection volume was 20㎕. All injection was performed in triplicate.

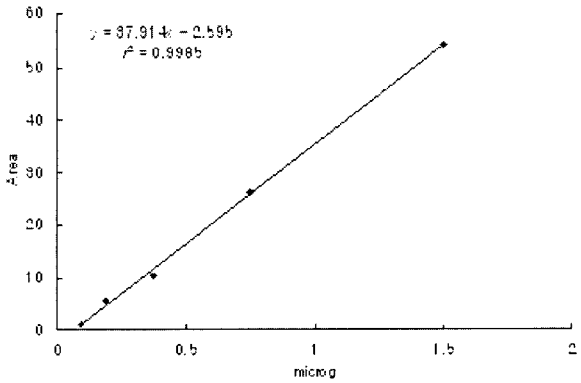



Fig. 1. Calibration curve of authentic chiisanoside (**1**).

## Results and Discussion

A chromatographic separation of MeOH extract from the leaves of *A. senticosus* led to the isolation of chiisanoside (Lee *et al.*, 2003). Chiisanoside is known to be the principle active compounds of *A. chiisanensis*. However, there are no comparative studies on the presence of chiisanoside in *A. senticosus* collected from various

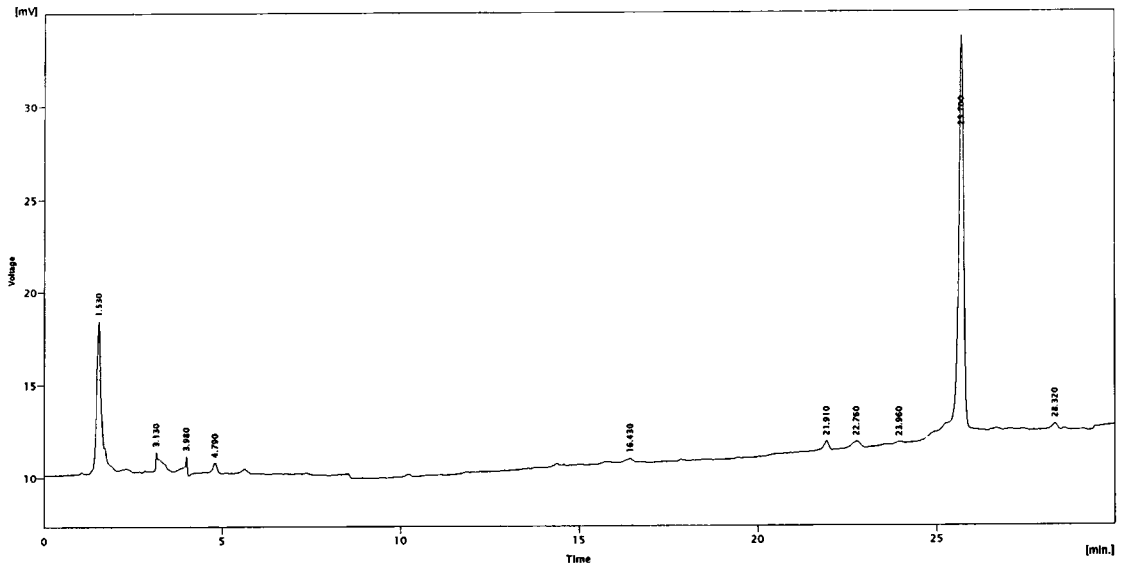



Fig. 2. HPLC chromatogram of authentic chiisanoside (**1**, $t_R$: 25.700 min).

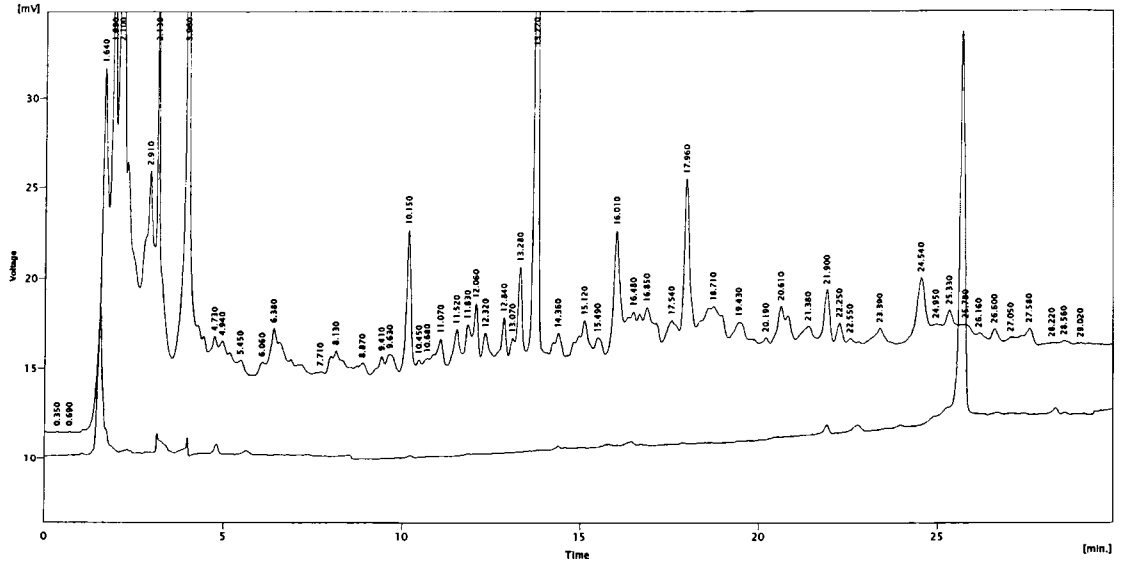



Fig. 3. HPLC chromatogram of the stem of *A. senticosus* collected from Mt. Baekdu, North Korea.

countries. Accordingly, the content of chiisanoside in the stem and root of *A. senticosus* collected from various countries was determined by HPLC.

As shown in Fig. 1, the standard curve for chiisanoside is Y = 37.914X−2.595 ($r^2$ = 0.9985). Chiisanoside was retained for about 25.700 min (Fig. 2). Fig. 3 demonstrates the satisfactory resolution achieved for chiisanoside in the stem of *A. senticosus* collected from Mt. Baekdu at North Korea. In the HPLC profile of the sample solution, the retention time of the expected peak of chiisanoside was the same as that of the standard compound. They were confirmed by the spiking test. Table 1 shows the content of chiisanoside in *A. senticosus* collected from various countries. The content of chiisanoside was measured in the stem and root of *A. senticosus* from Jeongseon at South Korea (4.13 and 3.03mg/g, respectively), Mt. Baekdu at North Korea (4.64 and 1.28mg/g, respectively), Yanbian at China (9.45 and 1.17mg/g, respectively) and Khabarovsk at Russia (3.10 and 1.93mg/g, respectively).

In a previous paper, *Acanthopanax* species could be classified into two groups of low concentration of chiisanoside, such as *A. senticosus* and *A. koreanus*, and high concentration of it, such as *A. senticosus f. inermis*, *A. divaricatus* var. *albeofructus* and *A. chiisanensis* (Kang *et al.*, 2003). All samples collected from various countries and used for our work could be classified into low concentration group of chiisanoside. The content of chiisanoside was same in *A. senticosus* collected from various countries. However, the content of chiisanoside in the root of *A. senticosus* collected from Jeongseon at South Korea was twice than that of other countries. Also, the content of chiisanoside in the stem of *A.*

Table 1. Concentration of chiisanoside (**1**) in *A. senticosus* by HPLC

| Sample | | **1** (mg/g) |
|---|---|---|
| *A. senticosus* collected from Jeongseon, South Korea | Stem | 4.13 ± 0.58 |
| | Root | 3.03 ± 0.91 |
| *A. senticosus* collected from Mt. Baekdu, North Korea | Stem | 4.64 ± 0.78 |
| | Root | 1.28 ± 0.26 |
| *A. senticosus* collected from Yanbian, China | Stem | 9.45 ± 1.32 |
| | Root | 1.27 ± 0.16 |
| *A. senticosus* collected from Khabarovsk, Russia | Stem | 3.10 ± 0.36 |
| | Root | 1.93 ± 0.31 |

Data are given as the mean ± S.D. (n = 3) in mg/g dried samples.

*senticosus* collected from Yanbian at China was twice than that of other countries.

Based on these results, it may be concluded that HPLC remains the method of choice for analyzing the most relevant chiisanoside of *A. senticosus*. Direct analysis by HPLC represents a valuable alternative to obtain typical fingerprints of *A. senticosus* and a reliable way to identify chiisanoside in *A. senticosus* collected from various countries. It is very important that chiisanoside as the main active compounds in *A. chiisanensis* have been identified in the stem and root of *A. senticosus* collected from various countries.

Accordingly, these results demonstrate that *A. senticosus* containing chiisanoside has promising potential as new additives to agricultural products for the development of fruit juice, food products and health supplements in Korea.

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