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Quantitative Analysis of Chiisanoside in *Acanthopanax* Species by HPLC

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Abstract – High performance liquid chromatography (HPLC) was used for the analysis of chiisanoside in *Acanthopanax* species. A reverse-phase system using a gradient of H_2O and acetonitrile as the mobile phase was developed and a wavelength of detection was at 210 nm. The analysis was successfully carried out for 30 min. Chiisanoside was measured in the fruit, stem and root of *A. sessiliflorus*, *A. koreanus*, *A. divaricatus* and *A. senticosus*.

Keywords – *Acanthopanax* species, 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 health supplements in Korea.

Many studies reported 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, the main component of *A. chiisanensis*, was reported to possess anti-inflammatory activity (Jung *et al.*, 2005; Won *et al.*, 2005), anti-aggregating activity (Jin *et al.*, 2004), anti-cancer (Yook *et al.*, 1996), anti-rotaviral activity (Bae *et al.*, 2001), pancreatic lipase activity (Jiang *et al.*, 2006; Yoshizumi *et al.*, 2006), an effect of mitogen-induced proliferation of lympocytes (Kim *et al.*, 1999) and anti-diabetic activity (Kim *et al.*, 1980).

Hence, the importance of chiisanoside has been increased for the development of clinically available medicine. Therefore, it is necessary to develop more efficient and simple analytical methods for the analysis of chiisanoside in *Acanthopanax* species. This report describes a simple HPLC method for analyzing chiisanoside in *Acanthopanax* species.

Experimental

Plant materials – *Acanthopanax* species (*A. sessiliflorus*, *A. koreanus*, *A. divaricatus* and *A. senticosus*) were cultivated and collected in the Gongju area in Autumn, 2004 and botanically identified by Prof. S. H. Cho, Gonju 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. 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 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 *Acanthopanax* species, each 10 g of the fruit, stem and root from *Acanthopanax* species was extracted with 20 mL of 50% MeOH by reflux and evaporated *in vacuo*. The residue was dissolved in 2 mL of 50% MeOH and filtered with a 0.45 μm filter. The resulting solution was

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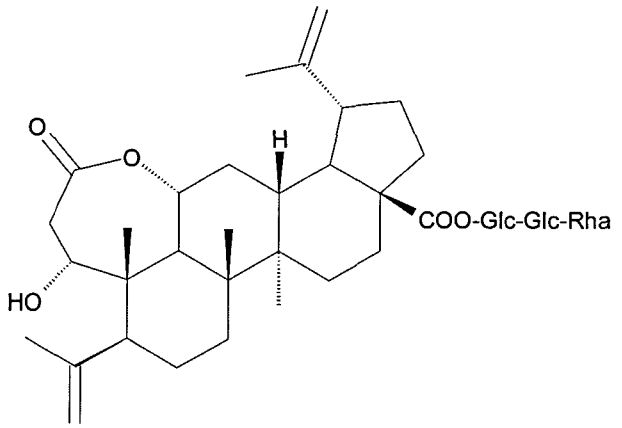
used for HPLC analysis.

HPLC condition – For the identification and quantification of chiisanoside *via* HPLC, the stationary phase used was a Mucleosil 100-5C18 (4.6 × 250 mm, 5 μm) 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 210 nm. The injection volume was 20 μL. All injection was performed in triplicate.



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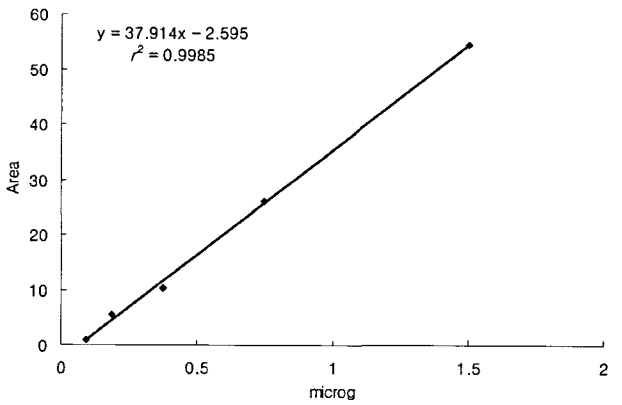

Fig. 1. Calibration curve of authentic chiisanoside.

Results and Discussion

A chromatographic separation of MeOH extract from the leaves of *A. senticosus* led to the isolation of chiisanoside. Chiisanoside is known to be a main bioactive compound of *A. chiisanensis*. However, there are no studies on the presence of chiisanoside in various parts of *Acanthopanax* species. Accordingly, the content of chiisanoside in various parts of *Acanthopanax* species was determined by HPLC.

The standard curve for chiisanoside is $Y = 37.914X - 2.595$ ($r^2 = 0.9985$) (Fig. 1). Chiisanoside was shown at the retention time 25.700 min (Fig. 2). Fig. 3 demonstrates the satisfactory resolution achieved for chiisanoside in the fruit of *A. sessiliflorus*. 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 various parts of *Acanthopanax* species. The content of chiisanoside was measured in the fruit, stem and root of *A. sessiliflorus* (4.64, 3.54 and 4.75 mg/g, respectively), *A. koreanus*

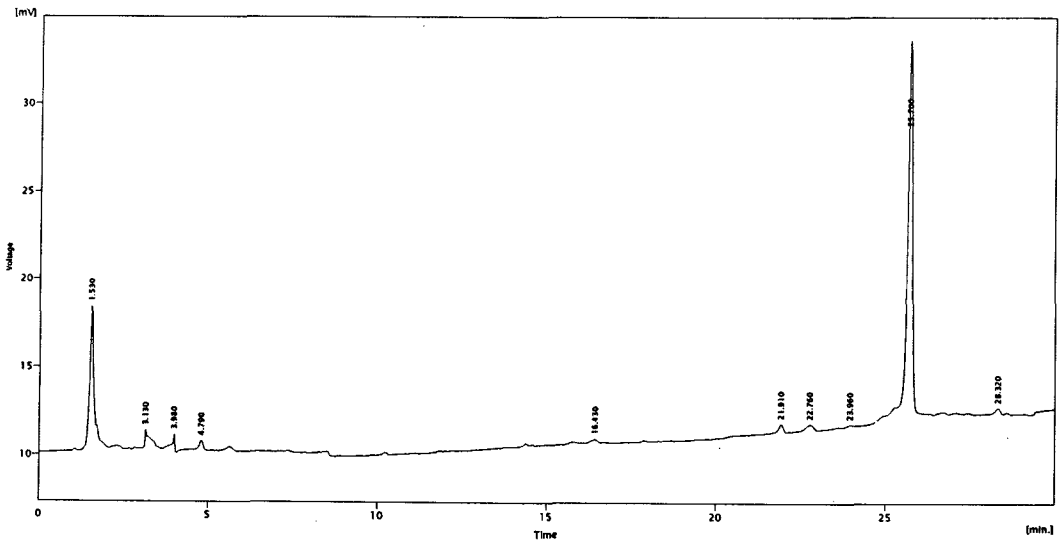

Fig. 2. HPLC chromatogram of authentic chiisanoside (t_R: 25.700 min).

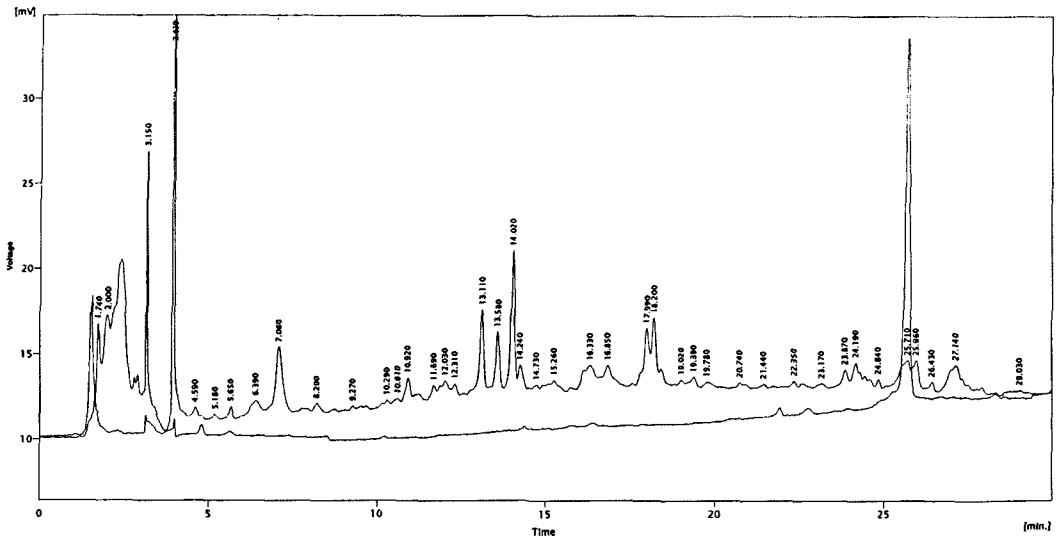

Fig. 3. HPLC chromatogram of the fruits of *A. sessiliflorus*.

Table 1. Concentration of chiisanoside (**1**) in *Acanthopanax* species by HPLC

Sample		**1** (mg/g)
A. sessiliflorus	Fruit	4.64 ± 0.35
	Stem	3.54 ± 1.05
	Root	4.75 ± 0.31
A. koreanus	Fruit	2.58 ± 0.15
	Stem	4.44 ± 1.62
	Root	1.24 ± 0.21
A. divaricatus	Fruit	4.94 ± 0.77
	Stem	8.12 ± 2.68
	Root	5.26 ± 1.63
A. senticosus	Fruit	2.62 ± 0.18
	Stem	2.58 ± 0.74
	Root	2.56 ± 0.52

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

(2.58, 4.44 and 1.24 mg/g, respectively), *A. divaricatus* (4.94, 8.12 and 5.26 mg/g, respectively) and *A. senticosus* (2.62, 2.58 and 2.56 mg/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 used for our work could be classified into low concentration group of chiisanoside. The content of chiisanoside was same in various parts of *A. sessiliflorus* and *A. senticosus*. However, in *A. koreanus* and *A. divaricatus*, the content of chiisanoside in stem was twice than that of fruit and root.

Based on these results, it may be concluded that HPLC remains the method of choice for analyzing the most relevant chiisanoside of *Acanthopanax* species. Direct analysis by HPLC represents a valuable alternative to obtain typical fingerprints of *Acanthopanax* species and a reliable way to identify chiisanoside in various parts of *Acanthopanax* species.

It is very important that chiisanoside as the main active compounds in *A. chiisanensis* have been identified in the fruit, stem and root of *A. sessiliflorus*, *A. koreanus*, *A. divaricatus* and *A. senticosus*. Specially, the presence of chiisanoside in the fruit of *Acanthopanax* species was very important in agricultural crop production for the development of increasing clinically available medicine and health supplements.

Accordingly, these results demonstrate that *Acanthopanax* species containing chiisanoside have promising potential as new additives to natural products for the development of fruit juice, food products and health supplements in Korea.

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