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Analysis of Lignans in *Acanthopanax sessiliflorus* Fruits and Their Fermented Wine by HPLC

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ABSTRACT : High performance liquid chromatography (HPLC) was used for the determination of lignans, eleutherosides B and E, in *Acanthopanax sessiliflorus* fruits and their fermented wine. The lignans were quantified by a reversed-phase system using a gradient of H_2O and acetonitrile as a mobile phase within 20 min. The analysis was successfully carried out within 20 min. The contents of eleutherosides B and E as main active principles of *Acanthopanax* species were measured in *A. sessiliflorus* fruits (1.15 and 8.49 ㎍/㎎, respectively), their fermented wine (0.45 and 1.33 ㎍/㎎, respectively) and wine residues (no detection).

Key words **:** *Acanthopanax sessiliflorus*, Araliaceae, fruit, wine, eleutheroside B, eleutheroside E, HPLC

INTRODUCTION

Approximately fifteen *Acanthopanax* species belonging to the family Araliaceae are known to be self-grown in the Korean peninsula. Among them, *A. senticosus* which is distributed in northern Asia has been traditionally used as a tonic and a sedative, as well as in the treatment of rheumatism and diabetes (Perry & Metzger, 1980; Yook, 1990).

There are many studies on *Acanthopanax* species. Many studies have shown that *Acanthopanax* species exhibits a variety of pharmacological activities such as anti-bacterial, anti-cancer, anti-inflammatory, anti-hyperglycemic, anti-oxidant, immunostimulatory and radioprotectant effects (Davydov & Krikorian, 2000; Shin & Lee, 2002). Chemical investigations of *Acanthopanax* species have afforded a diverse range of secondary metabolites such as lignans, coumarins, flavonoids and terpenes (Shin & Lee, 2002). Among them, lignans, eleutherosides B and E, are standard compounds of *Acanthopanax* species and both compounds in *Acanthopanax* species are determined by HPLC (Ahn *et al.*, 2000; Choi & Kim, 2002; Kang *et al.*, 2001; Kim *et al.*, 1996; Lee *et al.*, 2005; Slacanin *et al.*, 1991; Wagner *et al.*, 1982; Yat *et al.*, 1998).

Many fermented wine were manufactured by the stem and root of *Acanthopanax* species. However, there is no wine manufactured by the fruits of *Acanthopanax* species and there is no report on the comparison of both compound content in *A. sessiliflorus* fruits and their fermented wine.

Therefore, it is necessary to develop more efficient and simpler analytical methods for the determination of eleutherosides B and E in *A. sessiliflorus* fruits and their fermented wine. This report describes a simple HPLC determination method for both compounds in *A. sessiliflorus* fruits and their fermented wine.

MATERIALS AND METHODS

Plant materials

The fruits of *Acanthopanax sessiliflorus* were cultivated and collected at Gongju area in 2003 and botanically identified by Prof. S. H. Cho, Gongju National University of Education, Korea.

Instruments and reagents

MS spectrum was measured with a Jeol JMS-AX505WA mass spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded with a Bruker AVANCE 500 NMR spectrometer in DMSO using TMS as an internal standard. Chemical shifts were reported in parts per million (δ), and coupling constants (*J*) were expressed in hertz. TLC analysis was performed on Kieselgel 60 F_{254} (Merck) plates (silica gel, 0.25 ㎜ layer thick-

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ness), with compounds visualized by spraying with 20% H_2SO_4 followed by charring at 100 ℃. Silica gel (Merck, 200-400 mesh ASTM) was used for open column chromatography. HPLC chromatograms were recorded with a Gilson 305 HPLC system equipped with a Gilson UV 119. All other chemicals and reagents were analytical grade.

Extraction and isolation

The air-dried powder of *A. senticosus* stems was extracted with H_2O under reflux as earlier reported (Ryu *et al.*, 2003). The resultant extract was combined and lyophilized to afford a residues. The H_2O extract was re-suspended in H_2O and then extracted successively with equal volumes of $CHCl_3$, EtOAc, and *n*-BuOH. Each fraction was evaporated *in vacuo* to obtain $CHCl_3$ (14.8 g), EtOAc (23.6 g), *n*-BuOH (48.6 g), and H_2O (394.6 g) fractions. Among them, a portion of the *n*-BuOH fraction (10 g) was chromatographed on a silica gel (7 × 60 ㎝, No. 7734) column eluting with a gradient of $CHCl_3$-MeOH to afford eleutherosides B (**1**) (326 ㎎, 95 : 5) and E (**2**) (697 ㎎, 90 : 10).

Sample preparation

For the determination of eleutherosides B and E in *A. sessiliflorus* fruits and their fermented wine (Ogawine), each 10 g of *A. sessiliflorus* fruits and wine residues was extracted with 20 mL of 50% MeOH by reflux and evaporated *in vacuo*. Wine was evaporated *in vacuo*. The residues (extracts of *A. sessiliflorus* fruits, fermented wine and wine residues) were dissolved in 2 mL of 50% MeOH and filtered with 0.45 ㎜ filter. The resulting solutions were as sample solutions for the determination of lignans by HPLC analysis.

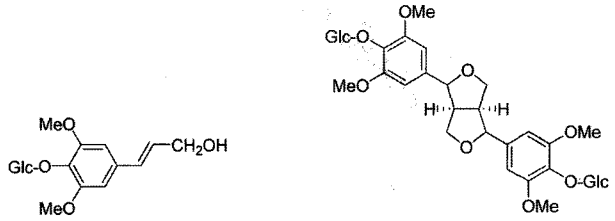
HPLC condition

For the identification and determination of eleutherosides B and E in HPLC, the stationary phase used was Mucleosil 100-5C18 (4.6 × 250 ㎜, 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 210 ㎚. The injection volume was 20 μL. All injection was performed in triplicate.

RESULTS AND DISCUSSION

A chromatographic separation of the MeOH extract from the stem of *A. senticosus* led to the isolation of lignans. Briefly, a portion of the *n*-BuOH fraction of this plant stem was chromatographed on a silica gel column eluting with a gradient of $CHCl_3$-MeOH to afford compounds **1** and **2** described in Materials and Methods. The structures of **1** and **2** were assigned from the ^1H- and ^{13}C-NMR signals derived hetero nuclear direct and long-range correlations. Accordingly, as shown in **Fig. 1**, compounds **1** and **2** were elucidated as eleutherosides B (= syringin) and E (= acanthoside D), respectively, by comparing with an authentic sample as described in the literature (Hong *et al.*, 2001; Kinjo *et al.*, 1990; Nishibe *et al.*, 1990; Ryu *et al.*, 2003; Sutarjadi *et al.*, 1978).

Lignans, eleutherosides B and E, are known to be main active principles of *Acanthopanax* species (Takasugi *et al.*, 1985). In previous papers, both compounds were detected in the stem and root of *A. senticosus* and *A. koreanum*. In the stem and root of *A. divaricatus* var. *albeofructus*, *A. senticosus* forma *inermis* and *A. chiisanensis*, only eleutheroside E was detected (Kang *et al.*, 2001). Eleutherosides B and E were measured in various parts of *Acanthopanax* species. There is no detection of eleutheroside B in the stem and root of *A. sessiliflorus* (Lee *et al.*, 2005). There are many papers focused on the contents of eleutherosides B and E in the stem and root of *Acanthopanax* species (Ahn *et al.*, 2000; Choi & Kim, 2002; Kang *et al.*, 2001; Kim *et al.*, 1996; Lee *et al.*, 2005; Slacanin *et al.*, 1991; Wagner *et al.*, 1982; Yat *et al.*, 1998). However, there is no research on the determination of both compounds in *A. sessiliflorus* fruits and their fermented wine. Accordingly,



1 2

Fig. 1. Structures of eleutherosides B (**1**) and E (**2**).

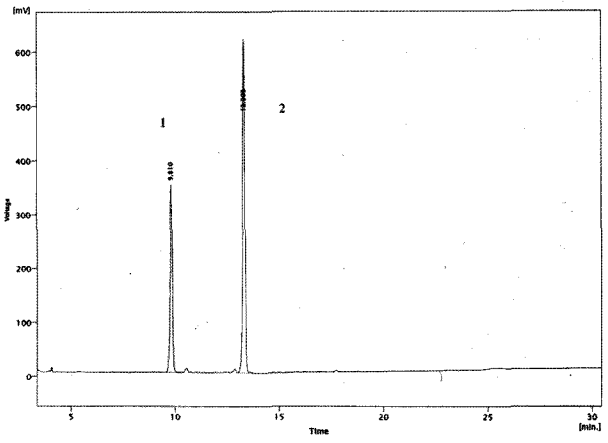


Fig. 2. HPLC chromatogram of eleutherosides B (**1**) and E (**2**).

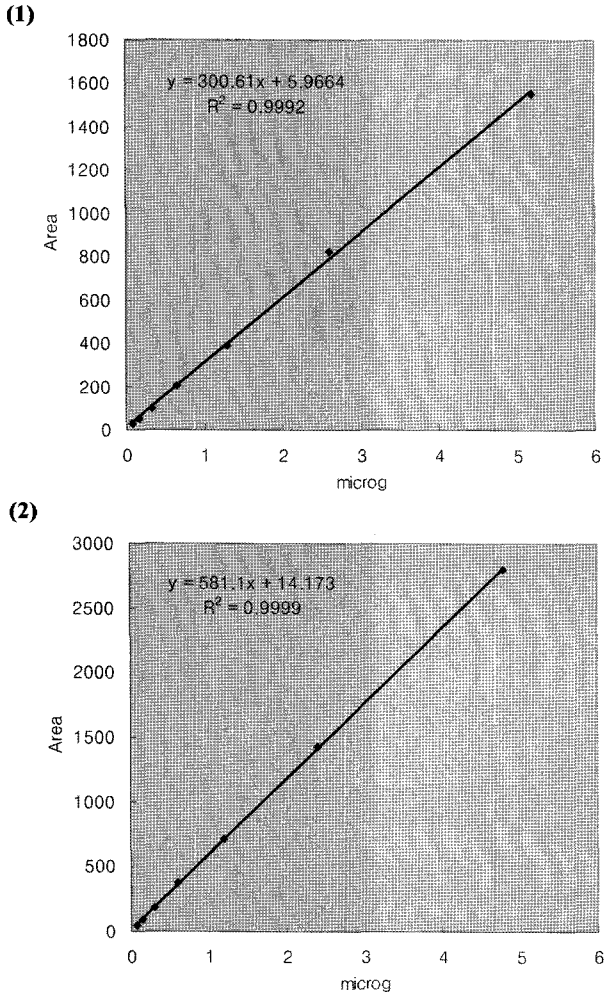


Fig. 3. Calibration curves for eleutherosides B (**1**) and E (**2**) - (X axis: μg/mL; Y axis: Area).

Table 1. Contents of eleutherosides B (**1**) and E (**2**) in *A. sessiliflorus* fruits (**A**), fermented wine (**B**) and wine residues (**C**).

Sample	Content (μg/mg)	
	1	**2**
A	1.15 ± 0.10	8.49 ± 0.22
B	0.45 ± 0.01	1.33 ± 0.05
C	0	0

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

eleutherosides B and E in *A. sessiliflorus* fruits and their fermented wine were determined by HPLC. **Fig. 2** demonstrates the satisfactory resolution achieved for the standard eleutherosides B and E. Eleutherosides B and E were retained at about 9.81 and 13.30 min, respectively. As shown in **Fig. 3**, the standard curves for eleutherosides B and E are $Y = 300.61X + 5.9664$ ($r^2 = 0.9992$) and $Y = 581.1X + 14.173$ ($r^2 = 0.9999$), respectively. In the HPLC profile of sample solution, the retention time of the expected eleutherosides B and E peak was the same that of standard compounds (**Fig. 4**). They are confirmed by spike test. **Table 1** showed the contents of eleutherosides B and E in *A. sessiliflorus* fruits and their fermented wine. The contents of eleutherosides B and E were measured in *A. sessiliflorus* fruits (1.15 and 8.49 μg/mg, respectively), their fermented wine (0.45 and 1.33 μg/mg, respectively) and wine residues (no detection).

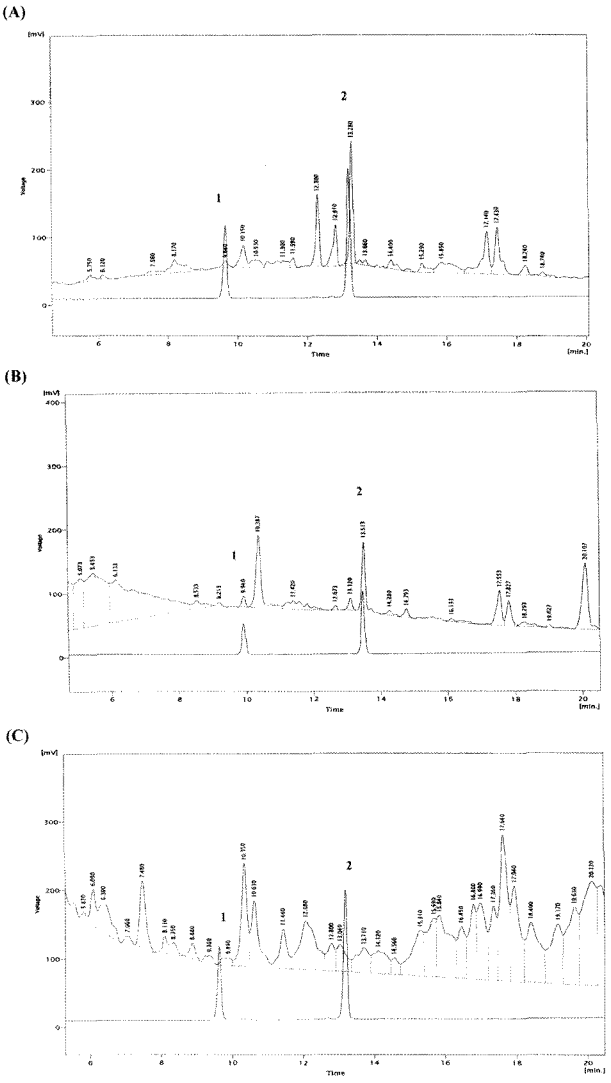


Fig. 4. HPLC chromatograms of *A. sessiliflorus* fruits (**A**), fermented wine (**B**) and wine residues (**C**) - (Below: HPLC chromatograms of standard compounds).

Based on the results, it may be concluded that HPLC remains the method of choice for the assay of most relevant eleutherosides B and E in *A. sessiliflorus* fruits and their fermented wine. Direct analysis by HPLC represents a valuable alternative to obtain typical fingerprints of *A. sessiliflorus* fruits and their fermented wine, and a reliable identification of

eleutherosides B and E in *A. sessiliflorus* fruits and their fermented wine. It is very important that eleutherosides B and E as main active principles of *Acanthopanax* species were identified in their fermented wine. Accordingly, these results demonstrate that the fruits of *A. sessiliflorus* containing eleutherosides B and E as main active principles have promising potential as new medicinal crop materials for the development of fruit juice, food products and health supplements.

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