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Quantitative Analysis of Lignans in the Fruits of *Acanthopanax* Species by HPLC

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Abstract High performance liquid chromatography (HPLC) was used for the analysis of the lignans eleutheroside B and eleutheroside E in the fruits of *Acanthopanax* species. A reverse-phase system using a gradient of H_2O and acetonitrile as the mobile phase was developed and detection was at 210 nm. The analysis was successfully carried out within 20 min. The content of eleutheroside B and eleutheroside E in *Acanthopanax* species was measured in the fruits of *A. senticosus* (0.58 and 1.66 μg/mg, respectively), *A. sessiliflorus* (1.15 and 8.49 μg/mg, respectively), *A. koreanum* (2.16 and 1.80 μg/mg, respectively), and *A. divaricatus* (1.06 and 7.08 μg/mg, respectively).

Keywords: *Acanthopanax* species, Araliaceae, eleutheroside B, eleutheroside E, HPLC

Introduction

Acanthopanax species belong to the family Araliaceae. Approximately fifteen *Acanthopanax* species are known to grow in the Korean peninsula. Among them, *A. senticosus* which is distributed throughout northern Asia has been traditionally used as a tonic and a sedative, as well as in the treatment of rheumatism and diabetes (1, 2).

Many studies 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 radio-protectant effects (3, 4). Chemical analysis of *Acanthopanax* species has revealed a diverse range of secondary metabolites such as lignans, coumarins, flavonoids and terpenes (5). Among these, the lignans eleutheroside B and eleutheroside E are the principle active compounds of *Acanthopanax* species, and both are found in the stems and roots of *Acanthopanax* species as determined by HPLC (6-12). However, there are no reports addressing the content of both compounds in the fruits of *Acanthopanax* species.

Therefore, it is necessary to develop more efficient and simpler analytical methods for the analysis of eleutheroside B and eleutheroside E in the fruits of *Acanthopanax* species. This report describes a simple HPLC method for analyzing both compounds in the fruits of *Acanthopanax* species.

Materials and Methods

Plant materials *Acanthopanax* species (*A. senticosus*, *A. sessiliflorus*, *A. koreanum*, and *A. divaricatus*) were cultivated and the fruits collected in the Gongju area in 2003 and botanically identified by Prof. S. H. Cho, Gonju National University of Education, Korea. The voucher specimen has been deposited at the Herbarium of Dept. of Applied Plant Science, Chung-Ang University, Korea.

Instruments and reagents The mass spectrometry (MS) spectra were measured with a Jeol JMS-AX505WA (Tokyo, Japan) mass spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded with a Bruker AVANCE 500 NMR (Karlsruhe, Germany) 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, Germany) plates (silica gel, 0.25 mm layer thickness), with compounds visualized by spraying with 20% H_2SO_4 followed by charring at 100°C. Silica gel (200-400 mesh ASTM; Merck) was used for open column chromatography. HPLC chromatograms were recorded with a Gilson 305 HPLC (Middleton, WI, USA) system equipped with a Gilson UV 119. All other chemicals and reagents were analytical grade.

Preparation of eleutheroside B and eleutheroside E Air-dried powder of *A. senticosus* stems was extracted with H_2O under reflux. The resultant extract was combined and lyophilized (13). 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 cm, No. 7734) column and eluted with a gradient of $CHCl_3$-MeOH to yield compounds **1** (326 mg, 95:5) and **2** (697 mg, 90:

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Eleutheroside B (**1**); FAB-MS: *m/z* 373 $[M + H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6): d 6.73 (2H, s, H-2,6), 6.46 (1H, d, J = 15.9 Hz, H-7), 6.33 (1H, dt, J = 15.9, 5.1 Hz, H-8), 4.84 (1H, d, J = 7.5 Hz, anomeric H-1'), 4.11 (1H, dd, J = 5.1, 1.4 Hz, H-9a), 4.09 (1H, dd, J = 5.1, 1.4 Hz, H-9b), 3.77 (6H, s, 2×OMe); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 152.7 (C-3,5), 133.0 (C-4), 131.0 (C-7), 129.0 (C-8), 128.1 (C-1), 104.5 (C-2,6), 103.1 (C-1'), 77.4 (C-5'), 76.5 (C-3'), 74.9 (C-2'), 71.0 (C-4'), 62.0 (C-9), 60.5 (C-6'), 56.3 (OMe).

Eleutheroside E (**2**); FAB-MS: *m/z* 743 $[M + H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 6.67 (4H, s, H-2',6'), 4.88 (2H, d, J = 7.3 Hz, anomeric H-1"), 4.67 (2H, d, J = 3.6 Hz, H-2), 4.28 (2H, dd, J = 8.5, 6.6 Hz, H-4_{eq}), 4.20 (2H, dd, J = 8.5, 3.0 Hz, H-4_{ax}), 3.76 (12H, s, 4×OMe), 3.19 (2 H, m, H-1); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 153.2 (C-3',5'), 138.1 (C-4'), 134.1 (C-1'), 104.6 (C-2',6'), 103.3 (C-1"), 85.7 (C-2), 77.5 (C-5"), 76.7 (C-3"), 74.5 (C-2"), 72.1 (C-4), 70.2 (C-4"), 61.2 (C-6"), 57.0 (OMe), 54.2 (C-1).

Sample preparation For the analysis of eleutheroside B and eleutheroside E in the fruits of *Acanthopanax* species, 10 g of fruits from each *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 used HPLC analysis.

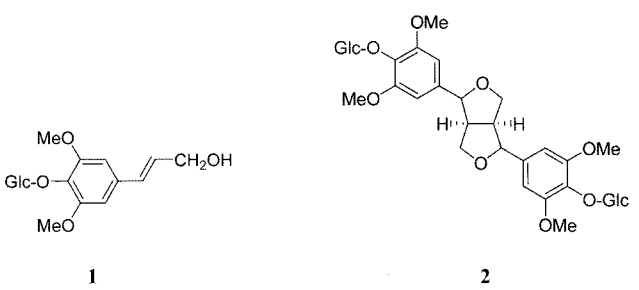
HPLC condition For the identification and quantification of eleutheroside B and eleutheroside E via HPLC, the stationary phase used was a Mucleosil 100-5C18 (4.6×250 mm, 5 μm) column and a mobile phase program was used, which started at 90:10 and then next 30 min to 50:50 in a linear gradient solvent 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.

Results and Discussion

A chromatographic separation of MeOH extract from the stem of *A. senticosus* led to the isolation of lignans. Briefly, a portion of the *n*-BuOH fraction of the MeOH extract from this plant stem was chromatographed on a silica gel column and eluted with a gradient of $CHCl_3$-MeOH to yield compounds **1** and **2** as described in Materials and Methods. Compound **1** was obtained as needles in MeOH. In the ^{1}H-NMR spectrum of **1**, an aromatic proton at δ 6.73 and methylene protons at δ 6.46 and 6.33 were observed. The configuration of the glycosidic linkage was determined as β on the basis of the $J_{1,2}$ value of the anomeric proton of 7.5 Hz (δ 4.84). The ^{13}C-NMR spectrum of **1** showed signals of an anomeric carbon at δ 103.1, methoxy carbons at δ 56.3 and a typical glucose moiety. The FAB-MS of **1** showed an $[M+H]^+$ ion at *m/z* 373. Compound **2** was obtained as needles in MeOH. In the ^{1}H-NMR spectrum of **2**, aromatic protons at δ 6.67 and methoxy protons at δ 3.76 were observed. The signals of oxymethine protons at δ 4.67 and methylene protons at δ 4.28 and 4.20 are characteristic of lignan compounds. The configuration of the glycosidic linkage was determined as β on the basis of the $J_{1,2}$ value of the anomeric proton of 7.3 Hz (δ 4.88). The ^{13}C-NMR spectrum of **2** showed an anomeric carbon at δ 103.3 and a typical glucose moiety. The FAB-MS of **2** showed an $[M+H]^+$ ion at *m/z* 743. Finally, the structures of **1** and **2** were assigned based on the ^{1}H- and ^{13}C-NMR signals derived hetero nuclear direct and long-range correlations. Accordingly, compounds **1** and **2** were determined to be eleutheroside B (= syringin) and eleutheroside E (= acanthoside D), respectively, by comparing with an authentic sample as described in the literature (Fig. 1) (13-17).

Lignans, specifically eleutheroside B and eleutheroside E, are known to be the principle active compounds of *Acanthopanax* species (18). 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 (7). Eleutheroside B and eleutheroside E were measured in various parts of *Acanthopanax* species. There is no evidence of eleutheroside B in the stem and root of *A. sessiliflorus* (9). There are many papers focused on the contents of eleutheroside B and eleutheroside E in the stem and root of *Acanthopanax* species (6-12). However, there are no studies on the presence of both compounds in the fruits of *Acanthopanax* species. Accordingly, the content of eleutheroside B and eleutheroside E in the fruits of *Acanthopanax* species were determined by HPLC.

The standard curves for eleutheroside B and eleutheroside E are Y = 300.61X + 5.9664 (R^2=0.9992) and Y = 581.1X + 14.173 (R^2=0.9999), respectively. Eleutheroside B and eleutheroside E were retained for about 9.81 and 13.30 min, respectively (Fig. 2). Figure 3 demonstrates the satisfactory resolution achieved for eleutheroside B and eleutheroside E of *A. senticosus* extract. In the HPLC profile of the sample solution, the retention time of the expected peaks of eleutheroside B and eleutheroside E was the same as that of standard compounds. They were confirmed by the spike test. Table 1 shows the eleutheroside B and eleutheroside E content in the fruits of *Acanthopanax* species. The contents of eleutheroside B and eleutheroside E were measured in the fruits of *A. senticosus* (0.58 and 1.66 μg/mg, respectively), *A. sessiliflorus* (1.15 and 8.49 μg/mg, respectively), *A. koreanum* (2.16 and 1.80 μg/mg, respectively) and *A. divaricatus* (1.06 and



1 2

Fig. 1. Structures of eleutheroside B (1) and eleutheroside E (2).

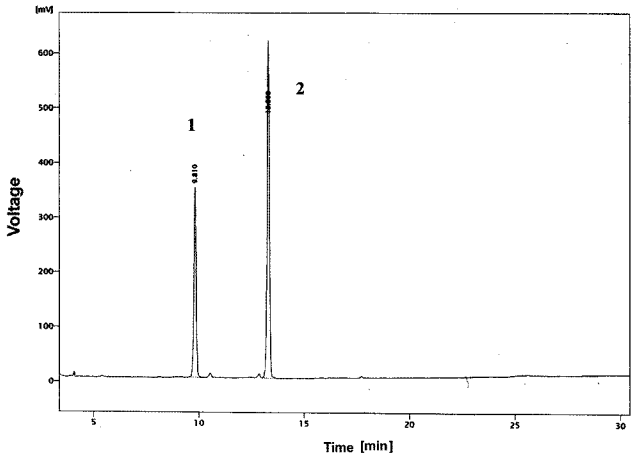


Fig. 2. HPLC chromatogram of eleutheroside B (1) and eleutheroside E (2).

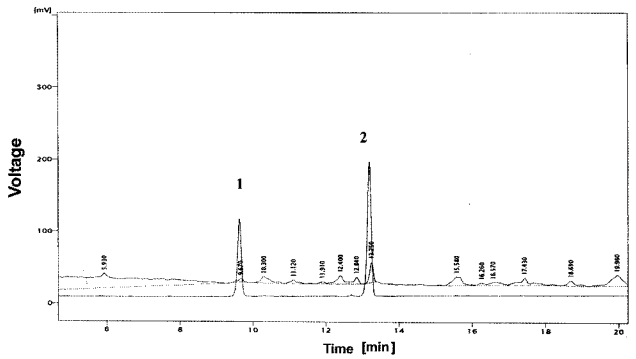


Fig. 3. HPLC chromatogram of the fruits of *A. senticosus*.

Table 1. Lignan content of the fruits of *Acanthopanax* species by HPLC

Sample	Content[1)] (μg/mg)	
	Eleutheroside B	Eleutheroside E
A. senticosus	0.58±0.10	1.66±0.05
A. sessiliflorus	1.15±0.10	8.49±0.22
A. koreanum	2.16±0.09	1.80±0.01
A. divaricatus	1.06±0.06	7.08±0.23

[1)]Data are given as the mean±SD (n = 3) in mg/mg dried samples.

7.08 μg/mg, respectively).

Based on these results, it may be concluded that HPLC remains the method of choice for analyzing the most relevant lignans, eleutheroside B and eleutheroside E, of *Acanthopanax* species. Direct analysis by HPLC represents a valuable alternative to obtain typical fingerprints of *Acanthopanax* species and a reliable way to identify eleutheroside B and eleutheroside E in the fruits of *Acanthopanax* species. It is very important that eleutheroside B and eleutheroside E, as the main active compounds, have been identified in the fruits of *A. senticosus*, *A. sessiliflorus*, *A. koreanum*, and *A. divaricatus*. Accordingly, these results demonstrate that the fruits of *Acanthopanax* species containing eleutheroside B and eleutheroside E have promising potential as new additives to natural products for the development of fruit juice, food products, and health supplements.

Acknowledgments

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