Semipreparative Separation and Determination of Eleutheroside E in *Acanthopanax giraldii Harms* by High-Performance Liquid Chromatography

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Abstract

A method for the isolation, purification, and determination of eleutheroside E in *Acanthopanax giraldii Harms*, collected in the Sichuan province (China), is established. The water extraction of *A. giraldii Harms* is pre-isolated using macroporous adsorption resin (D-101) and a C_{18} solid-phase extraction cartridge, and the enriched extract is purified to give eleutheroside E (syringaresinol-di-*O*-β-D-glucoside; liriodendrin) by semipreparative reversed-phase high-performance liquid chromatography. Structure identification is performed by a comparison of IR, ^{1}H-NMR, ^{13}C-NMR, and electrospray ionization-mass spectrometric data with the literature. The final purity of the compound is 97%. Quantitative determination of eleutheroside E in *A. giraldii Harms* is performed on a Zorbax SB C_{18} (150- × 4.6-mm i.d., 5 μm) column. The linear range of eleutheroside E is 4.85–194 mg/L (r = 0.9998), and the average recovery is 99.6–101%. The developed method is simple, reproducible, and easy to operate. It is useful for the evaluation of *Acanthopanax giraldii Harms.*

Introduction

The herbal plant *Acanthopanax giraldii Harms* is a deciduous shrub that is widely dispersed in the Sichuan, Gansu, and Ningxia provinces of China. The stem bark of this plant has long been used as both a treatment of rheumatism and a tonic (1). Extensive biological studies have been carried out. Reported pharmacological checks included tests for immunomodulation (2), antitumor (3), anti-inflammation (4), antivirus (5), and sedative properties (6). Chemical constituents studies on this herb revealed various constituents, which included nucleotide, lignan glucosides, triterpenoid saponins, and phenolic compounds (7–9).

Acanthopanax senticosus (Rupr. et Maxim) *Harms*, which is distributed in northeast China, and *Acanthopanax giraldii Harms*, which is distributed in northwest and southwest China, belong to the same family of Araliaceae. Extracts of *A. senticosus* are now widely used in various medical fields, but *A. giraldii Harms* is underdeveloped. Studies have shown that *A. giraldii Harms* and *A. senticosus* are similar in their constituents and biological activities (10).

Eleutheroside E (syringaresinol-di-*O*-β-D-glucoside, identical to liriodendrin and also known as eleutheroside D; chemical structure is shown in Figure 1) is thought to be the most pharmacologically active substance that increases the nonspecific resistance of an organism to the adverse influences while generating a normalizing action on bodily functions (11). It is reported to have a counteracting effect on stressed animals, androgenic effects in immature male mice, and an increase in the RNA content of the seminal vesicles and prostate (12). Eleutheroside E has been reported in both *A. senticosus* and *A. giraldii Harms* (9).

However, eleutheroside E was very difficult to isolate by common silica-gel chromatography. A simple, fast, cheap, less poisonous, and easy-to-operate method was established in this paper to isolate and purify eleutheroside E from *A. giraldii Harms* by semipreparative high-performance liquid chromatography (HPLC). Structure identification was performed by the

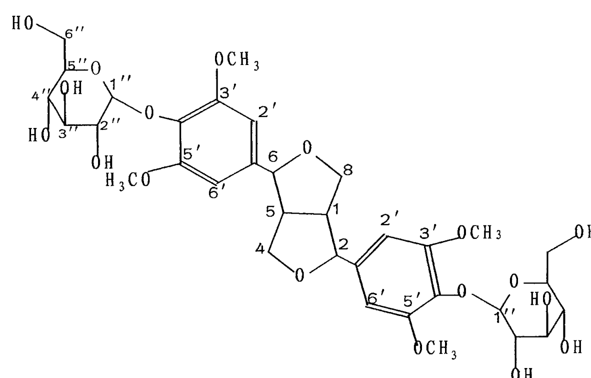

Figure 1. Chemical structure of eleutheroside E ($C_{34}H_{46}O_{18}$, M_w = 742).

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comparison of IR, ^{1}H NMR, ^{13}C NMR, and electrospray ionization (ESI)-mass spectrometry (MS) data with the literature. The contents of 18 batches of *A. giraldii Harms* and *A. senticosus Harms* were determined. It is useful for the evaluation of eleutheroside E in the Araliaceae family.

Experimental

Materials and reagents

The stem and root barks of *A. giraldii Harms,* collected from Sichuan and Ningxia provinces (China), and *A. senticosus Harms,* collected from northeast China, were identified by Prof. Qishi Sun (School of Traditional Chinese Medicine, Shenyang Pharmaceutical University, Shenyang, China). Eleutheroside E was prepared by semipreparative HPLC, and the purity was 97%. Acetonitrile was HPLC grade (Dikma, Beijing, China). The water used was a doubly-distilled pure water. Other reagents were of analytical grade.

Chromatographic system

The chromatographic system for semipreparation of eleutheroside E on a Hitachi 655 HPLC (Tokyo, Japan). The separation was performed on a Zorbax SB C_{18} column (100- × 9.4-mm i.d., 5 µm) (Agilent Technologies, Palo Alto, CA) with methanol–water (35:65) as the mobile phase. The flow rate was 2.5 mL/min, and the wavelength of the UV detector was 230 nm.

The chromatographic system for quantitative analysis was an Agilent 1100 series HPLC, which consisted of a quaternary pump, diode-array detector (DAD), online vacuum degasser, autosampler, and thermostatted column compartment (Agilent Technologies). The separation was performed on a Zorbax SB C_{18} column (150 × 4.6 mm, 5 µm). The column effluent was monitored by UV at 220 nm with a diode-array scan from 190 to 400 nm. The column temperature was maintained at 30°C. The injection volume was 10 µL. A gradient elution with acetonitrile and water at a flow rate of 1.0 mL/min was employed. The gradient elution program started with mobile phase of 6% acetonitrile for 2 min, and the linear gradient was then increased over to 17% acetonitrile over a period of 18 min.

Sample preparation

The dried stem barks of *A. giraldii Harms* were crushed, and 0.5 g of stem powder was extracted with 75% methanol (150 mL) under reflux for 1 h. The extract was evaporated under reduced pressure and the residue was dissolved with 10 mL of 75% methanol. The solution was filtered through a 0.45-µm filter before sample injection.

Instrumentation for NMR, MS, and IR

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker ARX 300 spectrometer (Bruker, Faellanden, Switzerland). Chemical shifts were reported on the d-scale relative to tetramethylsilane as an internal standard. MS spectrum was carried out on an Agilent ion-trap MS equipped with an ESI interface. The IR spectrum was obtained on a Bruker-IFS-55 instrument.

Isolation and purification

Dry crushed stem of *A giraldii Harms* (1000 g) was extracted three times with hot water. The water solution was evaporated to a small volume under reduced pressure. The water extract was chromatographed on a D-101 macroporous adsorption resin column, eluted with water and 15%, 25%, 75% ethanol, successively. The 25% eluted fraction was subjected to C_{18} solid-phase extraction cartridge (500 mg; Tianjin, China) eluted with water and 20% and 30% methanol, successively. The 30% methanol elute was purified by semipreparative HPLC to give eleutheroside E. To isolate this compound, the extract was injected repeatedly, and the fraction of the peak at the retention time of 5.7 min was pooled, evaporated to dryness, and stored in the desiccator before structural elucidation.

Results and Discussion

Structure identification

The relative molecular weight was inferred from the ESI-MS spectrum, which was very informative (Table I). In the positive

Table I. Attribution of MS Data of Eleutheroside E Isolated from *Acanthopanax giraldii Harms*

	m/z	Attribution
Positive mode	781	$[M+K]^+$
	765	$[M+Na]^+$
	735	$[M+Na–CH_2O]^+$
	603	$[M+Na–Glu]^+$
	441	$[M+Na–2Glu]^+$
Negative mode	777	$[M+Cl]^–$
	741	$[M–H]^–$
	579	$[M–H–Glu]^–$

Table II. Attribution of NMR Data of Eleutheroside E Isolated from *Acanthopanax giraldii Harms*

	^{13}C NMR (DMSO-d_6)		^{1}H NMR (DMSO-d_6)
No.	Literature value*	Test value	Test value
C-1, C-5	53.6	53.7	3.14 (m, 2H)
C-4, C-8	71.3	71.4	4.30 (m, 2H, αH), 4.17 (m, 2H, βH)
C-2, C-6	85.1	85.2	4.65 (d, 2H)
C-1'	133.9	133.8	
C-2', C-6'	104.3	104.3	6.65 (s, 4H)
C-3', C-5'	152.7	152.7	
C-4'	137.2	137.2	
$–OCH_3$	56.4	56.5	3.75 (s, 12H, 4 × OCH_3)
C-1''	102.7	102.7	4.9 (d, 2H, J = 7.5Hz)
C-2''	74.2	74.2	
C-3''	76.5	76.6	
C-4''	70.0	70.0	
C-5''	76.9	76.6	
C-6''	61.0	61.0	

* Taken from the literature (12).

mode, *m/z* 765 was identified as the $[M+Na]^+$ pseudomolecular ion of eleutheroside E (M_w = 742). The tandem MS spectrum of the *m/z* 765 ion that gave a prominent ion at *m/z* 603, which was 162 µm lower than the precursor ion, was thought to have arisen via the loss of a neutral molecule of glucose. In the negative mode, *m/z* 741 was identified as the deprotonated molecular ion of $[M–H]^-$, and the ion at *m/z* 579, which was 162 µm lower than the precursor ion, also corresponded to the loss of glucose.

The IR spectrum of the compound suggested the presence of hydroxyl groups with a broad band centered at 3395 cm^{-1}, a phenyl ring with an isolated H (1595,1502, 1463, and 810 cm^{-1}), CH_2–O linkage (1422 cm^{-1}), and methyl (2933 and 1334 cm^{-1}). This was supported by the ^1H and ^{13}C NMR spectra (Table II) of the compound, which clearly indicated a 2-fold symmetry. The ^{13}C NMR spectrum showed only 14 peaks for the 34 carbons, as expected for the structure of eleutheroside E. The IR, ^1H NMR, ^{13}C NMR, and ESI-MS spectra were in accord with the structure of eleutheroside E.

Eleutheroside E (syringaresinol-di-*O*-β-D-glucoside)

The colorless needle from methanol and the pseudomolecular ions shown in the ESI-MS spectra were *m/z* 765 $[M+Na]^+$ and 741 $[M–H]^-$, respectively. The melting point range was 256–258°C.

Extraction efficiency of eleutheroside E

The powdered stem bark of *A. giraldii Harms* was extracted with 0%, 25%, 50%, 75%, and 95% ethanol under reflux and sonic extraction, respectively. The highest extraction efficiency of eleutheroside E in *A. giraldii Harms* was achieved with 75% of ethanol under reflux. As shown in Figure 2, the amount of eleutheroside E with 75% ethanol under various extraction times indicated that 1 h is sufficient for extraction. It could be concluded from these experiments that the best solvent for the extraction of eleutheroside E in *A. giraldii Harms* was 75% ethanol with an extraction time of 1 h under reflux.

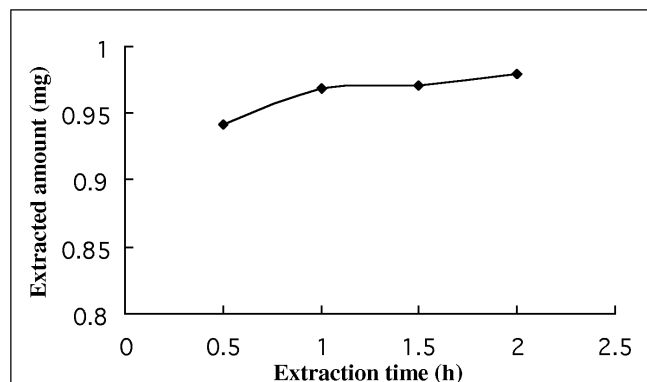

Figure 2. Extraction efficiency of eleutheroside E by the extraction time on the stem of *A. giraldii Harms*. Methanol (75%) was used as the extraction solvent.

Table III. Recovery of Eleutheroside E from the Stem of *A. giraldii Harms*

Added (µg)	Found (µg)	Recovery (%)	Average (%)	RSD (%)
15.1	15.0	99.2		
15.1	15.5	102	101	1.6
15.1	15.4	102		
25.2	25.0	99.0		
25.2	25.2	99.8	99.6	0.5
25.2	25.2	99.9		
40.4	40.8	101		
40.4	40.8	101	101	0.9
40.4	41.2	102		

Linearity, recovery, and limit of quantitation

The regression equation of eleutheroside E standard with peak area (Y) and concentration (X) was:

$$Y = 20.84X – 9.48 \quad \text{Eq. 1}$$

where r = 0.9998 over the concentration range 4.85 to 194 mg/L.

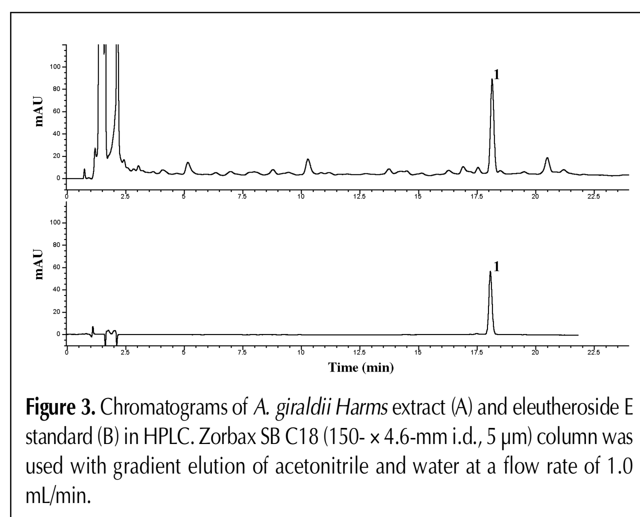

Figure 3. Chromatograms of *A. giraldii Harms* extract (A) and eleutheroside E standard (B) in HPLC. Zorbax SB C18 (150- × 4.6-mm i.d., 5 µm) column was used with gradient elution of acetonitrile and water at a flow rate of 1.0 mL/min.

Table IV. The Contents of Eleutheroside E in *A. giraldii Harms* and *A. senticosus Harms*

No. species	Parts	Habitats	Content (µg/g)
1 *A. giraldii Harms*	Stem bark	Jiuzhaigou, Sichuan	1174
2 *A. giraldii Harms*	Stem bark	Kangding, Sichuan	1319
3 *A. giraldii Harms*	Stem bark	Jinchuan, Sichuan	1491
4 *A. giraldii Harms*	Stem bark	Sanhetian(1), Sichuan	274
5 *A. giraldii Harms*	Stem bark	Sanhetian(2), Sichuan	316
6 *A. giraldii Harms*	Stem bark	Sichuan A	2546
7 *A. giraldii Harms*	Stem bark	Sichuan B	1063
8 *A. giraldii Harms*	Stem bark	Sichuan C	190
9 *A. giraldii Harms*	Stem bark	Yangpo, Ningxia	2050
10 *A. giraldii Harms*	Stem bark	Yingpo, Ningxia	2379
11 *A. giraldii Harms*	Stem	Ma'er'kang, Sichuan	1144
12 *A. giraldii Harms*	Stem	Ya'an'xiao'jin, Sichuan	1033
13 *A. giraldii Harms*	Root bark	Sichuan	3116
14 *A. giraldii Harms*	Root bark	Ningxia	2864
15 *A. senticosus Harms*	Stem	Heilong jiang	741
17 *A. senticosus Harms*	Stem	Heilong jiang	373
18 *A. senticosus Harms*	Stem	Hebei	281

Table V. The Contents of Eleutheroside E in Different Parts of *A. giraldii Harms*

No. species	Parts	Habitats	Content (μg/g)
1 *A. giraldii Harms*	Stem bark	Ya'an'xiao'jin, Sichuan	294.6
2 *A. giraldii Harms*	Stem xylem	Ya'an'xiao'jin, Sichuan	890.0
3 *A. giraldii Harms*	Stem center	Ya'an'xiao'jin, Sichuan	123.3

Nine samples from the same *A. giraldii Harms* with known eleutheroside E content were sampled, and certain volumes of standard eleutheroside E solution were added before they were extracted with the established method. The average recovery for the lowest, middle, and highest mass added to the samples were 99.6–101% (shown in Table III).

The limit of quantitation of eleutheroside E was 0.5 μg/mL at a signal-to-noise ratio of 10. The observed linearity and the results of recovery testing indicate that this HPLC method is suitable and applicable for the evaluation of eleutheroside E in the *Araliaceae* family.

Analysis of eleutheroside E in *A. giraldii Harms* and *A. senticosus Harms*

The content of eleutheroside E in the stems and roots of *A. giraldii Harms* and *A. senticosus Harms* was analyzed with the established HPLC method. The chromatograms of *A. giraldii Harms* are shown in Figure 3. The variation in the contents of eleutheroside E in different batches of *A. giraldii Harms* and *A. senticosus Harms* is presented in Table IV. Generally, the content of eleutheroside E was higher in the root than the stem for *A. giraldii Harms*. In this experiment, the content of eleutheroside E in most stems of *A. giraldii Harms* is higher than that of *A. senticosus Harms*.

The stem of *A. giraldii Harms* can be divided into three parts: bark, xylem, and center. As shown in Table V, the content of eleutheroside E in different parts of the stem has been investigated, and the results showed that the content of eleutheroside E in the stem xylem was three times that in the stem bark and seven times of that in the stem center. Traditionally, the stem, xylem, and center of *A. giraldii Harms* are thrown away, with only the stem bark being used as medicine. Thus, most of the eleutheroside E has been wasted. It is recommended that the entire stem be used for the extraction of eleutheroside E.

Conclusion

The stem and root of *A. giraldii Harms* have a large amount of eleutheroside E, and they could be resources for the development of androgenic effects and antifatigue drugs.

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