CNU Journal of Agricultural Science Vol. 38, No. 3, pp. 479-484, September 2011

Lignan contents in *Acanthopanax senticosus* by HPLC

Hye Min Kim[1], Seon Haeng Cho[2], Soon Ja Kook[3], Sanghyun Lee[1]*

[1]*Department of Integrative Plant Science, Chung-Ang University, Anseong 456-756, Korea*
[2]*Gongju National University of Education, Gongju 314-711, Korea*
[3]*Gimpo Agricultural Technology Extension Center, Gimpo 415-743, Korea*

고속액체크로마토그래피를 이용한 가시오갈피의 리그난 함량

김혜민[1] · 조선행[2] · 국순자[3] · 이상현[1]*

[1]중앙대학교 식물시스템과학과, [2]공주교육대학교, [3]김포시 농업기술센터

Received on 4 July 2011, revised on 22 July 2011, accepted on 19 September 2011

Abstract : A reverse-phase system of HPLC using a linear gradient of acetonitrile and deionized water was developed for the quantification lignans, eleutherosides B and E, in *Acanthopanax senticosus*. The HPLC system consisted of linear gradient of acetonitrile and deionized water, and UV/VIS detection was set at 210 nm. Both eleutherosides B and E contents in different parts of *A. senticosus* were determined. As a result, the contents of eleutherosides B and E were measured in the leaves (trace amounts and 0.029 mg/g, respectively), stems (0.107 and 1.015 mg/g, respectively), roots (0.026 and 0.390 mg/g, respectively), and fruits (0.022 and 0.043 mg/g, respectively). Moreover, eleutherosides B and E in the water extract were found 0.011 and 0.171 mg/g, respectively.

Key words **:** *Acanthopanax senticosus*, Eleutherosides B and E, HPLC, Water extract

I. Introduction

Acanthopanacis cortex (Araliaceae) is a well-recognised bush and widely distributed in the north-eastern region of Korea, China, and Japan, and the far-eastern region of Russia. The genus *Acanthopanax* includes ten species, three forma, and two varieties in Korea (Yook *et al.*, 1976). This herb is a typical oriental folk medicine and has been used clinically as a tonic, anti-rheumatic and prophylactic for anti-stress, chronic bronchitis, hypertension, ischemic heart disease, and gastric ulcer (Nishibe *et al.*, 1990; Fujikawa *et al.*, 1996). Particularly, the root and stem barks of Acanthopanacis cortex have been used as a tonic, and sedative as well as in the treatment of rheumatism, and diabetes (Perry and Metzger, 1980). The plant contains many kinds of functional compounds including eleuthero-sides A, B, B1, C, D, E, F, G, flavonoids, and poly-saccharides (Ovodov *et al.*, 1965; Xu, 1996). Among these compounds, eleutherosides B and E were main active components of *Acanthopanax* species (Takasugi *et al.*, 1985; Kang *et al.*, 2001; Kim *et al.*, 2006).

Eleutheroside B (= syringin) has an anti-fatigue function (Li *et al.*, 2008), and eleutheroside E (= acanthoside D) has the most pronounced anti-stress effect (Sandberg, 1973). Eleutheroside E was previously determined in Acanthopanacis cortex, which was collected from various locations, different cultivation periods, and various plant parts or diameters (roots and stems), by Ahn *et al.* (2000).

Among *Acanthopanax* species, *A. senticosus* (= *Eleutherococcus senticosus*, Siberian ginseng) is a woody shrub found only in northeast Asia. The water

***Corresponding author:** Tel: +82-31-670-4688
E-mail address: slee@cau.ac.kr

extracts of the root barks are commonly used as ingredients in health foods and for medicinal purposes (Slacanin *et al.*, 1991). The recent studies suggest that the fruits of *A. senticosus* were determined for its anti-microbial and anti-oxidant potential (Kim *et al.*, 2006) and the *n*-butanol fraction from the water extract of the stems was showed anti-oxidant activity (Lee *et al.*, 2004). The isolation and characterization of several constituents, such as (-)-sesamin, isofraxidin, 5-hydroxymethylfurfural, syringin, and acanthoside D, from the stem of *A. senticosus* has been described in the previous publications (Ryu *et al.*, 2003). Also anti-bacterial compounds, such as chiisanogenin, hyperin, and chiisanoside, were isolated from the leaves of this plant (Lee *et al.*, 2003).

Recent years have witnessed that the development of processed fermented products using ferment bacillus. Not many researches, however, were reported to be performed on the subject of fermented products of *A. senticosus*. In particular, there has been no previous report published to-date regarding the quantitative analysis of major active components such as eleutherosides B and E during fermentation process.

In the course of searching for the properties of fermented product, the contents of eleutherosides B and E in the water extract and fermented product of *A. senticosus* were measured by HPLC.

II. Materials and methods

1. Materials

The plant of *Acanthopanax senticosus* was collected at the farm of Gimpo Acantopanacis in 2010. Each plant part was extracted with methanol under reflux, and fermented product was gifted from Research Center of U Pharm in 2010.

2. Instruments and reagents

The mass spectrometry (MS) spectra were measured with a Jeol LMS-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. The HPLC system consisted of Water 1525 Binary HPLC pump, TCM column oven, and Waters 2489 UV/VIS detector all controlled by a computer using Empower Pro 2.0 software. Separation was carried on RP-type SunFire™ (C18, 3.5 μm R4.6 mm x 150 mm) HPLC column (Waters). The column temperature was maintained by at 33℃ in the experiment. 1st grade solvents for HPLC such as methanol and distilled water (J. T. Baker®, USA) were used as elution solution on HPLC.

3. Standard preparation

The air-dried powdered stems of *A. senticosus* was extracted with H_2O under reflux. The H_2O extract was resuspended in H_2O, and then fractionated successively with equal volumes of *n*-hexane, CH_3Cl, EtOAc, and *n*-BuOH. The *n*-BuOH fraction was chromatographed on silica gel eluting with a gradient of CH_3Cl-MeOH to yield compounds **1** and **2** (Fig. 1).

Eleutheroside B (**1**); FAB-MS: *m/z* 373 $[M + H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 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 $[M + H]^+$;

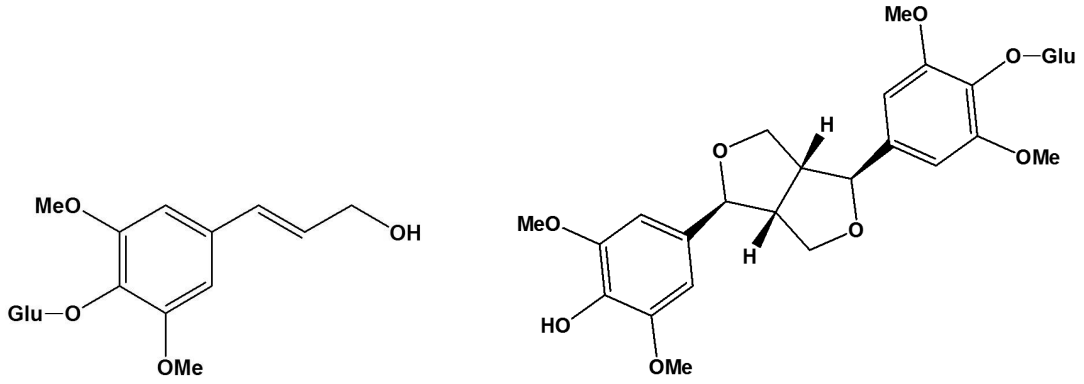

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Fig. 1. Structures of eleutherosides B (**1**) and E (**2**).

^{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 (2H, 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).

4. Sample preparation

For the analysis of eleutherosides B and E in parts of *A. senticosus* and two types of *A. senticosus* extract, each 3.0 g sample (leaves, stems, roots, fruits, and fermented products) was extracted three times for three hours with MeOH/water (1:1, v/v) by reflux. After filtered with a filter paper (Whatman No. 2, USA) and evaporated *in vacuo*. Each extract sample was performed by dissolving 3.0 mg in 2.0 mL 50% methanol in distilled water. For the injection, soluble samples through Whatman 0.45 μm PVDF syringe filter (Cat No. 6779 1304, USA). The resulting solvent was used for the HPLC analysis.

5. Chromatographic conditions

For the identification and quantification of eleutherosides B and E *via* HPLC, a mobile phase was used acetonitrile and distilled water. The elution consisted of a linear gradient program from 10 to 15% acetonitrile in distilled water over 30 min then ramped to 10% acetonitrile over 2 min, then returned to 15% acetonitrile water in distilled for 18 min and maintained at 15% acetonitrile for 10 min. The flow rate was 1 mL/min. Wavelength for detector was 210 nm and injection volume into the HPLC was 20 μL. All injection was performed in triplicate.

6. Calibration

Stock solutions of eleutherosides B and E (each 0.4 mg/0.8 mL) blended with 50% MeOH in distilled water, repeatedly. Of these was loaded into an HPLC for the preparation of the calibration functions. The calibration functions of eleutherosides B and E are calculated with peak area (Y), concentration (X, mg/mL), and mean values (n = 7) ± standard deviation.

III. Results and discussion

The optimum mobile phase for the analysis of eleutherosides B and E was by performing HPLC flow with gradient solvent (10 to 15% acetonitrile in distilled water) for 30 min. From the wavelengths monitored, two peaks of standard components were observed at

210 nm as shown in Fig. 2. The retention time of standard components such as eleutherosides B and E were showed at 7.41 and 21.52 min, respectively. In the HPLC profile of the sample solution, the retention times of the expected peaks of eleutherosides B and E were same as those of standard compounds (Fig. 3).

A calibration curve for eleutherosides B and E was constructed as shown in Fig. 4. The calibration equation for eleutheroside B was Y = 78,184,468.5745X − 17,763.0345 (r^2 = 0.9995) and eleutheroside E was Y = 127,927,178.4958X − 1,911.5345 (r^2 = 0.9996). The correlation faibratof a − compounds indeuthed 0.999 (Fig. 4). The contents of eleutherosides B and E in *A. senticosus* are shown in Fig. 5. The contents of

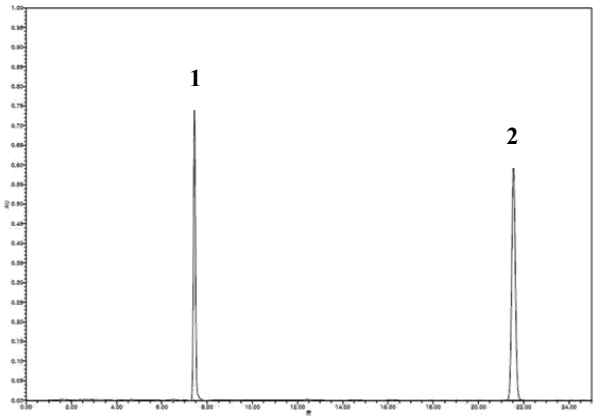

Fig. 2. HPLC chromatogram of eleutherosides B (**1**) and E (**2**) standards.

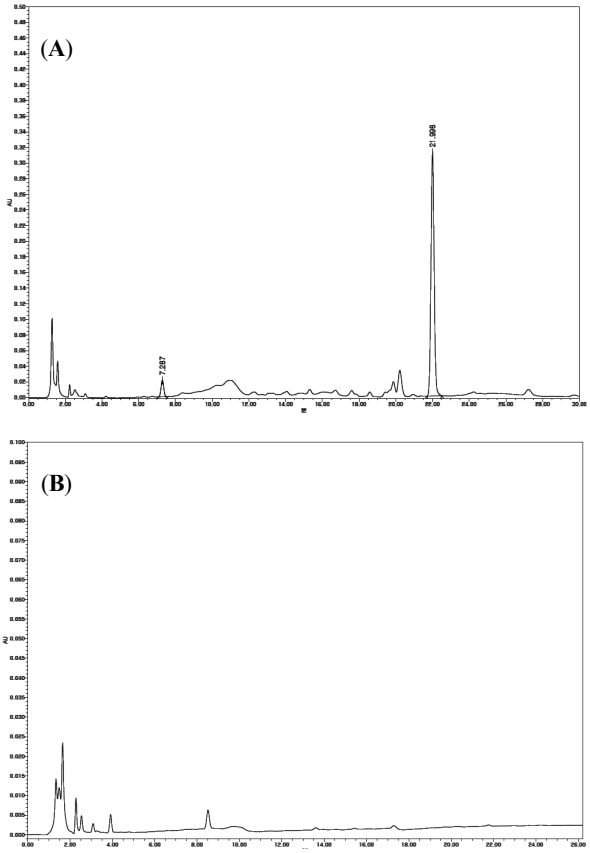

Fig. 3. HPLC chromatograms of the water extract (**A**) and fermented product **(B)** of *A. senticosus*.

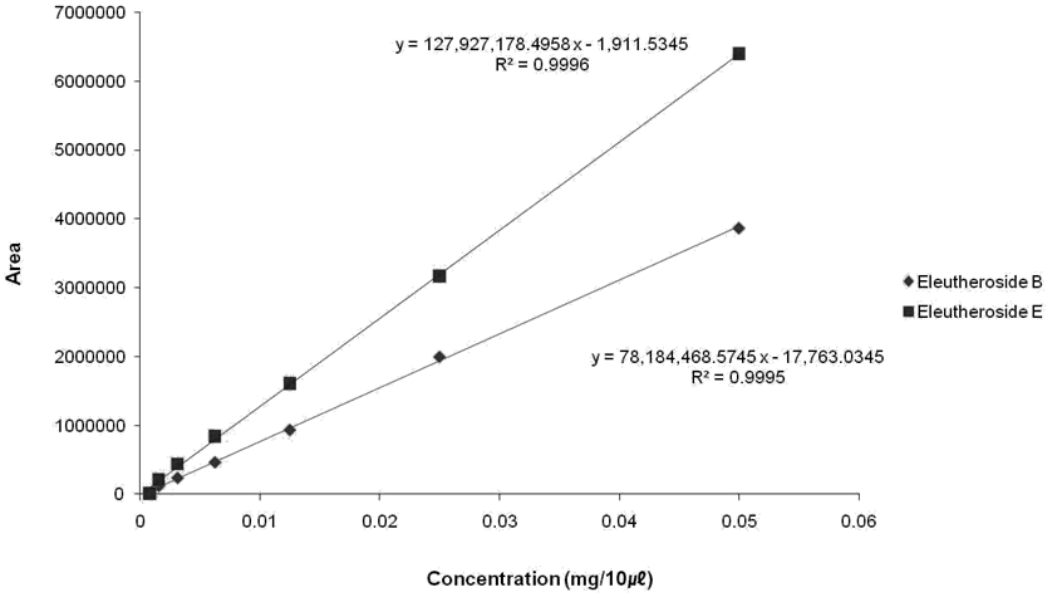

Fig. 4. Calibration curve for eleutherosides B and E.

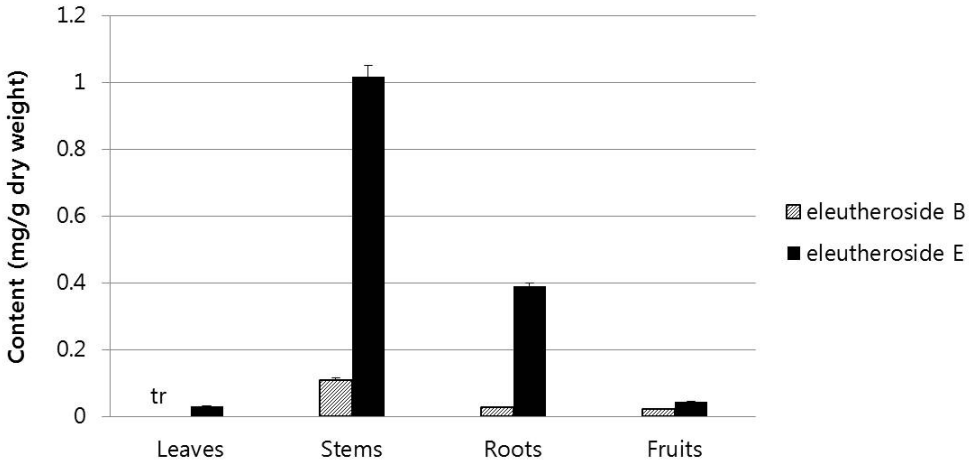

Fig. 5. Contents of eleutherosides B and E at different parts of *A. senticosus* cultivated at the farm of Gimpo Acantopanacis.

Table 1. Contents (mg/g dry weight) of eleutherosides B and E in the water extract and fermented product of *A. senticosus* cultivated at the farm of Gimpo Acantopanacis.

Samples	Eleutheroside B	Eleutheroside E
Water extract	0.011 ± 0.001	0.171 ± 0.002
Fermented product	n.d	n.d

Data are given as the mean ± S.D. (n = 3).
n.d = not detected.

eleutherosides B and E were measured in various of *A. senticosus* such as leaves, stems, roots, and fruits. The contents of eleutherosides B and E were measur995) athe leaves (trace amounts and 0.029 mg/g, respectively), stems (0.107 and 1.015 mg/g, respectively), roots (0.026 and 0.390 mg/g, respectively), and fruits (0.022 and 0.043 mg/g, respectively).

We detected eleutherosides B (0.011 mg/g) and E (0.171 mg/g) in water extract, but not in fermented product extract. The content of water extract and fermented products of *A. senticosus* are shown in Table 1.

These results indicated that the presence of eleutherosides B and E in fermented product are changed by fermentation process. According to previous research papers, flavonoid glycosides such as rutin, hesperidin, naringin and poncirin were transformed to their aglycones by the bacteria producing α-rhamnosidase (*Bacteroides* spp., *Pepetotreptococcus* spp., *Eubactrium* spp., and *Fusobacterium* spp.) and β-glucosidase (*Bacteroides* spp., *Streptococcus* spp., *Pepetotreptococcus* spp., *Eubactrium* spp., and *Fusobacterium* spp.) or endo-β-glucosidase, and baicalin, puerarin and daidzin were transformed to their aglycones by the bacteria producing β-glucuronidase (*Eubacterium* spp., *E. coil*, and *Bacteroides* spp.), C-glycosidase (*Bacteroides* spp.) and ramnosidase (*Bacteroides* spp., *Pepetotreptococcus* spp., *Eubactrium* spp., and *Fusobacterium* spp.) (Kim *et al.*, 1998; Moon *et al.*, 2004). According to these evidences in previous papers, aglycones, detached glucoside, is thought to be formed from eleutherosides B and E by the bacteria produced in the process of fermentation. Additional study on what causes the change of constituents in the process of fermentation should be carried out.

Acknowledgement

This work was supported by a grant from Gimpo Agricultural Technology Extension Center (2010), Gimpo, Korea.

References

Ahn JK, Lee WY, Oh SJ, Park YH, Hur SD, Choi MS. 2000. The contents of chlorogenic acid and eleutheroside E in *Eleutherococcus senticosus* (Rupr. et Maxim) Harms. J. Kor. For. Soc. 89: 216-222.

Fujikawa T, Yamaguchi A, Morita I, Takeda H, Nishibe S. 1996. Protective effects of *Acanthopanax senticosus* Harms from Hokkaido and its components on gastric ulcer in restrained cold water stressed rats. Biol. Pharm. Bull. 19: 1227-1230.

Kang JS, Linh PT, Cai XF, Kim HS, Lee JJ, Kim YH. 2001. Quantitative determination of eleutherosides B and E from *Acanthopanax* species by high performance liquid chromatography, Arch. Pharm. Res. 24: 407-411.

Kim DH, Jung EA, Sohng IS, Han JA, Kim TH, Han MJ. 1998. Intestinal bacterial metabolism of flavonoids and its relation to some biological activities. Arch. Pharm. Res. 21: 17-23.

Kim HM, Kim JS, Lee S, Lee SJ, Lee GP, Kang SS, Cho SH, Cheoi DS. 2006. Quantitative analysis of lignans in the fruits of *Acanthopanax* species by HPLC. Food Sci. Biotechnol. 15: 778-780.

Kim MK, Jin YS, Heo SI, Shim TH, Sa JH, Wang MH. 2006. Studies for component analysis and antioxidant effect, antimicrobial activity in *Acanthopanax senticosus* HARMS. Kor. J. Pharmacogn. 37: 151-156.

Lee S, Shin D-S, Oh K-B, Shin KH. 2003. Antibacterial compounds from the leaves of *Acanthopanax senticosus*. Arch. Pharm. Res. 26: 40-42.

Lee S, Son D, Ryu J, Lee YS, Jung SH, Kang J, Lee SY, Kim HS, Shin KH. 2004. Anti-oxidant activities of *Acanthopanax senticosus* stems and their lignin components. Arch. Pharm. Res. 27: 106-110.

Li C, Wang XY, Hu XW, Fang HT, Qiao SY. 2008. Determination of eleutheroside B in antifatigue fraction of *Acanthopanax senticosus* by HPLC. Zhongguo Zhong Yao Za Zhi 33: 2800-2802.

Moon SW, Kang SH, Jin YJ, Park, JG, Lee YD, Lee YK, Park DB, Kim SJ. 2004. Fermentation of *Citrus unshiu* Marc. and functional characteristics of the fermented products. Kor. J. Food Sci. Technol. 36: 669-676.

Nishibe S, Kinoshita H, Takeda H, Okano G. 1990. Phenolic compounds from stem bark of *Acanthopanax senticosus* and their pharmacological effect in chronic swimming stressed rats. Chem. Pharm. Bull. 38: 1763-1765.

Ovodov YS, Ovodova RG, Solov'eva TF, Elyakov GB, Kochetkov NK, Soedin KP. 1965. Glycoside from Eleutherococcus isolation and some properties of eleutherosides B and E. Arad. Nauk Uz. SSR. 1: 3-7.

Perry LM and Metzger J. 1980. *Medicinal Plants of East and Southeast of Asia.* pp. 4. MIT Press, Cambridge, MA, London.

Ryu J, Son D, Kang J, L SY, Kim H-S, Shin K H, Lee S. 2003. Phytochemical constituents of *Acanthopanax senticosus* (Rupr. & Maxim.) Harms stem. Kor. J. Medicinal Crop Sci. 11: 306-310.

Sandberg F. 1973. Two glycoside-containing genera of the Araliaceae family Panax and Eleutherococcus. Planta Med. 24: 392-396.

Slacanin I, Marston A, Hostettmann K, Guédon D, Abbe P. 1991. The isolation of *Eleutherococcus senticosus* constituents by centrifugal partition chromatography and their quantitative determination by high performance liquid chromatography. Phytochem. Anal. 2: 137-142.

Takasugi N, Moriguchi T, Fuwa T, Sanada S, Ida Y, Shoji J, Saito H. 1985. Effect of *Eleutherococcus senticosus* and its rectal temperature, body and grip tones, mortor coordination, and exploratory and spontaneous movement in acute stressed mice. Shoyakugaku Zasshi 39: 232-237.

Xu GJ. 1996. *Pharmacognosy*. 2nd ed. pp. 389-390. People Hygiene Publishers, Beijing.

Yook CS, Lee DH, Seo YK. 1976. A new forma of *Acanthopanax* species (I). Kor. J. Pharmacogn. 7: 179-190.