Standardization of Eleutherococcus species and HPLC Method Validation for Quantitative Analysis

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ABSTRACT

Objective: For the standardization and quality control of eleutheroside E in Eleutherococcus species, HPLC analysis was performed and eleutheroside E content was compared in 23 kinds of Eleutherococcus species collected from Korea and China.

Methods: The content of eleutheroside E in stem bark of Eleutherococcus species collected from Korea and China were analyzed by HPLC, 0.5% phosphoric acid and acetonitrile was used as mobile solvent, Validation of HPLC analysis method was confirmed by analyzing specificity, linearity, precision and accuracy following ICH guideline.

Results: Content of eleutheroside E was determined to be 1.0–1.6% and 0.5–0.8% in Korean and Chinese E. senticosus, respectively. Content of eleutheroside E in E. sessiliflorus was 0.7–1.1% and 0.2–0.4% respectively in Korean and Chinese origin. All calibration curves showed good linear regression. The method showed good precision and accuracy with intra-day and inter-day variations of 0.880–3.442% (RSD) and 0.606–3.328% (RSD), respectively, and average recovery was of 0.141–1.363% (RSD), for the eleutheroside E analyzed.

Conclusion: These results might be used to establish a criterion of eleutheroside E in Eleutherococcus species.

Key words: Eleutherococcus senticosus, Method validation, Standardization, Eleutheroside E, HPLC

INTRODUCTION

Eleutherococcus species were considered as useful medicinal herbal resource in Korea, China, Japan, and Russia from the time immemorial. In Traditional Korean Medicine (TKM), these have been used as a drug with adaptogenic activity, anti-tumor, anti-stress, fatigue and hypoglycemic. Root and stem bark of Eleutherococcus senticosus, known as Siberian Ginseng, has been often used to treat stroke as well as tonify qi, strengthen muscle and bone, tranquilize and dispel wind dampness. Recent researches have shown that E. senticosus exerted neuroprotective effect on amyloid beta induced neuritic atrophy, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine induced cell death, and transient focal cerebral ischemia.

Among various diterpenoids and triterpenoids reported from this plant, the lignan compounds, eleutheroside B (syringin) and eleutheroside E (−)-syringaresinol–di-O–13-D glucoside, are known to be main active principles. Beside these, E. senticosus contains chiisanoside, daucosterin, β-sitosterol and sesamin, which are responsible for its diverse biological activities.

Among 500 herbal medicines reported in Korean Herbal Pharmacopoeia and Herbal Pharmacopoeia, only 50 herbal medicines are available for evaluation.
quantitative analysis. On the other hand, 551 herbs listed in Chinese Herbal Pharmacopoeia. 215 herbal medicines can be possible through a quantitative analysis. The standardization and the quality control of the active constituents of many herbs are still lacking[15].

In this study, the content of eleutheroside E in 23 kinds of Eleutherococcus species collected from Korea and China were done by HPLC analysis. It was carried out HPLC method validation and quantity standardization according to the International Conference on Harmonization (ICH) guidelines.

Material and Methods

1. Plant materials

Dried stem barks of twenty three kinds of Eleutherococcus species were purchased from Kyung Dong Herbal Market, Jeji–dong Seoul, Korea and Cheolwon and Yang–gu, Gangwon–do, Korea. Samples were identified by Professor Dr. Hocheol Kim, Department of Herbal Pharmacology, College of Oriental Medicine, Kyung Hee University, Seoul, Korea.

2. Chemicals and Reagents

All reagents were of analytical grade. Acetonitrile was purchased from J. T. Baker (Philipsburg, NJ, USA), Eleutheroside E was obtained from Chromadex (purity ≥ 92.3%). Water was filtered through a 0.45 μm membrane (Millipore, Bedford, MA, USA).

3. Preparation of the crude extracts

At first, the dried stem barks of Eleutherococcus species were cut into the pieces, and then those were extracted with 70% ethanol for 3 hours at 82 ± 2°C in a reflux apparatus. The extracts were filtered, then the filtrate was evaporated in a rotary evaporator and the powders were lyophilized in a freeze–dryer (Operon™, Seoul, Korea).

4. Validation for HPLC analysis

1) Preparation of standard and sample solution

Stock solution of 1.0 mg/mL was prepared in 85% methanol for eleutheroside E. A serial dilution was made on each stock solution with 85% methanol to prepare standard solutions at concentrations of 0.5, 1, 5, 10, 50, 100, and 500 μg/mL from each of which 10 μL was used for plotting the standard curves for eleutheroside E. The E. senticosus extract (ESE) sample was accurately weighed (50.0 mg), placed in 5 mL of 50% methanol in aqueous solution in an ultrasonic device for 30 sec for extraction. This ESE solution was passed through a 0.45 μm syringe membrane filter and 10 μL of the filtrate was injected in triplicate the HPLC system for quantitative analysis.

2) Chromatographic conditions

Analysis was performed in a Waters instrument equipped with a Waters 600 pump, a Waters 717 autosampler and a Waters 996 PDA detector using a SunFire™ C18 column (5 μm : 4.6×250 mm : Ireland). The column was equilibrated with a 95 : 5 mixture of distilled water containing 0.5% phosphoric acid (solvent A) and acetonitrile (solvent B) at a flow rate of 1.0 mL/min. The column was eluted as follows : 0–60 min 5-50% solvent B, 60–61 min 50-70% solvent B, 61–80 min 70-70% solvent B. Column temperature was kept constant at 25 °C. The absorbance was measured at 205 nm for detection of eleutheroside E. The 2-D HPLC chromatogram of Eleutherococcus species is shown in Fig. 1.

![Fig. 1. 2-D HPLC Chromatogram for Standardization of Eleutherococcus Species.](image)

Detection was performed by using a photodiode array detector. X-axis is retention time; Y-axis is wavelength, and Z-axis is absorbance unit. Analytical conditions were as follows : column, SunFire™ C18 (5μm : 4×250 mm) ; mobile phase, solvent A (0.5% H₃PO₄) and solvent B (CH₃CN) ; Gradient program, 0–60 min 5–50% B ; 60–61 min 50–70% B ; 61–85 min 70% B. The column temperature was kept constant at 25 °C. The flow rate was set at 1.0 mL/min and the injection volume was fixed at 10 μL. The UV wavelength was monitored at 205 nm.

3) Linearity and range

To assess the linearity of standard curve, seven different concentrations of standard were prepared