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Full Length Research Paper

Effect of ethanol concentrations and extraction time on acanthoside-D and total polyphenol contents and antioxidant activities in ethanol extracts of eleuthero

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Eleutherococcus senticosus **has been used as a traditional medicine with tonic and sedative effects. Preliminary data would be necessary for eleuthero products to find out better biological functions. The effect of ethanol concentration and extraction time on the contents of acanthoside-D, polyphenols and antioxidant activities in ethanol extracts of eleuthero (*Eleutherococcus senticosus*) was evaluated to provide preliminary data for making eleuthero products that may have better biological functions. Bark of eleuthero stem was cut into 0.5 cm long pieces, which were then put in 20, 40, 60 or 80% ethanol (75 g in one L ethanol) and stored for 30, 60 and 90 days at room temperature. Acanthoside-D content in the ethanol extracts from eleuthero (EEE) extracted in 40% ethanol was higher than those in the other samples ($p<0.05$). Total polyphenol content (TPC) in the EEE extracted in 20 and 40% ethanol were significantly less than in the others on the 60th day ($p<0.05$). Extracting time had little influence on the acanthoside-D content and TPC in the EEE. Antioxidant activities of the EEE, determining DPPH and ABTS free radical scavenging activities were not significantly different among the samples regardless of ethanol concentration and extracting time. The eleuthero extracts with the initial ethanol concentrations of more than 40% had generally more acanthoside-D contents and total polyphenols and stronger antioxidant activities than the others. Extracting time little influenced on them in the EEE.**

Key words: Eleuthero (*Eleutherococcus senticosus*), polyphenol, acanthoside-D, antioxidant activity, radical scavenging capacity.

INTRODUCTION

Eleuthero (*Eleutherococcus senticosus*; formerly *Acanthopanax senticosus*), which belongs to the Araliaceae family, is widely distributed in Russia, Korea and northeast China. Eleuthero, also called "Siberian ginseng", has been used as a traditional medicine with tonic and sedative effects. According to traditional Oriental medicine books, eleuthero is known to be effective in treating palsy, hypertension and diabetes (Kim et al., 2006). It has been studied extensively and shown to have anti-mutagenic activity (Kim et al., 2000; Chung et al., 1988), anti-cancer effect, especially from the stems (Yook et al., 1996; Jeoun et al., 2005), immunomodulating and toxicity reducing effects (Lee et al., 2007) and anti-hypertensive activity (Rho et al., 2006). Davydov and Krikorian (2000) reported that eleuthero also has high antioxidant activity. Kim et al. (2002) found that antioxidant activity in ethanol extracts of eleuthero was much higher than that of butylated hydroxytoluene (BHT).

It has been demonstrated that many effective compounds are present in eleuthero, such as sesamin, savinin, acanthoside A, B, C and D, chiisanoside, polyacetylene, β-sitosterol, stigmasterol, campesterol,

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vitamins and minerals (Yook et al., 1996; Kim et al., 2002; Tang and Eisenbrand, 1992; Ovodov et al., 1967). Particularly, acanthoside-D, also called eleutheroside E, acankoreoside D, (+)-syringaresinol and di-O-β-D-glucoside, was shown to increase the growth of T-cell and the activity of the prostate and to energize the human body (Yook et al., 1996; Choi et al., 2008; Hong et al., 2002; Song et al., 2003). The chemical analyses of eleuthero using various methods have also been studied (Song et al., 2003; Lee et al., 2001).

Methanol extracts of different parts of the tree had different levels of acanthoside-D, which was found more in stems (653 ppm) than roots (531 ppm), and both increased with the age of the tree (Jwa et al., 2000). A comparison study on extraction yields of acanthoside-D from eleuthero using various solutions showed that the ethanol solution was the best (69.7% yield) (Jwa et al., 2001). Optimum extraction conditions for acanthoside-D extraction were also investigated using preparative recycle chromatography (Hong et al., 2002), supercritical fluid extraction system and response surface methodology (Kim et al., 2008).

This study was aimed to evaluate the effect of ethanol concentration and extraction time on the contents of acanthoside-D, polyphenols and antioxidant activities in ethanol extracts of eleuthero and to provide preliminary data for making eleuthero products that may have better biological functions.

MATERIALS AND METHODS

Plant materials

Dried bark of eleuthero stem (about 30 cm long pieces) was purchased from a market in Seoul, Korea in 2008 and stored at 4°C until used within one year of purchase.

Preparation of ethanol extracts of eleuthero

The bark of eleuthero stem was cut into 0.5 cm long pieces, which were than put in 20, 40, 60 and 80% ethanol (Ducksan Pure Chemicals, Seoul, Korea) (75 g in one L ethanol). The ethanol extracts from eleuthero (EEE) were stored at room temperature in the dark and extracted for 30, 60 and 90 days. The EEE were filtered through one piece of Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, England).

Acanthoside-D assay using HPLC

The EEE was filtered through a 0.50 µm syringe filter (Advantec Toyo Roshi Kaisa, Ltd., Tokyo, Japan). Acanthoside-D contents in the EEE were analyzed using an Agilent 1100 series system (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with µ-Bondapak C_{18} column (3.9 x 300 mm internal diameter, 10 µm, Waters, Milford, Ireland). Isocratic elution was employed for determining the contents of acanthoside-D in the EEE using 15% acetonitrile (HPLC grade, J.T. Baker, Phillipsburg, NJ, USA). Flow rate was 1.0 mL/min and injection volume was 20 µL. Detection wavelength was 210 nm. Calibration curves were obtained using an acanthoside-D standard prepared in our lab for the quantitative analysis (Hong et al., 2002; Jwa et al., 2000).

Determination of total polyphenol content (TPC)

TPC in the EEE was measured by the Folin-Ciocalteu method (Singleton et al., 1999). 20 µL of the EEE was mixed with 1.58 mL distilled water and 100 µL of 2 N Folin-Ciocalteu reagent (Sigma Chemical Co., St. Louis, MO, USA) at ambient temperature. Three min later, 300 µL of 20% Na_2CO_3 was added and the mixture was allowed to stand for 30 min at 40°C. The absorbance of each sample was measured at 765 nm using a spectrophotometer (Beckman DU® 530, Beckman Coulter Inc., Fullerton, CA, USA). TPC was expressed as gallic acid equivalents (GAE) using gallic acid (Sigma Chemical Co.) for the calibration curve.

DPPH assay

DPPH free radical scavenging activity for the EEE was determined according to the method of Brand-Williams et al. (1995). The EEE was vacuum evaporated to dryness using a rotary evaporator (N-1000-VW, EYELA Co., Tokyo, Japan) and diluted with methanol (Samchun Pure Chemicals, Pyeongtaek, Korea) (50, 100, 200, 300, 500 and 700 µg EEE concentrate/mL), 1 mL of which was mixed with 2 mL of 0.1 mM 2,2´-diphenyl-2-picrylhydrazyl (DPPH; Sigma Chemical Co.) in methanol and allowed to stand in the dark for 30 min at ambient temperature. Absorbance was measured at 517 nm using the spectrophotometer. DPPH free radical scavenging activity (%) was calculated as follows:

$$\text{DPPH free radical scavenging activity (\%)} = \frac{\text{Absorbance of the control - absorbance of the sample}}{\text{Absorbance of the control}} \times 100$$

The DPPH IC_{50} value was defined as the amount of antioxidant required to decrease the initial DPPH radical concentration by 50%.

ABTS assay

ABTS free radical scavenging activity of the EEE was measured by the method of Brand-Williams et al. (1995). 2,2'-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammounium salt (ABTS; Sigma Chemical Co.) at 3.84 mg/mL, distilled water and potassium persulfate (Sigma Chemical Co.) at 0.66 mg/mL were mixed to make 10 mL of 7 mM ABTS solution, which was then allowed to stand overnight at room temperature in the dark. This solution was diluted with ethanol (Samchun Pure Chemicals) to reach the proper absorbance value (0.70±0.05). Four hundred micro-litre of the EEE, which had been vacuum evaporated and then diluted with ethanol (100, 300, 500, 700 and 1000 µg EEE concentrate/mL), were mixed with 3.6 mL of the diluted ABTS solution. After 1 min, absorbance was measured at 734 nm using the spectrophotometer. ABTS free radical scavenging activity (%) was calculated as follows:

$$\text{ABTS free radical scavenging activity (\%)} = \frac{\text{Absorbance of the control - absorbance of the sample}}{\text{Absorbance of the control}} \times 100$$

The ABTS IC_{50} value was defined as the amount of antioxidant required to decrease the initial ABTS radical concentration by 50%.

Statistical analyses

All experiments were carried out in triplicate. Statistical analyses

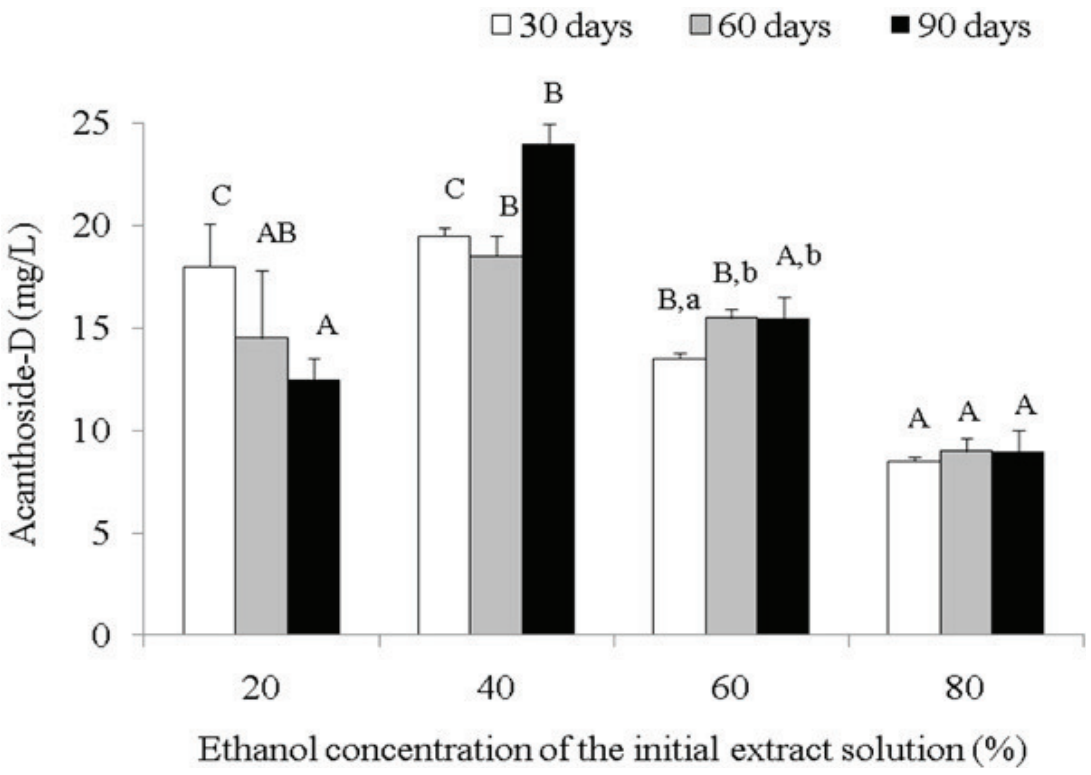

Figure 1. Acanthoside-D contents in ethanol extracts from eleuthero extracted with different conditions. Bars represent means ± SE (n=3). Different capital and small letters represent significant differences among different ethanol concentrations of the initial extracting solution at the same extraction time and among different extraction times of the same ethanol concentrations of the initial extracting solution, respectively (p<0.05; one-way ANOVA and Duncan's multiple range test).

were performed using the SPSS program (SPSS version 12.0, SPSS Inc., Chicago, IL, USA) and expressed as mean ± standard error. When p values for the repeated one-way ANOVA were significant (p<0.05), differences in the means were determined using Duncan's multiple range tests (p<0.05).

RESULTS AND DISCUSSION

Acanthoside-D content in ethanol extracts from eleuthero

Acanthoside-D contents in the EEE extracted with different ethanol concentrations and extracting time are shown in Figure 1. Extracting time had little effect on the acanthoside-D content in the EEE; however, the acanthoside-D content in the EEE extracted in 60% ethanol for 30 days was significantly less than those of the samples with the other extracting times (p<0.05). Acanthoside-D content in the EEE extracted in 80% ethanol was 8.5 to 9.0 mg/L, significantly lower than those of the samples with the other ethanol concentrations (p<0.05). Acanthoside-D content in the EEE extracted in 40% ethanol was 18.5 to 24.0 mg/L, significantly higher than those of the samples with the other ethanol concentrations (p<0.05). The result suggests that the EEE extracted in 40% ethanol had more acanthoside-D and extracting time less than 30 days worked fine.

A previous study showed that acanthoside-D content in methanol extract from ground eleuthero was 631 mg from one kg of the raw material (Jwa et al., 2001), which was about twice as much as in the EEE extracted in 40% ethanol for 90 days (320 mg from one kg of the raw material) in our study. The difference in the solutions and of the starting materials might make a difference in the acanthoside-D contents in the extracts. In our study acanthoside-D content in the EEE extracted in 40% ethanol was about twice as much as in the EEE extracted in 80% ethanol. Hong et al. (2002) and Song et al. (2003) reported that acanthoside-D was more easily extracted in a polar solvent such as water than in a less polar solvent like ethanol, which may explain why acanthoside-D content in the EEE extracted in 80% ethanol in our study was lower than those in other studies.

Total polyphenol content in ethanol extracts from eleuthero

TPC in the EEE extracted with different conditions is

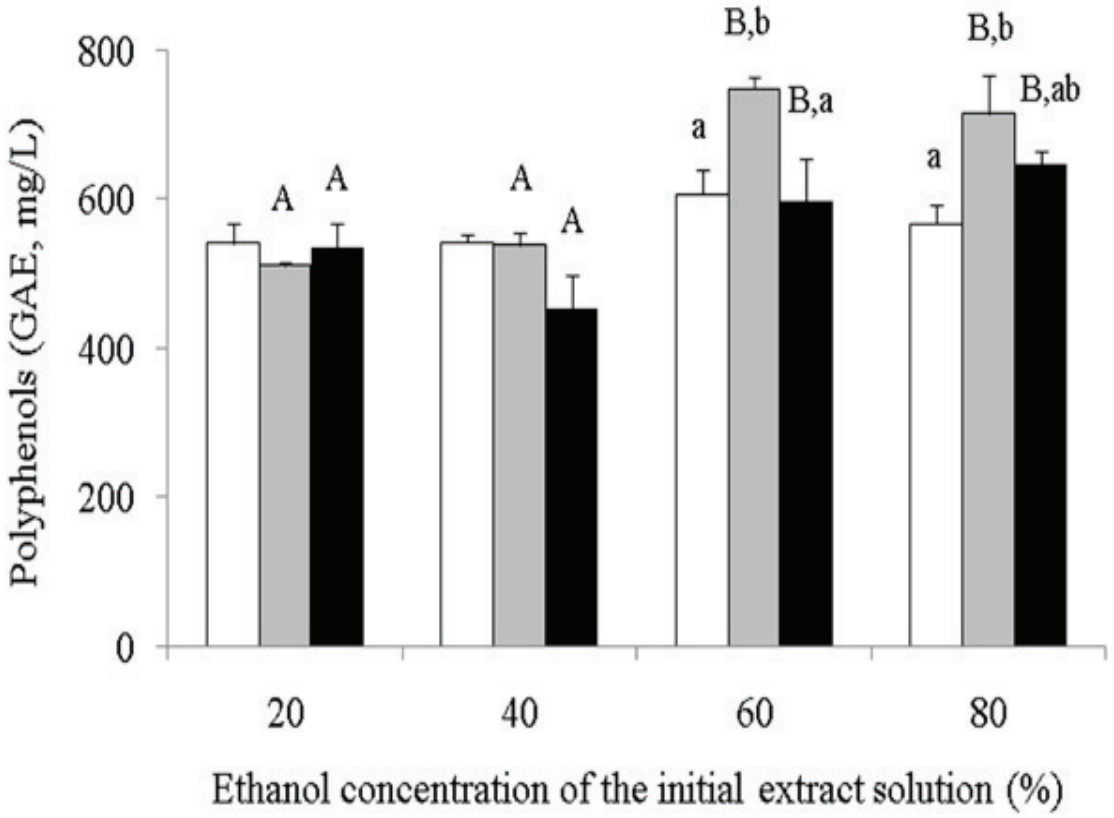

Figure 2. Total polyphenol contents in ethanol extracts from eleuthero extracted with different conditions. GAE: gallic acid equivalent. Bars represent means ± SE (n=3). Different capital and small letters represent significant differences among different ethanol concentrations of the initial extracting solution at the same extraction time and among different extraction times of the same ethanol concentrations of the initial extracting solution, respectively (p<0.05; one-way ANOVA and Duncan's multiple range test).

Table 1. DPPH IC_{50}[a] values of ethanol extracts from eleuthero extracted with different conditions. (unit: μg/ml)[b]

Ethanol concentration[c] (%)	Extraction time (days)			Significance
	30	60	90	
20	173±11	172±15	206±20	ns[g]
40	145±13[d]	156±15[de]	187±8[ef]	
60	157±29	141±9	165±34	ns
80	146±15	139±10	184±32	ns
Significance	ns	ns	ns	

[a] 50% DPPH radical scavenging activity. [b] Values are means ± SE (n=3). [c] Ethanol concentration of the initial extracting solution (%). [d,e,f] Values with different superscripts within the same rows are significantly different (p<0.05; one-way ANOVA and Duncan's multiple range test). [g] not significant within the same columns or rows (p>0.05; one-way ANOVA).

shown in Figure 2. The samples with the initial ethanol concentrations of 20 and 40% had lower total polyphenols (510 to 538 mg GAE/L) than the other samples. Extracting time had little influence on the TPC in the EEE. These results concur with a former study reporting that the lower the ethanol concentration for black raspberry extractions, the less TPC was present in the black raspberry extract (Lee et al., 2009). This may be due to the fact that the EEE may have more nonpolar polyphenols than polar ones thus favoring the higher level of less polar solvent.

Antioxidant activity of ethanol extracts from eleuthero

Table 1 shows the DPPH IC_{50} values of the EEE extracted with different conditions. DPPH IC_{50} values in

Table 2. ABTS IC_{50}[a] values of ethanol extracts from eleuthero extracted with different conditions. (unit: μg/ml) [b].

Ethanol concentration[c] (%)	Extraction time (days)			Significance
	30	60	90	
20	302±14[d]	321±63	307±7	ns[d]
40	245±31	305±33	293±14	ns
60	294±67	263±20	274±28	ns
80	348±86	302±51	318±21	ns
Significance	ns	ns	ns	

[a] 50% ABTS radical scavenging activity. [b] Values are means ± SE (n=3). [c] Ethanol concentration of the initial extracting solution (%). [d] not significant.

the EEE extracted in 20% ethanol were lower than the other samples for 90 days, although not significantly different (p>0.05). Extracting time had little effect on the antioxidant activities of the EEE except for the sample with the initial ethanol concentration of 40%. DPPH IC_{50} value of the EEE extracted in 40% ethanol for 30 days (145 μg/mL) was significantly lower than that for 90 days (187 μg/mL) (p<0.05).The ABTS IC_{50} values of the EEE extracted with different conditions are shown in Table 2. ABTS IC_{50} values of the EEE showed little difference among the samples regardless of ethanol concentration and extracting time. ABTS IC_{50} values of the EEE were 245 to 348 μg/mL, similar to the ABTS IC_{50} values in the black raspberry extracts (213 to 288 μg/mL) (Lee et al., 2009).

Antioxidant activity of the EEE was not necessarily fully correlated with acanthoside-D contents. This observation was similar to that found with the previous study, which reported that acanthoside-D in the EEE had little influence on DPPH radical scavenging activity (Lee et al., 2004). The samples with an initial ethanol concentration of 20% had less TPC and lower antioxidant activity than the other samples in this study. However, the correlation between TPC and antioxidant activity of the EEE was low. The reason for this result could be that the differences in TPC in each EEE were too low to affect antioxidant activities. This agrees with the previous study reporting that no significant correlations could be found between the TPC and antioxidant activities of 92 plant extracts (Kahkonen et al., 1999). The eleuthero extracts with the initial ethanol concentrations of more than 40% had generally more acanthoside-D contents and total polyphenols and stronger antioxidant activities than the others. Extracting time little influenced on them in the EEE.

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