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Comparison of Ginsenoside and Phenolic Ingredient Contents in Hydroponically-cultivated Ginseng Leaves, Fruits, and Roots

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In this study, hydroponically-cultivated ginseng leaves, fruits, and roots were respectively extracted with ethanol. The contents of 12 ginsenosides and three phenolics in the extracts were quantitatively analyzed and the free radical scavenging activities were measured and compared. Hydroponically-cultivated ginseng leaves contained higher levels of gensenosides (Rg1, Rg2+Rh1, Rd, and Rg3) and *p*-coumaric acid than the other parts of the ginseng plants. The 2,2'-azino-di-(3-ethylbenzothiazoline)-6-sulfonic acid radical scavenging activities of leaves were also the highest. Accordingly, hydroponically-grown ginseng leaves were shown to hold promise for use as an environmentally-friendly natural anti-oxidant.

Keywords: *Panax ginseng*, Hydroponic cultivation, Leaves, Ginsenoside, Phenolic compound

INTRODUCTION

Ginseng plants, *Panax ginseng* are cultivated in soil for 4 to 6 yr, and ginseng roots are mostly used as medicine and food, whereas all other parts of the plant are discarded. More recently, non-pesticides and hydroponically-grown ginseng leaves have been used in salads. Thus, there is a need to study the active ingredients in hydroponically-grown ginseng plants. There have been many studies on the ingredient contents of ginseng plants grown in soil [1-4]; however, very little research has been conducted on the ingredient contents and antioxidative activities of hydroponically-grown ginseng plants. Accordingly, in this study, hydroponically-cultivated ginseng leaves, fruits and roots were separated and the contents of 12 ginsenosides and three phenolics were quantitatively analyzed. In addition, the free radical scavenging activities of the extracts were measured and compared.

MATERIALS AND METHODS

Materials

For this study, hydroponically-grown ginseng plants were purchased in May 2011 from the Hydroponic Ginseng Agricultural Union Corporation located in Seocheon-gun, Chungcheongnam-do Province, Korea. The corporation cultivated 1- to 2-year-old ginseng plants for 120 d with a hydroponic cultivation system without using pesticides until the plants grew to a weight of 5 to 20 g.

Production of extract specimens

Whole ginseng plants were cleansed with distilled water. Then the leaves, fruits, and roots of the plants were separated and heat air-dried at 50°C for 36 h. The dried plants were then ground to powder. Each powder specimen was mixed with ethanol and extracted three times. The obtained supernatants were filtered and concentrated in vacuum to produce extract specimens.

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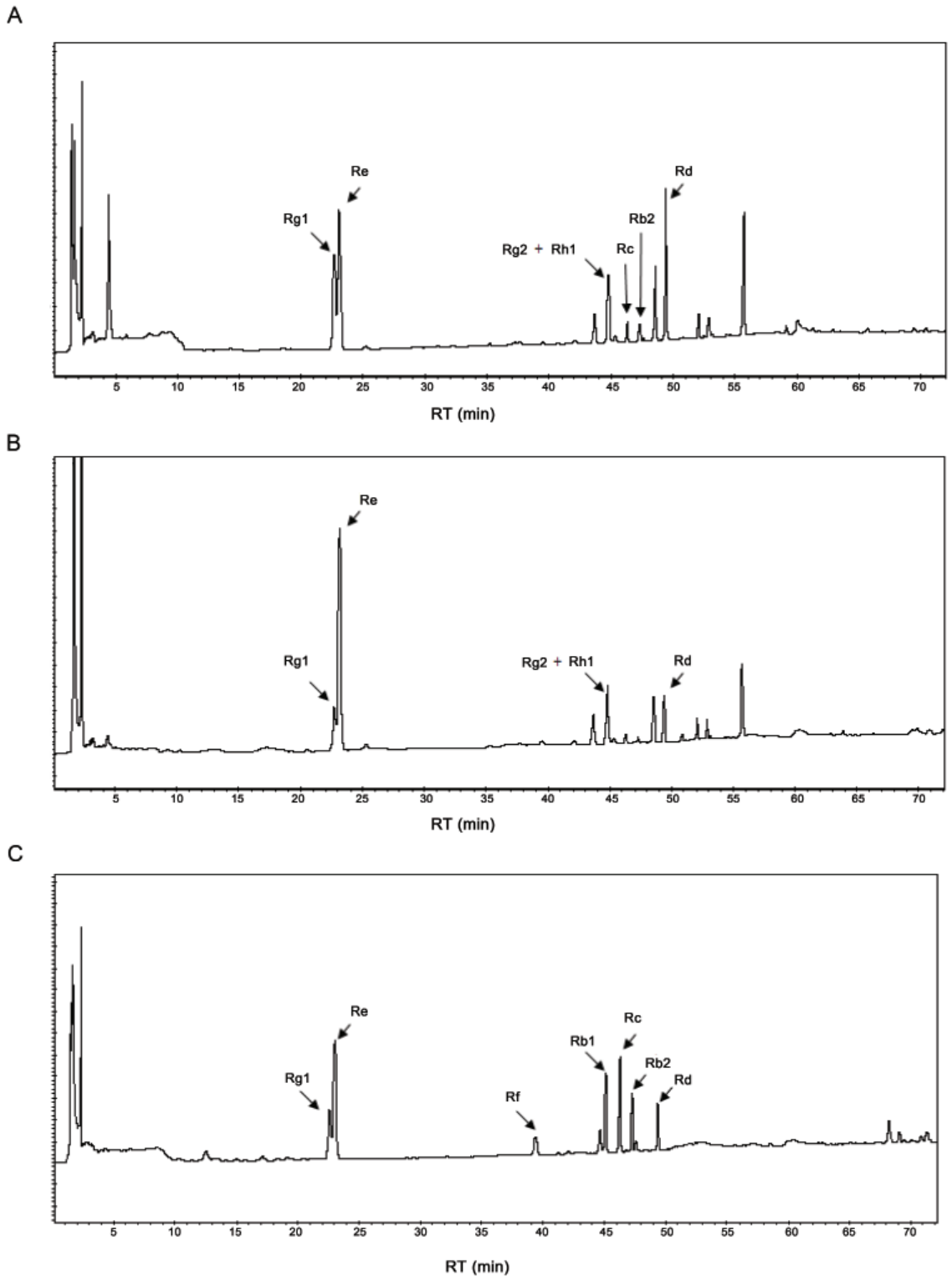

Fig. 1. Chromatogram of ginsenosides in hydroponically-cultivated ginseng leaf (A), fruit (B), and root (C). RT, retention time.

The analysis of ginsenosides

The content of ginsenosides was quantitatively analyzed with an HPLC system (Jasco, Tokyo, Japan). Using a bondapak C_{18} column (10 μm, 3.9×300 mm), water (solvent A) and acetonitrile (solvent B) were mixed in 80% solvent A in the beginning of the analysis, then after

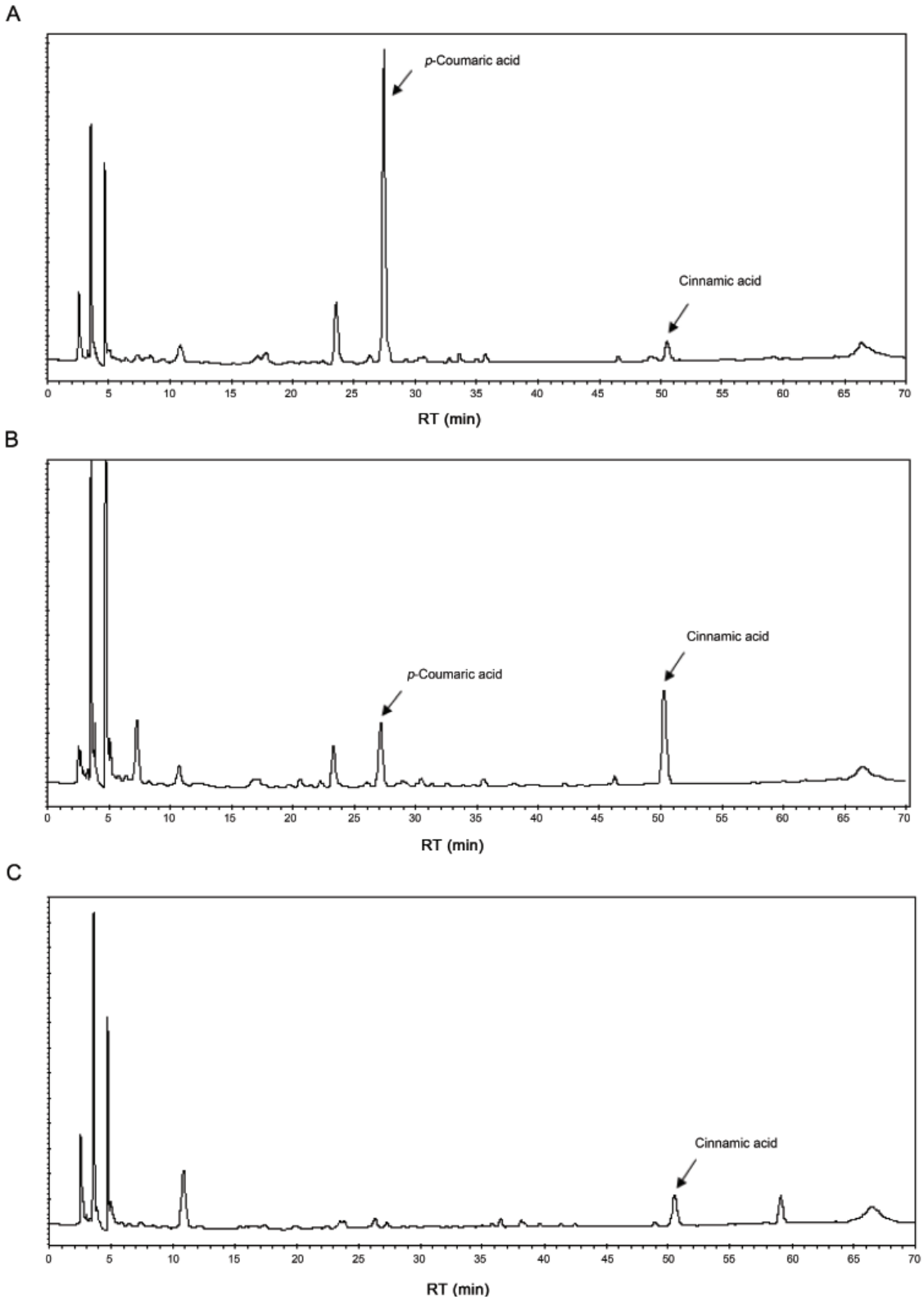

Fig. 2. Chromatogram of phenolic compounds in hydroponically-cultivated ginseng leaf (A), fruit (B), and root (C). RT, retention time.

70 min of the analysis, the solution was switched to a 0% solvent A by a gradient mobile phase. The elution speed was maintained at 1.0 mL/min, the temperature of the column was maintained at 25°C, and the absorbance was

Table 1. Ginsenoside composition of hydroponically-cultivated ginseng fruit, leaf, and root ethanol extracts (mg/g)

	Rg1	Re	Rf	Rg2+Rh1	Rb1	Rc	Rb2	Rb3	Rd	Rg3 (S,R)	Rh2
Leaf	36.2	61.3	0.6	17.4	3.1	6.1	5.2	0.5	38.0	2.2	-
Fruit	15.8	101.0	1.5	14.7	1.3	2.8	1.7	0.2	12.0	-	-
Root	19.4	54.9	7.0	6.3	29.2	31.8	18.7	3.0	12.7	-	-

S, S form; R, R form.

measured at 203 nm. To obtain specimens for analysis, the concentrated extracts were diluted with methanol to 10 mg/mL and filtered with a 0.45 μm syringe filter (Millipore, Bedford, MA, USA) [5,6].

The analysis of phenolic compounds

The contents of three phenolics (maltol, *p*-coumaric acid, and cinnamic acid) were measured with an HPLC system (Jasco, Tokyo, Japan). For quantitative analysis of the phenolic content using HPLC, 2% acetic acid-containing water (solvent A) and 0.5% acetic acid-containing 50% acetonitrile (solvent B) were mixed in 100% solvent A in the beginning of the analysis, then after 70 min of the analysis, the solution was mixed in 45% solvent A by a gradient mobile phase, using a bondapak C_{18} column (4 μm, 3.9×300 mm). The elution speed was maintained at 0.8 mL/min, the temperature of the column was maintained at 40°C, and the absorbance were measured at 280 nm. To obtain specimens for analyses, the concentrated extracts were diluted with methanol to 10 mg/mL, and filtered with a 0.45 μm syringe filter (Millipore, Billerica, USA).

2,2′-Azino-di-(3-ethylbenzothiazoline)-6-sulfonic acid scavenging effects

2,2'-Azino-di-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) radical scavenging activities were measured as described by Van den Berg et al. [7], with slight modifications. 2.5 mM ABTS and 1.0 mM 2,2'-azobis(2-methylpropionamidine) dihydrocholide was mixed in 0.1 M phosphate buffered saline (PBS, pH 7.4). The mixture was reacted in a darkroom at 68°C for 12 min, then rapidly cooled to produce the $ABTS^+$ solution. Twenty microliter of the PBS-melted ginseng extract was added to 980 μL of the $ABTS^+$ solution and incubated at 37°C for 10 min, and the optical density of the solution was measured at 734 nm.

RESULTS AND DISCUSSION

Quantification of ginsendoside and phenolic ingredient contents

In the ginsenoside content analyses of hydroponically-cultivated ginseng plants, the roots were shown to contain high amounts of ginsenoside Rf, Rb1, Rc, Rb2, and Rb3, whereas the fruits contained high amounts of ginsenoside Re (101.0 mg/g in ethanol extract). Compared to the other parts of the plants, the leaves contained higher amounts of gensenoside Rg1, Rg2+Rh1, Rd, and Rg3 (Table 1 and Fig. 1). These results were similar with those of other studies on soil-cultivated ginseng plants, which reported that soil-cultivated ginseng fruits contained particularly high amounts of ginsenoside Re [8], and soil-cultivated ginseng leaves contained high amounts of ginsenoside Rg1, Re, and Rd [9]. Accordingly, there was no difference between soil-cultivated ginseng leaves and hydroponically-cultivated ginseng leaves in terms of ginsenoside composition.

Table 2. Phenolic ingredient composition of hydroponically-cultivated ginseng fruit, leaf, and root ethanol extracts (μg/g)

	Matol	*p*-Coumaric acid	Cinnamic acid
Fruit	-	247.8	212.8
Leaf	-	965.9	46.4
Root	-	14.8	69.9

Based on the quantitative analysis of the phenolic contents, hydroponically-cultivated ginseng leaves were shown to contain high amounts of *p*-coumaric acid (965.9 μg/g in ethanol extract), whereas the fruits contained high amounts of cinnamic acid (Table 2 and Fig. 2). In a study by Lee et al. [10], even though a different extraction solution was used, soil-cultivated ginseng leaves were reported to contain higher amounts of cinnamic acid than *p*-coumaric acid. However, the result of this study showed that hydroponically-cultivated ginseng leaves contained significantly higher amounts of *p*-coumaric acid.

Radical scavenging effects

Based on the ABTS radical scavenging activities of ethanol extracts from hydroponically-cultivated ginseng plants, the leaves were shown to display an activity of 76.7% at 100 μg/g, whereas the activity of the fruits and roots were 39.5% and 53.2%, respectively (Fig. 3). These results demonstrated that the leaves showed the highest

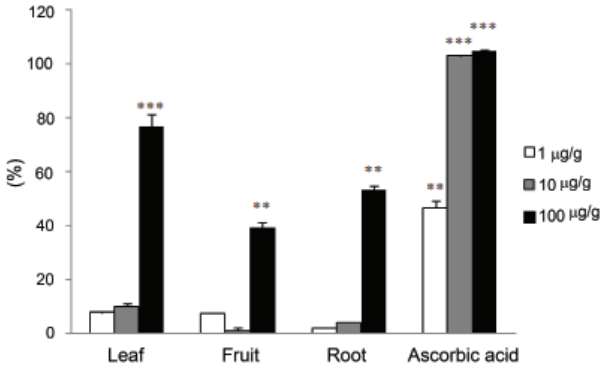

Fig. 3. 2,2'-Azino-di-(3-ethylbenzothiazoline)-6-sulfonic acid radical scavenging activity of the ginseng ethanol extracts of leaf, fruit, and root. Data are expressed as mean±SD of three experiments. Ascorbic acid was used as a positive control. **p<0.01, ***p<0.001.

free radical scavenging activity. The results of a study by Nenadis et al. [11] showed that *p*-coumaric acid most effectively eliminated ABTS among the 11 cinnamic acid derivatives. The *p*-coumaric acid content, which was present in high concentrations in hydroponically-cultivated ginseng leaves, seemed to be the major substance responsible for the ABTS radical scavenging activity. These results imply that hydroponically-cultivated ginseng leaves hold promise for use as an environmentally-friendly anti-oxidant.

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