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Research article

High-performance liquid chromatography analysis of phytosterols in *Panax ginseng* root grown under different conditions

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ABSTRACT

Background: The *Panax ginseng* plant is used as an herbal medicine. Phytosterols of *P. ginseng* have inhibitory effects on inflammation-related factors in HepG2 cells.
Methods: Phytosterols (e.g., stigmasterol and β-sitosterol) in the roots of *P. ginseng* grown under various conditions were analyzed using high-performance liquid chromatography. The *P. ginseng* roots analyzed in this study were collected from three cultivation areas in Korea (i.e., Geumsan, Yeongju, and Jinan) and differed by cultivation year (i.e., 4 years, 5 years, and 6 years) and production process (i.e., straight ginseng, red ginseng, and white ginseng).
Results: The concentrations of stigmasterol and β-sitosterol in *P. ginseng* roots were 2.22–23.04 mg/g and 7.35–59.09 mg/g, respectively. The highest concentrations of stigmasterol and β-sitosterol were in the roots of 6-year-old *P. ginseng* cultivated in Jinan (82.14 mg/g and 53.23 mg/g, respectively).
Conclusion: Six-year-old white ginseng and white ginseng cultivated in Jinan containing stigmasterol and β-sitosterol are potentially a new source of income in agriculture.

1. Introduction

The *Panax ginseng* plant is a very widely used herbal medicine globally. It was first mentioned in East Asian folklore more than 4,000 years ago [1,2]. Dried *P. ginseng* has diverse pharmacological benefits on the central nervous system and cardiovascular system and has immune-modulating functions. *Panax ginseng* enhances lymphocyte proliferation, stimulates macrophages in cytokine production, and improves the phagocytic activity of polymorphonuclear leukocytes, and is beneficial in the treatment of diabetes, inflammation in aging, oxidative damage, and cancer [1,3–12].

The increasing demand for alternative health remedies has expanded the global nutraceutical market. Plant sterols (i.e., phytosterols) represent an important group of compounds in unsaponifiable plant oils that confer biological activities to the oils [13]. Phytosterols reduce serum low-density lipoprotein cholesterol levels by decreasing intestinal cholesterol absorption [14]. Therefore, phytosterol-enriched food products have been engineered and marketed to lower serum cholesterol and reduce cardiovascular risks [15,16]. In addition to their cholesterol-lowering effect, phytosterols possess anti-inflammatory, antifungal, antiulcerative, antibacterial, and antitumor activities [17–20]. Phytosterols are also valuable in the treatment of benign prostatic hyperplasia and colon cancer [21,22].

To date, much research has focused on the analysis of saponins in *P. ginseng*, but only few studies have focused on phytosterols in *P. ginseng*. In this paper, we aimed to analyze the phytosterol content in the roots of *P. ginseng* grown under different conditions using high-performance liquid chromatography (HPLC).

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2. Materials and methods

2.1. Plant materials

The dried and powdered roots of *P. ginseng* from different cultivation areas in Korea (i.e., Geumsan, Yeongju, and Jinan), cultivation years (i.e., 4 years, 5 years, and 6 years), and production processes (i.e., straight ginseng, red ginseng, and white ginseng) were obtained from Korea Food Research Institute in Sungnam, Korea. In brief, the cultivation conditions of ginseng in the experiment consisted of the expected annual average temperature of 4–10°C and average precipitation of 800–900 mm. Ginseng requires 80% shade to thrive and prefers a deep rich loamy soil. The roots can be harvested once in the fall after the leaves have died. White ginseng was produced by sun-drying fresh ginseng.

2.2. Instruments and reagents

For thin layer chromatography (TLC) analysis, a TLC precoated silica gel 60 F_{254} (No. 5715; Merck Co., Darmstadt, Germany) was used. We used silica gel (No. 7734; Merck Co.) as the stationary phase for repeated column chromatography. A medium-pressure liquid chromatography (MPLC) system (Biotage, Uppsala, Sweden) was used; it was equipped with KP-SIL cartridges (39 mm × 225 mm; Biotage). The HPLC analysis was conducted using the Waters 1525 Binary HPLC Pump (Waters, Miami, FL, USA), which was equipped with a UV/visible spectroscopy detector (model 2489; Waters, Miami, FL, USA). The water and acetonitrile were of HPLC grade, and all other reagents were of analytical grade.

2.3. Extraction, fractionation, and isolation of Compounds 1 and 2

The dried and powdered root of *P. ginseng* (7.0 kg) was extracted with ethanol (3 × 21 L) and heated to reflux. The extracts were combined and concentrated to a brown residue (139 g). The residue was dissolved in water (7 L) and partitioned successively with *n*-hexane, trichloromethane ($CHCl_3$), ethyl acetate (EtOAc), and *n*-butanol (*n*-BuOH) to yield *n*-hexane-soluble (50 g), $CHCl_3$-soluble (11 g), EtOAc-soluble (11 g), and *n*-BuOH-soluble (50 g) fractions. A portion of the $CHCl_3$ fraction was separated by MPLC with a gradient system of *n*-hexane-EtOAc and EtOAc-methanol (EtOAc-MeOH). The flow rate was maintained at 20.0 mL/min. The peaks in MPLC were detected by UV absorbance at 254 nm. The $CHCl_3$ fraction gained 38 subfractions. Compound 1 was isolated from subfraction 6 (*n*-hexane:EtOAc was 85:15). Compound 2 was isolated from subfraction 7 (*n*-hexane:EtOAc was 80:20) [23,24].

2.4. The preparation of Compounds 1 and 2 and the samples for HPLC

To quantify the amounts of Compounds 1 and 2, we dissolved 3 mg of each compound in 300 μL of $CHCl_3$. The dried and powdered roots of *P. ginseng* were extracted by refluxing using ethanol (300 mL × 3). The extracts were then combined and evaporated to obtain a brown residue. The brown residue was dissolved in 2 mg of 1 mL MeOH. The resultant solutions were filtered through a 0.45-μm filter (Cat No. 6779 1304, USA), and used for HPLC analysis.

2.5. The HPLC conditions

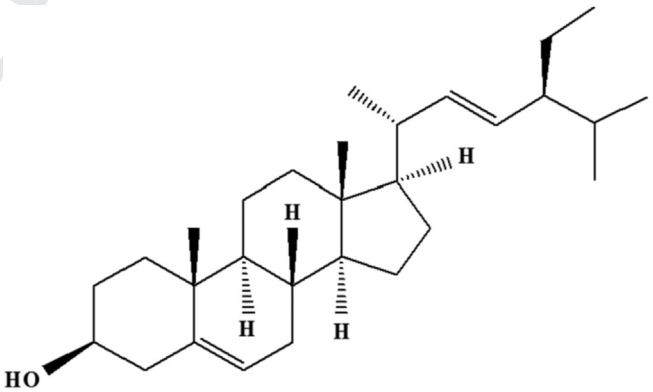
The HPLC separation of Compounds 1 and 2 for qualitative and quantitative analyses was performed using a reverse phase system. A reverse phase column (SunFire C-18 stainless steel, 2.1 mm × 50 mm, 5 μm; Waters Corporation, Milford, MA, USA) was used with MeOH and acetonitrile. The gradient solvent system was initially 30:70, and was increased in a linear gradient to 30:70 for 20 minutes, 0:100 for 10 minutes, and finally 30:70 for 15 minutes. UV detection was conducted at 210 nm. The injection amount was 10 μL and the flow rate was 1.0 mL/min. All injections were performed three times.

2.6. Calibration

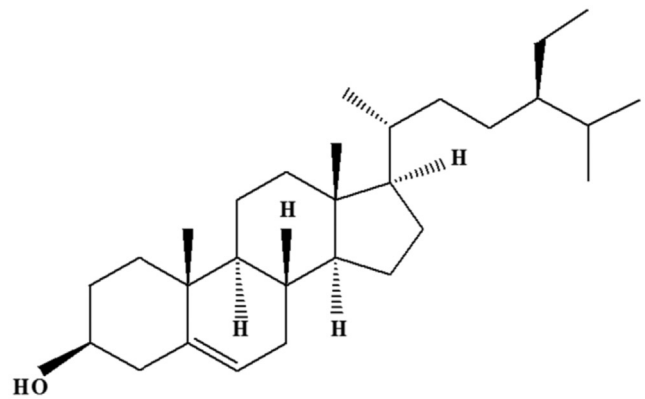
A stock solution (1 mg/mL) of Compounds 1 and 2 was prepared using $CHCl_3$. The concentration of the analytes was determined, based on the corresponding calibration curves. The peak area (Y), concentration (X; mg/10 μL), and the mean concentration value (of three measurements) ± standard deviation were calculated.

3. Results and discussion

Compounds 1 and 2 were obtained in the form of a white powder. These compounds were phytosterols, based on mass spectrometry and proton (^1H) nuclear magnetic resonance (NMR) and carbon-13 (^{13}C)-NMR analyses. The typical ^1H-NMR and ^{13}C-NMR spectral patterns of Compounds 1 and 2 indicated the presence of a sterol skeleton. The structures of Compounds 1 and 2 were stigmasterol and β-sitosterol, respectively (Fig. 1), based on the interpretation of spectroscopic data in the literature [25,26].



Compound 1



Compound 2

Fig. 1. The chemical structure of stigmasterol (i.e., Compound 1) and β-sitosterol (i.e., Compound 2). Stigmasterol and β-sitosterol are representative phytosterols in the plants and have the chemical structure of a terpenoid compound.

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