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Extraction of Panaxynol and Panaxydol Compounds from Korean Ginseng

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Abstract The extraction of panaxynol and panaxydol from Korean ginseng was found to be optimal at 55°C for the shaking method, 80°C for the soxhlet method, and 65°C for the supercritical extraction method. The amount of extracted panaxydol and panaxynol, and their ratio increased over a period of 12 h with the shaking method. The soxhlet method produced an extract with the largest panaxydol/panaxynol ratio. A reduced particle size enhanced extraction, however, the ratio of panaxydol/panaxynol decreased. Swelling in water was found to be detrimental for the extraction of panaxydol and panaxynol.

Keywords : panaxynol, panaxydol, shaking, soxhlet, supercritical extraction

INTRODUCTION

Ginseng has been used for medical purposes for more than 4000 years. *Panax ginseng* C. A. Meyer (*Araliaceae*) is known as the most valuable medicinal plant because of its minimal side-effects on the human body. Previous reports on the efficacy of ginseng include the recovery of liver functions [1], detoxification of toxic materials, decrease of blood sugar levels, anti-fatigue effects [2], prevention of arteriosclerosis [3], and a deterring effect on aging processes.

Recent investigations on the medical effects of ginseng have focused on its saponin and protein compounds. As a result, ginsenoside-Rh_2 [4] and polyacetylene [5] have been found to be effective in suppressing the multiplication of cancer cells. Polyacetylene is a kind of unsaturated polyalcohol with triple bonds and is widely distributed in *Araliaceae* and *Umbelliferae*. The structure of polyacetylene includes C_{17} compounds with a basic structure of hept-1-ene-4,6-diyne-3-ol. Twenty kinds of polyacetylene have already been isolated from white and red ginseng [6]. Among the three major compounds of polyacetylene (panaxydol, panaxynol, and panaxytriol) panaxydol exhibits the strongest cytotoxicity against cancer cells [7]. Since panaxytriol has only been found in red ginseng [8], the current study focused on panaxydol and panaxynol, and their ratio.

The solvents that have been previously used to extract polyacetylene from ginseng are alcohol or a mixture of alcohol and water [9,10]. Occasionally, ether and ethyl acetate have also been used [11,12]. Silica gel col umn chromatography has been used for purification. The extraction methods that have been used in previous studies include solvent extraction using shakers [13] soxhlet [14], and supercritical fluid extraction using CO_2 [15]. Solvents with different polarities have been used to compare the extraction efficiency of the solvents and the extraction methods [14]. In supercritical fluid extraction the effects of temperature and pressure have already been reported [15]. However, the current work would appear to be the first report on the effects of the extraction time, size of the ginseng particles, and water swelling time on the amount of extracted panaxynol and panaxydol. This is also the first study to compare three different extraction methods (shaking, soxhlet, and supercritical extraction) at various extraction temperatures.

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MATERIALS AND METHODS

Chemicals

Four-year-old white ginseng roots from Kumsan, Korea were used. The ether and petroleum ether were purchased from Sigma, and the other solvents were from Duksam Pharmaceutical Co., Ltd. Methanol was used as the extraction solvent since it has been reported to be the most effective solvent [14]. Silica gel 60 with a diameter of 0.063-0.2 mm (Merck) was used for the silica gel chromatography. Plus the thin layer chromatography (TLC) used a silica gel 60F254 plate backed by 0.2 mm thick aluminum sheet (Merck).

Preparation of Polyacetylene Standards

Polyacetylene was extracted from powdered ginseng (900 g) by an ultrasonic cleaner using methanol as the solvent. The material was extracted for 2 h in 2 liters of

methanol. This procedure was repeated three times, then the extract was vacuum concentrated. The concentrate was separated in a funnel after being dissolved in 400 mL of a mixture of distilled water and n-hexane:ethylacetate (1:1, v/v). The n-hexane:ethylacetate layer was dehydrated using magnesium sulfate anhydrous, concentrated in a vacuum, and then separated by silica gel column chromatography. The solvent from the column was collected and expanded on a TLC plate using n-hexane:ethylacetate (6:1, v/v). The samples were collected in sequence and concentrated in a vacuum and weighed. Panaxynol and panaxydol were identified by ^{13}C NMR FT-Wide Bore (400 MHz) AVANCE.

Preparation of Standard Solutions and GC Analysis

The analysis of the polyacetylene compounds followed the procedure of Nho [16]. Standard solutions of panaxynol and panaxydol were prepared at concentrations of 20, 50, 100, 200, 500, and 1000 ppm using ether as the solvent. The concentrations of panaxynol and panaxydol were determined by a gas chromatography (HP 5890 II) equipped with an FID detector and HP-5 column (0.2 mm i.d. × 25 m length, HP). The injector and detector temperatures were 200°C and 250°C, respectively. The oven temperature was increased from 100°C to 200°C at a rate of 10°C per minute, and then maintained at the final temperature (200°C) for 8 minutes. The sample injection volume was 4 μL.

Effect of Extraction Time

Five grams of ginseng powder (mesh 60) was placed in twelve 250-mL flasks each containing with 100 mL of methanol, and shaken at 120 rpm. The whole solvent (100 mL) was then taken from each flask after 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 20, and 24 hrs of shaking at 35°C. The samples were filtered using Whatman 2 filter paper and concentrated in a vacuum. Next, the filtrates were extracted in 50 mL of a 4:1 (v/v) petroleum ether-ether mixture after being dissolved in 25 mL of distilled water. The residue was then extracted again in 50 mL of a 4:1 (v/v) petroleum ether-ether mixture. The extracts from the first and second extractions were combined and vacuum concentrated. The concentrate was finally dissolved in 10 mL of ether, and 4 μL was taken for a GC analysis.

Effect of Extraction Temperature

For the shaking method, ten grams of ginseng powder (mesh 60) was placed in a 250-mL flask with 100 mL of methanol. The polyacetylene compounds were extracted in a shaker at 120 rpm at different temperatures. After 6 h of extraction at 25, 35, 45, and 55°C, the supernatant was decanted and 100 mL of fresh methanol was added for another 6 h of extraction. The decanted solutions from both extractions were combined for analysis. For the soxhlet method, ten grams of ginseng powder was placed in a 300-mL round-bottom flask with 100 mL of methanol and extracted at 65, 80, and 95°C for 12 h.

For both methods the extracted solutions were filtered and concentrated in a vacuum. Next, the filtrates were extracted in 50 mL of a 4:1 (v/v) petroleum ether-ether mixture after being dissolved in 25 mL of distilled water. The residue was extracted again in 50 mL of 4:1 (v/v) petroleum ether-ether mixture. The extracts from the first and second extractions were combined and vacuum concentrated. The concentrate was finally dissolved in 10 mL of ether, and 4 μL was taken for a GC analysis.

Supercritical fluid extraction was performed at 55, 65, and 75°C for 30 min. The amount of ginseng powder was 10 g and the pressure was 4,500 psi. The temperature was controlled by an air-driven oven (DS-99001, Dosung Science, Korea). CO_2 was circulated by an HPLC pump (MCPV-110, Burbank, USA) in a constant flow mode by supplying 2 mL/min of CO_2.

Effect of Particle Size

One hundred ml of methanol was added to 10 g of ginseng powder with a mesh size of 20, 40, and 60, respectively. Assuming a spherical shape and uniform size for the ginseng particles, the surface area per unit volume was calculated as follows.

$$\frac{A}{V} = \frac{4\pi r^2}{\frac{4}{3}\pi r^3} = \frac{3}{r} = \frac{6}{d} \quad (1)$$

where A is the surface area, V is the volume, r is the radius of the particle, and d is the diameter of the particle. From the Tyler Standard Screen Scale [17], the mesh sizes of 20, 40, and 60 corresponded to clear openings of 0.0833, 0.0375, and 0.0246 cm, respectively. From Equ. (1), the surface area per unit volume for the mesh sizes of 20, 40, and 60 was 72.03, 160.0, and 243.9 cm^{-1}, respectively.

For the shaking method, the extraction was performed for 6 h at 35°C and 120 rpm. Fresh methanol (100 mL) was then added to the supernatant and the extraction continued for another 6 hrs at 120 rpm. The decanted solutions from both extractions were combined for analysis. For the soxhlet method, 10 g of ginseng powder was extracted for 12 h at 80°C in a 300 mL round-bottom flask with 100 mL of methanol.

For both methods, the extracted solutions were filtered and then concentrated in a vacuum. Next, the filtrates were extracted in 50 mL of a 4:1 (v/v) petroleum ether-ether mixture after being dissolved in 25 mL of distilled water. The residue was extracted again in 50 mL of a 4:1 (v/v) petroleum ether-ether mixture. The extracts from the first and second extractions were combined and vacuum concentrated. The concentrate was finally dissolved in 10 mL of ether, and 4 μL was taken for a GC analysis.

Supercritical fluid extraction was performed on 10 g

of ginseng powder for 30 min at 65°C and 4,500 psi with a 2 mL/min CO_2 supply.

Effect of Water Swelling

Ten grams of ginseng powder (mesh 60) was swollen in water for 1, 6, and 12 h before extraction. Ten grams of ginseng powder was extracted by the soxhlet method using 100 mL of methanol in a 300 mL round-bottom flask for 12 h at 80°C.

The extracted solutions were filtered and concentrated in a vacuum. Next, the filtrates were extracted in 50 mL of a 4:1 (v/v) petroleum ether-ether mixture after being dissolved in 25 mL of distilled water. The residue was extracted again in 50 mL of a 4:1 (v/v) petroleum ether-ether mixture. The extracts from the first and second extractions were combined and vacuum concentrated. The concentrate was finally dissolved in 10 mL of ether, and 4 μL was taken for a GC analysis.

RESULTS AND DISCUSSION

Temporal Profile of Polyacetylene in Shaking Method

In the shaker experiments the amount of extracted panaxynol and panaxydol was determined every 2 to 4 hrs at 35°C (Fig. 1). After 12 h of extraction the panaxynol and panaxydol concentrations reached maximum values of 35.5 ppm and 64.3 ppm, respectively. The ratio of panaxydol to panaxynol steadily increased from 1.22 after 1 h to 1.81 after 12 h.

The profiles of the panaxynol and panaxydol concentrations can be approximated using the following equation.

$$C = at^b \tag{2}$$

where C is the concentration of panaxynol or panaxydol in ppm and t is time in hrs. By taking the logarithm on both sides of the Eq. (2), a linear relationship between $\log C$ and $\log t$ is obtained as follows.

$$\log C = \log a + \log t \tag{3}$$

Linear regressions on the data in Fig. 1 were made using Eq. (3), and values of a and b for panaxynol and panaxydol were determined (Table 1).

Effect of Temperature

In the shaking experiments, the amounts of extracted panaxynol and panaxydol increased in proportion to the temperature and reached 68.9 and 76.5 ppm, respectively, at 55°C. When compared to the amounts extracted at 25°C, the panaxynol and panaxydol concentrations at 55°C were 51% and 53% higher, respectively.

In the soxhlet experiments, the amount of panaxynol and panaxydol increased up to 57.7 and 79.6 ppm, respectively at 80°C. At 95°C the amount of panaxydol remained essentially the same (79.4 ppm), yet the panaxynol decreased to 52.0 ppm. This result was similar to a previous report where saponin extraction from red ginseng was best at 80°C [18].

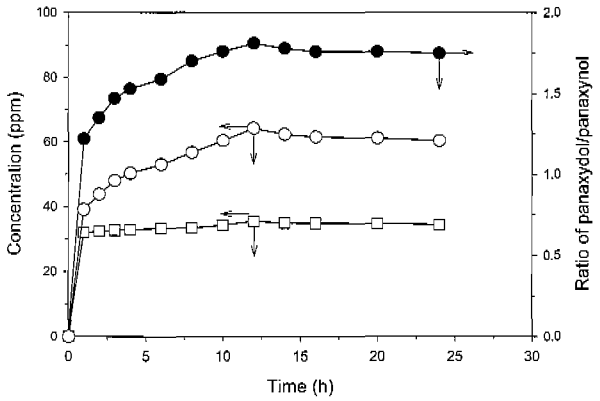

Fig. 1. Time dependence of extracted amount of panaxydol (○) and panaxynol (□), and their ratio (●) in shaking method.

Table 1. Constants a and b for panaxynol and panaxydol in Eq. (2)

Polyacetylene	a	b
Panaxynol	31.7146	0.0822
Panaxydol	40.2393	0.155

In the supercritical fluid extraction, the panaxynol and panaxydol contents were highest at 65°C (panaxynol, 43.2 ppm and panaxydol, 52.7 ppm). A Further increase in temperature to 75°C decreased both the panaxynol (41.7 ppm) and the panaxydol (43.6 ppm) contents.

When considering all three methods of extraction, the maximum amount of panaxynol and panaxydol was extracted using the shaking method at 55°C and the soxhlet method at 80°C, respectively. The ratios of panaxydol to panaxynol were 1.47, 1.17, and 1.12 for the soxhlet, shaking, and supercritical extraction methods, respectively. This suggests that soxhlet extraction would appear to be the best method for obtaining an extract with the strongest cytotoxicity. The reason for the lowest ratio of panaxydol to panaxynol with supercritical extraction was possibly due to an enhanced reactivity between the epoxy ring of the panaxydol and other materials in the matrix under the facilitated diffusion of supercritical CO_2 [19].

Effect of Particle Size

The extraction of panaxynol and panaxydol was enhanced with a smaller particle size (larger mesh number) with all three extraction methods used in the current study (Fig. 3). With the soxhlet method at 80°C,

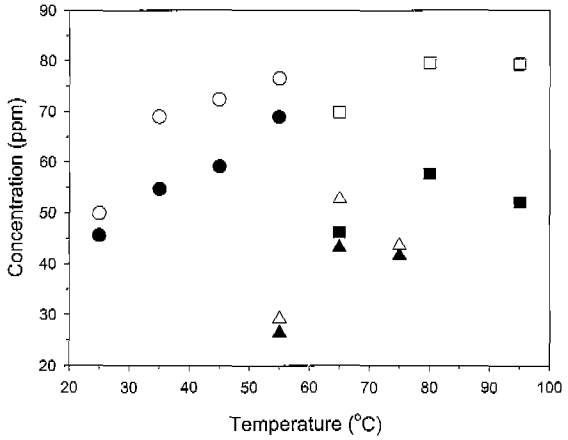

Fig. 2. Effect of temperature on extracted amount of panaxydol (open) and panaxynol (solid) using shaking (circle), supercritical (triangle), and soxhlet (square) methods.

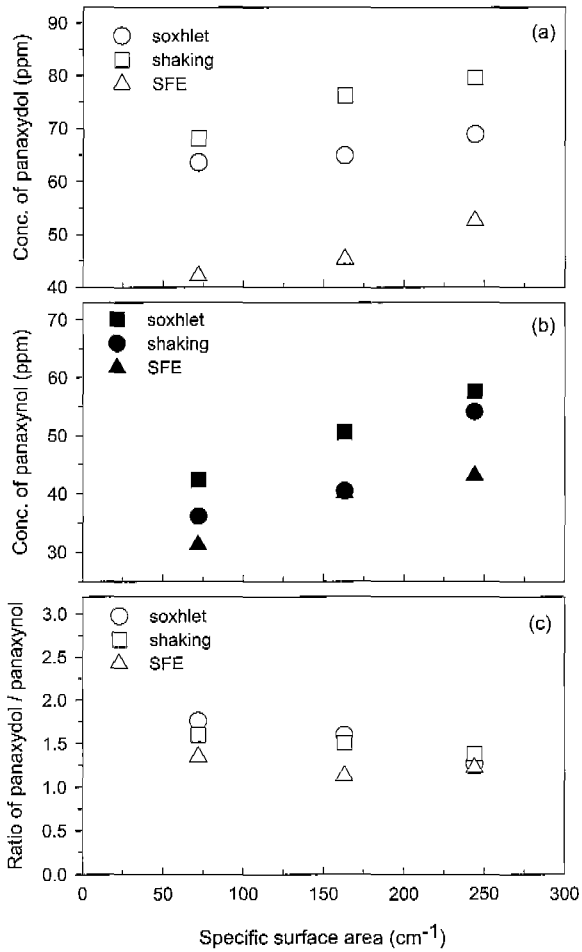

Fig. 3. Effect of particle size on extracted amount of panaxydol (a) and panaxynol (b), and their ratio (c) using shaking, supercritical, and soxhlet methods.

the amount of extracted panaxynol and panaxydol was

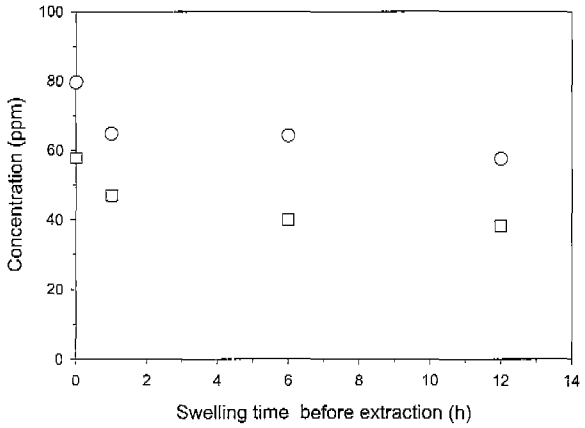

Fig. 4. Effect of water swelling on extracted amount of panaxydol (–○–) and panaxynol (–□–) with soxhlet method.

57.7 and 79.6 ppm, respectively, for a mesh size of 60, which was 54% and 17% higher, respectively, than the values for a mesh size of 20. This can be explained by the increase in the specific surface area with the smaller particle size. In the shaking experiments at 35°C, the amount of extracted panaxynol and panaxydol was 54.2 and 69.0 ppm, respectively, for a mesh size of 60. This was 50% and 85% higher as compared to the mesh size of 20. In the supercritical fluid extraction at 65°C, the amount of extracted panaxynol and panaxydol was 43.2 and 52.7 ppm, respectively, for a mesh size of 60. This was 38% and 25% higher as compared to the mesh size of 20. The ratio of panaxydol to panaxynol was higher with a larger particle size. Plus the ratio was lowest with supercritical extraction.

Effect of water swelling

A lower amount of panaxynol and panaxydol was extracted after a longer period of swelling in water before extraction (Fig. 4). The amount of extracted panaxynol and panaxydol was 38.1 and 57.5 ppm, respectively after 12 h of swelling, which was 51% and 39% lower, respectively, as compared to without swelling.

CONCLUSION

The extraction of panaxydol and panaxynol from Korean ginseng was found to be dependent on temperature, and the optimum temperatures for maximum extraction were 55°C for the shaking method, 80°C for the soxhlet method, and 65°C for the supercritical extraction method. When considering the ratio of pana-xydol to panaxynol, the soxhlet method was the best method to obtain an extract with the strongest cytotoxicity against cancer cells. Smaller particles were advantageous to increase the amount of extraction, yet the extract would most likely have a decreased cytotoxicity

because the ratio of panaxydol/panaxynol was decreased. A longer period of swelling in water before extraction decreased the amount of panaxydol and panaxynol extracted.

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