Free Radical Scavenging Capacity as Related to Antioxidant Activity and Ginsenoside Composition of Asian and North American Ginseng Extracts

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ABSTRACT: Different *Panax* species derived from Asia (*Panax ginseng* C.A. Meyer) and North America (*Panax quinquefolium* L.) were extracted by methanol and evaluated for relative ginsenoside composition and antioxidant activities. Ginseng root contained a greater proportion of total ginsenoside compared to ginseng hair analyzed by high-performance liquid chromatography. North American ginseng root was characterized with undetectable ginsenoside Rf and greater Rb_1/Rb_2 than Asian ginseng root. *Panax quinquefolium* exhibited a relatively higher ($P < 0.05$) affinity to scavenge free radical than *panax* ginseng using the 2,2-azobis (3-ethylbenzothine-6-thine-6-surfonic acid) radical model. In a bilayer lamella suspension oxidation model induced by peroxyl radicals, ginseng samples exhibited notable antioxidant activity. Specifically, however, the *P. quinquefolium* extracts delayed lipid peroxidation longer ($P < 0.05$) than the *P. ginseng* extracts. Ginseng extracts from both *Panax* species protected human low-density lipoprotein against cupric ion-mediated oxidation. Similar protection was observed against peroxyl radical-induced supercoiled DNA breakage. A pure ginsenoside standard (e.g., Rb_1) produced similar results. The antioxidant activities of different ginseng species and specific plant parts include free radical scavenging and may be related to ginsenoside Rb_1/Rb_2 content.

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KEY WORDS: Antioxidant, DNA, ginseng, *Panax* ginseng, *Panax* quinquefolium.

Panax ginseng C.A. Meyer (Asian ginseng, mainly produced in northeast China and Korea) has been used extensively in traditional Chinese medicine for more than 2,000 yr (1). The species *Panax quinquefolium* L., known as North American ginseng and currently cultivated in British Columbia, Canada, and Wisconsin (USA), represents an important additional source of ginseng. In addition to *P. ginseng* and *P. quinquefolium*, there are three minor species of ginseng, namely *P. notoginseng, P. japonicus,* and *P. vietnamensis*. Triterpenoid dammarane derivatives (ginsenosides) are believed to be the active components of ginseng (2).

Both Asian ginseng extract (3) and North American ginseng extract (4) have been reported to quench highly reactive hydroxyl radical *in vitro* and to delay oxygen consumption in a ferrous-induced linoleic acid emulsion oxidation. In an *ex vivo* test, ginsenosides Rb_1 and Rg_1 inhibited lipid peroxidation of rat liver and brain microsomes (5). Ginsenoside monomers Rb_1, Rb_2, Rb_3, Rc, Re, Rg_1, Rg_2, and Rh_1 decreased the superoxide signal monitored by electron spin resonance in an *in vitro* xanthine-xanthine oxidase system (6). Ginseng leaf contains ginsenoside comparable to root (7,8). Recently, a ginseng saponin mixture isolated from leaves and stems of Chinese-cultivated *P. quinquefolium* was reported to inhibit low-density lipoprotein (LDL) oxidation in the presence of cupric ion (9). The important finding was that peroxidation indices of LDL, such as thiobarbituric acid-reactive substances (TBARS) and electrophoretic mobility, were suppressed at a concentration range of 0.5–1.0 mg/mL *P. quinquefolium* saponin. Moreover, oxidized LDL incubated with *P. quinquefolium* saponin resulted in a decreased conversion of phosphatidylcholine to lysophosphatidylcholine compared with fully oxidized LDL. However, the effect of standardized ginseng extracts on other reactive oxygen radicals, for example, peroxyl radical, is unknown.

The aim of the present study was to investigate the antioxidant activities of *Panax* species using different *in vitro* assay model systems (4,10,11). The antioxidant activities of *Panax* extracts associated in both polar and nonpolar media, as well as the characterization of relative free radical scavenging activities, were evaluated relative to the chemical composition of each ginseng species.

MATERIALS AND METHODS

Cultivated North American ginseng (*P. quinquefolium* L., 10 individual samples each estimated to be *ca.* 2 yr old) root and root hairs, and Asian ginseng (*P. ginseng* C.A. Meyer, 10 individual samples with an estimated age of 4 yr) root and root hairs were obtained from a Chinese herb importer in Vancouver, Canada. Ginseng was chopped to a thickness of 1–2 mm and frozen until used. Human-LDL (h-LDL), pBR322 plasmid DNA (from *Escherichia coli* strain RRI), α-phosphatidylcholine, Chelex-100 chelating resin, barbital buffer (50 mM, pH 8.6), 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), diammonium salt (ABTS), potassium persulfate, 2-deoxyribose, 2-thiobarbituric acid, ginsenoside R_{b1}, and Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxyic

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acid) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). The 2,2′-azobis(2-amidinopropane)dihydrochloride (AAPH) was purchased from Wako Chemicals USA Inc. (Richmond, VA). Agarose (electrophoresis grade) was purchased from Bio-Rad Laboratories (Richmond, CA). All the other chemicals used were analytical grade. Phosphate buffers made from distilled deionized water were eluted through Chelex-100 chelating resin column to eliminate the presence of transition metal ions prior to use.

Extraction procedure. All ginseng samples were soaked with methanol overnight, followed by extraction with methanol for 9 h using a Soxhlet extractor. Methanolic ginseng extracts were further vacuum-concentrated (<40°C), and final concentration of ginseng extract was expressed as mg ginseng/mL. Samples were extracted in duplicate, and the procedural analysis was performed in triplicate.

Evaluation of ABTS radical cation-scavenging activities of ginseng extracts. The ABTS radical cation was generated by the reaction between ABTS and potassium persulfate (12). ABTS radical stock solution (50 μL) was mixed with ginseng extracts in 1000 μL ethanol solution. The inhibiting effect was calculated according to the absorbance of sample and blank control at 734 nm.

Evaluation of hydroxyl radical scavenging activities of ginseng extracts. Cleavage of deoxyribose by hydroxyl radical generated in an ascorbic acid-mediated Fenton reaction (13) was used to evaluate the efficiency of ginseng extract on the scavenging of hydroxyl radical (10). Briefly, deoxyribose (3.6 mM), $FeCl_3$ (100 μM), L-ascorbic acid (100 μM), EDTA (100 μM), and ginseng extracts were mixed and incubated at 37°C for 1 h. The reaction mixture was mixed with an equal volume of 10% trichloroacetic acid and 0.5% of 2-thiobarbituric acid containing 0.02% butylated hydroxytoluene, followed by incubation in boiling water for 15 min. Absorbance at 532 nm was taken. The scavenging effect of ginseng extracts on hydroxyl radical was calculated as:

$$\% \text{ scavenging} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100 \quad [1]$$

where, $\text{Abs}_{\text{sample}}$ and $\text{Abs}_{\text{control}}$ represented the absorbance at 532 nm with and without sample, respectively.

Antioxidant activities of ginseng extracts in peroxyl radical-initiated bilayer lamella suspension peroxidation. The method of Hu and Kitts (10) was used for measuring peroxyl radical-induced phospholipid-based bilayer lamella suspension peroxidation. Briefly, a bilayer lamella stock suspension (10 mg/mL) was made from soybean α-phosphatidylcholine in 10 mM phosphate-buffered saline (PBS) (pH 7.4, 0.9% of NaCl, wt/vol) by sonication in ice-water bath. Peroxidation of bilayer suspension was performed using 75 μg/mL phosphatidylcholine and absorbance at 234 nm was recorded for 2 h at 37°C after initiation by 0.5 mM AAPH. A molar extinction coefficient of 29,500 M^{-1} cm^{-1} at 234 nm was used to calculate conjugate diene hydroperoxide of phosphatidylcholine (14). The lag phase of oxidation was determined from an intersection point identified between two best fit linear regression lines for both the induction period and the propagation period for the plotted conjugated diene-time course data. Antioxidant activity of different ginseng samples was also characterized by the rate of propagation, which was defined as the change in concentration of conjugate diene/minute during the propagation phase. A control incubation in which phosphate buffer was added to the bilayer lamella suspension was run in parallel with suspension exposed to different ginseng samples. Positive antioxidant control was run with Trolox under the same condition.

Antioxidant activity evaluation in human LDL oxidation model. The h-LDL was dialyzed (6,000–8,000 Da cut-off) against 10 mM PBS (pH 7.4) at 4°C for 24 h with nitrogen diffusion (10). The h-LDL (0.5 mg protein/mL) was used as the substrate for forced peroxidation by 10 μmol/L of $CuCl_2$. Ginseng extracts were mixed with h-LDL and $CuCl_2$ in 10 mM PBS (pH 7.4) for 20 h at 37°C with shaking, under air in darkness. Oxidative modification of h-LDL was evaluated using agarose (0.6%, wt/vol) gel electrophoresis in 50 mM of barbital buffer (pH 8.6) employing a horizontal electrophoresis apparatus (EC Apparatus Corp., St. Petersburg, FL). Native and oxidized LDL were visualized with Sudan black B.

Effects of ginseng extracts on supercoiled DNA breakage induced by peroxyl radical. Our previous method was used to evaluate peroxyl radical-induced pBR322 plasmid DNA nicking (11). Briefly, 17 ng/mL DNA in 10 mM PBS (pH 7.4) was mixed with different amounts of ginseng extracts. AAPH (5 mM) was added to initiate reaction (total volume was 12 μL) at 37°C for 2 h. After 2 μL of loading dye (containing 0.25% bromophenol blue, 0.25% xylene cyanole, and 40% sucrose in water) was added, the reaction mixtures were loaded to an agarose gel (0.7%, wt/vol). Electrophoresis was conducted in Tris-acetic acid-EDTA buffer (40 mM Tris-acetate, 2 mM EDTA, pH 8.5) with horizontal electrophoresis apparatus (EC Apparatus Corp.) at constant voltage (3 V/cm) for 1.5 h. DNA strand was visualized by 0.5 μg/mL ethidium bromide. The image was analyzed by densitometer (GS-670; Bio-Rad Laboratories, Richmond, CA). The inhibitory effect of ginseng extract on scission of supercoiled DNA strand was calculated according to Equation 2:

$$\% \text{ retention} = \frac{\text{Amount}_{\text{sample}}}{\text{Amount}_{\text{native}}} \times 100 \quad [2]$$

where $\text{Amount}_{\text{native}}$ represented the amount of supercoiled DNA strand treated without peroxyl radical initiator, and $\text{Amount}_{\text{sample}}$ represented the amount of supercoiled DNA strand treated with peroxyl radical initiator and ginseng extract.

Analysis of reducing power, total phenolics, and ginsenoside content. Reducing power was evaluated according to the method of Yen *et al.* (15). The amount of ginseng equivalent to reducing agent, such as L-ascorbic acid, was expressed as μg of ascorbic acid/mg of ginseng. The total phenolic concentration was assessed according to the method of Shahidi and Nazck (16). Total phenolics were calculated and expressed as rutin equivalent (RE) (mg rutin/g of ginseng) or

caffeic acid equivalent (CAE) (mg caffeic acid/g of ginseng). Ginsenoside was analyzed with high-performance liquid chromatography (HPLC) (Hewlett-Packard, Palo Alto, CA) equipped with an ODS column (250 × 4.6 mm, 5 μ) and diode detector operated at 203 nm. The mobile phase used was acetonitrile and water at flow rate of 1.5 mL/min (7). Methyl fatty acid was prepared by BF_3/CH_3OH and analyzed using Shimadzu (GC-17A) gas chromatograph-flame-ionization detector (Kyoto, Japan) with an Omegawax 320 fused-silica capillary column (0.32 mm × 30 m, 0.25 μm; Supelco, Bellefonte, PA).

Statistics. Results were expressed as mean ± SD. An analysis of variance test was used to analyze the difference between each samples with significance level set as $P < 0.05$. Pearson coefficient of the relation between lag-phase duration and ginsenoside contents was also obtained (SPSS for Windows 10.0; SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

All samples tested exhibited marked reducing power which was attributed to the total phenolic contents ($P < 0.05$) (Table 1). A typical HPLC chromatogram of *P. ginseng* and *P. quinquefolium* is shown in Figure 1, indicating that the total ginsenoside content varied among the different tested samples (Table 1). Typical different characteristics for these two *Panax* species were the higher ginsenoside Rb_1/Rb_2 ratio in the *P. quinquefolium* root compared to *P. ginseng*, and the presence of ginsenoside Rf in *P. ginseng* (7,8). However, a similar ginsenoside profile was noted between *P. ginseng* hair and *P. quinquefolium* hair (Table 1).

Earlier reports addressed the fact that crude plant extracts and individual phytochemicals provide antioxidant activities in various *in vitro* model systems, among which, phenolics and flavonoids inhibit lipid peroxidation of liposome (17,18) and LDL oxidation (19). Similar plausible evidence shows that ginseng protects against lipid oxidation (4). Phenolics with a high reducing power are often good candidates for possessing antioxidant activity; however, prooxidant behavior is also possible if transition metals coexist in free form (15). In the present study, a human LDL oxidation induced by Cu^{2+} was used to evaluate the effectiveness of ginseng extracts to protect against oxidation using agarose electrophoresis. Incubation of h-LDL with Cu^{2+} resulted in an increase in h-LDL electrophoretic migration distance on the agarose gel (Fig. 2, lane 2), reflecting the increase in the negative charge of LDL-apolipoprotein during the metal-induced oxidation. Ginseng extracts at 30 mg/mL suppressed the change in negative charge of Cu^{2+}-induced h-LDL oxidation, as shown in Figure 2 (lanes 3–6). Combined with a previous report (9), these results show that methanolic extracts derived from different ginseng species protect LDL from oxidative deterioration induced by Cu^{2+} *in vitro*. A similar effect has not been confirmed *in vivo*, which may be attributed to the bioavailability issues associated with the antioxidant constituents present in ginseng. Low phenolic content and reducing power were also found among all ginseng samples tested, thus confirming that ginseng was relatively inactive as a prooxidant. Previous results from our laboratory have also shown that North American ginseng (*P. quinquefolium*) has a greater affinity to sequester free metal ions than it has activity in redox reactions (4).

The Fenton reaction generates hydroxyl radical, which results in the cleavage of deoxyribose. Ginseng extracts derived from the two *Panax* sources showed scavenging activity of hydroxyl radical generated by Fenton reactants ($P < 0.05$) (Table 2). The affinity of ginseng to scavenge free radicals confirms our previous finding with a specific North American ginseng extract (4). By using the same model system, Trolox, a water-soluble analog of tocopherol, was shown to inhibit 81.0 ± 1.6% of the deoxyribose cleavage at a concentration of 10 μg/mL. In addition to the notable scavenging activities of ginseng extracts on hydroxyl radicals, all four ginseng extracts exhibited significant ($P < 0.05$) ABTS radical scavenging activities within a distinctive concentration-dependent range (Fig. 3). Of these samples, both root and hair derived

TABLE 1
Comparison of Reducing Powers, Total Phenolic Amounts and Ginsenoside Contents of Roots and Root Hairs of Asian Ginseng (*Panax ginseng*) and North American Ginseng (*P. quinquefolium*) (mean – SD)

	P. ginseng hair	*P. quinquefolium* hair	*P. ginseng* root	*P. quinquefolium* root
Reducing power[a]	0.64 ± 0.02	0.91 ± 0.02	1.11 ± 0.03	1.00 ± 0.02
RE[b]	0.155 ± 0.00	0.16 ± 0.00	0.33 ± 0.01	0.46 ± 0.00
CAE[c]	0.06 ± 0.00	0.07 ± 0.00	0.14 ± 0.00	0.17 ± 0.02
Ginsenoside content[d]	11.3 ± 0.6	23.0 ± 1.4	13.3 ± 0.5	39.4 ± 2.2
Rg_1 (%)[e]	5.3 ± 0.3	5.0 ± 0.6	23.0 ± 1.0	6.5 ± 0.2
Re (%)[e]	14.9 ± 1.0	19.0 ± 1.9	6.1 ± 0.3	30.8 ± 3.0
Rf (%)[e]	1.9 ± 0.1	2.9 ± 0.1	4.2 ± 0.5	ND
Rb_1 (%)[e]	25.7 ± 1.2	25.3 ± 2.2	37.6 ± 3.0	47.8 ± 3.7
Rc (%)[e]	22.0 ± 1.5	22.4 ± 1.9	17.0 ± 1.4	7.3 ± 0.4
Rb_2 (%)[e]	17.6 ± 1.2	14.9 ± 1.2	7.3 ± 0.8	2.0 ± 0.1
Rd (%)[e]	12.7 ± 0.9	10.6 ± 0.9	4.9 ± 0.4	5.7 ± 0.2

[a]Reducing power expressed as μg of ascorbic acid/mg of ginseng; [b]RE, rutin equivalent (a measure of total phenolics content) expressed as mg of rutin/g of ginseng; [c]CAE, caffeic acid equivalent (a measure of total phenolics content) expressed as mg of caffeic acid/g of ginseng. [d]Ginsenoside content expressed as mg/g dry sample. [e]Relative percentage of respective individual ginsenosides in total ginsenosides; [f]ND = not detectable.

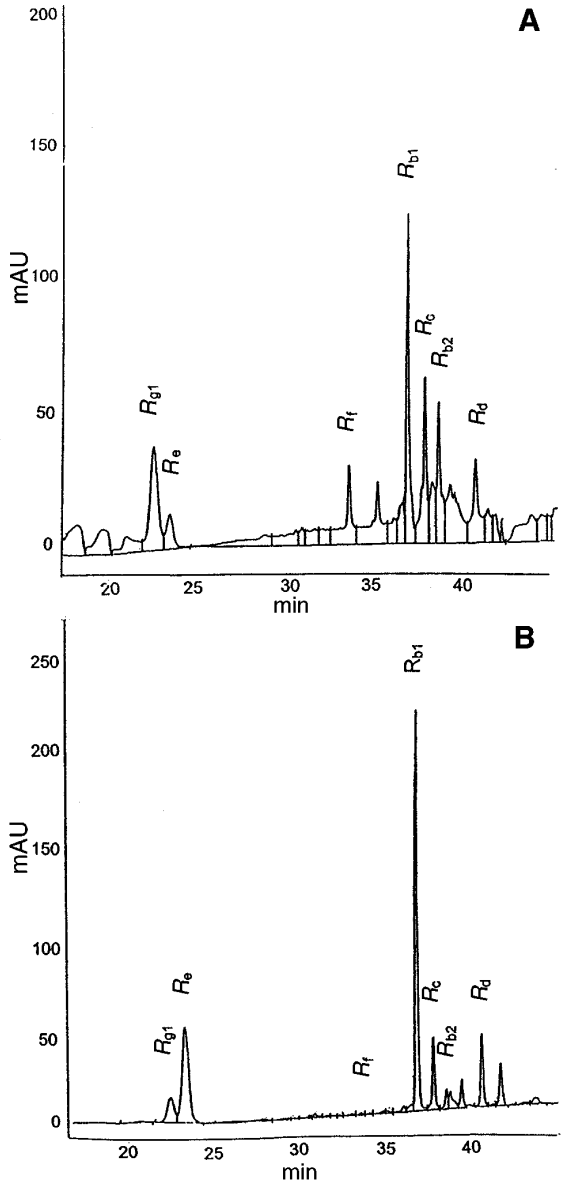

FIG. 1. High-performance liquid chromatograms of (A) *Panax ginseng* roots and (B) *P. quinquefolium* roots.

from *P. quinquefolium* showed a higher ($P < 0.05$) affinity for scavenging ABTS radical than the respective part of *P. ginseng*. The use of Trolox (10 μg/mL) as a positive control in this system resulted in 66.5 ± 0.7% inhibition of ABTS radical.

In addition to assessing both the hydroxyl radical and ABTS radical scavenging capacities of ginseng, similar experiments were performed with ginseng extracts on quenching peroxyl radical. Peroxyl radical was generated at a constant rate by the thermal decomposition of AAPH at 37°C (20), and the formation of conjugated diene in a phosphatidylcholine bilayer model was measured spectrometrically as the end point parameter of oxidation. Linoleic acid (59.8 ± 1.6%) was the predominant fatty acid in this soybean α-phosphatidylcholine, followed by palmitic acid (16.6 ± 0.8%),

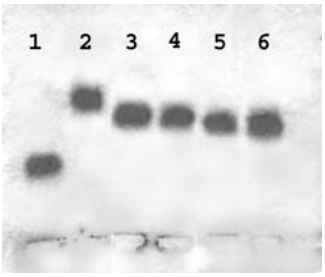

FIG. 2. Effect of ginseng extract at the protection of human-low-density lipoprotein (h-LDL) from oxidation induced by cupric ion at 37°C [(lane 1 = native h-LDL without oxidative treatment; lane 2 = h-LDL with oxidative treatment; lane 3 = h-LDL with *Panax ginseng* C.A. Meyer root extract (30 mg/mL), lane 4 = h-LDL with *P. ginseng* C.A. Meyer root extract (30 mg/mL), lane 5 = h-LDL with *P. quinquefolium* root extract (30 mg/mL), lane 6 = h-LDL with *P. quinquefolium* root extract (30 mg/mL)].

linolenic acid (6.4 ± 0.7%), stearic acid (4.4 ± 0.7%), and palmitoleic acid (0.1 ± 0.0%). The high percentage of polyunsaturated fatty acid made the phosphatidylcholine bilayer prone to oxidation. Linoleic acid oxidation induced by peroxyl radical will follow a kinetic process which includes oxygen uptake, formation of hydroperoxide, and the consumption of antioxidants (21). Generating peroxyl radical resulted in the peroxidation of the phosphatidylcholine bilayer, which was suppressed by the antioxidant Trolox as shown by the extended lag phase duration (Fig. 3).

A notable difference in the antioxidant activity between different ginseng species was observed when extracts were evaluated using a peroxyl radical-induced bilayer lamella suspension peroxidation model (Table 3, Fig. 4). A relatively higher antioxidant activity was found to be characteristic of the *P. quinquefolium* root ($P < 0.05$) when relative lag phase durations were compared. *Panax quinquefolium* extracts had a longer characteristic lag phase compared to *P. ginseng* counterparts at both concentrations tested ($P < 0.05$). It is also important to note that the formation of conjugate diene hydroperoxide in the presence of the *P. quinquefolium* extracts was much lower ($P < 0.01$) than that of the control. The propagation reaction rate was notably lower ($P < 0.01$) for the *P. quinquefolium* compared to the control and other ginseng extracts. This effect was related to the total ginsenoside ($P < 0.01$) and total phenolic content in these extracts ($P < 0.05$). Despite the marked difference in the lag-phase duration and

TABLE 2
Effect of Various Ginseng Extracts in the Inhibition of Deoxyribose Degradation Induced by Hydroxyl Radical[a]

Inhibiting percentage (%)	1 mg/mL	5 mg/mL
Panax ginseng C.A. Meyer hair	44.4 ± 0.3[a]	60.4 ± 1.3[a]
Panax quinquefolium L. hair	42.2 ± 0.7[a]	55.4 ± 2.2[b]
P. ginseng C.A. Meyer root	52.2 ± 0.2[b]	64.7 ± 2.1[a]
P. quinquefolium L. root	53.7 ± 2.5[b]	52.1 ± 1.5[b]

[a]Value represents mean ± SD ($n = 3$); data with different roman letter superscripts within the same column are significantly different ($P < 0.05$, analysis of variance).

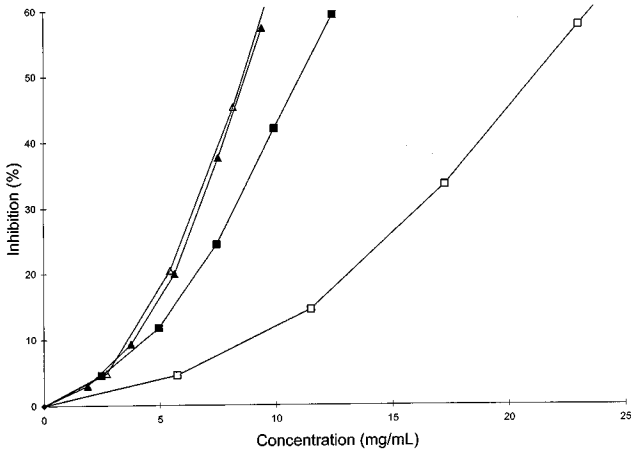

FIG. 3. The 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging effects of North American and Asian ginseng extracts, □ = *Panax ginseng* C.A. Meyer hair, ■ = *P. ginseng* C.A. Meyer root, △ = *P. quinquefolium* hair, ▲ = *P. quinquefolium* root.

propagation rate, a ginseng concentration-dependent lag-phase prolongation was common among all ginseng samples in preventing peroxyl radical-induced liposomal peroxidation (Fig. 2A and 2B). However, the antioxidant properties of extracts were less ($P < 0.01$) than that obtained from positive Trolox control. The presence of *P. ginseng* and *P. quinquefolium* extracts in these reactions resulted in prolongation of the lag phase, thus reflecting the nature of the chain-breaking antioxidant activity (22) of ginseng extracts. It is particularly noteworthy that the efficiency with which the specific ginseng extracts elicited this response was characteristically different and corresponded to both ginsenoside and total phenolic composition. Some reports which have emphasized the antioxidant activity of monoginsenosides have produced evidence that 40 µg/mL ginsenoside will prevent endothelial cell free radical injury *in vitro* (23). Ginsenoside Rb_1, Rb_2, Rb_3, and Rc (30 µg/mL) were effective at protecting against xanthine-xanthine oxidase-generated superoxide radical-induced injury to cultured cardiac myocytes (24). On the other hand, Suh *et al.* (25) also reported that maltol, derived from Korean red ginseng (*P. pseudoginsen*), was the principal constituent of ginseng that protected DNA from hydroxyl radical-induced degradation.

TABLE 3
Lag-Phase Duration (min) Produced by Ginseng Extracts on Peroxyl Radical-Induced Peroxidation of Phosphatidylcholine Bilayer[a]

Lag-phase duration		125 µg/mL	250 µg/mL
Control	27.5 ± 0.6		
Panax ginseng C.A. Meyer hair		36.7 ± 0.8*	56.6 ± 1.3***
P. quinquefolium L. hair		49.3 ± 0.6*	80.5 ± 1.0***
P. ginseng C.A. Meyer root		42.1 ± 0.4*	70.5 ± 2.8***
P. quinquefolium L. root		56.4 ± 1.4**	83.1 ± 0.3***

[a]*, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$ vs. control.

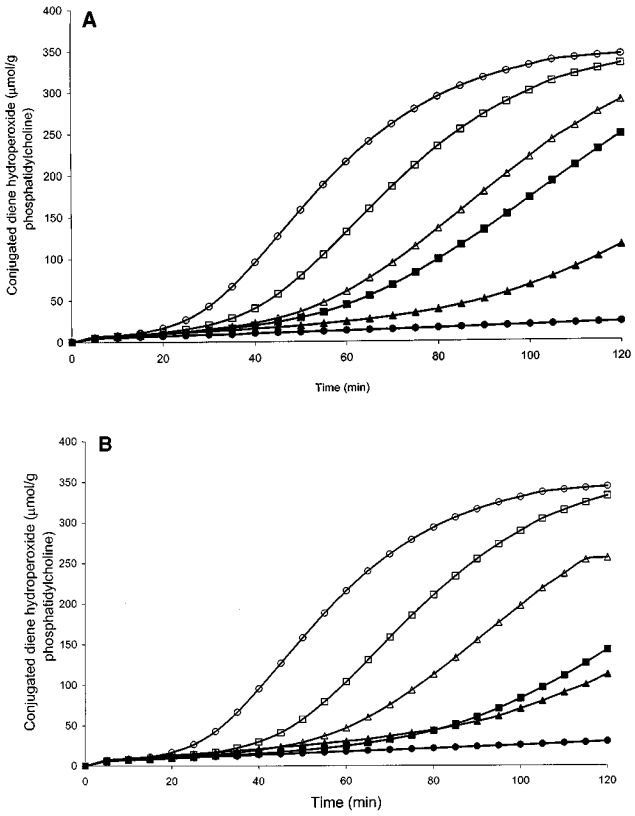

FIG. 4. (A) Effect of North American ginseng and Asian ginseng hair extracts on the protection of bilayer lamella suspension peroxidation induced by 2,2′-azobis(2-amidino propane) dihydrochloride (AAPH) at 37°C. ○ = control, □ = 125 µg/mL *Panax ginseng* C.A. Meyer hair extract, △ =125 µg/mL *P. quinquefolium* hair extract, ■ = 250 µg/mL *P. ginseng* C.A. Meyer hair extract, ▲ = 250 µg/mL *P. quinquefolium* hair extract, ● = 2.5 µg/mL Trolox. B: Effect of North American ginseng and Asian ginseng root extracts on the protection of bilayer lamella suspension peroxidation induced by AAPH at 37°C. ○ = control, □ = 125 µg/mL *P. ginseng* C.A. Meyer root extract, △ = 125 µg/mL *P. quinquefolium* root extract, ■ = 250 µg/mL *P. ginseng* C.A. Meyer root extract, ▲ = 250 µg/mL *P. quinquefolium* root extract, ● = 2.5 µg/mL Trolox.

Peroxyl radicals generated under thermal decomposition of AAPH resulted in breakage of supercoiled DNA (Fig. 5, lane 2). A concentration-dependent DNA protection pattern for the four ginseng samples tested occurred over a range of 1–10 mg/mL (Table 3). The protective effect of ginsenoside Rb_1 was also tested in this model. DNA scission in the presence of peroxyl radical was inhibited at a minimum concentration of 83 µg/mL. Trolox (2 µg/mL) resulted in a 92.6 ± 2.6% protection against peroxyl radical-induced supercoiled DNA nicking using the same experimental conditions.

The affinity of reactive oxygen species to initiate DNA damage has been well characterized *in vitro*, although further studies have only recently shown the same effect with peroxyl radical (10). In our previous report, a North American ginseng standardized extract was shown to protect pBR322 DNA from hydroxyl radical oxidation catalyzed by the presence of transitional metal ion (4). Our current result was ob-

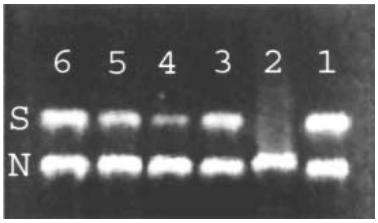

FIG. 5. A representative electrophoresis of supercoiled DNA under peroxyl radical-induced oxidation and the protective effect of ginseng extracts. Lane 1 = DNA incubated without AAPH, lane 2 = DNA incubated with AAPH, lane 3 = DNA incubated with AAPH and 10 mg/mL of *P. ginseng* C.A. Meyer root extract, lane 4 = DNA incubated with AAPH and 10 mg/mL of *P. ginseng* C.A. Meyer root extract, lane 5 = DNA incubated with AAPH and 10 mg/mL of *P. quinquefolium* root extract, lane 6 = DNA incubated with AAPH and 10 mg/mL of *P. quinquefolium* root extract. S = supercoiled DNA strand, N = nicking DNA strand. See Figure 4 for other abbreviation.

tained by modifying this assay to include peroxyl radical. Using this different approach to initiate scission of DNA base pairs, we confirm our previous finding that ginseng extracts are effective at preventing supercoiled DNA from oxidation damage. *Panax quinquefolium* hair, which contained a relatively higher content of ginsenoside, exhibited a relatively higher protection for DNA scission induced by peroxyl radical, compared to *P. ginseng* hair ($P < 0.05$). Similar levels of protection were achieved with root extracts obtained from both *P. ginseng* and *P. quinquefolium* (Table 4). Root extract from both *P. ginseng* and *P. quinquefolium* exhibited a higher affinity to prevent supercoiled DNA strand breakage than respective hair extracts. Ginsenoside Rb_1 was also found to have notable protection on DNA against breakage at 83 μg/mL under the same experimental conditions. Although the DNA test model provides a simple and fast screening method for evaluating natural antioxidants, it is still unclear at this stage if the ginsenosides individually, or when present as a mixture, elicit similar protection *in vivo*. Also, it is not sure at this time whether other individual ginsenoside except the tested Rb_1 have similar *in vitro* protection against oxidative damage.

Other studies have added to the controversy of whether the ginsenoside(s) act alone, or in concert with other active constituents of ginseng in eliciting antioxidant activity. For example, Huong *et al.* (26) reported that *P. vietnamensis* saponin inhibited the formation of TBARS in mouse tissue homogenates *in vitro,* but individual ginsenosides Rg_1 and Rb_1 failed to do so in a hydroxyl radical model system. Our current result shows that the free radical scavenging effect of ginsenoside Rb_1 in a peroxyl radical-induced DNA breakage model produced antioxidant activity similar to that reported in different model systems (6,24). This finding is important from the standpoint that standardization of ginseng extracts, in accordance with a general agreement on the specific test method to be used for evaluating ginseng antioxidant activity, is required in order to evaluate interlaboratory research findings designed to assess the biological activities of ginseng (2).

The intent of this study was not to make a direct comparison of the superiority of different ginseng species in terms of antioxidant activity. This would be an unfair comparison simply by the fact that the two sources of *Panax* species likely varied in physiological age. Rather, we have demonstrated for the first time that the different antioxidant activities derived from a battery of test methods specific to ginseng were in fact different between Asian and North American ginseng. These differences may also be related, at least in part, to relative differences in specific ginsenosides. For example, the contents of both ginsenosides Re and Rb_1 were positively related to the duration of the lag phase in a peroxyl radical induced-bilayer lamella peroxidation ($r = 0.80$ and 0.56, respectively). Although considerable effort was given in this study to examine multiple samples of the two different *Panax* species for characteristic antioxidant activity and ginsenoside content, the differences in ginseng growing environment, harvesting conditions, and processing technology are additional factors that can also influence both absolute total and individual ginsenoside levels (8,27). Although we do acknowledge the fact that these variables obviously exceed the number of samples tested in this study, our work is the first attempt simply to relate the variable antioxidant activity noted herein between Asian and North American ginseng with major ginsenoside compositional differences.

TABLE 4
Protection of Ginseng Extracts Against Peroxyl Radical-Induced Scission of Supercoiled DNA

Inhibition (%)[a]	1 mg/mL	5 mg/mL	10 mg/mL
Panax ginseng C.A. Meyer hair	1.3 ± 0^a	8.5 ± 0.5^a	51.7 ± 5.7^a
P. quinquefolium L. hair	2.6 ± 1.8^a	17.0 ± 5.2^b	71.4 ± 1.8^b
P. ginseng C.A. Meyer root	7.6 ± 3.4^b	44.0 ± 9.8^c	78.5 ± 1.4^b
P. quinquefolium L. root	7.1 ± 1.9^b	45.9 ± 7.4^c	90.1 ± 0.6^c

[a]Calculation of inhibition (%) is described in the Materials and Methods section (mean ± SD, $n = 3$). Data within same column with different roman superscripts are significantly different ($P < 0.05$, analysis of variance).

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