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Differential antioxidant and quinone reductase inducing activity of American, Asian, and Siberian ginseng ☆

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ABSTRACT

The antioxidant and quinone reductase (QR) inducing activities of American, Asian, and Siberian ginseng have been reported using various plant materials, solvents, and assays. To directly establish their comparative bioactivity, the effects of extracts obtained from acidified methanol (MeOH), a gastrointestinal mimic (GI), and hexane (Hex) on free radical scavenging and QR induction were tested. Siberian ginseng-MeOH had the highest total phenolic content at 52.6 μmol gallic acid equivalents/g. GI liberated ⩾50% more phenolics than MeOH and Hex from American and Asian ginseng. Siberian ginseng-Hex was most effective at inducing QR activity in Hepa1c1c7 cells. GI and MeOH extracts of American and Asian ginseng exhibited comparable HOCl scavenging activity, but were ⩾4.6-fold more potent than Siberian ginseng. Siberian ginseng was the most effective scavenger of $ONOO^-$. Siberian-MeOH had the highest ferric reducing antioxidant power (FRAP), and American ginseng-GI had the highest oxygen radical absorbance capacity (ORAC). Thus, the components in ginseng have antioxidant and QR-inducing activity which are dependent upon the dose, method of extraction, radical species, and plant species.

1. Introduction

The roots of Asian ginseng (*Panax ginseng* C. A. Meyer) have long been one of the most common components of general tonics employed in traditional herbal medicines throughout Asia due to their putative benefits in general health promotion, vitality, stamina, restoration of homeostasis, chemoprevention, wound healing, longevity, and other indications (Kitts & Hu, 2000). A recent nationwide survey of US adults found the most common form of complimentary and alternative medicine therapy was the use of natural products and, among users, 14% reported using ginseng in the past 30 days (Barnes, Bloom, & Nahin, 2008). However, the survey did not specify which ginseng species were consumed. In the US, "ginseng" can refer to either Asian ginseng, American ginseng (*Panax quinquefolius* L.), or Siberian ginseng [*Eleutherococcus senticosus* (Rupr. and Maxim.) Maxim; botanical syn. *Acanthopanax senticosus* (Rupr. & Maxim.) Harms] (McGuffin, Kartesz, Leung, & Tucker, 2000). Although the preferred common name of *E. sentiosus* is now Eleuthero, it is often referred to as Siberian ginseng in the published literature. All three species of ginseng are members of the Araliaceae family, and used primarily as adaptogens (McGuffin et al., 2000) in alternative and complementary medicines in Western countries, particularly among older adults (Gardiner et al., 2007; Yeh, Davis, & Phillips, 2006). A diverse range of bioactivity and clinical therapeutics have been reported for ginseng, including the reduction of blood lipids, immuno-stimulation, anti-inflammation, vasodilation, anti-stress, chemoprevention, and antioxidation (Buettner, Yeh, Phillips, Mittleman, & Kaptchuk, 2006; Hasegawa et al., 2002; Kaneko & Nakanishi, 2004; Keum et al., 2000). The active constituents in ginseng include, but are not limited to, ginsenosides, polysaccharides, peptides, polyacetylenes, vitamins, phenols, and enzymes (Xiang, Shang, Gao, & Zhang, 2008).

While each ginseng species exhibits several different mechanisms of action potentially related to health promotion and disease prevention, there is a need to better characterise their relative antioxidant capacity as reactive oxygen, nitrogen, and halide reactive species ("free radicals") contribute to the pathogenesis of age-related chronic diseases, including atherosclerosis, cancer, and neurodegenerative conditions. Indeed, a growing body of evidence suggests the medicinal efficacy of the different ginseng species is mediated via their antioxidant actions (Davydov & Krikorian, 2000; Kim, Guo, & Packer, 2002), e.g., inhibiting lipid peroxidation and oxidative injury to DNA (Lee, Lee, & Kim, 1998; Lee et al., 2008; Naval, Gómez-Serranillos, Carretero, & Villar, 2007). Several reports indicate the different ginseng species are effective in scavenging free radicals, inducing antioxidant enzyme activity, and stimulating glutathione synthesis (Kang, Kim, Pyo, & Yokozawa, 2006; Keum et al., 2000; Liu et al., 2003; Naval et al., 2007).

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Both American and Asian ginseng contain a group of saponins generally referred to as ginsenosides. American ginseng has been used as a tonic for indications similar to those of Asian ginseng, but the two species differ in their respective quantities of the specific ginsenosides (Yun, Lee, Kwon, & Choi, 1996). Siberian ginseng has been used widely as a tonic or adaptogen to increase stamina, but has not been found to contain ginsenosides (Hartz et al., 2004).

Despite the overlapping therapeutic indications of these ginseng species, the marked variations in their phytochemical profiles (Attele, Wu, & Yuan, 1999) suggest the value of a direct comparison between them using different methods of extraction and tests of bioactivity. While such an effort may help elucidate differences in their application and efficacy, no reports of this type have been published. Thus, the objective of this study was to characterise *in vitro* the bioactivity of Asian, American, and Siberian ginseng species similarly extracted with three solvent systems: [1] acidified methanol (MeOH), [2] a gastrointestinal mimic (GI), and [3] hexane (Hex). For each extract, three related measures of bioactivity were tested: [1] the capacity to quench free radical reactions generated by hypochlorite (HOCl), peroxynitrite ($ONOO^-$), superoxide anions ($O_2^{\bullet-}$), and 2,2-diphenyl-1-picrylhydrazyl (DPPH); [2] the potential to scavenge peroxyl radicals ($ROO^\bullet$) generated in the oxygen radical absorbance capacity (ORAC) assay of electron donating potential, and catalyse the reduction of Fe^{3+} to Fe^{2+} in the ferric reducing antioxidant power (FRAP) assay; and [3] the ability to induce quinone reductase in cultured mouse hepatocytes. Our goal was to help generate new hypotheses regarding the mechanisms of action of the different ginseng species, and inform the design of *in vivo* studies.

2. Experimental

2.1. Chemicals

Mouse hepatoma 1c1c7 (Hepa1c1c7) cells (CRL-2026™) and Dubelco's Modified Eagle medium (DMEM) were obtained from American Type Culture Collection (ATCC) Manassas, VA; foetal bovine serum (FBS) from Hyclone (Logan, UT); $ONOO^-$ in 0.3 M NaOH from Cayman (Ann Arbor, MI); 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) from Wako Chemicals (Richmond, VA); NaOH, methanol, and HCl from Fisher Scientific (Pittsburgh, PA). All other chemicals were purchased from Sigma Chemical (St. Louis, MO).

2.2. Ginseng samples

Certified Asian, American, and Siberian ginseng root reference materials in powdered form were obtained from Chromadex (Irvine, CA). Based on results of macroscopic, microscopic and thin layer chromatography analyses, all ginseng products were authenticated by Chromadex as conforming to identity standards for the correct species and plant part. After receipt, all ginseng species were stored in the dark at −20 °C. The colour of the Asian, American, and Sabrina ginseng powders were tan, cream, and beige, respectively. The Chromadex Biomass Reference Material lot numbers for Asian, American, and Siberian ginseng were 30303-055, 30676-120, and 30688-139, respectively.

2.3. Extraction of ginseng constituents

Extraction by MeOH and Hex was achieved using a Dionex Accelerated Solvent Extraction System 200 (Sunnyvale, CA), according to the methods of Chen and Blumberg (Chen & Blumberg, 2008). Each ginseng powder was sequentially extracted with 90%, 60%, and 30% aqueous MeOH (acidified with 5% acetic acid) for 3 cycles of 15 min each, respectively, at 1500 psi and 100 °C. Aliquots of combined extracts were dried under purified N_2 gas. Hex extraction was achieved with 9 cycles of 15 min each. Aliquots of the dried residues were stored at −20 °C.

The GI extraction, which may extract more water-soluble compounds than MeOH or Hex, as well as simulate the impact of digestive enzymes, was performed as described by Chen and Blumberg (2008). Briefly, each ginseng powder was subjected to a simulated gastric and intestinal digestion using pepsin and a pancreatin-bile solution, respectively. An aliquot of the GI extract was mixed with an equal volume of methanol to remove protein (which interferes with radical scavenging assays), spun at 10,000*g* for 10 min, dried under N_2 gas, and stored at −20 °C. Because butylated hydroxytoluene (BHT) was added to prevent any potential degradation of ginseng constituents during the GI extraction, a GI blank including BHT but absent ginseng powder was generated and used as a blank control for all assays described below.

Just prior to use in the assays, the dried MeOH extract was reconstituted in 60% methanol containing 5% acetic acid, while the dried GI extract was reconstituted in water. The dried Hex extract was dissolved in acetone due to its capacity for complete solubilisation. The organic solvents used in each reconstitution did not interfere with the determination of total phenols (data not shown).

2.4. Total phenols

The total phenol content of each ginseng extract served as the basis for determining their antioxidant activity and QR induction. Phenolic compounds, including both simple phenols and polyphenols, appear to contribute substantially to the bioactivity of botanical medicines and plant foods and are frequently employed as a reference standard in their evaluation, particularly with regard to antioxidant capacity (Dávalos, Gomez-Cordoves, & Bartolome, 2003; Singleton, Orthofer, & Lamuela-Ravent, 1999; Tang, Whiteman, Peng, Jenner, Yong, & Halliwell, 2004). The total phenol content was determined with the Folin–Ciocalteu reagent, according to the method of Singleton et al. (1999). All results are expressed as μmol/L gallic acid equivalents (GAE) as gallic acid has been commonly employed as a standard in the Folin–Ciocalteu assay in the literature. The limit of quantification for the determination of total phenols was 47.0 μmol/L (80.6 μg/mL).

GAE of MeOH, Hex, and GI extracts at 0.1, 1, 10, and 100 μmol/L were selected for all antioxidant assays to reflect the range of concentrations potentially present in cells, plasma, and the gastrointestinal tract after ingestion. Further, blanks containing corresponding amounts of organic solvents present in the ginseng extracts were included to adjust for their potential contribution to the assay. Due to its toxicity to cells, the 100 μmol/L GAE dose was not employed in the assay for QR induction; no impact on Hepa1c1c7 cell viability was observed with 2% methanol, 1% acetone or 5% GI blanks.

2.5. Induction of quinone reductase activity

The modulation of QR activity in Hepa1c1c7 cells *in vitro* has been widely employed to examine the potential chemopreventive activity of phytochemicals. Hepa1c1c7 cells were cultured until confluent in Minimum Essential Medium Eagle α modified supplemented with 10% heat inactivated, charcoal treated FBS, 1% penicillin/streptomycin, and 1% L-glutamine in a Napco incubator with 5% CO_2 at 37 °C. After confluence, cells were plated at a density of 2×10^4 cells/well and allowed to settle for 24 h. After the medium was aspirated, cells were treated with the ginseng extract at 0.1–10 μmol/L GAE in medium for 48 h. QR activity was measured by an NADPH-generating system, coupling the oxidation of menadi-

one to the reduction of the dye 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide according to Chen and Blumberg (2008). The protein content of cells in each well was determined by a bicinchoninic acid (BCA) protein kit (Pierce, Rockford, IL). The resulting blue-brown colour was measured at 570 nm using a FLUOstar Optima plate reader (BMG LABTECH GmbH, Offenburg, Germany). After adjusting for protein content, the result of QR induction was expressed as a percentage of the negative control. β-Napthoflavone (BNF) at a concentration of 1 μmol/L was employed as a positive control and increased QR activity by 2.4 ± 0.5-fold of the negative control (absent ginseng extracts).

2.6. Radical scavenging activity

The antioxidant activity of each ginseng extract against physiologically relevant radicals, $ONOO^-$, $O_2^{\cdot-}$, and HOCl was determined according to Chen and Blumberg (2008). Briefly, $ONOO^-$ scavenging activity was measured by monitoring the increase in fluorescence intensity from the oxidation of dihydrorhodamine 123 at 485 nm excitation and 530 nm emission using a FLUOstar Optima plate reader. Intra- and inter-day coefficients of variation (CV) were 4.7% and 3.6%, respectively. Scavenging activity against HOCl was assessed via the oxidation of ferrocyanide $[Fe(II)CN)_6]$ by HOCl at 420 nm using a Shimadzu UV1601 spectrophotometer (Kyoto, Japan). Intra- and inter-day CV were 0.9% and 2.9%, respectively. Scavenging activity against $O_2^{\cdot-}$ was measured in a xanthine/xanthine-oxidase system with spectrophotometric determination of the reduction product of nitroblue tetrazolium at 560 nm using the spectrophotometer. Intra- and inter-day CV were 1.93% and 7.70%, respectively. Inhibition of xanthine oxidase activity by each extract was monitored by the spectrophotometric determination of uric acid production. DPPH scavenging activity was performed according to Brand-Williams, Cuvelier, and Berset (1995). Briefly, 900 μL of 100 μmol/L DPPH in ethanol were mixed with 100 μL of selected concentrations of each ginseng extract, and the absorbance at 520 nm was measured after 30 min of incubation at room temperature in the dark. Intra- and inter-day CV were 1.4% and 7.6%, respectively.

Results of radical scavenging activity are expressed as a percentage of the appropriate negative control (with no ginseng extract), and the IC_{50} (concentration of ginseng extract required to scavenge radicals by 50%) in μmol/L GAE was calculated using a spline function.

2.7. Total antioxidant activity

The "total antioxidant activity" of each ginseng was assessed with the ORAC and FRAP assays. The ORAC assay was conducted according to Ou, Hampsch-Woodill, and Prior (2001). Briefly, the ORAC assay employs the area under the curve of the magnitude and time to the oxidation of fluorescein due to $ROO^{\cdot}$ radicals generated by the addition of AAPH in the presence of added antioxidants. The assay was carried out on a FLUOstar OPTIMA plate reader (BMG LABTECH GmbH, Offenburg, Germany) utilising fluorescence filters with 485 nm excitation and 520 nm emission. ORAC values of the ginseng extracts were calculated based on standard curves established using Trolox at 5–50 μmol/L. Intra- and inter-day CV were 3.0% and 7.3%, respectively.

The FRAP assay measures the capability of antioxidants to act as reductants in a redox-linked colorimetric reaction of the reduction of Fe^{3+}-2,4,6-tri-pyridyl-S-triazine to a blue-coloured Fe^{2+} complex at low pH, which is measured spectrophotometrically at 593 nm (Chen & Blumberg, 2008). The ginseng extracts were incubated at room temperature with the FRAP reagent, and the absorbance was recorded after 1 h. FRAP values were calculated based on standard curves established using Trolox at 31.25–500 μmol/L. Intra- and inter-day CV were 0.7% and 4.2%, respectively.

2.8. Statistical analyses

All assays were performed in triplicate. Results of the total phenol, HOCl, $ONOO^-$, FRAP, ORAC assays are presented as liquid extracts (μmol/L GAE) and ginseng dry weight (g) in a given volume of extraction solvent. Since the antioxidant assays performed were based on their total phenol content in liquid extracts, results expressed as ginseng dry weight represent the quotient of antioxidant activity (μmol/L GAE) by total phenol content (μmol/g). All results are reported as means ± SE. When statistical significance was obtained using a 2-way ANOVA with ginseng type and extraction solvent as independent variables, the Tukey–Kramer honestly significant difference (HSD) test was performed on the results of total phenol, $ONOO^-$, HOCl, FRAP, and ORAC assays. The statistical significance of ginseng type, extraction, and concentration on QR activity were evaluated using a multi-factor ANOVA test. Student's *t*-test was employed to assess the significance of QR induction by the ginseng extracts relative to the negative control. $P < 0.05$ was considered significant. The JMP IN 4 statistical software package (SAS Institute Inc., Cary, NC) was used to perform all statistical analyses.

3. Results and discussion

3.1. The effect of extractions on total phenols

The total phenol content of plant foods is often employed to approximate their concentration of antioxidant constituents (Tang et al., 2004; Wu et al., 2004). We found the levels of total phenols in the powdered ginseng products were comparable to those reported for apple, berries, onion, and ginger, but ~10-fold less than ground cinnamon and clove (Wu et al., 2004). The total phenol content of each ginseng was dependent on its species and the extraction method ($P < 0.0001$) (Fig. 1A and B). As simple phenols and polyphenols are typically hydrophilic in nature, it was not surprising to find that Hex extracts had >4-fold lower in total phenol content than either the MeOH or GI extracts. Though hexane may not be as efficient as methanol in extracting phenols and polyphenols, lignans (gomisins), trilinolein, polyacetylenes, and phenolic acids have all been reported in the hexane extracts of ginseng (Hirakura, Morita, Nakajima, Ikeya, & Mitsuhashi, 1992; Huh, Lee, & Han, 1990). Though organic solvents may be more efficient at extracting bioactive compounds from plant material, the GI extraction method may better reflect the *in vivo* accessibility of the constituents of the different ginseng species when consumed as a dried plant. Thus, it is interesting that the efficiency of the GI method was greater than MeOH in extracting phenolic constituents from American and Asian ginseng, while MeOH was more efficient for Siberian ginseng. This discrepancy may be attributed to variations in the solubility of the different phenols present in each ginseng species.

3.2. The effect of ginseng species on total phenols

The total phenol content of the American ginseng powder tested after MeOH extraction was comparable to that reported by Kim et al. (2007) who steamed the roots before extracting with 80% methanol. Using MeOH and GI, the total phenol content of Asian ginseng was >21% higher than American ginseng, in contrast to Kang et al. (2007) who found comparable values at ~20 mg/g GAE. Overall, MeOH extract of Siberian ginseng provided the highest amount of total phenols. Thus, the concentration of phenolic

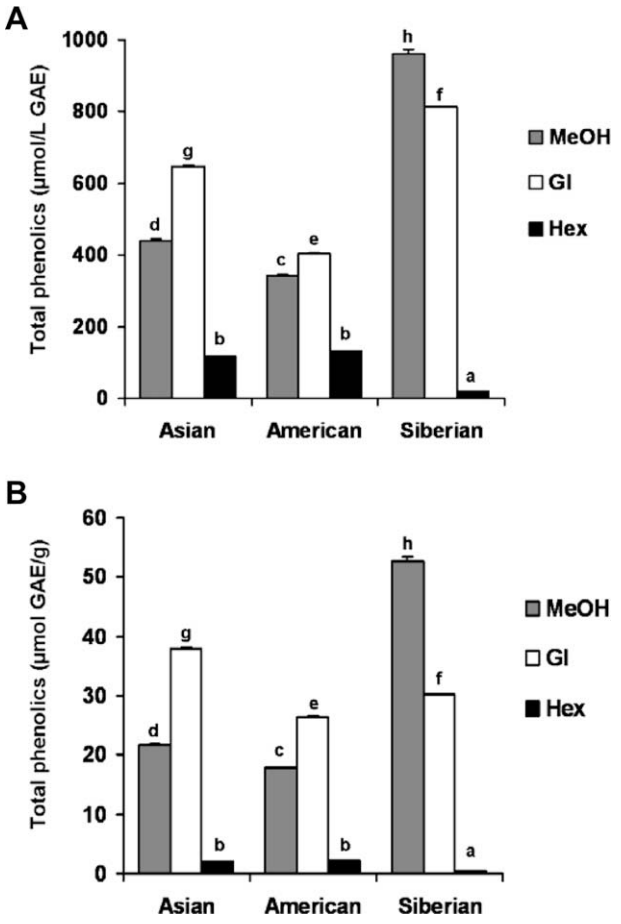

Fig. 1. Total phenol content in American, Asian, and Siberian ginseng. (A) Data are expressed as gallic acid equivalents (GAE) in extracts. (B) Data are expressed as ginseng dry weight. Means without the same letter differ, determined by Tukey's HSD test, $P \leqslant 0.05$.

antioxidants in ginseng is dependent upon the species and extraction method used. Further, within each ginseng species, the total phenol content is also subject to the influence of cultivation practice, growing season, and geographic origin.

3.3. Induction of quinone reductase activity

QR, a phase II enzyme, plays an important role in both detoxification pathways and antioxidant defenses. The effect of ginseng on QR induction has not been well studied (Lee et al., 2008). Our results show that the extraction solvent, ginseng species, and dose each had a different degree of impact on QR induction ($P < 0.0001$) (Table 1). GI extracts of Asian and American ginseng at tested concentrations $\leqslant$10 μmol/L did not significantly affect QR. However, the GI extract of Siberian ginseng, which belongs to a different genus than Asian and American species, did promote QR induction at 10 μmol/L GAE. MeOH extracts of each ginseng species induced QR only at the 10 μmol/L GAE dose. Our results are comparable to those of Lee et al. (2008) who found that 2 mg/mL ethanol extracts of American ginseng doubled QR activity. As noted, Hex is not an effective solvent for extracting phenols, so it was unexpected that they were more potent QR inducers than MeOH and GI. However, 10 μmol/L GAE Hex extracts of American and Siberian ginseng resulted in cell death. A recent study showed that ginsenosides were not active in QR induction, while polyacetylenes were the most active (Lee et al., 2009). Since Hex principally extracts hydrophobic compounds, such as polyacetylenes (Hirakura et al., 1992), it is likely that these compounds were responsible for QR induction in the Hex extracts. Of course, interpreting results of *in vitro* experiments to actions *in vivo* always requires caution as factors such as bioaccessibility, bioavailability, and metabolism are absent.

3.4. Radical scavenging activity

While American, Asian, and Siberian ginseng species have each been reported to scavenge free radicals, chelate transition minerals, and/or increase the resistance of lipoproteins to oxidation (Kang et al., 2007; Kitts, Wijewickreme, & Hu, 2000; McGuffin et al., 2000), no direct comparison of antioxidant activity among these three botanicals has been published. For radical quenching assays, we selected three physiologically relevant species, HOCl, $O_2^{\cdot-}$, and $ONOO^-$, and the synthetic nitrogen radical DPPH because of its wide application in testing for antioxidant potential in botanical and food products.

3.5. Dpph scavenging activity

Kim et al. (2007) reported that phenolic constituents in American and Asian ginseng scavenge DPPH. Kang et al. (2007) observed that a water-extracted powder of American ginseng was $\geqslant$2-fold more potent than Asian ginseng in quenching DPPH. In contrast, we found that extracts of American and Asian ginseng were not potent in this test. MeOH, GI, and Hex extracts of American ginseng at 100 μmol/L GAE scavenged only 37.2 ± 0.2%, 0.0%, and 8.9 ± 0.1% of DPPH, respectively; similarly, the same extracts of Asian ginseng scavenged only 20.4 ± 1.8%, 25.5 ± 0.2%, and 7.0 ± 0.6% of DPPH, respectively. However, MeOH and Hex extracts of Siberian ginseng revealed greater potency against DPPH with an IC50 of 40.0 ± 0.2 and 35.2 ± 0.5 μmol/L GAE, respectively. An IC50 could not be generated for the GI extract of Siberian ginseng against DPPH because doses $\geqslant$25 μmol/L GAE created opacity that interfered with the determination of absorbance.

3.6. Superoxide scavenging activity

Similar to their activity against DPPH, extracts of American and Asian ginseng were not potent scavengers of $O_2^{\cdot-}$ generated by a xanthine/xanthine oxidase reaction. Only the MeOH extract of Asian ginseng showed marked activity with 48.3 ± 1.8 μmol/L GAE quenching 50% of $O_2^{\cdot-}$. The American ginseng extracts had IC50 $\geqslant$100 μmol/L GAE. In contrast, Kang et al. (2007) reported water-extracted powders of American and Asian ginseng both scavenged $O_2^{\cdot-}$ at 50 μg/mL. GI and MeOH extracts of Siberian ginseng were potent quenchers of $O_2^{\cdot-}$ with IC50 of 7.0 ± 0.1 and 7.9 ± 0.3 μmol/L GAE, respectively. As uric acid production in the assay was not affected, the $O_2^{\cdot-}$ scavenging activity observed was not an artifact arising from the inhibition of the xanthine/xanthine oxidase reaction. Hex extracts of all tested ginseng species were found to interfere with the $O_2^{\cdot-}$ assay.

3.7. Hypochlorite scavenging activity

The GI and MeOH extracts of American and Asian ginseng were potent inhibitors against HOCl with an IC50 range of 1.5–2.5 μmol/L GAE (Fig. 2). However, GI and MeOH extracts of Siberian ginseng were $\geqslant$4.6-fold less effective than similar extracts of the other two ginseng species. Ginseng species and extraction solvent were significant determinants of HOCl scavenging activity ($P < 0.0001$). Asian ginseng was the most potent HOCl scavenger when expressed as either liquid extract (μmol/L GAE) or ginseng dry weight (g/100 mL).

Table 1
Induction of quinone reductase (QR) activity by extracts of American, Asian, and Siberian ginseng in Hepa 1c1c7 cells.

Ginseng	Concentration (μmol/L GAE)			P[a]
	0.1	1	10	
Quinone reductase activity (% induction of control)				
American				
MeOH	102 ± 3	105 ± 3	203 ± 7*	⩽ 0.0001
GI	98 ± 5	109 ± 2	102 ± 3	0.159
Hex	98 ± 5	180 ± 4*	Toxic[b]	⩽ 0.0001
Asian				
MeOH	99 ± 4	103 ± 4	183 ± 7*	⩽ 0.0001
GI	91 ± 3	102 ± 5	106 ± 6	0.188
Hex	107 ± 4	147 ± 6*	189 ± 5*	⩽ 0.0001
Siberian				
MeOH	98 ± 1	101 ± 3	146 ± 2*	⩽ 0.0001
GI	105 ± 2	101 ± 2	135 ± 2*	⩽ 0.0001
Hex	141 ± 3*	381 ± 8*	Toxic	⩽ 0.0001

[a] *P* value for means in the same row were obtained from multi-factor ANOVA.
[b] Exposure to constituents in American and Siberian ginseng from Hex led to 72.9 and 49.3% cell death, respectively.
* Values are significantly different from the negative control, $P < 0.05$, using Student's t-test.

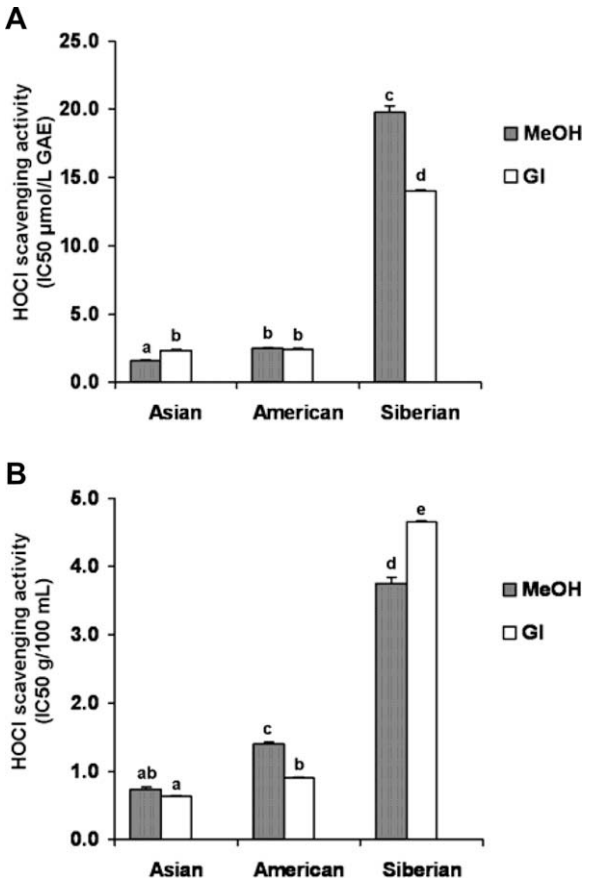

Fig. 2. Hypochlorite (HOCl) scavenging activity of American, Asian, and Siberian ginseng. (A) Data are expressed as gallic acid equivalents (GAE) in extracts. (B) Data are expressed as ginseng dry weight. The IC50 of Hex extracts were ⩾100 μmol/L GAE. Means without the same letter differ, determined by Tukey's HSD test, $P \leqslant 0.05$.

3.8. Peroxynitrite scavenging activity

$ONOO^-$, formed from a reaction between nitric oxide and $O_2^{\cdot-}$, can rapidly nitrify and oxidise thiols, lipids, carbohydrates, and nucleic acids. Kang et al. (2007) reported that American ginseng was a more potent scavenger of $ONOO^-$ than Asian ginseng. We found $ONOO^-$ scavenging activity was dependent on both the ginseng species ($P < 0.0001$) and extraction method ($P \leqslant 0.0003$) (Fig. 3). Extracts of Siberian ginseng were ⩾100% stronger than American and Asian ginseng in quenching $ONOO^-$, the converse of our results with HOCl. Surprisingly, we found the Hex extracts were effective scavengers of $ONOO^-$ even though their total phenol concentrations were quite low. The Hex extracts of Asian and Siberian ginseng were more potent than the GI and MeOH extracts ($P \leqslant 0.05$). However, because of the relatively lower total phenol content in Hex than the GI and MeOH extracts, the statistical significance of the former disappears when results are expressed as ginseng dry weight (antioxidant quantity). The antioxidant quality of 1.0 μmol/L GAE of Hex extracts against $ONOO^-$ was superior to that of MeOH and GI extracts. Importantly, such ranking did not reflect on antioxidant quantity, an index of the total antioxidant power in a given amount of ginseng.

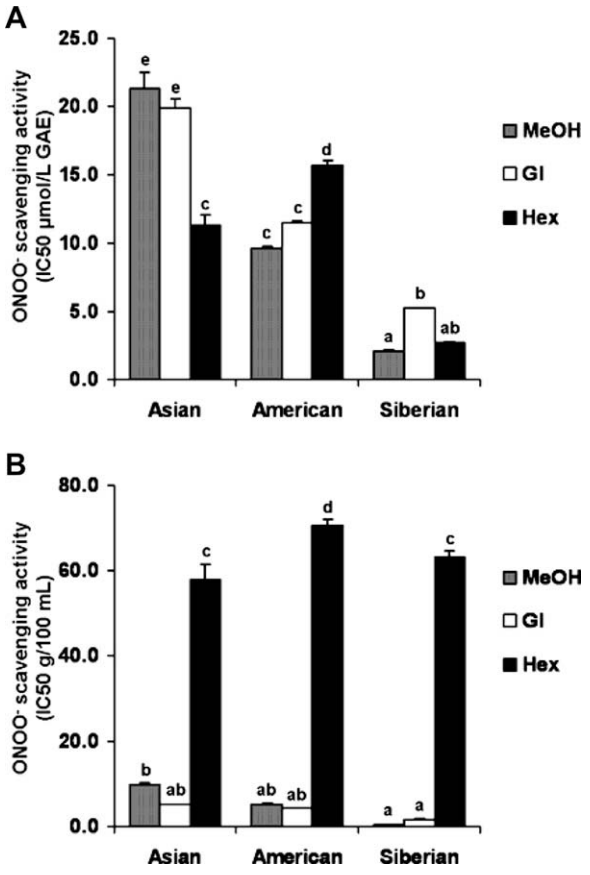

Fig. 3. Peroxynitrite ($ONOO^-$) scavenging activity of American, Asian, and Siberian ginseng. (A) Data are expressed as gallic acid equivalents (GAE) in extracts. (B) Data are expressed as ginseng dry weight. Means without the same letter differ, determined by Tukey's HSD test, $P \leqslant 0.05$.

3.9. Total antioxidant activity

In vitro assays of "total antioxidant activity" have been applied widely to assess the potential overall antioxidant value of botanical and food products. However, the relationship between these assays is not always consistent (Chen & Blumberg, 2008; Ou, Huang, Hampsch-Woodill, Flanagan, & Deemer, 2002) and, in our study, we did not find a direct correlation between the results from the FRAP and ORAC assays. The rank order of results from the ORAC assay was dependent on the ginseng species and extraction method

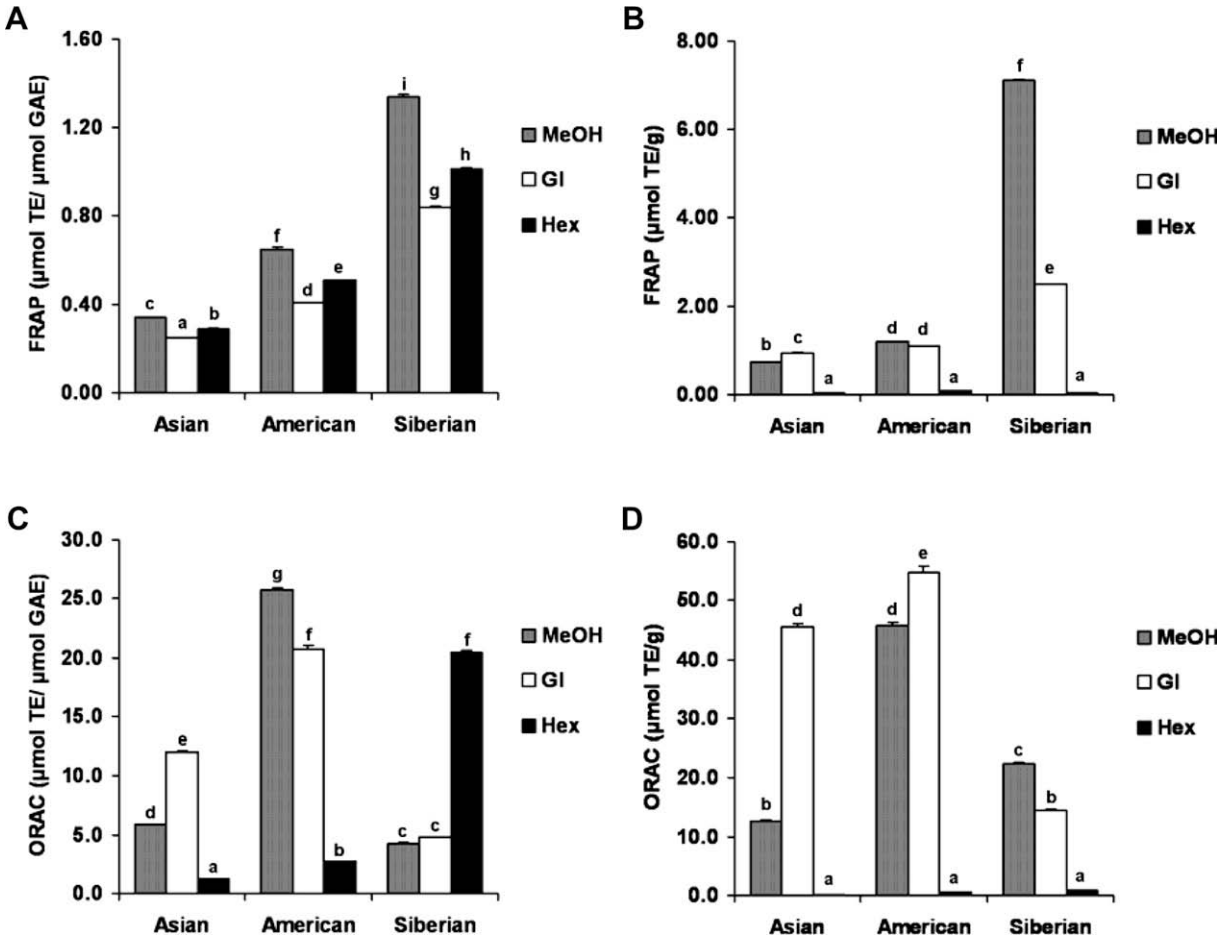

Fig. 4. Total antioxidant activity of American, Asian, and Siberian ginseng. (A, C) Values are expressed as gallic acid equivalents (GAE) in extracts. (B, D) Values are expressed as ginseng dry weight. Means without the same letter differ, determined by Tukey's HSD test, $P \leqslant 0.05$.

(Fig. 4C). Regardless of extraction solvent, the Siberian ginseng extract had ⩾2-fold higher FRAP values than either the American or Asian ginseng when the results are adjusted for total phenol content (1 μmol/L GAE) (Fig. 4A). Further, the GI extract of the American ginseng had the highest ORAC value while the MeOH extract of the Siberian ginseng had the highest FRAP value. Surprisingly, the Hex extract of the Siberian ginseng showed a high ORAC value, indicating the presence of potent hexane-soluble antioxidants against $ROO^{\bullet}$. Wu et al. (2004) have suggested that a high ratio of total antioxidant activity to total phenol content (as expressed in Fig. 4A and C) reflects either a significant contribution of non-phenolic compounds or assays that are particularly sensitive to phenols. Thus, 1.0 μmol/L GAE of the MeOH extract of American ginseng may either contain more non-phenolic antioxidants than Asian ginseng or possess more potent phenolic antioxidants. Additionally, synergistic interactions between the constituents in the extracts may underlie some of the diversity in these measures of total antioxidant activity. When the results are expressed as ginseng dry weight (antioxidant quantity), the GI and MeOH extracts of American and Asian ginseng have comparable FRAP values, though they are ⩾100% lower than those of Siberian ginseng (Fig. 4B). The GI extract of American ginseng and the MeOH extract of Siberian ginseng have the highest ORAC and FRAP values, respectively, based on ginseng dry weight. When comparing our data with the MeOH extracts of plant foods tested by Wu et al. (2004), the American ginseng has higher ORAC values than apples, grapes, and carrots, but lower values than pecans and walnuts; our ORAC values for Asian ginseng are comparable to those reported for cashews, potatoes, and broccoli.

4. Conclusions

American, Asian, and Siberian ginseng roots possess a wide range of potency in quenching free radicals and up-regulating QR. This activity is dependent on the plant species, extraction method, and dose, as well as the free radical scavenging or antioxidant assay employed. Of these three species, the GI extracts, a more physiologically relevant solvent, had comparable antioxidant activity against HOCl and $ONOO^-$ and total antioxidant activity to the MeOH extracts, but had little effect on QR activity. Although they contained fewer phenolic compounds, the Hex extracts were more effective at inducing QR activity than either the GI or MeOH extracts. The Asian ginseng was the most potent scavenger of HOCl while the Siberian ginseng was most potent against $ONOO^-$. The Siberian and American ginseng species had the highest FRAP and ORAC values, respectively. The results of this study suggest that a panel of assays incorporating different extraction methods is necessary to fully characterise and compare the relative antioxidant actions of botanical products, like ginseng, and elucidate their potential mechanisms of action. Further studies to examine the impact of other variables, including plant age, root size, cultivation method, and post-harvest processing, on the antioxidant and QR-inducing activity of ginseng are warranted.

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