

The antioxidant effects of aqueous and organic extracts of *Panax quinquefolium*, *Panax notoginseng*, *Codonopsis pilosula*, *Pseudostellaria heterophylla* and *Glehnia littoralis*

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Abstract

The roots of *Panax quinquefolium*, *Panax notoginseng*, *Glehnia littoralis*, *Codonopsis pilosula* and *Pseudostellaria heterophylla* were extracted with an aqueous extraction method and also with an organic extraction method. The aqueous extracts of *Glehnia littoralis* and *Codonopsis pilosula* were the most potent in inhibiting erythrocyte hemolysis. The aqueous extracts of *Panax quinquefolium* and *Panax notoginseng* had lower potencies while the aqueous extract of *Pseudostellaria heterophylla* and the organic extract of *Panax quinquefolium* were only weakly active. The organic extracts of *Glehnia littoralis*, *Panax heterophylla* and *Panax quinquefolium* were potent in inhibiting lipid peroxidation while the organic extracts of *Codonopsis pilosula* and *Panax notoginseng* had weaker potencies. The aqueous extracts possessed much lower potencies the corresponding organic extracts. However, the *Glehnia littoralis* extract was the most potent aqueous extract. The results suggest that *Glehnia littoralis*, *Codonopsis pilosula*, *Panax notoginseng* and *Panax heterophylla* are cheaper substitutes of *Panax quinquefolium* with regard to antioxidant activity.

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1. Introduction

The American ginseng *Panax quinquefolium* is well-known for its antioxidant and free radical scavenging activities (Li et al., 1999, 2000; Kitts et al., 2000). *Codonopsis pilosula* is used as a less costly substitute of the Korean ginseng *Panax ginseng* because the former also possesses pharmacological activities similar to *Panax ginseng* such as antifatigue and immunomodulatory activities (Wang et al., 1996). It also demonstrates antiulcer activity (Wang et al., 1997), inhibits platelet aggregation (Xu et al., 1995), inhibits phosphodiesterase activity and elevates cAMP level in rat myocardial cells (Qin et al., 1994), and improves survival and inhibits anti-dsDNA antibody production in lupus mice (Chen et al., 1993). The sanchi ginseng *Panax notoginseng* reportedly exerts beneficial effects on the heart and its constituents are ginsenosides similar to those found in *Panax quinquefolium*

and *Panax ginseng* (Chuang, 1999). The total ginseng saponins from *Panax notoginseng* may act as a novel and selective Ca²⁺ antagonist that does not interact with the L-type Ca²⁺ channel (e.g. in KCl-induced contraction) but may interact with the putative receptor operated Ca²⁺ channel (e.g. in phenylephrine-induced contraction) (Kwan, 1995).

Cyclic peptides have been isolated as chemical constituents of *Pseudostellaria heterophylla* (Tan et al., 1993; Morita et al., 1994a,b,c, 1995). Polysaccharides from *Pseudostellaria heterophylla* express immunoenhancing and antitumor activities (Wong et al., 1992, 1994a–c).

The presence of polyacetylenes with antiproliferative activity against tumor cell lines MK-1, HeLa and B16 F10 was suggested in *Glehnia littoralis* fruits (Nakano et al., 1998). Antibacterial and antifungal polyene compounds (Matsuura et al., 1996) and a potent allelochemical falcilindiol (Satoh et al., 1996) have been reported from *Glehnia littoralis*. *Glehnia littoralis* inhibits the synthesis of thromboxane A₂ and PGI₂ (Wang et al., 1993).

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Liu and Ng (2000) screened the extracts of 12 medicinal herbs for antioxidant and free radical scavenging activities. Four herbs including *Coptis chinensis*, *Paeonia suffruticosa*, *Prunella vulgaris* and *Senecio scandens* were found to possess the highest activities. The roots of *Panax quinquefolium*, *Panax notoginseng*, *Codonopsis pilosula*, *Pseudostellaria heterophylla* and *Glehnia littorales* are consumed as tonics in China in the form of soup or medicine. It was deemed worthwhile to compare the antioxidant and free radical scavenging activities of the extracts of these five species especially when the species, with the exception of *Panax quinquefolium* and *Panax notoginseng*, have not been previously so examined.

2. Materials and methods

2.1. Materials

The roots of *Panax quinquefolium* L., *Panax notoginseng* (Burk.) F.H. Chen, *Codonopsis pilosula* (Franch.) Nannf., *Pseudostellaria heterophylla* (Miq.) Pax et Hoffm. and *Glehnia littoralis* Fr. Schmidt ex Miq. were purchased from a local wholesaler of Chinese medicinal materials.

2.2. Aqueous extraction

The medicinal herbs examined in this investigation were purchased from local vendors. The dried herbs were cut into small pieces, soaked in water at room temperature overnight and then boiled under reflux for 2 h. The extract was decanted and centrifuged at $500 \times g$ for 10 min. The supernatant was lyophilized, and the resulting powder was used in the present study (Liu and Ng, 2000).

2.3. Organic extraction

The dried herbs were cut into small pieces and extracted with *n*-butanol which was saturated with water. The butanolic extract was then removed and dried down by rotary evaporation.

2.4. Assay for erythrocyte hemolysis

Blood was obtained from Sprague–Dawley rats by cardiac puncture and collected in heparinized tubes. Erythrocytes were separated from plasma and the buffer coat and washed three-times with 10 volumes of 0.15 M NaCl. During the last washing, the erythrocytes were centrifuged at 2500 rpm for 10 min to obtain a constantly packed cell preparation (Miki et al., 1987).

Erythrocyte hemolysis was mediated by peroxy radicals in this assay system (Sugiyama et al., 1993) (26). A 10% suspension of erythrocytes in phosphate buffered saline pH 7.4 (PBS) was added to the same volume of 200 mM 2,2'-azobis (2-amidinopropane) dihydrochloride solution (in PBS)

containing samples to be tested at different concentrations. The reaction mixture was incubated with shaking at 37 °C for 2 h, diluted with 8 volumes of PBS and centrifuged at 2500 rpm for 10 min. The absorbance A of the supernatant was read at 540 nm. Similarly, the reaction mixture was treated with 8 volumes of distilled water to achieve complete hemolysis, and the absorbance B of the supernatant obtained after centrifugation was measured at 540 nm. The percentage hemolysis was calculated by using the equation $(1 - A/B) \times 100\%$. The data were expressed as mean \pm standard deviation. L-Ascorbic acid was used as a positive control.

2.5. Assays of lipid peroxidation using brain homogenates

For the in vitro studies, the brains of normal rats were dissected and homogenized in ice-cold Tris–HCl buffer (20 mM, pH 7.4). The homogenate was centrifuged at 14,000 rpm for 15 min. One milliliter aliquots of the supernatant were incubated with the test samples in the presence of 10 μ M FeSO₄ and 0.1 mM ascorbic acid at 37 °C for 1 h. The reaction was stopped by addition of 1.0 ml 28% trichloroacetic acid and 1.5 ml 1% thiobarbituric acid (TBA) in succession, and the solution was then heated at 100 °C for 15 min. After centrifugation, the color of the malondialdehyde–TBA complex was detected at OD 532 nm. Butylated hydroxyanisole was used as a positive control.

The inhibition percentage (%) was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{A - A_1}{A} \times 100\%$$

where A was the absorbance of the control, and A₁ was the absorbance of the test sample (Liu and Ng, 2000).

3. Results

The effects of aqueous and organic extracts of *Panax quinquefolium*, *Glehnia littoralis*, *Codonopsis pilosula*, *Pseudostellaria heterophylla* and *Panax notoginseng* in inhibiting hemolysis of rat erythrocyte are presented in Table 1 and their effects in inhibiting lipid peroxidation in rat brain homogenate are recorded in Table 2. The aqueous extracts of *Glehnia littoralis* and *Codonopsis pilosula* exhibited the highest inhibitory potency followed by the aqueous extract of *Panax quinquefolium*. The aqueous extract of *Pseudostellaria heterophylla* did not elicit a marked inhibition of hemolysis. The organic extract of *Panax quinquefolium* weakly inhibited hemolysis while the organic extracts of the other aforementioned species failed to inhibit hemolysis (Table 1). The organic extracts of *Glehnia littoralis* and *Pseudostellaria heterophylla* potently inhibited lipid peroxidation followed by the organic extract of

Table 1
Inhibitory effects of extracts of medicinal herbs on hemolysis of rat erythrocytes

Sample	Concentration (µg/ml)	Inhibition (%)	
		Water extract	Organic extract
Control	H ₂ O or DMSO (1%)	0 ± 0.5	0 ± 0.2
<i>Panax quinquefolium</i>	500	16.9 ± 1.0	6.3 ± 1.7
	100	9.3 ± 2.4	8.5 ± 0.2
<i>Glehnia littoralis</i>	500	37.8 ± 4.2	-9.3 ± 2.6
	100	18.9 ± 1.9	-4.7 ± 0.7
<i>Pseudostellaria heterophylla</i>	500	5.5 ± 0.8	-8.2 ± 1.4
	100	5.9 ± 1.0	-2.7 ± 0.5
<i>Panax notoginseng</i>	500	21.0 ± 1.8	-0.9 ± 0.2
	100	11.8 ± 3.3	-1.7 ± 0.1
Vitamin C	500	89.3 ± 4.8	89.3 ± 4.8

The values are mean ± S.D. (n = 3).

Table 2
Inhibitory effects of extracts from medicinal herbs on lipid peroxidation in brain homogenates

Sample	Concentration (µg/ml)	Inhibition (%)	
		Water extract	Organic extract
Control	H ₂ O or DMSO (1%)	0	0
<i>Panax quinquefolium</i>	500	10.5 ± 3.3	65.1 ± 1.9
	100	8.7 ± 2.8	36.4 ± 2.3
<i>Glehnia littoralis</i>	500	20.8 ± 0.8	92.0 ± 1.7
	100	14.1 ± 1.5	89.7 ± 3.5
<i>Codonopsis pilosula</i>	500	8.3 ± 1.7	30.4 ± 2.5
	100	9.8 ± 0.6	16.7 ± 3.3
<i>Pseudostellaria heterophylla</i>	500	12.1 ± 1.1	91.5 ± 5.4
	100	5.3 ± 0.8	84.3 ± 4.7
<i>Panax notoginseng</i>	500	5.8 ± 0.7	28.6 ± 4.6
	100	6.2 ± 1.6	17.0 ± 0.4
Butylated hydroxyanisole	500	91.9 ± 2.7	91.9 ± 2.7

The values are mean ± S.D. (n = 3).

Panax quinquefolium. The organic extracts of *Codonopsis pilosula* and *Panax notoginseng* exerted some inhibitory action. The aqueous extract of *Glehnia littoralis* produced some inhibition. The inhibitory action of the other species was even weaker (Table 2).

4. Discussion

An extract of North American ginseng containing ginsenosides exhibited free radical scavenging activity (Kitts et al., 2000). *Panax quinquefolium* saponins protected low density lipoprotein from oxidation (Li et al., 1999). The antioxidant activity of *Panax quinquefolium* saponins at low

concentrations was enhanced by Vitamin C (Li et al., 2000). The triacylglycerol trilinolein from *Panax notoginseng* exerted its antioxidant effect through potentiation of superoxide dismutase (SOD), especially CuZn-SOD during hypoxia (Chan et al., 1997; Chan and Tomlinson, 2000). However, the antioxidant activity of *Codonopsis pilosula*, *Pseudostellaria heterophylla* and *Glehnia littoralis* has not been reported.

The method of extraction using organic solvents was applied for extracting *Panax quinquefolium*, *Panax notoginseng*, *Codonopsis pilosula*, *Pseudostellaria heterophylla* and *Glehnia littoralis* in the present investigation. Only the *Panax quinquefolium* extract was effective in slightly inhibiting erythrocyte hemolysis. The other extracts were ineffective. The organic extracts of *Glehnia littoralis* and *Pseudostellaria heterophylla* were highly potent in suppressing lipid peroxidation in rat brain homogenates, and indeed more so than the organic extract of *Panax quinquefolium* and much more so than the organic extracts of *Panax notoginseng* and *Codonopsis pilosula*. The chemical natures and structures of the antioxidant principles present in these organic extracts await elucidation but the principles may include steryl glycosides and lipids.

The aqueous extracts of the aforementioned plant species were much less effective than the corresponding organic extracts in inhibiting lipid peroxidation in rat brain homogenates. Even so, the organic extract of *Glehnia littoralis*, like its corresponding aqueous extract, was the most potent in inhibiting lipid peroxidation. The aqueous extract of *Glehnia littoralis* was more potent than while the aqueous extract of *Pseudostellaria heterophylla* was approximately equipotent to that of *Panax quinquefolium* in suppressing lipid peroxidation. The organic extracts of *Panax notoginseng* and *Codonopsis pilosula* possessed only meager inhibitory activity against lipid peroxidation. The difference in potency between the organic extracts and aqueous extracts in inhibiting lipid peroxidation was caused by the different compounds present in these extracts. This was again borne out by the observation that the aqueous extracts were more potent than the corresponding organic extracts in inhibiting erythrocyte hemolysis. The aqueous extracts of *Glehnia littoralis* and *Codonopsis pilosula* were more potent than and the aqueous extract of *Panax notoginseng* had about the same activity as that of *Panax quinquefolium* in inhibiting erythrocyte hemolysis. The types of compounds present in the aqueous extracts responsible for the antioxidant activity and their structures await elucidation. A review of the results of this investigation would suggest that *Glehnia littoralis* extracts seem to be more potent than *Panax quinquefolium* extracts in antioxidant action.

The roots of *Panax notoginseng* and *Codonopsis pilosula* are cheaper while those of *Glehnia littoralis* and *Pseudostellaria heterophylla* are much cheaper than those of *Panax quinquefolium*. As far as antioxidant and free radical scavenging activities are concerned, it might be a cheaper alternative to use *Panax notoginseng*, *Codonopsis pilosula*,

Glehnia littoralis or *Pseudostellaria heterophylla* instead of the expensive *Panax quinquefolium*.

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