Phytomedicine 9: 398–404, 2002

http://www.urbanfischer.de/journals/phytomed

Phytomedicine

Extractable polysaccharides of *Panax quinquefolius* L. (North American ginseng) root stimulate TNFα production by alveolar macrophages

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Summary

We have investigated the immunostimulatory activity of the medicinal plant *Panax quinquefolius* L. (North American ginseng). Rat alveolar macrophages were treated with different extracts from 4-year old roots, and tumour necrosis factor alpha (TNF) production was used as a measure of immunostimulatory activity. Aqueous extracts of *P. quinquefolius* root (1–100 µg/ml) were found to significantly stimulate alveolar macrophage TNF release. Both a *P. quinquefolius* methanol extract containing ginsenosides (but no polysaccharides), and pure ginsenoside-Rb1, the major ginsenoside present in *P. quinquefolius*, were found to be inactive as TNF-stimulating agents. Significant TNF-stimulating activity was found in the extractable polysaccharide fraction, which was hydrolyzed and found to contain glucose, galactose, arabinose, rhamnose, and mannose. This represents the first evidence that North American ginseng exerts cytokine – stimulating activity on macrophages.

Key words: *Panax quinquefolius*, North American ginseng, TNFα, ginsenosides, polysaccharides, macrophage activation

Introduction

Asian ginseng (*Panax ginseng* C.A. Meyer, Araliaceae) is known for its anti-stress and adaptogenic properties (Liu and Xiao, 1992), and for its pharmacological actions including cardioprotection (Maffei Facino et al., 1999), vasorelaxation (Rimar et al., 1996) and stimulation of the central nervous system (Liu and Xiao, 1992). One of the major effects of *P. ginseng* is the enhancement of host resistance against infection, in both experimental animals (Song et al., 1997) and in humans (Scaglione et al., 1994). This effect is most likely due to the stimulation of innate or non-specific immunity. *P. ginseng* stimulates non-specific immune response, notably by activating various macrophage functions including phagocytosis (Tomoda et al., 1994), intracellular killing and anti-candida activity (Akagawa et al., 1996; Scaglione et al., 1994), and production of various cytokines such as interleukin 1α (IL-α) (Kim et al., 1998), interleukin 8 (IL-8) (Sonoda et al., 1998) and interleukin 6 (IL-6) (Shin et al., 1998).

North American ginseng (*Panax quinquefolius* L., Araliaceae), a closely related species originating in deciduous forest habitats of eastern North America, is a distinct medicinal plant in Asian traditions (Foster, 1996). *P. quinquefolius* has been used in traditional medicine by First Nation peoples to decrease fever, stomach pain and hemorrhage (Arnason et al., 1981). In recent studies, *P. quinquefolius* has been shown to exhibit estrogen-like activity (Duda et al., 1996), and to exert beneficial effects on the central nervous system (Yuan et al., 1998; Li et al., 1999). *P. quinquefolius* may produce effects similar to *P. ginseng*,

0944-7113/02/09/05-398 $ 15.00/0

and both are consumed for general well-being (Eissenberg et al., 1993). However, relatively few studies have been performed with North American ginseng, and its effects as an immunomodulator are at present unknown.

In this study, we investigated *P. quinquefolius*' immunostimulating activity *in vitro* by testing the ability of alveolar macrophages (AM) to produce tumour necrosis factor alpha (TNF) in response to different extracts of *P. quinquefolius* root. TNF is an important cytokine produced by activated macrophages during inflammatory response (Beutler, 1999), and as part of host defence mechanisms against tumour (Srividya et al., 2000) and infection (Souto et al., 2000).

Materials and Methods

Animals

Male Wistar rats (250–300 g each; 8 to 9 weeks old) were obtained from Charles River Canada, Inc. (St-Constant QC) where they were raised in a pathogen-free colony. The rats were shipped behind filter barriers, and upon arrival, housed in isolated, temperature-controlled quarters, in a horizontal laminar flow isolator (John Scientific Inc., Toronto ON). They were given standard lab chow and water *ad libitum*, and were used within two weeks.

Plant materials and extraction

Four-year old roots of *P. quinquefolius* were obtained from Northern Lights Ginseng Farm (Quyon QC) and dried at room temperature. A voucher specimen has been deposited in the herbarium at the University of Ottawa (Voucher No. 19511). Pure ginsenoside-Rb1 (Rb1) was obtained from Indofine Chemical Co. (Somerville NJ).

To prepare an aqueous root extract, whole *P. quinquefolius* root was milled to No. 40 mesh (Thomas-Wiley laboratory mill, Philadelphia PA) and 1 g of the ground root was sonicated at room temperature for 1 h in 10 ml water. The water-soluble fraction was filtered (Whatman No. 1, Clifton NJ) and lyophilized to yield 365 mg of crude extract (Fig. 1). To prepare a polyacetylene fraction, ~10 g of ground root was extracted in 100 ml of 95% ethanol for 2 h on a shaker, and suction filtered (Whatman No. 1). The ethanol fraction was mixed with 100 ml of hexane in a separatory funnel. The hexane fraction was reduced to 5 ml in a rotoevaporator, and the UV spectrum was scanned in quartz cuvettes in a Beckman double-beam spectrophotometer with hexane as a blank. To prepare a ginsenoside-rich fraction, ~1 g of the ground *P. quinquefolius* root was extracted (Fig. 2) in 200 ml methanol (MeOH) at room temperature for 24 h to remove soluble ginsenosides. Methanol extraction was performed twice, and the methanol-soluble fractions were combined, filtered (Whatman No. 1) then rotoevaporated to yield a total ginsenoside-enriched fraction (432 mg). The plant residue obtained from the methanol-insoluble fraction was further extracted twice in 200 ml hot water (95 °C) to remove soluble cell-wall polysaccharides, and the combined water-soluble fractions were suction-filtered (Whatman No. 1) and lyophilized to yield total extractable polysaccharides (98 mg). These extracts, as well as the Rb1, were resolubilized in water and diluted at appropriate concentrations in culture medium to stimulate the cells. The possible presence of bacterial endotoxin (lipopolysaccharide) in the extract was verified using the Limulus amebocyte lysate assay (Associates of Cape Cod Inc., Falmouth, MA) (Lindsay et al., 1989). The Pyrochrome assay can detect >0.005 EU/ml and was performed as per manufacturer's instructions.

Ginsenoside analysis

A Beckman reversed-phase High Pressure Liquid Chromatography (HPLC) system was used for ginsenoside analyses. The system consists of a module 168 diode-

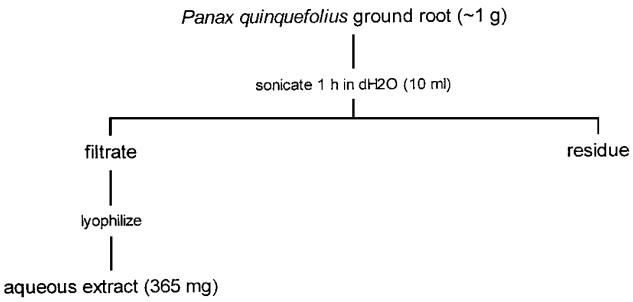

Fig. 1. Extraction scheme of the aqueous extract from *Panax quinquefolius* root.

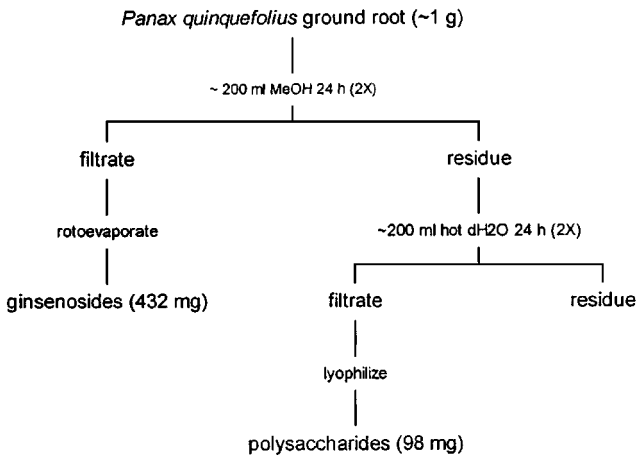

Fig. 2. Extraction scheme for ginsenoside and extractable-polysaccharide fractions from *Panax quinquefolius* root.

array detector, a module 126 solvent delivery system, a module 502 autosampler, and a computer equipped with System Gold software. The ginsenosides Rg1, Re, Rf, Rb1, Rc, Rb2, and Rd (Indofine Chemical Co., Somerville NJ) were mixed together as pure standards at concentrations of 0.01–0.1 mg/ml in MeOH. Analyses based on peak area (AU) were performed and the response factors (mg/ml/AU) calculated and incorporated into the method. Response factors were continuously monitored and updated by bracketing the standard mixture between 6–10 samples. The separation of the ginsenosides was achieved with a reversed-phase Beckman ultrasphere C-18, 5 µm octadecylsilane, 250 × 4.6 mm column (Beckman Coulter Canda Inc., Mississauga ON) connected to a LiChroCart LiChrospher 100 RP-18.4 × 4 mm, 5 µm guard column (EM Science, Cherry Hill NJ). The mobile phase was water (A) and acetonitrile (B) at a constant composition of 21% B from 0–20 min followed by a linear gradient to 42% from 20–60 min. The column was flushed and equilibrated after each analysis. The flow rate was 1.6 ml/min, and the detector was set at 203 nm. The residue from the MeOH extraction was dissolved in 10 ml MeOH, then filtered using a 0.2-µm nylon filter (Chromatographic Specialties Inc., Brockville ON) and 5 µl were injected into the HPLC system.

All solvents used in the extractions and HPLC analyses were HPLC grade (Omnisolv; EM Science, Gibbstown NJ), and the water was distilled and de-ionized (Milli-Q Reagent Water System; Millipore Canada Ltd., Nepean ON).

Analysis of crude polysaccharide extract

The extractable polysaccharide extract (50 mg) was hydrolyzed in hot 1 M sulfuric acid (H_2SO_4) (3 ml, 97 °C) for 5 h with mixing every 30 min. After cooling, 0.5 ml of the extract was mixed with 1.5 ml water, filtered, and then analyzed by HPLC. The neutral monosaccharides were separated on a DIONEX 4 × 250-mm PA-10 column fitted with a 4 × 50-mm PA-10 guard column. The HPLC system included Waters Millennium 32 software, a Waters 626 pump and 600S controller, a Waters 717plus Autosampler, and a Waters 464 pulsed amperometric detector. The solvent systsem, at a constant flow rate of 1 ml/min, consisted of isocratic 2% 100 mM sodium hydroxide and 90% water for 32 min, followed by a 6-min linear gradient increase to 100% 300 mM sodium hydroxide maintained for 22 min. An external standard contained fucose, arabinose, rhamnose, galactose, glucose, xylose and mannose. Hydrolysates from similar extractions but using 12 M H_2SO_4 were also obtained, and uronic acids were determined from this preparation (Englyst et al., 1992). The analyses were calibrated against galacturonic acid and the absorbance measured at 400 and 450 nm to determine the difference in absorbance due to uronic acids.

Simple starch tests were conducted by placing extracts on a filter and staining them with an iodine reagent (0.66% KI and 0.33% I_2).

Isolation of alveolar macrophages and stimulation

Alveolar macrophages (AM) were recovered from normal rats by bronchoalveolar lavage (BAL) (Lemaire, 1991). The lungs were lavaged with seven 7-ml aliquots of sterile phosphate buffered saline (pH 7.4; Wisent, St. Bruno QC), and BAL cells were obtained by centrifugation at 200 × g at 4 °C for 5 min. The cells were resuspended in RPMI 1640 medium (Wisent, St Bruno QC) supplemented with 0.5% dialysed fetal bovine serum (Wisent, St Bruno QC), 0.005% gentamycin (Schering Canada Inc., Pointe Claire QC) and 0.8% HEPES (Sigma Chemical Co., St Louis MO). Cells were counted in a hemocytometer chamber, and cell viability (>98%) was determined by trypan blue exclusion. Differential counts of BAL cells made from cytocentrifuge smears stained with Wright-Giemsa indicated that greater than 98% of the BAL cell population was of macrophage morphology. AM (2×10^5) were incubated in 200 µl of complete medium in the presence and absence of various extracts of *P. quinquefolius* root or Rb1 at different concentrations (0.1–250 µg/ml) for 20 h at 37 °C in a humidified atmosphere containing 5% CO_2. Lipopolysaccharide (LPS, 1 µg/ml) (*Escherichia coli* Ser1027:B8; Sigma) was used as a positive control. Following incubation, no significant cytotoxicity (necrosis or apoptosis) was seen in treated cells as compared to controls (87–92% viability) except for LPS (70% viability) as determined by propidium iodide (PI) and Hoechst staining. The culture supernatants were collected by centrifugation and frozen at –80 °C until assayed for TNF.

TNF assay

Immunoreactive TNF was measured in culture supernatants as described (Reasor et al., 1996) with a solid phase enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis MN) employing the multiple antibody sandwich principle (Pizarro et al., 1993). Culture supernatants were added to each well of 96-well microtiter plates, precoated with polyclonal antibody specific for TNF and incubated for 2 h at room temperature. After washing away (5×) unbounded substances, the culture was incubated for an additional 2 h with polyclonal anti-murine TNF antibody conjugated to horseradish peroxidase. After washing (5×) to remove unbound antibody-enzyme reagent, tetramethylbenzidine was added as a peroxidase substrate and the reaction stopped after 30 min. Absorbance was read at 450 nm using an automated Spectra Count microplate reader (Packard Instrument Co., Meridan CT). The minimum detectable dose of TNF is typically less than

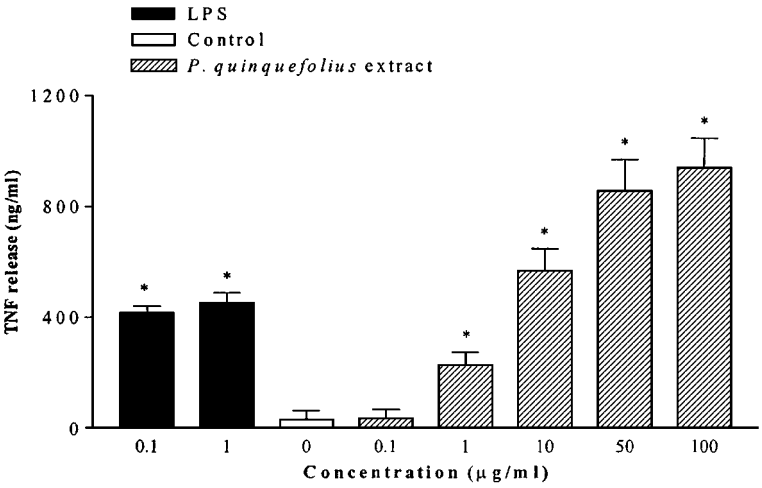

Fig. 3. Effects of *Panax quinquefolius* aqueous root extract on TNF production by rat alveolar macrophages. LPS (0.1 and 1 μg/ml) was the positive control. Results are means ± SEM of 6 separate experiments performed in triplicate. *Significant difference measured by Tukey test from the non-stimulated control ($P < 0.05$).

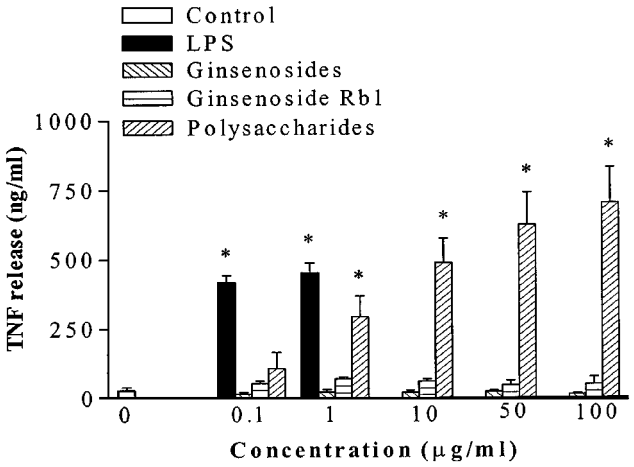

Fig. 4. Effects of various concentrations of ginsenoside and polysaccharide fractions from *Panax quinquefolius* root, and ginsenoside-Rb1 on TNF production by rat alveolar macrophages. The positive control was LPS (0.1 and 1 μg/ml). Results are mean ± SEM of 5 separate experiments performed in triplicate. *Significant difference from the non-stimulated control measured by Tukey test ($P < 0.05$).

5.1 pg/ml in this assay with inter and intra assay variation of 4.3 to 9%.

Statistical analysis

Each experiment was performed at least three times using AM from a different rat. Results are expressed as mean values ± SEM. Mean values were tested using Tukey's multiple range test and statistical significance wa set at $p < 0.05$ using SYSTAT software.

Results and Discussion

Incubation of AM with the aqueous extract from *P. quinquefolius* root significantly stimulated the release of immunoreactive TNF in the culture supernatants. As shown in Fig. 3, stimulation was apparent at 1 μg/ml and increased in a dose-dependent fashion, with maximal effect observed at 50 μg/ml, thus demonstrating the macrophage-activating potential of North American ginseng. Higher concentrations of *P. quinquefolius* root extract (250 μg/ml) retained significant

Table 1. Ginsenoside content in different extracts of *Panax quinquefolius* root.

Extract	Ginsenoside content (% W/W)[1]						
	Rg1	Re	Rb1	Rc	Rb2	Rd	Total
Aqueous extract[2]	0.00	0.25	0.97	0.84	0.03	0.94	3.22
Methanol fraction[3]	0.09	1.83	2.07	0.52	0.09	0.75	5.34
Water-soluble fraction[4]	0.00	0.00	0.00	0.00	0.00	0.00	0.00

[1] Content is based on the percentage of ginsenoside weight to the sample weight.
[2] Ground root sonicated in water.
[3] Enriched ginsenoside fraction.
[4] Enriched polysaccharide fraction.

Table 2. Composition of crude polysaccharide fraction from 4-year old *Panax quinquefolius* root following acid hydrolyses[a].

Monosaccharide (%)					
Glucose	Galactose	Arabinose	Fucose	Rhamnose	Mannose
85.09	7.48	5.89	0.09	0.79	0.41

[a] From 1 M H2SO4 hydrolysis of crude polysaccharide extract from 3 different roots.

stimulatory activity (albeit to a lower level). This was not related to a cytotoxic effect of the extract as determined by propidium iodide and Hoechst staining, but more likely due to the presence of potential inhibitors in more concentrated extracts.

Next, we attempted to identify the class of phytochemical responsible for TNF-stimulating activity. The hexane extract showed sharp multiple absorbance bands in the UV characteristic of ginseng polyacetylenes (Kwon et al., 19997; Bohlmann et al., 1973), but based on the extinction coefficients of these molecules, only trace amounts of these substances were detected (>0.1 µg/g). Polyacetylenes are unstable, and are particularly prone to degradation in dried plant materials. The yield from our dried ginseng root was judged too low to contribute to biological activity, and was not subjected to bioassay. A fraction enriched in ginsenosides, obtained by extraction in MeOH, contained a high level of the ginsenosides characteristic of *Panax* spp. (Table 1). The lack of an Rf peak and the high Rb1/Rg1 ratio confirmed that authentic *P. quinquefolius* and not *P. ginseng* roots were used. This fraction probably contained other components, but was free of high-molecular-weight polysaccharides that are insoluble in alcohols. As evidence of this, the characteristic iodine test for starch was negative. A hot water extraction of the insoluble residue from the MeOH extraction yielded a soluble polysaccharide-enriched fraction. This extraction removed starch and extractable cell-wall polysaccharides (pectins and hemicellulose, but not cellulose). After lyophilization, the resulting fraction was a white cottony substance (10% of the dried root sample), which tested positive for starch with iodine. To investigate the composition of the polysaccharides, acid hydrolysis analyses were undertaken. The 1 M H_2SO_4 hydrolysis revealed glucose, galactose, arabinose and rhamnose in the approximate ratio 85:8:6:1, and two other monosaccharides, fucose and mannose, in small amounts (Table 2). Hydrolysis with 12 M H_2SO_4 indicated the presence of approximately 9% uronic acid in the extractable polysaccharide fraction. The acid hydrolysis did not investigate methoxy and acetoxy substitutions, and linkages were not identified. These results are consistent with the presence of starch and extractable neutral and/or acidic cell-wall heteroglycans as found in other higher plants (Lien and Gao, 1990). The findings are similar to those of *P. ginseng* studies in which two acid polysaccharides (ginsenan PA and ginsenan PB) composed of arabinose, galactose, rhamnose, galacturonic and glucuronic acid were identified (Tomada et al., 1994).

When the enriched fractions were incubated with AM (Fig. 4), the extractable polysaccharides significantly stimulated TNF release at a level comparable to that observed for aqueous root extracts. However, *P. quinquefolius* polysaccharides at concentrations between 0.1 and 1 µg/ml had TNF-stimulating effects of lower magnitude that the bacterial antigen LPS, a potent activator of macrophages. On a dose-response basis, 0.1 µg/ml LPS, the lowest concentration used in our study, was as effective as 10 µg/ml *P. quinquefolius* extract in stimulating TNF. Thus our polysaccharide extract was at least 100 times less potent than LPS in our cell-culture system. This is consistent with a beneficial immunomodulatory effect without the occurrence of significant toxicity. In support of this, our polysaccharide extract did not exert a cytotoxic effect on AM, in contrast to LPS, which induces macrophage death during cell activation (72% cell viability as compared to 92% for controls), an effect also reported by others (Bingisser et al., 1996). It is notable that the potent biological toxicity of endotoxin (LPS), which may lead to endotoxic shock, is due to the lipid A portion of LPS (Raetz, 1990), a molecular entity unlikely to be present at significant levels in our plant-derived polysaccharide fraction. In agreement with this, polysaccharide extracts of *P. quinquefolius* did not contain levels of endotoxin (<0.01 EU/ml) sufficient to cause AM stimulation under our experimental conditions.

By contrast, the fraction containing total ginsenosides had little activity on TNF release by AM. To rule out the possibility that our ginsenoside preparation had lost activity during the fractionation procedure, we tested the effects of Rb1, which represents the major ginsenoside present in *P. quinquefolius* (Table 1). As was the case with total ginsenosides from *P. quinquefolius*, Rb1 did not significantly stimulate TNF production by resting macrophages. In fact, as reported by Cho et al. (2001), we observed that ginsenosides and Rb1 inhibit LPS-induced TNF production by AM (in preparation). Therefore, the TNF-stimulating activity of *P. quinquefolius* root is associated almost exclusively with the soluble polysaccharide fraction. These observations are consistent with previous work, which showed that various polysaccharides from higher plants stimulate and modulate the immune system, notably through macrophage activation (Lien and Gao, 1990). Starch is inactive in these bioassays, and the immunomodulatory activity in

other plants is normally associated with the soluble cell-wall polysaccharides. In addition, polysaccharides from *P. ginseng* have been shown to be potent inducers of macrophage cytokines IL-8 (Sonoda et al., 1998), IL-6 (Shin et al., 1998) and IL-1α (Kim et al., 1998).

Further work is required to characterize the specific polysaccharide or combination of polysaccharides from *P. quinquefolius* that stimulate TNF production. Previous studies have shown that in *P. ginseng*, the two acid polysaccharides, ginsenan PA and PB, have immunomodulating activity in carbon-clearance tests (Tomoda et al., 1993). Acidic arabinogalactans that induce peritoneal macrophages to secrete cytokines were isolated from *Echinacea purpurea* L. Moench. (Purple coneflower, Asteraceae), and were particularly effective in stimulating TNF production (Luettig et al., 1989). In a more recent study, rhamnogalacturonan II (RG-II) from *P. ginseng* has been shown to be responsible for cytokine production (Shin et al., 1998).

In conclusion, our study demonstrates that *P. quinquefolius* shares with its Asian relative, *P. ginseng*, the ability to stimulate macrophage cytokine secretion, a crucial component of innate immunity. North American ginseng is a potent inducer of TNF, an important cytokine in normal host response to infection (Souto et al., 2000; Srividya et al., 2000; Beutler et al., 1999), suggesting that it may augment the immune response. Whether North American ginseng also exerts other macrophage-activating effects is currently under investigation. Our observation that TNF stimulation resides with the polysaccharide fraction further indicates that the macrophage-stimulating activity of North American ginseng preparations may be standardized partially according to their polysaccharide content.

Acknowledgement

This work was funded by a University of Ottawa interfaculty award, and Natural Sciences and Engineering Research Council grants.

References

Akagawa G, Abe S, Tansho S, Uchida K, Yamaguchi H (1996) Protection of C3H/HE J mice from development of *Candida albicans* infection by oral administration of Juzentaiho-to and its component, *Ginseng radix*: possible roles of macrophages in the host defense mechanisms. Immunopharmacol. Immunotoxicol 18: 73–89

Arnason T, Hebda R J, Johns T (1981) Use of plants for food and medicine by Native Peoples of eastern Canada. Can, J Botany 59: 2189–2325

Beutler BA (1999) The role of tumor necrosis factor in health and disease. J Rheumatol 26: 16–21

Bingisser R, Stey C, Weller M, Groscurth P, Russi E, Frei K (1996) Apoptosis in human alveolar macrophages is induced by endotoxin and is modulated by cytokines. Am Rev Respir Cell Mol Biol 15: 64–70

Bohlmann F, Burkhardt T, Zdero C (1973) Naturally occurring acetylenes. Academic Press, New York YN

Cho HY, Yoo ES, Baik KU, Park MH (2001) *In vitro* inhibitory effect of protopanaxidiol ginsenosides on tumour necrosis factor (TNF)-alpha production and its modulation by known TNF alpha antagonists. Planta Med 67: 213–218

Duda RB, Taback B, Kessel B, Dooley DD, Yang H, Marchiori J, Slomovic BM, Alvarez JG (1996) pS2 expression induced by American ginseng in MCF-7 breast cancer cells. Ann Surg Oncol 3: 515–520

Eissenberg DM, Kessler RC, Foster C, Norlock FE, Calkins DR, Delbanco TL (1993) Unconventional medicine in the United States. N Engl J Med 328: 246–252

Englyst HN, Quigly ME, Hudson GJ, Cummings JH (1992) Determination of dietary fibre as non starch polysaccharides by gas chromatography. Analyst 117: 1707–1714

Foster S (1996) American Ginseng, *Panax quinquefolius*. American Botanical Council Botanical Series No. 308

Kim KH, Lee YS, Jung IS, Park SY, Chung HY, Lee IR, Yun YS (1998) Acidic polysaccharide from *Panax ginseng*, Ginsan, induces Th1 cell and macrophage cytokines and generates LAK cells in synergy with rIL-2. Planta Med 64: 110–115

Kwon BM, Roe SH, Kim MK, Nam JY, Jung HJ, Lee LR, Kim YK, Bok SH (1997) Polyacetylene analogues, isolated from hairy roots of *Panax ginseng*, inhibit Acyl-CoA: cholesterol acyltransferase. Planta Med 63: 552–553

Lemaire I (1991) Selective differences in macrophage populations and monokine production in resolving pulmonary granuloma and fibrosis. Am J Pathol 138: 487–495

Li Z, Guo YY, Wu CF, Li X, Wang JH (1999) Protective effects of pseudoginsenoside-F11 on scopolamine-induced memory impairment in mice and rats. J Pharm Pharmacol 51: 435–440

Lindsay GK, Rolansky PF, Novitski T (1989) Single-step, chromogenic Limulus Amebocyte Lysate assay for endotoxin. J Clinc Microbiol 27: 947–951

Lien EJ, Gao H (1990) Higher plant polysaccharides and their pharmacological activities. Int J Oriental Med 15: 123–140

Liu CX, Xiao PG (1992) Recent advances on ginseng research in China. J Ethnopharmacol 36: 27–38

Luettig B, Steinmüller C, Gifford GE, Wagner H, Lohmann-Matthes ML (1989) Macrophage activation by the polysaccharide arabinogalactan from plant cell cultures of *Echinacea purpurea*. J Natl Cancer Inst 81: 669–675

Maffei Facino RM, Carini M, Aldeni G, Berti F, Rossoni G (1999) *Panax ginseng* administration in the rat prevents myocardial ischemia – reperfusion damage induced by hyperbaric oxygen: evidence for an antioxidant intervention. Planta Med 65: 614–619

Pizzaro TT, Malinowska K, Kovacs EJ, Clanecy J Jr, Robinson J, Piccini LA (1993) Induction of TNF-alpha and TNF-beta gene expression in rat. Transplant 56: 399–404

Raetz CRH (1996) Biochemistry of endotoxins. Annu Rev Biochem 59: 129–170

Reasor MJ, McLoud CM, DiMatteo M, Schafer R, Ima A, Lemaire I (1996) Effects of amiodarone-induced phospho-

lipidosis on pulmonary host defense functions in rats. Proc Soc Exp Biol Med 211: 346–352
Rimar S, Lee-Mengel M, Gillis CN (1996) Pulmonary protective and vasodilator effects of a standardized *Panax Ginseng* preparation following artificial gastric digestion. Pulmonary Pharmacol 9: 205–209
Scaglione F, Cogo R, Cocuzza C, Arcidiacono M, Beretta A (1994) Immunomodulatory effects of *Panax ginseng* C.A. Meyer (G115) on alveolar macrophages from patients suffering with chronic bronchitis. Int J Immunother 10: 21–24
Shin KS, Kiyohara H, Matsumoto T, Yamada H (1998) Rhamnogalacturonan II dimers crosslinked by borate diesters from the leaves of *Panax ginseng* C.A. Meyer are responsible for expression of their IL-6 production enhancing activities. Carbohydrate Res 307: 97–106
Song ZJ, Johansen HK, Faber V, Hoiby N (1997) Ginseng treatment enhances bacterial clearance and decreases lung pathology in athymic rats with chronic *P. aeruginosa* pneumonia. APMIS 105: 438–444
Sonoda Y, Kasahara T, Mukaida N, Shimizu N, Tomoda M, Takeda T (1998) Stimulation of interleukin-8 production by acidic polysaccharides from the root of *Panax ginseng*. Immunopharmacol 38: 287–294
Souto JT, Figueiredo F, Furlanetto A, Pfeffer K, Rossi MA, Silva JS (2000) Interferon-gamma and tumor necrosis factor-alpha determine resistance to paracoccidioides brasiliensis infection in mice. Am J Pathol 156: 1811–1820
Srividya S, Roy RP, Basu SK, Mukhopadhyay A (2000) Scavenger receptor-mediated delivery of muramyl dipeptide activates antitumor efficacy of macrophages by enhanced secretion of tumor-suppressive cytokines. J Leukoc Biol 67: 683–690
Tomoda M, Hirabayashi K, Shimizu N, Gonda R, Ohara N (1994) The core structure of ginsenan PA, a phagocytosis-activating polysaccharide from the root of *Panax ginseng*. Biol Pharm Bull 17: 1287–1291
Tomoda M, Takeda K, Shimizu N, Gonda R, Ohara N, Takeda K, Hirabayashi K (1993) Characterization of two acidic polysaccharides having immunological activities from the root of *Panax ginseng*. Biol Pharm Bull 16: 22–25
Yuan CS, Attele AS, Wu JA, Liu D (1998) Modulation of American ginseng on brainstem GABAergic effects in rats. J Ethnopharmacol 62: 215–222

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