JPP Journal of Pharmacy and Pharmacology

JPP 2006, 58: 1399–1403

Received May 10, 2006
Accepted June 23, 2006
DOI 10.1211/jpp.58.10.0014
ISSN 0022-3573

Novel selective cytotoxicity of wild sarsaparilla rhizome extract

Y. G. Huang, Q. Z. Li, G. Ivanochko and R. Wang

Abstract

Among six fractions, including total extract and fractions of hexane, ethyl acetate, butanol, water and boiling water extracted and separated from wild sarsaparilla rhizome, the hexane fraction (HRW) was the most effective in eliminating four different human cancer cell lines with cellular viability less than 6.8%. HRW exhibited the highest potency against human leukaemia cells with an IC50 (concentration that inhibited the growth rate of cells by 50%) of $3.3 \pm 0.3\,\mu g\,mL^{-1}$, which was 17.6-fold smaller than that against normal human umbilical vein endothelial cells (IC50, $58.0 \pm 1.5\,\mu g\,mL^{-1}$). For its rich natural resources, simple extraction procedure and high yield (3.2%), HRW has the potential to be developed as a selective anti-cancer nutraceutical or pharmaceutical natural health product with low side effects and high economical return.

Introduction

An increased morbidity of cancer is foreseen at an alarming rate from 10 million new cases globally in 2000 to 15 million in 2020 (Mignogna et al 2004). This trend indicates that the present cancer therapies, including surgical operation, radiotherapy and chemotherapy, are still not effective enough to combat the cancer epidemic. It is imperative to continue the search for novel anti-cancer agents with higher effectiveness, less side effects and reduced economical cost.

Aralia nudicaulis L. in the ginseng family of Araliacea is commonly known as wild sarsaparilla and grows widespread in shady, rich woodland across Canada and northern USA (Johnson et al 1995). Traditional uses of this plant as medicines by Aboriginal peoples of Canada's northwestern boreal forest have been recorded (Marles et al 2000). To date, no extracts have been prepared from *A. nudicaulis* and the cytotoxic and anti-cancer properties of this plant have not been explored.

Previous studies on plant species in the same genus as *A. nudicaulis*, such as *A. elata* (Hernandez et al 1988) and *A. cordata* (Okuyama et al 1991), have revealed the anti-ulcer and analgesic effects of these plants. The liver-protective activity of leaf extract from *A. elata* (Saito et al 1993) and of root and cortex extract from *A. taibaiensis* (Wang et al 1997) have also been studied. In addition, the antioxidant properties of *Aralia sp*. (Bol'shakova et al 1997) and the anti-diabetic activity of cortex extract from *Aralia sp*. (Lee et al 2000) have been explored. The root bark extract from *A. dasyphylla* (Xiao et al 1999) and the shoot extract from *A. elata* (Tomatsu et al 2003) also exhibit cytotoxic activity.

The objective of this research was to extract and separate some selective natural anti-cancer components from wild sarsaparilla — a local medicinal plant in Canada and northern USA. The cytotoxic activity of different fractions from the rhizome of *A. nudicaulis* was investigated with several human cancer and normal cell lines in the in-vitro test by MTT assay (Denizot & Lang 1986).

Materials and Methods

Reagents

Methanol, hexane, ethyl acetate, butanol, sodium bicarbonate and dimethyl sulfoxide (DMSO) were the products of Merck (Darmstadt, Germany). RPMI-1640 medium, Nutrient Mixture F-12

Department of Physiology, College of Medicine, University of Saskatchewan, 107 Wiggins Road, Saskatoon, Saskatchewan, S7N 5E5, Canada

Y. G. Huang, Q. Z. Li, R. Wang

Saskatchewan Agriculture and Food, La Ronge, Saskatchewan, S0J 1L0, Canada

G. Ivanochko

Correspondence: R. Wang, Department of Physiology, College of Medicine, University of Saskatchewan, 107 Wiggins Road, Saskatoon, Saskatchewan, S7N 5E5, Canada. E-mail: rwang@lakeheadu.ca

Acknowledgment: The authors would like to thank Ms Kirsten Remarchuk, W. P. Fraser Herbarium, Department of Plant Science, College of Agriculture, University of Saskatchewan, Saskatoon, Saskatchewan, Canada for her verification of the plant species.

Ham Kaighn's Modification (F12K), polyoxyethylenesorbitan monooleate (Tween 80), cell freezing medium-DMSO, trypsin-EDTA solution, endothelial cell growth supplement, insulin, heparin and penicillin/streptomycin (P/S) solution were purchased from Sigma-Aldrich Co. (St Louis, MO). C, N-Diphenyl-N′-4,5-dimethyl thiazol-2-yl tetrazolium bromide (MTT) was from Promega Corporation (Madison, WI). Dulbecco's modified Eagle medium (DMEM) and fetal bovine serum (FBS) were from Invitrogen Canada Inc. (Burlington, ON).

Plant material and extract preparation

The rhizomes of wild sarsaparilla were collected at the boreal forest near La Ronge, Saskatchewan, Canada and the plant species was verified by a botanist at the W. P. Fraser Herbarium, Department of Plant Science, College of Agriculture, University of Saskatchewan, Canada.

The collected rhizomes of wild sarsaparilla were dried at room temperature for two weeks and then in an oven at 45°C for 48 h. The dried rhizomes were ground to powder using a grinder. The powder was soaked with methanol by the extracting method for total composition from natural medicinal materials (Chen 1993) and the total extract was obtained by recovering the methanol with an evaporator (Buchi Rotavapor R-2000; CH-9230 Flawil, Switzerland) at 45°C. The total extract was further separated with hexane, ethyl acetate, butanol, water and boiling water using the partitioning method by gradually increasing the solvent polarity (Huang et al 1997a).

Yield of the fractions

The yields (weight of dried extract/weight of raw plant rhizome powder, g/g × 100%) were 15.0% for the total extract, 3.2% for hexane fraction, 0.5% for ethyl acetate fraction, 1.8% for butanol fraction, 8.4% for water fraction and 4.0% for boiling water fraction.

Cell culture

T-470 cells from human breast ductal carcinoma were cultured with RPMI1640 supplemented with insulin (0.2 IU mL^{-1}), 10% FBS and 1% P/S. HT29 cells from human colon adenocarcinoma were cultured with DMEM supplemented with 10% FBS and 1% P/S. WiDr from human colon adenocarcinoma and Molt from human T-cell leukaemia were cultured with RPMI1640 plus 10% FBS and 1% P/S. Normal non-cancer cells of HUV-EC (human umbilical vein endothelial cells) were cultured with F12K supplemented with 0.1 g L^{-1} of heparin, 0.06 g L^{-1} of ECGS, 2.5 g L^{-1} of $NaHCO_3$, 15% FBS, and 1% P/S. All cancer cell lines were provided by Saskatoon Cancer Centre and HUV-EC were obtained from American Type Culture Collection. The culture media were changed twice a week (Bae et al 1994). The cells were grown in 25-cm^2 tissue culture flasks and maintained in a humidified (95% air and 5% CO_2) incubator at 37°C.

Cytotoxicity assay in-vitro

Each rhizome fraction of wild sarsaparilla was added to culture-medium-containing wells of the 96-well plates to reach different final concentrations. The highest final concentration of DMSO as the solvent was smaller than 0.1%. Thereafter, 5000 cells were added to each well and incubated for 48 h at 37°C in a humidified environment containing 5% CO_2. After that, 15 μL of MTT solution was added into every well and incubated for another 4 h. After adding 100 μL of solubilization/stop solution to each well, the 96-well plates were left at 4°C overnight. Finally, the absorbance of each well was recorded at the wavelength of 595 nm and reference wavelength of 655 nm with a plate reader (Model 1500; Multiskan Spectrum, ON). Blank control was included by mixing media and MTT in the absence of cells. Adding cells and MTT to the media without plant extracts constituted the negative control. The yellow-coloured MTT dye (low absorbance) becomes blue-coloured formazan product (high absorbance) by the mitochondrial enzyme succinate dehydrogenase. The conversion takes place only in living cells and the amount of formazan produced, the absorbance, is proportional to the number of cells present (Denizot & Lang 1986). The lower the absorbance values, the lesser cellular viability of the tested cells.

Data calculation

All data were expressed as means ± s.e.m. from at least 3 repetitions performed in duplicate. Statistical analyses were done using unpaired Student's *t*-test in conjunction with the Newman–Keuls test and analysis of variance for repeated measures where appropriate. Cellular viability differences between rhizome extract treatment groups and negative control group were considered statistically significant at the level of $^{*}P < 0.05$ and $^{**}P < 0.01$. IC50 values, the concentration of an extract to inhibit the growth rate of cells by 50%, were determined with the polynomial regression equation composed of the logarithmic values of four or six graded concentrations and the viabilities of cells induced by the rhizome extracts of wild sarsaparilla. Viability was calculated based on the formula of V (%) = T/C × 100, where V represents viability or survival rate, T represents absorbance value in extract-treated group and C, absorbance value in non-treated negative control group. The background MTT absorbance obtained from the blank control group was subtracted from all test groups.

Results

The cytotoxic effects of HRW against human cancer cell lines

IC50 values of the four tested human cancer cell lines induced by the six fractions extracted and separated from the rhizome of *A. nudicaulis* L. are listed in Table 1. The top one fraction with the highest cytotoxicity against WiDr and Molt cancer cells from the 6 fractions selected through in-vitro bioassay was HRW (hexane fraction from the rhizome of wild sarsaparilla), which significantly ($P < 0.01$) concentration-dependently reduced the viability or survival rates of these two cancer cell lines tested. The IC50 of HRW against WiDr

Table 1 Cytotoxic activity of six fractions extracted and separated from rhizome of *Aralia nudicaulis* L. (IC50; $\mu g\,mL^{-1}$)

Fractions	WiDr	Molt	T-470	HT29
T	130.5 ± 6.2	22.6 ± 1.2	65.0 ± 2.9	108.8 ± 7.8
H	21.9 ± 5.3	3.3 ± 0.3	17.6 ± 2.0	35.3 ± 3.7
EA	> 200	> 200	> 200	> 200
Bu	> 200	179.5 ± 19.2	> 200	> 200
W	> 200	> 200	> 200	> 200
Bo	> 200	> 200	> 200	> 200

T, total extract; H, hexane fraction; EA, ethyl acetate fraction; W, water fraction; Bo, boiling water fraction; WiDr, human colon cancer cell line; Molt, human leukaemia cell line; T-470, human breast cancer cell line; and HT29, human colon cancer cell line.

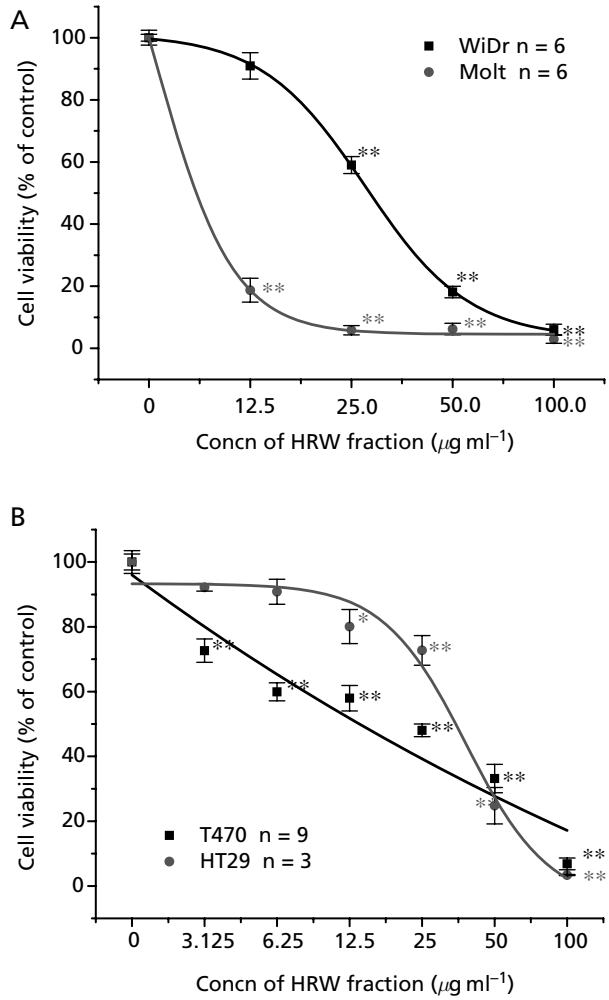

Figure 1 Viability of WiDr (human colon cancer cells) and Molt (human leukaemia cells) (A) and T-470 (human breast cancer cells) and HT29 (human colon cancer cells) (B) induced by HRW (hexane fraction extracted and separated from the rhizome of wild sarsaparilla). *$P < 0.05$ and **$P < 0.01$ vs negative control (0 $\mu g\,mL^{-1}$ of HRW); data are expressed as means ± s.e.m. from at least 3 repetitions performed in duplicate.

and Molt human cancer cells was $21.9 \pm 5.3\,\mu g\,mL^{-1}$ and $3.3 \pm 0.3\,\mu g\,mL^{-1}$, respectively. The minimum survival rates of WiDr and Molt human cancer cells induced by HRW were $6.0 \pm 1.7\%$ ($100\,\mu g\,mL^{-1}$) and $2.9 \pm 1.3\%$ ($100\,\mu g\,mL^{-1}$) (Figure 1A). HRW significantly ($P < 0.01$) eliminated the proliferation of T-470 and HT29 cells as well (Figure 1B). The minimum viabilities of these two cancer cells induced by HRW were $6.8 \pm 1.8\%$ ($100\,\mu g\,mL^{-1}$) with the IC50 of $17.6 \pm 2.0\,\mu g\,mL^{-1}$ for T-470 and $3.4 \pm 0.2\%$ ($100\,\mu g\,mL^{-1}$) with the IC50 of $35.3 \pm 3.7\,\mu g\,mL^{-1}$ for HT29.

The selective cytotoxic effect of HRW against normal human cells

HRW was also cytotoxic to the human normal cell line of HUV-EC in a concentration-dependent fashion (Figure 2A). For example, HUV-EC exhibited a viability of $11.6 \pm 1.5\%$ in the presence of 100 μg/ml HRW. The IC50 was $58.0 \pm 1.5\,\mu g\,mL^{-1}$ for HRW to induce cytotoxicity to HUV-EC, which was significantly greater than for the tested human cancer cells (Figure 2B). In other words, the concentration of HRW needed to kill normal HUV-EC was significantly ($P < 0.01$) higher than that needed to kill cancer cells.

Results showed that the viabilities of the tested human cancer cell lines were significantly reduced by some of the fractions. More specifically, HRW was identified as the most effective and selective anti-cancer cell natural product.

Discussion

Our results showed that the most effective anti-WiDr (human colon cancer cell line) and anti-Molt (human leukaemia cell line) fraction was HRW (IC50 = $21.9 \pm 5.3\,\mu g\,mL^{-1}$ and $3.3 \pm 0.3\,\mu g\,mL^{-1}$, respectively). HRW was also extremely potent in exterminating other types of human cancer cells lines, such as breast cancer (T-470) and colon cancer (HT-29) (Figure 1). More interesting was the selective cytotoxicity of HRW. Figure 2B showed that the IC50 of HRW for normal HUV-EC was 17.6 and 3.3 times greater than that for Molt and T-470 human cancer cells, respectively. Therefore, application of HRW to Molt and T-470 cells will have a great safety margin and effectiveness.

Even though the selective mechanism has not yet been clarified, it appears that the HRW particularly acted on cells with faster growth rate. The faster the cells grew, the higher were the cytotoxic effects induced by HRW. Molt cells grew very fast and 5×10^5 cells for the bioassay could be obtained in only 3–4 days, and HRW was very potent in killing these cells. On the other hand, the HUV-EC grew very slowly and the same amount of cells for the bioassay could not be obtained until culturing the cells for more than 30 days and, correspondingly, HRW was less potent in killing these cells in comparison with its effect on Molt cells.

Wild sarsaparilla is widespread and common across Canada and northern USA. In many locations, it forms the major part of herbaceous vegetation in forest. Wild sarsaparilla is a

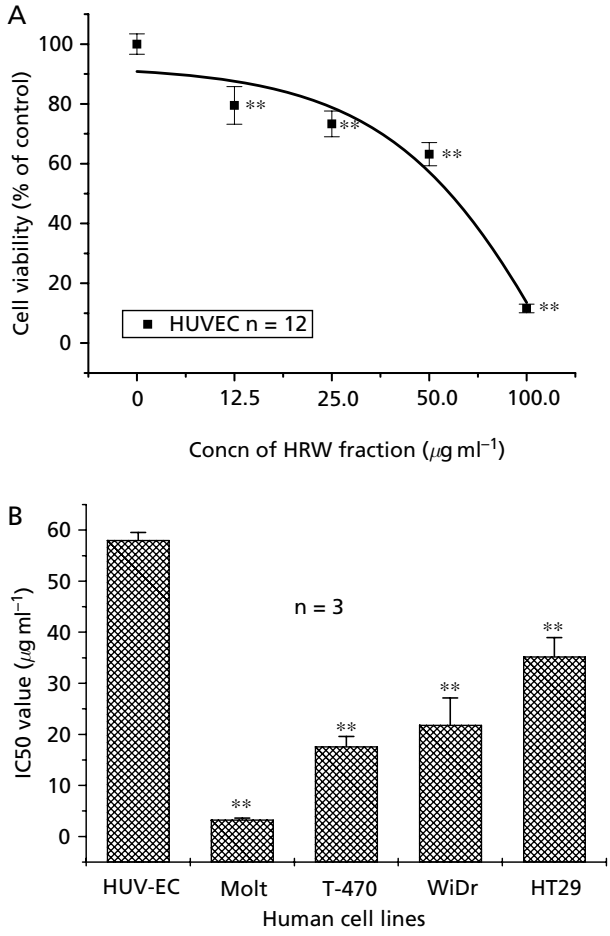

Figure 2 A. Viability of HUV-EC (normal human cells) induced by HRW (hexane fraction extracted and separated from the rhizome of wild sarsaparilla). $^{**}P < 0.01$ vs negative control (0 μg mL^{-1} of HRW); data are expressed as means ± s.e.m. from at least 3 repetitions performed in duplicate. B. Comparison of IC50 values induced by HRW among HUV-EC (normal human cells) and Molt (human leukaemia cells), T-470 (human breast cancer cells), WiDr (human colon cancer cells) and HT29 (human colon cancer cells). $^{**}P < 0.01$ vs HUV-EC; data are expressed as means ± s.e.m. from at least 3 repetitions performed in duplicate.

characteristic feature of parkland groves and wooded ravines of the prairie area in all parts except the extreme south and south-west (Vance et al 1999). In addition, the extraction procedure for obtaining HRW was simple (Huang et al 1997a) and the yield of 3.2% for HRW was high. These advantages make HRW an excellent candidate as a natural health product with great economical value.

The research for natural anti-cancer products from plants (Hsu 1980), mushrooms (Kodama et al 2003), insects and animals (Huang et al 1997b), and marine products (Nagle et al 2004) have been conducted for many years. Among the major obstacles for the development of natural anti-cancer products is the low selectivity between tumour and normal cells (Blagosklonny et al 2000). Arlin, a novel protein extracted from the shoots of *A. elata*, selectively induces apoptosis of HELA cells (Tomatsu et al 2003). Although *A. elata* is in the same genus as *A. nudicaulis*, HRW extracted from *A. nudicaulis* in our study was not the same as aralin obtained from *A. elata*. Not only are the species of *A. nudicaulis* and *A. elata* different, but also their distribution, plant parts from which the compounds are extracted and particularly the functional constituents differ. Firstly, *A. elata* mainly distributes in northeast of China and scarcely distributes in North Korea, Japan and Russia; *A. nudicaulis* mainly distributes in Canada with some in northern parts of the USA. Secondly, arlin was obtained from the shoots of *A. elata* but HRW was extracted from the rhizome of *A. nudicaulis*. Thirdly, aralin is a protein with A and B chains at 29.1 kD and 32.2 kD, respectively (Tomatsu et al 2003); in contrast, HRW is an extract obtained with the solvent hexane. Therefore, the main constituents in the hexane fraction would be oil, chlorophyll, phytosterol, terpenes, etc., and their molecular weights should be much smaller than those of proteins that exist in the water fraction (Chen 1993).

Several interesting observations have been made in this study. Firstly, the best extract among the 6 fractions extracted and separated from the rhizome of wild sarsaparilla was HRW, which had the highest potency and efficiency against the tested cancer cell lines. Secondly, not all tested cancer cell lines reacted to HRW equally. Although HRW exhibited potent cytotoxic activity against various types of human cancer cell lines, Molt cells proved to be the most sensitive to HRW. Thirdly, the potency and efficiency of HRW against normal HUV-EC cells were significantly lower than those for selective human cancer cells. It is thus expected that the side effects of HRW application in treating certain types of cancers would be significantly less. Finally, the plant resources of HRW are rich. The process procedure of HRW was simple and the yield of HRW was quite high. Taking all these in account, the costs for producing HRW would be much lower than for producing other synthetic anti-cancer compounds. Wild sarsaparilla, *A. nudicaulis*, has existed in Canada and northern America for millions of years. HRW extracted from this plant could be developed as a selective anti-cancer nutraceutical or pharmaceutical product with low side effects and low cost.

In the future, HRW will be purified and the chemical structures of the purified compounds with the maximum effectiveness will be identified. The cytotoxicity of the identified compounds will also be compared with that of known anti-cancer drugs presently used clinically. In-vivo testing with different doses of the compounds will be carried out as well.

References

Bae, K. H., Ji, J. M., Kang, J. S., Ahn, B. Z. (1994) A cytotoxic component from *Angelicae koreanae* Radis against L1210 and HL-60 cells. *Arch. Pharm. Res.* **17**: 45–48

Blagosklonny, M. V., Bishop, P. C., Robey, R., Fojo, T., Bates, S. E. (2000) Loss of cell cycle control allows selective microtubule-active drug-induced Bcl-2 phosphorylation and cytotoxicity in autonomous cancer cells. *Cancer Res.* **60**: 3425–3428

Bol'shakova, I. V., Lozovskaia, E. L., Sapezhinskii, I. I. (1997) Antioxidant properties of a series of extracts from medicinal plants. *Biofizika* **42**: 480–483

Chen, Q. (1993) *Methodology of pharmacological research for traditional Chinese medicine*. People's Health Press, Beijing, China, pp 65–70

Denizot, F., Lang, R. (1986) Rapid colorimetric assay for cell growth and survival modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. *J. Immunol. Methods* **89**: 271–277

Hernandez, D. E., Hancke, J. L., Wikman, G. (1988) Evaluation of the anti-ulcer and antisecretory activity of extracts of *Aralia elata* root and *Schizandra chinensis* fruit in the rat. *J. Ethnopharmacol*. **23**: 109–114

Hsu, B. (1980) The use of herbs as anticancer agents. *Am. J. Chin. Med*. **8**: 301–306

Huang, Y. G., Kang, J. K., Liu, R. S., Oh, K. W., Nam, C. J., Kim, H. S. (1997a) Cytotoxic activities of various fractions extracted from some pharmaceutical insect relatives. *Arch. Pharm. Res*. **20**: 110–114

Huang, Y. G., Kang, J. K., Liu, R. S., Oh, K. W., Nam, C. J., Lee, K. S., Kim, H. S. (1997b) Cytotoxic effects of various fractions extracted from some pharmaceutical insects and centipede. *Nat. Med*. **51**: 471–474

Johnson, D., Kershaw, L., MacKinnon, A., Pojar, J. (1995) *Plants of the western boreal forest & aspen parkland*. Lone Pine Publishing, Edmonton, Alberta, Canada, p. 196

Kodama, N., Komuta, K., Nanba, H. (2003) Effect of Maitake (*Grifola frondosa*) D-fraction on the activation of NK cells in cancer patients. *J. Med. Food* **6**: 371–377

Lee, Y. M., Kim, H., Hong, E. K., Kang, B. H., Kim, S. J. (2000) Water extract of 1:1 mixture of *Phellodendron cortex* and *Aralia* cortex has inhibitory effects on oxidative stress in kidney of diabetic rats. *J. Ethnopharmacol*. **73**: 429–436

Marles, R. J., Clavelle, C., Monteleone, L., Tays, N., Burns, D. (2000) *Aboriginal plant use in Canada's northwest boreal forest*. UBC Press, Vancouver, BC, Canada, pp 113–114

Mignogna, M. D., Fedele, S., Russo, L. L. (2004) The world cancer report and the burden of oral cancer. *Eur. J. Cancer Prev*. **13**: 139–142

Nagle, D. G., Zhou, Y. D., Mora, F. D., Mohammed, K. A., Kim, Y. P. (2004) Mechanism targeted discovery of antitumor marine natural products. *Curr. Med. Chem*. **11**: 1725–1756

Okuyama, E., Nishimura, S., Yamazaki, M. (1991) Analgesic principles from *Aralia cordata* Thunb. *Chem. Pharm. Bull*. **39**: 405–407

Saito, S., Ebashi, J., Sumita, S., Furumoto, T., Nagamura, Y., Nishida, K., Isiguro, I. (1993) Comparison of cytoprotective effects of saponins isolated from leaves of *Aralia elata* Seem. (Araliaceae) with synthesized bisdesmosides of oleanoic acid and hederagenin on carbon tetrachloride-induced hepatic injury. *Chem. Pharm. Bull*. **41**: 1395–1401

Tomatsu, M., Ohnishi-Kameyama, M., Shibamoto, N. (2003) Aralin, a new cytotoxic protein from *Aralia elata*, inducing apoptosis in human cancer cells. *Cancer Lett*. **199**: 19–25

Vance, F. R., Jowsey, J. R., Mclean, J. S., Switzer, F. A. (1999) *Wildflowers across the prairies*. GreyStone books, Douglas & Mcintyre Publishing Group, Vancouver, Toronto, Canada, p. 185

Wang, Z., Hu, J., Su, Z., Li. C., Li, R., Tang, H., Yi, Y. (1997) Liver-protective activity of *Aralia taibaiensis* Z. Z. Wang et H. C. *Zheng. Zhongguo Zhong Yao Za Zhi* **22**: 307–308

Xiao, K., Yi, Y. H., Wang, Z. Z., Tang, H. F., Li, Y. Q., Lin, H. W. (1999) A cytotoxic triterpene saponin from the root bark of *Aralia dasyphylla. J. Nat. Prod*. **62**: 1030–1032