Anticancer Effect of Extracts from a North American Medicinal Plant – Wild Sarsaparilla

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Abstract. *The wild sarsaparilla (Aralia nudicaulis) plant is richly distributed in North America, mainly in Canada. In the present study, 24 extracts were obtained from the rhizome, stem, leaf and fruit of wild sarsaparilla. In the presence of RH (hexane fraction from the rhizome), the survival rate of WiDr (human colon cancer cell) was $3.5\pm2.7\%$ ($IC_{50}=30.1\pm3.5$ μg/ml) and that of Molt (human leukemia cell) was $2.4\pm2.8\%$ ($IC_{50}=7.0\pm0.6$ μg/ml). The survival rate of HELA (human cervix cancer cell) was only $1.8\pm0.9\%$ in the presence of FH (hexane fraction from the fruit of wild sarsaparilla) ($IC_{50}=33.3\pm2.7$ μg/ml). The cytotoxicities of RH and FH against normal human umbilical vein endothelial cells were significantly lower than against the tested human cancer cells. RH appeared to be the best extract against WiDr and Molt, whereas FH was the most effective against HELA. Because of the rich natural supply, simple extraction procedure and high yield, RH and FH of wild sarsaparilla have the potential to be developed into selective anticancer nutraceutical and/or pharmaceutical products with few side-effects and low cost.*

It has been estimated that the morbidity of cancer will keep increasing at an alarming rate from 10 million new cases globally in 2000 to 15 million in 2020 (1). While surgical treatment cannot be applied once cancer has spread, radiotherapy and chemotherapy do not distinguish normal cells from cancer cells and can induce serious side-effects. Therefore, these anticancer therapies in practice

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cannot cope with the increasing cancer incidence. Novel anticancer agents with improved effectiveness, few side-effects and reduced cost are in great demand.

Aralia nudicaulis L., a member of the ginseng family of Araliacea, is commonly known as wild sarsaparilla, and grows abundantly in shady rich woodland across Canada and northern USA (2). Traditional uses of this plant as a medicine by Aboriginal peoples of Canada's northwestern boreal forest have been recorded. The rhizome can be chewed or made into a tea to treat "heart pain", chronic chest trouble, upset stomach, liver problems and sore throat (2). The Woods Cree used wild sarsaparilla in boiled teas to treat infected gums of teething children, venereal disease, pneumonia and low milk production in nursing mothers. Poultices made from bruised bark or chewed woody rhizomes of wild sarsaparilla were applied to wounds to promote healing and to draw out infection (3). To date, the anticancer properties of this plant has not been explored.

Previous studies on the plant species in the same genus, such as *Aralia elata* (4) and *Aralia cordata* (5), have revealed their anti-ulcer and analgesic effects. The liver-protective activities of the leaf extract from *Aralia elata* (6) and of the root and cortex extract from *Aralia taibaiensis* (7) have also been studied. In addition, the antioxidant property of *Aralia elata* (8) and anti-diabetic activity of the root extract from *Aralia cachemirica* (9) have been explored. The bark extract from *Aralia decaisneana* (10) and the shoot extract from *Aralia elata* (11) also exhibit cytotoxic activities.

The anticancer activities of different extracts from different plant parts of *Aralia nudicaulis* were investigated and compared. The viabilities of three types of human cancer cells were determined after exposure to different plant extracts. The hexane fraction extracted and separated from the rhizome of wild sarsaparilla (RH) and the hexane fraction from the fruit (FH) of this plant were identified as the most effective and selective anticancer products for specific types of cancer cells.

Materials and Methods

Plant materials. Wild sarsaparilla was collected from the boreal forest near La Ronge, Saskatchewan, Canada, and the plants were processed and divided into rhizomes, stems, leaves and fruit. 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.

Reagents. Methanol, hexane, ethyl acetate, butanol, sodium bicarbonate and dimethyl sulfoxide (DMSO) were obtained from Merck (Darmstadt, Germany). RPMI-1640 medium, Nutrient Mixture F-12 Ham Kaighn's Modification (F12K), polyoxyethylenesorbitan monooleate (Tween 80), cell freezing medium-DMSO, trypsin-EDTA solution, endothelial cell growth supplement, heparin and the penicillin/streptomycin (P/S) solution were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). C,N-diphenyl-N'-4,5-dimethyl thiazol-2-yl tetrazolium bromide (MTT) was from Promega Corporation (Madison, WI, USA), while fetal bovine serum (FBS) was from Invitrogen Canada Inc. (Burlington, Ontario, Canada).

Extract preparation. The collected rhizomes, stems, leaves and fruit of wild sarsaparilla were dried at room temperature for 2 weeks and then in an oven at 45°C for 48 h. The water content of each plant part was calculated by the formula: WC (%)=(W1 – W2) / W1 x 100, where WC represents the water content and W1 and W2 represent the fresh and dry weight of the plant part, respectively. The dried materials of the four plant parts were ground to powder using a blender (ChampHP3, Model ES-3, K-TEC, Orem, UT, USA). The powder was soaked with methanol and the total extract of each plant part was obtained by recovering the methanol with an evaporator (Buchi Rotavapor R-2000, CH-9230 Flawil, Switzerland) at 45°C. The total extracts of rhizome, stem, leaf and fruit were further partitioned with hexane, ethyl acetate, butanol and water, respectively. The residue, after filtration, was processed with boiling water to obtain the boiling water fraction. The extraction flow chart is shown in Figure 1 (12).

Cell culture. WiDr cells from human colon adenocarcinoma, Molt cells from human T-cell leukemia and HELA cells from human cervix epitheloid carcinoma were cultured with RPMI-1640 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 of heparin, 0.06 g/L of ECGS, 2.5 g/L of NaHCO$_3$, 15% FBS and 1% P/S. All the cancer cell lines had been provided by the Saskatoon Cancer Centre and HUV-EC was obtained from the American Type Culture Collection. The culture media were changed twice a week (13). The cells were grown in 25-cm^2 tissue culture flasks (Becton Dickinson Labware, Franklin Lakes, NJ, USA) and maintained in a humidified (95% air and 5% CO$_2$) incubator (Model 3110, ThermoForma, Marietta, OH, USA) at 37°C.

Cytotoxicity assay in vitro. Each plant fraction of wild sarsaparilla was added to culture medium in 96-well plates (MICROTEST™96, Becton Dickinson and Company) to reach different final concentrations. Thereafter, 5000 cells were added to each well and incubated for 48 h at 37°C in a humidified environment containing 5% CO$_2$. Subsequently, 15 μl of MTT solution were added to every well followed by an additional 4 h of incubation. 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 a wavelength of 595 nm and reference wavelength of 655 nm with a plate reader (Model 1500, Multiskan Spectrum, Thermo Labsystems, Nepean, ON, Canada). A 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-colored MTT dye (low absorbance) becomes the blue-colored formazan product (high absorbance) by the mitochondrial enzyme succinate-dehydrogenase. This conversion only takes place in living cells and the amount of formazan produced is proportional to the number of vital cells present (14). The lower the absorbance values, the fewer cells survive and the lower viabilities of the tested cells. The highest final concentration of DMSO as the solvent was less than 0.1%. At this concentration, DSMO did not alter the cell viability (data not shown).

Data calculation. All data were expressed as means±SE from three repetitions performed in duplicate. The statistical analyses were carried out using the unpaired Student's *t*-test in conjunction with the Newman-Keuls test and analysis of variance for repeated measures ANOVA where appropriate. Differences in cellular viabilities, or survival rate, between treatment groups and the negative control group were considered statistically significant at the level of *$p<0.05$ and ** $p<0.01$. IC$_{50}$ values, the concentration of an extract to cause 50% cell death, were determined with the polynomial regression equation composed of the logarithmic values of four graded concentrations and the viabilities of cells induced by the plant extracts of wild sarsaparilla. Viability was calculated based on the formula: V (%)=T / C x 100, where V represents the viability or survival rate, T represents the absorbance value in the extract-treated group, and C the absorbance value in the non-treated negative control group. The background MTT absorbance obtained from the blank control group was subtracted from all the test groups.

Results

Water content of each plant part and yield of the fractions. The water contents were 60.9% for rhizomes, 70.8% for stems, 72.2% for leaves and 45.3% for fruit (Figure 2A). The yields of total extracts (weight of dried total extract / weight of raw plant powder, g/g x 100%) were 15.0% for the rhizome total extract, 15.0% for the stem total extract, 21.0% for the leaf total extract and 56.0% for the fruit total extract. The yields of the hexane, ethyl acetate, butanol and water fractions (weight of dried fraction / weight of dried total extract from which the fraction is partitioned, g/g x 100%) and boiling water fractions (weight of dried boiling water fraction / weight of dried residue after filtration, g/g x 100%) are provided in Figure 2B. For example, the yields of RH (rhizome hexane fraction) and FH (fruit hexane fraction) were 21.0% and 2.4%, respectively.

Cytotoxic effects of selected fractions. The top six fractions with the highest cytotoxicities against WiDr, Molt and HELA cancer cells and average IC$_{50}$ values lower than 135.7±37.4 μg/ml were selected from the 24 fractions by
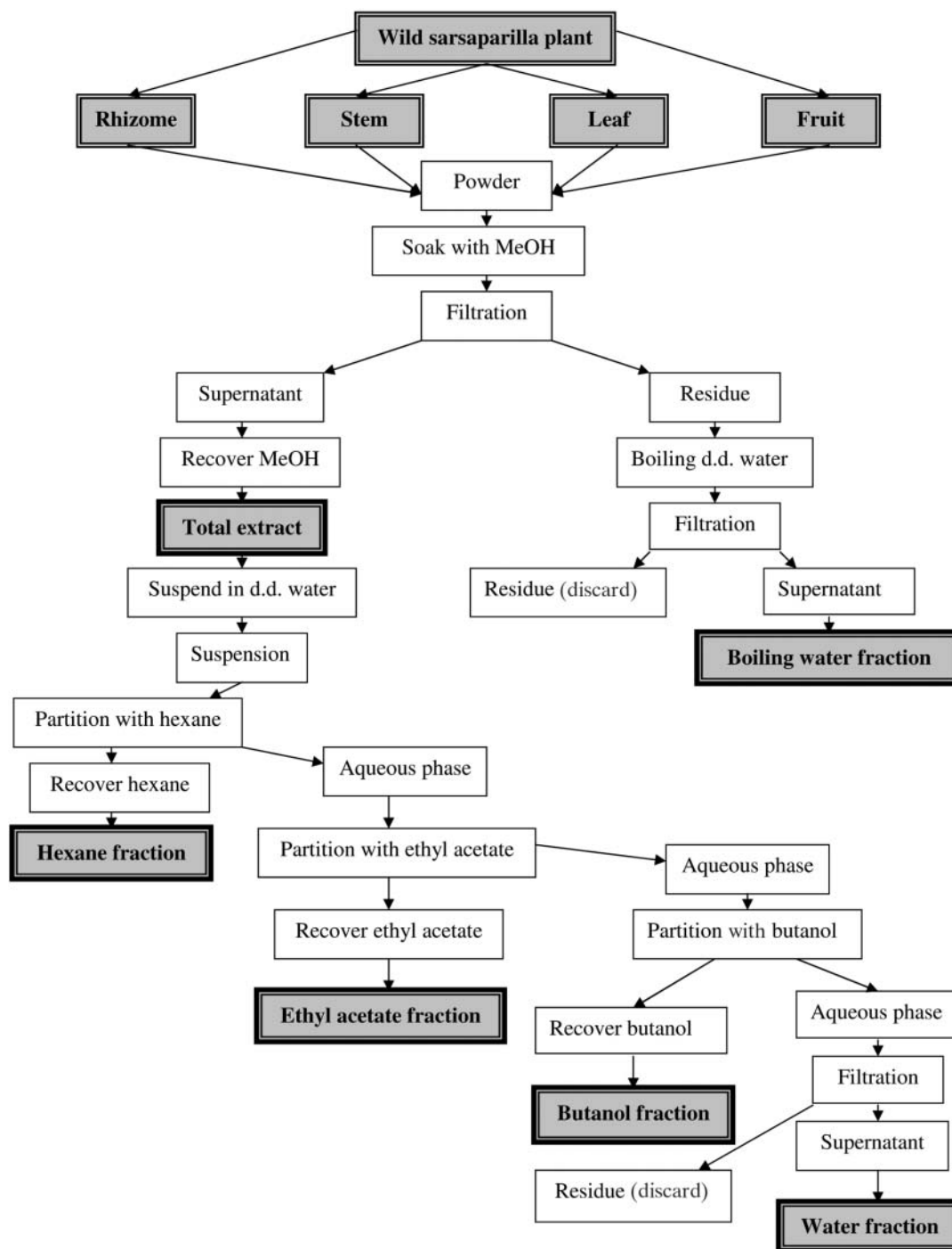
Figure 1. *Flow chart for the preparation of the total extract and the hexane, ethyl acetate, butanol, water and boiling water fractions from the rhizome, stem, leaf and fruit of wild sarsaparilla.*

means of an *in vitro* bioassay. All of these six fractions concentration-dependently reduced the viabilities or survival rates of the three cancer cell lines tested. The average IC_{50} of these fractions against the cancer cell lines were 26.0 ± 10.0 μg/ml for RH, 48.0 ± 11.3 μg/ml for FH, 44.6 ± 14.5 μg/ml for the stem extract in hexane (SH), 70.5 ± 9.1 μg/ml for the leaf extract in hexane (LH), 87.1 ± 33.0 μg/ml for the rhizome total extract (RT) and 135.7 ± 37.4 μg/ml for the stem extract in ethyl acetate (SEA), respectively.
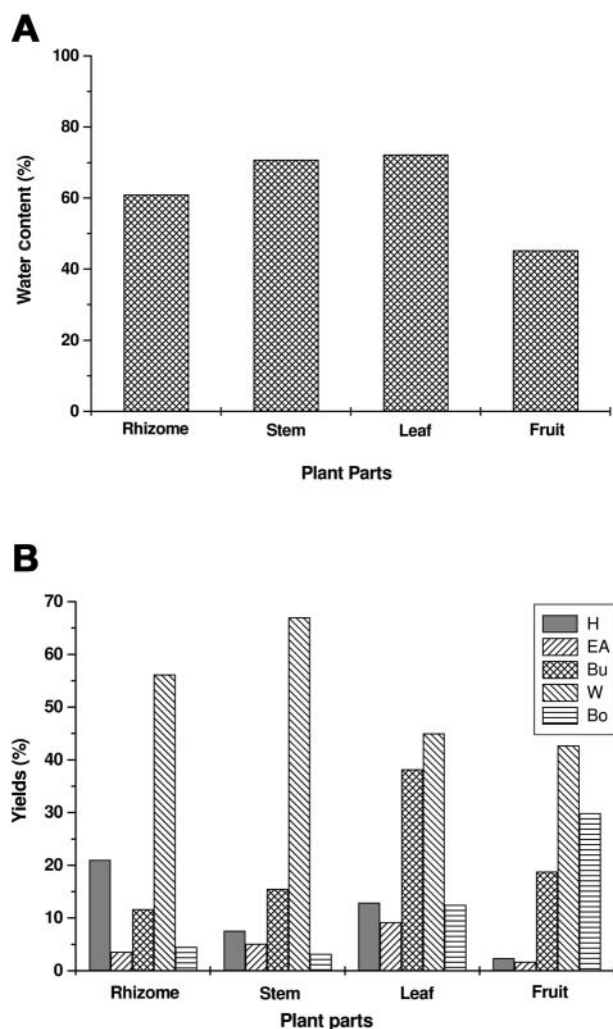
A

B

Figure 2. *(A) Water contents of the four plant parts and (B) yields of fractions obtained from the total extracts (that of boiling water fractions was from residue) of four plant parts. H (hexane fraction), EA (ethyl acetate fraction), Bu (butanol fraction), W (water fraction) and Bo (boiling water fraction).*
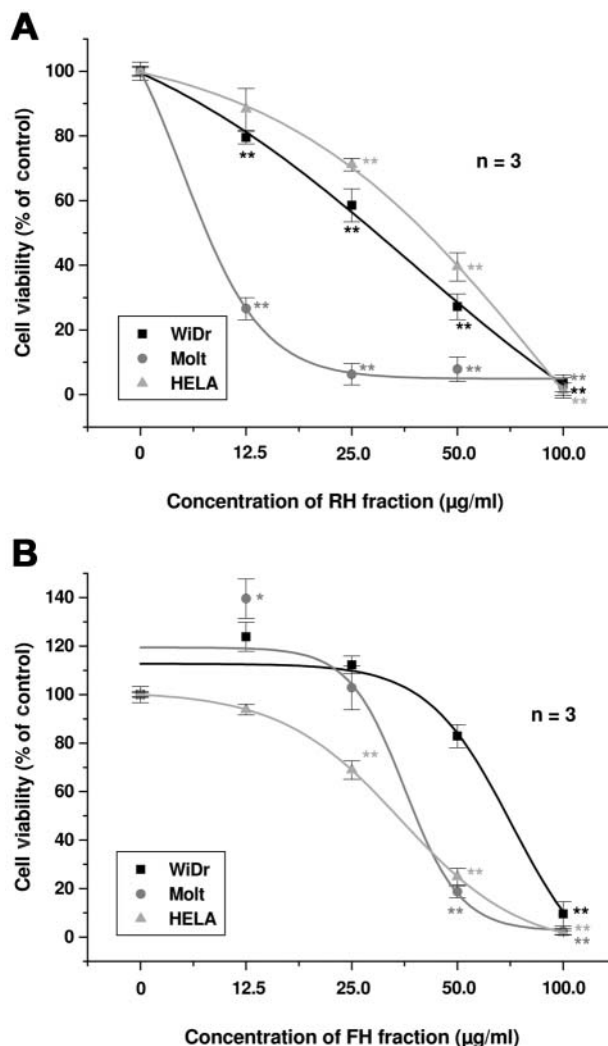
A

B

Figure 3. *Viabilities of WiDr – human colon cancer cells, Molt – human leukemia cells and HELA – human cervix cancer cells induced by RH (rhizome hexane fraction) (A) and FH (fruit hexane fraction) (B). *$p<0.05$ and **$p<0.01$ vs. negative control.*

The minimum survival rates of the WiDr, Molt and HELA cancer cells induced by RH were 3.5±2.7, 2.4±2.8 and 1.3±2.3% with IC_{50}s of 30.1±3.5, 7.0±0.6 and 40.8±2.1 μg/ml (Figure 3A). With FH at 100 μg/ml, the survival rates were 9.6±5.0, 2.4±1.3 and 1.8±0.9% for WiDr, Molt and HELA with IC_{50}s of 70.1±3.3, 40.5±1.9 and 33.3±2.7 μg/ml, respectively (Figure 3B).

The SH and LH also proved toxic to the tested cancer cells. The IC_{50}s of SH against WiDr, Molt and HELA were 73.1±2.9, 25.7±1.4 and 35.2±1.7 μg/ml, with minimum survival rates of 4.6±3.6, 5.3±3.2 and 2.7±1.6%, respectively (Figure 4A). The IC_{50}s of LH against WiDr, Molt and HELA were 83.0±7.3, 52.7±1.2 and 75.2±2.0 μg/ml, with

minimum survival rates of 35.2±6.2, 1.1±0.6 and 39.3±2.0%, respectively (Figure 4B). Although the RT and SEA extracts were effective against the three human cancer cell lines, their cytotoxic effects were significantly less than those of the hexane fractions (Figure 5A and 5B). The cytotoxic effects of another 18 fractions extracted from the plant parts against the human cancer cell lines were minimal (data are not presented).

Selective cytotoxicity of RH and FH against normal human cells. The most effective fractions against the WiDr, Molt and HELA cancer cells were RH and FH. However, the minimum survival rates of non-cancer HUV-EC cells
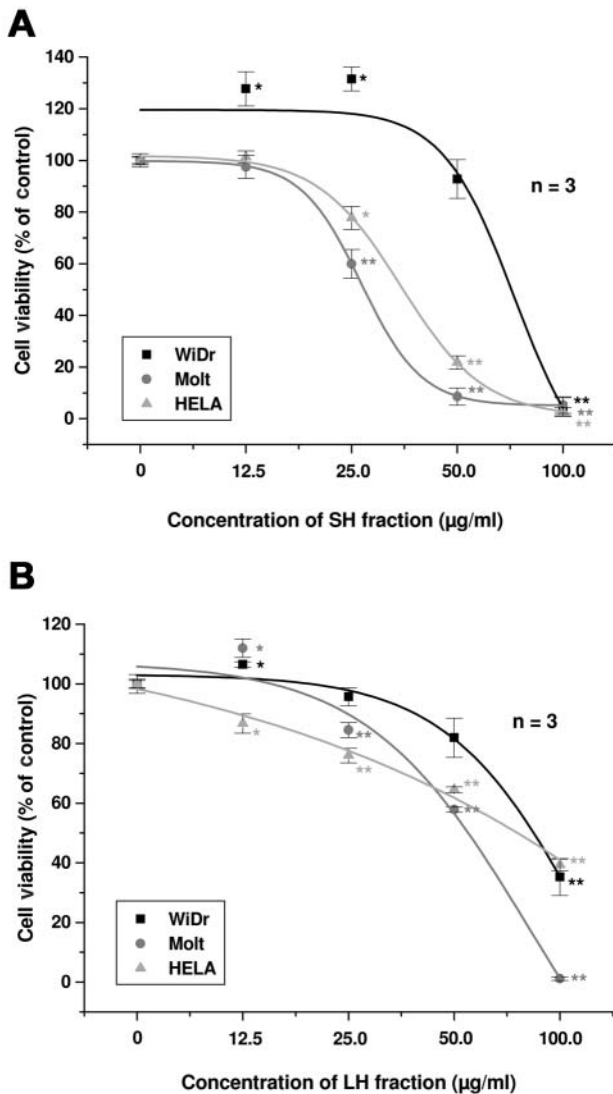
Figure 4. *Viabilities of WiDr – human colon cancer cells, Molt – human leukemia cells and HELA – human cervix cancer cells induced by the SH (stem hexane fraction) (A) and LH (leaf hexane fraction) (B). *p<0.05 and **p<0.01 vs. negative control.*

Figure 5. *Viabilities of WiDr – human colon cancer cells, Molt – human leukemia cells and HELA – human cervix cancer cells induced by RT (rhizome total extract) (A) and SEA (stem ethyl acetate fraction) (B). *p<0.05 and **p<0.01 vs. negative control.*

induced by RH and FH were as high as 90.3±9.6% and 78.1±14.1%, respectively. These survival rates were not significantly different from the negative control ($p>0.05$) (Figure 6A).

The IC_{50} of RH was 393.0±36.4 μg/ml for cytotoxicity against HUV-EC, which was significantly greater ($p<0.01$) than those for the tested human cancer cells. The IC_{50} of FH against HUV-EC was 206.0±48.1 μg/ml, which was also significantly greater ($p<0.05$) than those for Molt, WiDr and HELA (Figure 6B). The concentrations of RH and FH required for normal HUV-EC cell death was significantly higher than those required for the cancer cells.
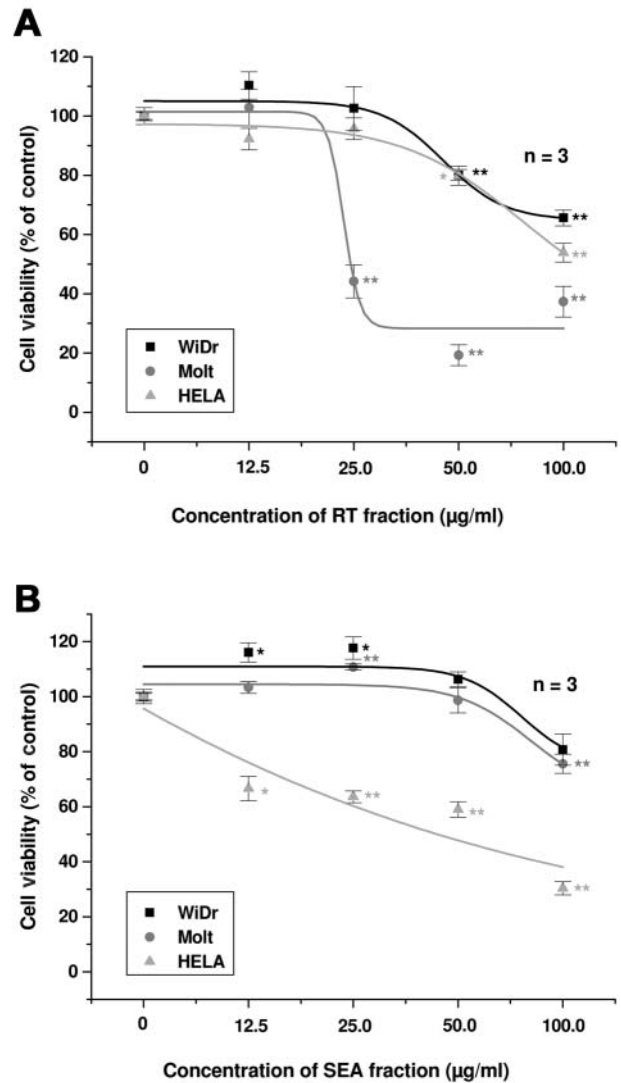
Discussion

The plant of wild sarsaparilla is widespread and common across Canada and northern USA. In many locations, it forms the major part of the herbaceous vegetation in forests. Wild sarsaparilla is a characteristic feature of parkland groves and wooded ravines of the prairie area in all parts except the extreme South and Southwest (15). In our study, different fractions were extracted from wild sarsaparilla and their anticancer effects were tested on cultured human cancer cell lines. It was found that four hexane fractions showed the highest anticancer potency
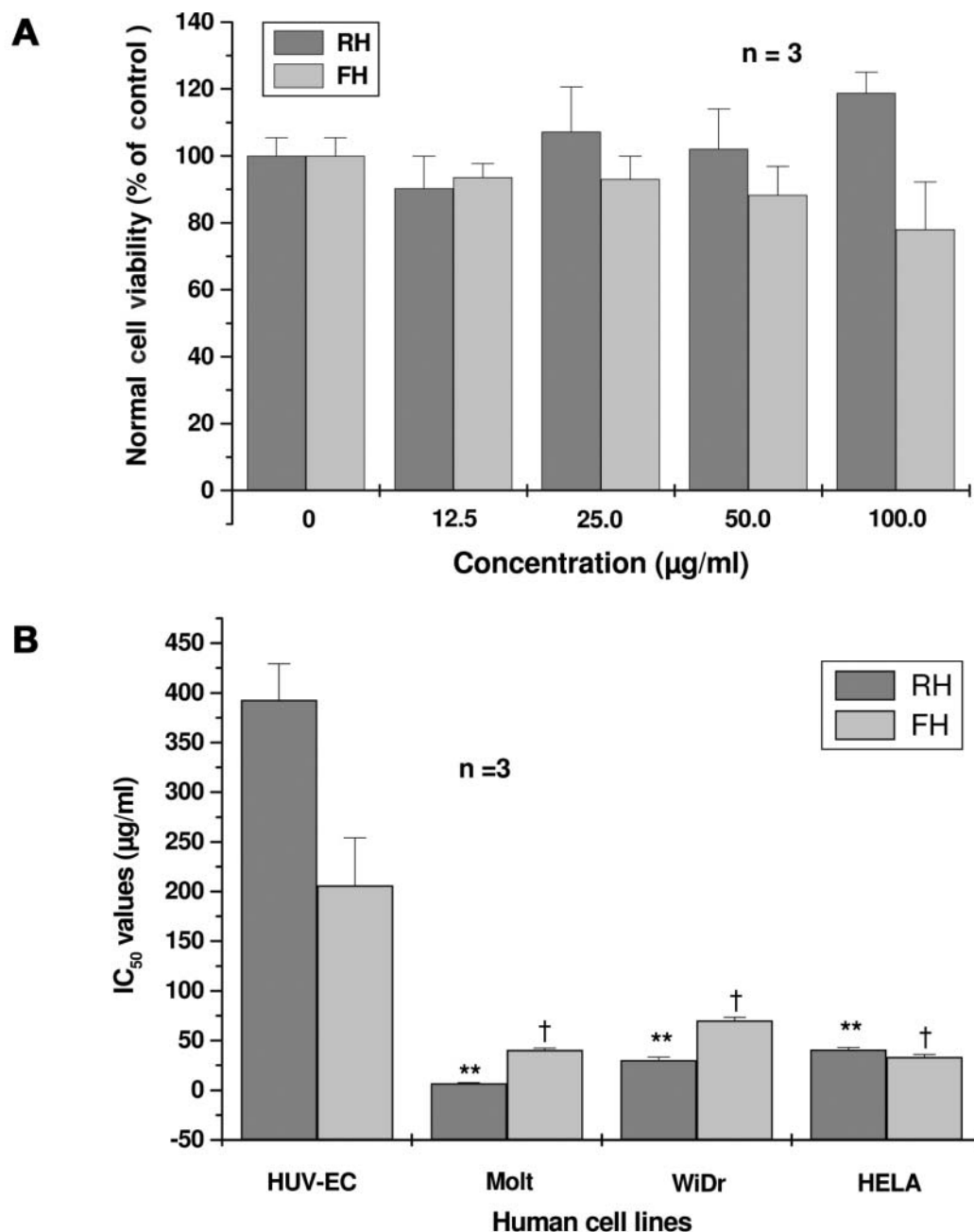
Figure 6. *(A) Viabilities of HUV-EC – normal human cells induced by RH (rhizome hexane fraction) and FH (fruit hexane fraction). (B) Comparison of IC$_{50}$ values induced by RH and FH against a normal human cell and human cancer cells lines. ** p<0.01 vs. normal HUV-EC cells for RH and †p<0.05 vs. normal HUV-EC cells for FH.*

among the 24 extracts. The highest yield among these fractions from each total extract was RH (21.0%), followed by LH (12.9%), SH (7.6%) and FH (2.4%) (Figure 2B).

The smaller the IC$_{50}$ value, the more potent the anticancer effect of the fraction. Our results showed that the most effective anti-WiDr and anti-Molt fraction was RH (IC$_{50}$=30.1±3.5 μg/ml and 7.0±0.6 μg/ml) and the most

effective anti-HELA fraction was FH (IC$_{50}$=33.3±2.7 μg/ml). On the other hand, RH and FH displayed no significant cytotoxicity to normal HUV-EC cells. The IC$_{50}$ of RH for normal HUV-EC was approximately 56.1, 13.1 and 9.6 times greater than that for Molt, WiDr and HELA, respectively. Similarly, the IC$_{50}$ of FH for normal HUV-EC was about 5.1, 2.9 and 6.2 times larger than that for Molt, WiDr and HELA

cells, respectively. Therefore, the application of RH and FH to Molt, WiDr and HELA cells demonstrated a great safety margin and effectiveness.

Even though the selective anticancer mechanisms have not yet been clarified, it appears that RH and FH acted particularly on cells with fast growth rates. The faster the cells grew, the greater the cytotoxic effects induced by RH and FH were. Molt cells grew very fast and 5×10^5 cells for the bioassay could be obtained in only 3-4 days; both RH and FH were very potent in killing these cells. On the other hand, the HUV-EC cells grew very slowly, requiring more than 30-day culture to obtain the same number of cells for the bioassay. Correspondingly, RH and FH were much less potent in killing these cells in comparison with their effects on Molt cells.

Comparing RH and FH, we suggest that RH should be further developed. Both the anti-Molt and anti-WiDr activities of RH were significantly stronger ($p < 0.01$) than those of FH in terms of the relative IC_{50} values. However, no significant difference ($p > 0.05$) in anti-HELA activity between RH ($IC_{50} = 40.8 \pm 2.1$ μg/ml) and FH ($IC_{50} = 33.3 \pm 2.7$ μg/ml) was found (Figure 3A, B). In addition, the cytotoxicity of RH against normal cells with a IC_{50} of 393.0 ± 36.4 μg/ml was significantly less ($p < 0.05$) than that of FH with a IC_{50} of 206 ± 48.1 μg/ml (Figure 6B). The yield from the total extract for RH (21.0%) was also about 8.8 times higher than that for FH (2.4%) (Figure 2B).

Investigation of natural anticancer agents from plants (16), mushrooms (17), insects and animals (18) and marine products (19) have been conducted for many years. However, the cytotoxic and anticancer effects of the natural extracts from wild sarsaparilla have not, to our knowledge, been investigated. Among the major obstacles for the development of natural anticancer agents is the low selectivity against tumor and normal cells (20). Aralin, a protein, extracted from the shoots of *Aralia elata,* selectively induced apoptosis of HELA cells (11). Although *Aralia elata* is in the same genus as *Aralia nudicaulis*, the RH and FH extracted from *Aralia nudicaulis* in our study did not contain Aralin obtained from *Aralia elata*. Not only the species of *Aralia nudicaulis* and *Aralia elata* are different, but their distribution, plant parts from which the compounds are extracted and, particularly, the functional constituents differ as well. *Aralia elata* is mainly distributed in Northeast China, and is scarcely distributed in North Korea, Japan and Russia. *Aralia nudicaulis* is found in Canada and some in Northern USA. Aralin was obtained from the shoots of *Aralia elata*, but the RH and FH were extracted from the rhizome and fruit of *Aralia nudicaulis*. Aralin is a protein with A and B chains of 29.1 kDa and 32.2 kDa, respectively (11). In contrast, the RH and FH hexane extracts contain oil, chlorophyll, phytosterol, terpene, *etc*., with much lower weights than those of proteins that exist in aqueous fractions.

The plant resources of RH and FH are rich and the procedure for RH and FH extraction was simple and afforded high yields. RH and FH extracted from wild sarsaparilla could be developed as selective anticancer nutraceutical and/or pharmaceutical natural health products with few side-effects and low cost. Future studies will include the purification of RH and FH and the identification of the chemical structures of the purified compounds with maximum effectiveness. The anticancer properties of the identified compounds will also be compared with that of known anticancer drugs currently in clinical use.

Acknowledgements

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