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The Cytotoxic Effect of Two Chemotypes of Essential Oils from the Fruits of *Angelica archangelica* L.

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Abstract. *Background: The aim of this work was to study the constituents and cytotoxicity of the essential oils from the fruits of Angelica archangelica growing in Iceland. Materials and Methods: Three samples of essential oils were prepared by steam distillation. Their composition was established with GC/MS. The effects of the oils were examined in PANC-1 human pancreas cancer cells and Crl mouse breast cancer cells in concentrations ranging from 10-400 μg/ml, measuring the reduction of the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium (MTS) by mitochondrial enzymes. Results: Two types of essential oils were found, differing mainly in the absence or presence of β-phellandrene.* The *ED_{50} of the oils ranged from 48.6 μg/ml to 108.3 μg/ml for PANC-1 and 48.0 μg/ml to 91.8 μg/ml for Crl cells. Conclusion: The cytotoxic activity of the essential oils was independent of the quantity of their main components.*

Angelica archangelica has been long and widely used in folk medicine, and is one of the most respected medicinal herbs in northern countries, where it was cultivated during the Middle Ages, and exported to other parts of Europe. The most characteristic secondary metabolites of its fruits are essential oils and furanocoumarins.

Monoterpene hydrocarbons are the main constituents of the essential oils from the fruit of *A. archangelica* (1, 2). β-Phellandrene is usually considered to be the main compound of the essential oils of *A. archangelica* fruit and root (3). The essential oils of *A. archangelica* fruit growing in Iceland have not been previously studied. The essential oil of *A. archangelica* root of Icelandic origin has, however, been studied, showing that the Icelandic roots differ from Finnish roots by their low β-phellandrene content (4).

Cytotoxicity has been reported for many essential oils (5-8). Some monoterpenes, particularly limonene and perillyl alcohol, have been extensively studied in connection with cancer, and have been shown to be cytotoxic against various tumour cell lines (9, 10). The monoterpenes have been reported to affect the cells in various ways – for example perillyl alcohol, as well as geraniol and farnesol, induce apoptosis (11), but it has also been shown to cause cytostasis (12).

In this study, we demonstrate two chemotypes of *A. archangelica* L. with significantly different essential oil composition and cytotoxicity.

Materials and Methods

Essential oils. Fruit samples A and B were collected in Reykjavik, from separate locations. Each sample was collected from one plant. There was no apparent morphological difference between the plants or their fruits. Fruit sample C was collected outside Reykjavik, and was a pooled sample from about 50 individual plants. When collected, the fruits had typically reached their full size, but had not yet dried. The fruits were dried at room temperature. The essential oils were isolated by steam distillation, by passing steam through the fruits (70 g), condensing and collecting in a special trap. After 5 hours, the oils were separated from the water-phase. For cytotoxicity assays, stock solutions of the oils were made, approximately 1% in 60% aqueous ethanol, and stored under nitrogen at room temperature in the dark.

GC/MS. Essential oils were diluted 10,000-fold in n-hexane prior to analysis. Mass spectrometric identification was performed on an HP 6890 GC coupled to an HP 5973 quadrupole mass selective detector operated at 70 eV, using a scan rate of 1100 amu/s, electron multiplier voltage of 1600 V and ion source temperature of 250˚C. The GC/MS was controlled by the program HP ChemStation B.02.05. The fractions were analyzed on fused silica column DB-225 30 m x 0.25 i.d. The oven temperature was programmed from 40˚C isothermal 3 min, 40-80˚C, rate 5˚C/min, 80-210˚C, rate 10˚C/min, and finally isothermal at 210˚C 4.5 min, with an injection temperature of 250˚C. Helium was used as the

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Key Words: A. archangelica, essential oils, cytotoxicity, Crl, β-phellandrene.

Table I. *Content of essential oils and the most prominent compounds in the fruit samples.*

Fruit sample	Oil content (%)	most prominent compound
A	0.17	α-pinene
C	0.32	β-phellandrene
D	0.51	β-phellandrene

carrier gas (flow-rate 1 ml/min). Identification of the constituents was based on the retention time and on computer matching against the spectra library Wiley275. The relative quantity of compounds was estimated by integrating the total ion content of the individual peaks.

Cancer cells. The human pancreas cancer cell line PANC-1 and the mouse breast cancer cell line Crl (American Type Culture Collection, Rockville, MD, USA) were cultured in RPMI-1640 medium with 10% foetal calf serum, 50 I.U. ml^{-1} penicillin, 50 μg ml^{-1} streptomycin, 0.01 M HEPES-buffer and 0.2 M L-glutamine (all from Gibco, Paisley, UK). The cultures were incubated at 37˚C in 95% humidity and 5% CO_2. The cultures were passed once a week by trypsinization using a 1:30 dilution of standard Gibco trypsin-EDTA solution.

Assessment of cytotoxicity. The cells were trypsinized, counted and placed in 96-well plates at 10^4 cells per well. The test compound was added at the start, having been dissolved and serially diluted in 60% (v/v) ethanol. The ethanol concentration in each culture, including the controls, was 3% (v/v). In each test, the highest concentration of test sample was 300-400 μg/ml, which was then diluted three-fold at each step, the third dilution thus being 11-15 μg/ml, depending on the stock solution in question. Survival and proliferation of the cancer cells was assessed 24 h later with the CellTiter 96TM AQ_{ueuous} Non-Radioactive Cell Proliferation Assay (SDS, Falkenberg, Sweden), as described by the manufacturer. The 490-nm absorbance of formazan was followed for 3 h after the addition of MTS/PMS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulpho-phenyl)-2*H*-tetrazolium/phenazine methosulphate). Cytotoxicity was observed as the reduced change in absorbance in cultures containing sample at 490 nm as compared with control cultures.

Results

Oil quantity and composition. Due to the relatively small quantity of fruits steam-distilled, the quantity of distilled oil could not be established with high accuracy. However, it was clear that the oil content of the fruit sample containing most oil was about three times higher than that of the sample of the lowest oil content, ranging from 0.17-0.51%, as shown in Table I. The composition of the oils was different, falling into two categories characterized by the presence or absence of β-phellandrene. In B and C, β-phellandrene was the prevalent compound, whereas it was absent in A.

Table II. *Relative amount of known compounds in oils A, B and C (%).*

Rt	Compound	A	B	C
5.493	α-pinene*	41.4	28.9	14.4
7.462	β-pinene*	1.7	1.2	0.7
7.781	sabinene	2.1	0.3	2.4
8.274	myrcene	0	0	2.1
8.48	α-phellandrene	0	1.7	3.4
9.13	limonene	0.5	1.2	1.7
9.743	β-phellandrene	0	37.8	55.2
15.698	bicycloelemene	3.2	0.8	0.8
15.831	α-copaene	0.6	0.3	0.3
17.256	γ-elemene	2.4	0.2	0.9
18.135	α-zingiberene	3.6	0.4	0.6
18.566	AR-curcumene	0.9	0	0.2
18.748	β-sesquiphellandrene	0.7	0	0.1
18.828	bicyclogermacrene	10.1	3.0	3.0
19.68	germacrene B	1.3	0.1	0.6
21.808	(+) spathulenol	1.6	0.3	0.7
	Sum	70.2	76.1	87.1

*identification confirmed with authentic standard
Rt = retention time

The composition of the oils is shown in Table II. Compounds that accounted for less than 0.5% in all oils, and those that could not be identified with reasonable certainty, were neglected.

Cytotoxicity. The cytotoxicity of the oils on the two cancer cell lines is shown in Figures 1 and 2. The ED_{50} values are given in Table III.

The cell lines reacted to the oils in a similar manner. Oils A and C had most activity on both cell lines, A being more active on the Crl cells, whereas C had more cytotoxic effect on the PANC-1 cells. Oil B, with a composition similar to that of oil C, had less effect on both cell lines.

Discussion

Although each oil sample was unique, the chemotypes fell into two major categories, depending on the presence or absence of β-phellandrene. Preliminary studies, in which 20 plants from two locations in Reykjavık were screened for absence of β-phellandrene, indicated that 10-30% of *A. archangelica* plants in those locations are devoid of β-phellandrene (results not shown). β-phellandrene is usually one of the major compounds in *A. archangelica* essential oils, both in the fruit as well as in the root. Sample A was chosen for its lack of β-phellandrene. The fact that Icelandic *A. archangelica* roots seem to contain much less β-phellandrene than their Finnish counterparts (4) could

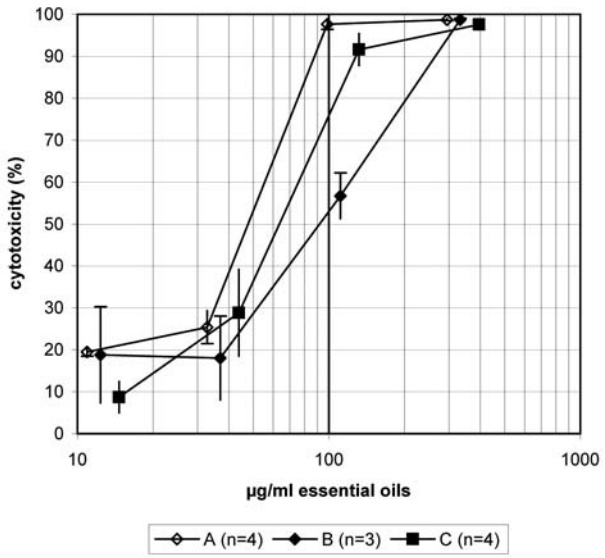

Figure 1. *The dose-dependent cytotoxic effect on Crl by the essential oil samples A, B and C. Cytotoxicity was measured as the reduced change in absorbance in cultures containing sample at 490 nm as compared with control cultures. Each point represents the average from three or four separate measurements, each done in triplicate, and the standard deviation of the mean.*

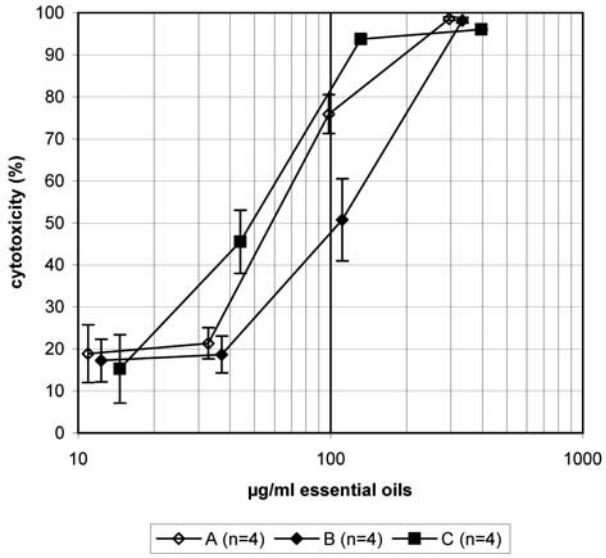

Figure 2. *The dose-dependent cytotoxic effect on PANC-1 by the essential oil samples A, B and C. Cytotoxicity was measured as the reduced change in absorbance in cultures containing sample at 490 nm as compared with control cultures. Each point represents the average from three or four separate measurements, each done in triplicate, and the standard deviation of the mean.*

indicate that Icelandic *A. archangelica* is unusual in this respect. It is worth mentioning that one study has reported low β-phellandrene in Scandinavian *A. archangelica* roots (13).

The cell lines were similar in their response to the oils. However, there was some difference in the reaction to oils A and C, A affecting the mouse mammary cancer cells (Crl) most, whereas oil C had the greatest effect on the human pancreas cancer cells (PANC-1). Oils A and C, which differed markedly in composition, were most active, with EC_{50} of 47-65 µg/ml. The activity of oil B was considerably less, although the composition was similar to C, with regards to the predominant compounds of the oils.

If the activity were due to a single component, it would be expected to be found in similar amounts in oils A and C, and at about half that amount in oil B. Comparing the chromatograms, no such component, identified or unidentified, could be found among those present in more than 0.5% in any oil. The alternative explanation is that more than one compounds act together, synergistically or cumulatively. This possibility has been discussed in the literature, where it was pointed out that different components have been shown to affect cells in a different manner (6). Furthermore, one cannot exclude that the activity pertains to a component occurring at less than 0.5%. However, its EC_{50} would thus be about 0.25 µg/ml or less, which would make it an extremely active compound.

Table III. *Cytotoxic activity of essential oil samples. EC_{50} in µg/ml.*

	PANC-1	Crl
A	58.4	47.7
B	108.3	91.8
C	48.6.0	63.6

Conclusion

At least two highly different chemotypes of *A. archangelica* exist in Iceland. The cytotoxicity of the essential oils of both chemotypes was similar.

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