Antiproliferative Effect of *Angelica archangelica* Fruits

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The aim of this work was to study the antiproliferative effect of a tincture from fruits of *Angelica archangelica* and the active components using the human pancreas cancer cell line PANC-1 as a model. Significant dose-dependent antiproliferative activity was observed in the tincture with an EC_{50} value of 28.6 μg/ml. Strong antiproliferative activity resulted from the two most abundant furanocoumarins in the tincture, imperatorin and xanthotoxin. The contribution of terpenes to this activity was insignificant. Imperatorin and xanthotoxin proved to be highly antiproliferative, with EC_{50} values of 2.7 μg/ml and 3.7 μg/ml, respectively, equivalent to 10 and 17 μM. The results indicate that furanocoumarins account for most of the antiproliferative activity of the tincture.

Key words: Angelica archangelica, Xanthotoxin, Imperatorin

Introduction

Angelica archangelica has been long and widely used in folk medicine, and it is one of the most respected medicinal herbs in Nordic 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 (Newall *et al.,* 1996).

Xanthotoxin (8-methoxypsoralen) and imperatorin (8-isopentenyloxypsoralen), shown in Fig. 1, are linear furanocoumarins occurring in a number of plants. Furanocoumarins have been extensively studied and photosensitization very well documented (Murray *et al.,* 1982). Phototoxicity of xanthotoxin is much higher than that of imperatorin (Colombain *et al.,* 2001). Light-independent activity has also been the subject of numerous studies. Most recently, furanocoumarins were studied for

Fig. 1. Structures of imperatorin (a) and xanthotoxin (b).

their inhibiting effect on cytochrome P450, resulting in drug-interactions (Guo *et al.,* 2000; Koenigs and Trager, 1998; Zhang *et al.,* 2001). Imperatorin has also been found to decrease chemically induced DNA adduct formation and may thus possess chemopreventive activity by impairing the conversion of polycyclic hydrocarbons to carcinogenic products (Kleiner *et al.,* 2001).

Xanthotoxin and imperatorin have been shown to inhibit proliferation in HeLa cells by measuring their effect on colony formation, xanthotoxin being more active than imperatorin (Gawron and Glowniak, 1987). In another study, xanthotoxin has been found to be cytotoxic to three cancer cell lines, whereas no activity could be demonstrated for imperatorin (Saqib *et al.,* 1990). In the same study lethality to brine shrimp larvae was demonstrated for xanthotoxin whereas none was found in the case of imperatorin. In more recent studies antiproliferative activity of xanthotoxin against MK-1, HeLa, and B16F10 cell lines has been reported (Fujioka *et al.,* 1999) and imperatorin was shown to inhibit proliferation of several cancer cell lines [human lung cancer (A549), melanin pigment producing mouse melanoma (B16 melanoma 4A5), human T-cell leukemia (CCRF-HSB-2) and human gastric cancer, lymph node metastasized (TGBC11TKB)] whereas normal human cell lines [human umbilical vein endothelial cells (HUVE)
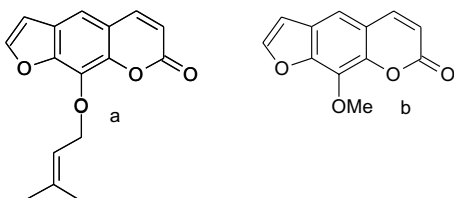
and normal human foreskin keratinocytes (HFK)] were less affected (Kawaii *et al.,* 2001). Furthermore, imperatorin has been shown to induce apoptosis of Hʟ-60 cells at micromolar concentrations (Pae *et al.,* 2002).

The aim of this study was to evaluate the antiproliferative activity of a tincture from *A. archangelica* fruits, and to identify its active components.

Material and Methods

Cancer cells

The pancreas-cancer cell line PANC-1 (American Type Culture Collection, Rockville, MD) was cultured in RPMI-1640 medium with 10% foetal calf serum, 50 int. units ml^{-1} penicillin, 50 μg ml^{-1} streptomycin, 0.01 ᴍ *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid)-buffer (HEPES) and 0.2 ᴍ ʟ-glutamine (all from Gibco, Paisley, UK). The cultures were incubated at 37 °C in 95% humidity and 5% CO$_2$. The cultures were passaged once a week by trypsinization using a 1:30 dilution of standard Gibco trypsin-EDTA solution.

Tincture

60 g of dried fruits from *A. archangelica*, growing in the southwest of Iceland, were ground. The resulting powder was then incubated in the dark at room temperature in 45% ethanol, 10 ml for each gram of fruits. After six weeks the tincture was filtered and stored at room temperature. The weight of the dried residue of the tincture was 18.8 mg/ml.

Furanocoumarin compounds

Imperatorin was isolated by soxhlet-extraction with *n*-hexane and isolated on a silica gel column, eluted with increasing amounts of diethyl ether in hexane. It was recrystallized twice from a mixture of *n*-hexane and diethyl ether and its purity was more than 95%. Xanthotoxin was purchased from Aldrich, MI, USA.

Assessment of furanocoumarin in the tincture with HPLC

HP-1100 with a quaternary pump was used for the mobile phase. The column was from Pecosphere (Perkin-Elmer Labs, Norwalk, CT, USA) with a C18 stationary phase with a particle size of 3 μm, 33 × 4.6 mm I.D. Gradient elution: 0–8 min from 40–60% MeOH in water, detection at 305 and 309 nm at room temperature, with a flow rate of 1.0 ml min^{-1}. The tincture was diluted tenfold before measurement, and its signal at 305 and 309 nm was monitored and compared to standard curves with known amounts of xanthotoxin and imperatorin. In the case of the other furanocoumarins, the same relationship of signal to concentration was assumed as for xanthotoxin and imperatorin, correcting with the respective extinction coefficients at their maxima at about 300 nm (Lee and Soine, 1969).

Removal of essential oils or volatile compounds from the tincture

The essential oils present in the tincture were also examined separately. The tincture was slowly distilled, until 55% of the volume of the original tincture had been evaporated, and the boiling temperature had reached 93 °C, removing the volatile oil components and ethanol. The destillate was then diluted again with water to ensure that the destillate contained the volatile oil compounds in the same concentration as originally present in the tincture.

Assessment of DNA synthesis

The cells were trypsinized, counted and placed in 96-well plates at 10^4 cells per well. The test compounds were dissolved, serially diluted before they were added at the start of the culture. The tincture and the volatile compounds from the tincture were diluted in 45% ethanol (v/v), the isolated furanocoumarins in 60% (v/v) ethanol. The ethanol concentration in each culture, including the controls, was 3% (v/v). After 24 h of culture [^{3}H]-thymidine was added at 37,000 Bq per well, and 4 h later the cells were washed and trypsinized, and harvested in a Skatron Cell Harvester (Skatron Instruments, Inc., Sterling, VA, USA) on to a glass fibre filter (Titertek, Huntsville, AL, USA). These were dried and the radioactivity was counted in a liquid scintillation counter.

Results

Concentrations of imperatorin and xanthotoxin in the tincture

The most abundant furanocoumarins in the tincture were imperatorin and xanthotoxin, 0.90 mg/ml and 0.32 mg/ml, respectively. Other detectable

furanocoumarins were identified as isoimperatorin (0.29 mg/ml), isopimpinellin (0.17 mg/ml), oxypeucedanin (0.14 mg/ml) and bergapten (0.13 mg/ml). The dry weight of the tincture was 18.8 mg/ml, so imperatorin accounted for almost 5% of its dry weight and the total furanocoumarin-content was estimated as 1.96 mg/ml, or 10–11% of the dry material of the tincture.

Inhibition of cell proliferation

The dose-dependent inhibition of cancer cell proliferation by the tincture was measured in three separate experiments, each done in triplicate. The tincture was diluted serially in 45% ethanol, fourfold each time. The results are shown in Fig. 2. The highest concentration tested was 307 μg/ml, with almost 100% inhibition. The inhibition decreased continually with dilution, and at 16-fold dilution (19 μg/ml) the inhibition was about 40%. The EC_{50} value was estimated as 28.6 μg/ml.

The fraction containing the essential oil or volatile compounds, diluted in the same way as the tincture, had no measurable effect on cell proliferation.

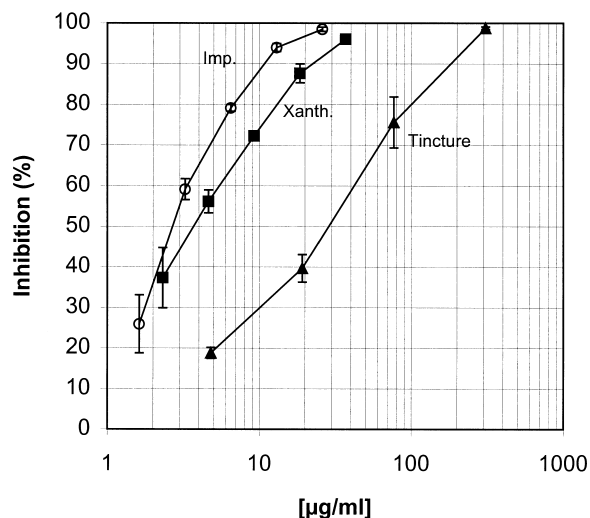The furanocoumarins, imperatorin and xanthotoxin, were serially diluted, twofold in each step.

Fig. 2. The dose-dependent inhibition of PANC-1 cell proliferation by a tincture from *A. archangelica* fruits and its ingredients xanthotoxin and imperatorin, relative to control. Each point represents the average from three (tincture) or four (xanthotoxin and imperatorin) separate measurements, each done in triplicate, and the standard deviation of the mean. The control rate of proliferation in the various experiments was 20–35,000 counts per minute.
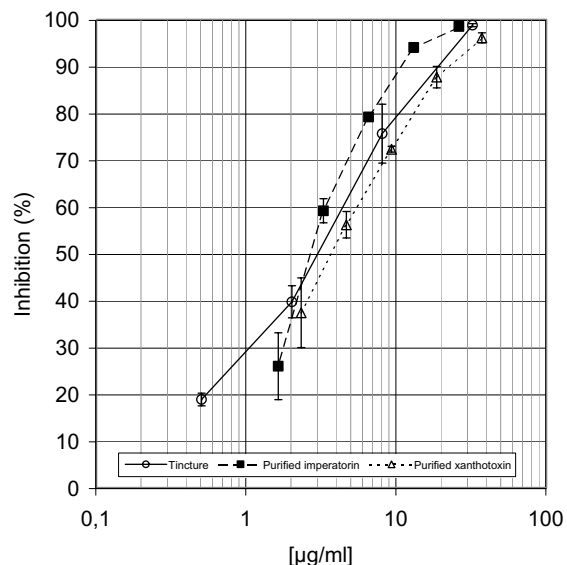

Fig. 3. The dose-dependent inhibition of PANC-1 cell proliferation by total furanocoumarins in the tincture, assuming that these compounds are responsible for its activity, compared with that of imperatorin and xanthotoxin.

The concentration-range was 1.6–26 and 2.3–37 μg/ml for imperatorin and xanthotoxin, respectively. Both compounds inhibited cell-proliferation in a dose-dependent manner, as shown in Fig. 2. Imperatorin showed more activity, its EC_{50} value being estimated as 2.7 μg/ml and that of xanthotoxin as 3.7 μg/ml, or 10 and 17 μM, respectively.

Fig. 3 compares the antiproliferative activity of the tincture, as a function of its furanocoumarin-content, with that of imperatorin and xanthotoxin. Assuming that furanocoumarins were the only compounds in the tincture contributing to its activity, the EC_{50} value of total furanocoumarins is about 3.0 μg/ml, as seen in Fig. 3.

Discussion

The tincture from *A. archangelica* fruits was antiproliferative with an EC_{50} value estimated as 28.6 μg/ml. Furanocoumarins and essential oils were considered to be its most likely active ingredients.

The volatile compounds were separated from the tincture, and their activity measured, in the same concentration as they occurred in the tests in the tincture as a whole. The volatile oils were present in the tincture in a very low concentration, or less than 35 μg/ml, that is less than 0.5 μg/ml in

the highest tincture concentration tested. As no activity was found, the essential oils could be excluded as significant contributors to this activity.

Regarding the furanocoumarins, the activity of two compounds, imperatorin and xanthotoxin, was studied. Imperatorin was by far the most abundant furanocoumarin in the tincture, whereas xanthotoxin was the second most abundant furanocoumarin. Xanthotoxin differs from imperatorin only by a methoxy-group where imperatorin has an isopentenyloxy-group.

If one assumes, as in Fig. 3, that the antiproliferative activity is solely due to furanocoumarins, the EC_{50} value for the furanocoumarins would be about 3.0 μg/ml. This value is between the EC_{50} values for imperatorin and xanthotoxin, of 2.7 and 3.7 μg/ml, respectively. This is plausible, since various studies have demonstrated antiproliferative and/or cytotoxic activity of numerous furanocoumarins of similar magnitude (Fujioka *et al.*, 1999; Gawron and Glowniak, 1987; Saqib *et al.*, 1990). The results thus indicate that furanocoumarins could account for the antiproliferative activity of the tincture.

A recent study has demonstrated antiproliferative activtity of imperatorin on various cancer cell lines, but much less activity against normal cell lines (Kawaii *et al.*, 2001). This study, in which antiproliferative activity was assessed by measuring metabolic activity of cell cultures after a 3-day exposure to the test compounds, compares various coumarins, including furanocoumarins, and the results suggest that the isopentenyl-group is important for the antiproliferative activity of simple coumarins. This was demonstrated by comparing the activity of pairs of compounds differing only in the presence or absence of this group. The present results seem to confirm the importance of the isopentenyl-group in imperatorin.

Direct comparison of the antiproliferative activity of xanthotoxin and imperatorin at 25 μg/ml has been done with HeLa cells, comparing the ability of incubated cells to form colonies and the formation of protein in incubated cultures. Xanthotoxin was found to be more active in both cases (Gawron and Glowniak, 1987). In the present study, both furanocoumarins were highly antiproliferative, imperatorin being more potent than xanthotoxin.

Preliminary studies indicated a similar antiproliferative activity of xanthotoxin and imperatorin on the human breast cancer cell line T47D and the human colon cancer cells WiDr (results not shown).

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