In vitro Antiproliferative and Antifungal Activity of Essential Oils from *Erigeron acris* L. and *Erigeron annuus* (L.) Pers.

Jolanta Nazaruk[a,*], Ewa Karna[b], Piotr Wieczorek[c], Paweł Sacha[c], and Elżbieta Tryniszewska[c]

[a] Department of Pharmacognosy, Medical University of Białystok, Mickiewicza 2a Str., 15-089 Białystok, Poland. E-mail: jolanta.nazaruk@umwb.edu.pl
[b] Department of Medicinal Chemistry, Medical University of Białystok, Kilińskiego 1 Str., 15-089 Białystok, Poland
[c] Department of Microbiological Diagnostics, Medical University of Białystok, Waszyngtona 15A Str., 15-269 Białystok, Poland

* Author for correspondence and reprint requests

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Antiproliferative and antifungal activities of essential oils from *Erigeron acris* root and herb and from *Erigeron annuus* herb were investigated. The cell viability assay was performed in cultured fibroblasts, cancer cell lines (MCF-7 and MDA-MBA-231), and endometrial adenocarcinoma (Ishikawa) cells as well as colon adenocarcinoma (DLD-1) cells using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The essential oil from *E. acris* root showed the highest antiproliferative activity in the MCF-7 cell line with an IC_{50} value of 14.5 μg/mL. No effect of the essential oil on normal cells at that concentration was found. Antifungal activity against various strains of five *Candida* species, *i.e. C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. parapsilosis*, was tested by the microdilution method. It was found that all examined oils can be useful as antifungal agents against the abovementioned species, but the essential oil of *E. acris* herb was the most active. Their minimum inhibitory concentrations (MIC) ranged from 30 to 0.4 μL/mL. The data presented suggest that essential oils from *E. acris* and *E. annuus* possess antifungal activity against *Candida* spp. and antiproliferative activity against breast cancer MCF-7 cells.

Key words: Erigeron spp., Antiproliferative, Antifungal

Introduction

Erigeron acris L. (blue fleabane) is an annual, biennial or rarely perennial herb and *Erigeron annuus (*L.*)* Pers. (daisy fleabane) is an annual plant. Both species, belonging to the family Asteraceae, grow on dry, stony or sandy places. They are distributed almost throughout Europe (Tutin, 1976). *E. acris* is occasionally used in health care. In the Spanish province Castellón it has been traditionally used for digestive infusions (de Santayana *et al*., 2005). In Italy the roots of *E. acris* are traditionally used for the treatment of toothache, bruises, and arthritis (Pieroni *et al*., 2004). *In vitro* studies showed that diethyl ether and ethyl acetate extracts from leaves, inflorescences, and roots of *E. acris* possess antioxidant activity (Nalewajko-Sieliwoniuk *et al*., 2008). *E. annuus* has been used in Chinese folk medicine for the treatment of indigestion, enteritis, epidemic hepatitis, and haematuria (Li *et al*., 2005). Root extracts were found to possess antiproliferative activity (Réthy *et al*., 2007).

The chemical composition of the essential oils from *E. acris* and *E. annuus* is well known. The volatile fractions of *E. acris* and *E. annuus* herbs consist of over 60 components. The major constituents are monoterpenes and sesquiterpenes (Nazaruk *et al.*, 2006; Lis *et al*., 2008). In the essential oil from the root of *E. acris* 54 compounds were identified, among them polyacetylenes predominate (Nazaruk and Kalemba, 2009).

Essential oils, secondary metabolites of plants with volatile properties and specific odour, are mixtures of small-molecular chemical compounds, mainly terpenes and phenols, and possess a broad spectrum of biological action. The antispasmodic, analgesic, sedative, and anti-inflammatory effects of essential oils have been well established (Bakkali *et al*., 2008). In some tests they showed cancer suppressive activity and exhibited chemopreventive efficacy (Edris, 2007). Essential oils are natu-

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Table I. *In vitro* antiproliferative effect of essential oil from *Erigeron acris* root on tumour and normal cells.

Cell line	Histotype	IC_{50} [μg/mL]
Tumour cells		
MCF-7	Breast adenocarcinoma	14.5 ± 2
MDA-MBA-231	Breast adenocarcinoma	>50
Ishikawa	Human endometrial adenocarcinoma	>50
DLD-1	Human colon adenocarcinoma	>50
Normal cells		
CRL-1474	Normal skin fibroblasts	>50

ral antimicrobial agents able to act on bacteria, viruses, and fungi, and many trials have been performed in this field (Bakkali *et al*., 2008; Kalemba and Kunicka, 2003). There is increasing interest in antimicrobial plant sources because of the growing resistance of microbes to synthetic compounds. Mycoses are particularly difficult to cure. Importantly, there has been a recent increase in infections due to non-*albicans Candida* spp., such as *C. glabrata*, *C. krusei*, *C. tropicalis*, and *C. parapsilosis*. The important issue is to search for antifungal agents with alternative mechanisms of action and less toxicity (Singh, 2001).

So far the activity of essential oils from *E. acris* and *E. annuus* has not been studied. Therefore, we sought to examine the influence of the oil from *E. acris* root and herb on cancer cell proliferation, and the toxicity of the oils from *E. acris* root and herb and *E. annuus* herb against randomly chosen strains of fungi from the genus *Candida*.

Results and Discussion

Breast adenocarcinoma cells (MCF-7, MDA-MBA-231), human endometrial adenocarcinoma cells (Ishikawa cell line), and human colon adenocarcinoma cells (DLD-1) (each at $1 \cdot 10^5$ cells/well) were treated with the essential oil samples at different concentrations. In the experiment DMSO, added as a solubilizing agent at the maximum content of 0.4%, did not have any significant effect on cell proliferation. Doxorubicin was used as positive control. Its IC_{50} value for MCF-7 cells was 44.5 μg/mL. In the MCF-7 cell line, the essential oil from *E. acris* root showed the highest antiproliferative activity, the IC_{50} value being 14.5 μg/mL. In the other cell lines this value was higher than 50 μg/mL (Table I). Similar studies were performed on normal skin fibroblasts which were found not to be susceptible to the antiproliferative activity of the essential oils at the concentrations active in the MCF-7 cell line (Table II).

In case of the essential oil from the herb of *E. acris*, there was no influence on proliferation in all cell lines studied (data not shown).

Compared with doxorubicin, the IC_{50} value of essential oil from the root of *E. acris* is low, so it was recognized as a potentially active agent for breast cancer MCF-7 cells.

Essential oils from *E. acris* root and herb and from *E. annuus* herb were tested for their toxicity against various strains of fungi from the genus *Candida* (*C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, and the control strain *C. parapsilosis* ATCC 22019) applying the serial microdilution method. Table III shows the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) for each oil and fungal strain. We conclude that the examined oils can be used as antifungal agents against the species mentioned above. The oil of *E. acris* herb seems to be most active since its MIC values are relatively low (in the 3.75–0.4 μL/mL range), however, large variations are observed for individual strains.

Table II. Viability of confluent human fibroblasts incubated for 22 h with different concentrations of *E. acris* root essential oil. The slight cytotoxic effect of DMSO (0.4%) was subtracted from control values, and the viability of cells in 0.4% DMSO was considered 100%.

Concentration [μg of oil/mL]	Viability of fibroblasts (% of control)
0	100
5	100 ± 1
10	100 ± 2
15	98 ± 2
25	93 ± 3
50	54 ± 5*
100	33 ± 4*
1000	32 ± 7*

Mean values ± SD from three independent experiments done in duplicate are presented; *$P < 0.05$.

Table III. *In vitro* activity of the essential oils from *E. acris* root, *E. acris* herb, and *E. annuus* herb against fungi of the genus *Candida* (MIC and MFC in μL/mL).

Species	Strain number	*E. acris* root		*E. acris* herb		*E. annuus* herb	
		MIC	MFC	MIC	MFC	MIC	MFC
C. albicans	919057/09	1.8	3.75	0.9	1.8	15	30
	918053/2/09	≤ 0.4	≤ 0.4	0.9	0.9	15	30
	918214/1/09	3.75	3.75	1.8	3.75	15	30
	919091/1/09	≤ 0.4	3.75	≤ 0.4	0.9	3.75	15
	918994/1/09	1.8	3.75	1.8	1.8	15	30
C. glabrata	918018/09	15	30	1.8	7.5	30	> 30
	919016/2/09	15	15	3.75	15	30	> 30
	918761/2/09	15	15	1.8	3.75	30	30
	919099/3/09	15	30	3.75	15	30	> 30
	918214/2/09	15	30	1.8	3.75	30	30
C. tropicalis	918104/2/09	≤ 0.4	1.8	1.8	3.75	15	30
	918581/3/09	≤ 0.4	≤ 0. 4	1.8	3.75	15	30
	918406/2/09	≤ 0.4	7.5	1.8	3.75	15	30
	918253/4/09	≤ 0.4	≤ 0.4	1.8	1.8	15	30
	919100/09	0.9	15	1.8	3.75	15	15
C. krusei	917806/2/09	ND	ND	0.9	1.8	15	15
	918177/1/09	ND	ND	0.9	0.9	15	15
	919091/2/09	ND	ND	0.9	1.8	1.8	15
	918451/4/09	ND	ND	≤ 0.4	1.8	1.8	7.5
	917447/2/09	ND	ND	0.9	0.9	7.5	15
C. parapsilosis	919144/09	ND	ND	0.9	1.8	3.75	30
	919014/3/09	ND	ND	0.9	3.75	1.8	30
	919013/3/09	ND	ND	0.9	3.75	3.75	30
	918412/1/09	ND	ND	0.9	3.75	3.75	30
	918994/2/09	ND	ND	0.9	3.75	3.75	> 30
C. parapsilosis	ATCC 22019	3.75	> 30	0.9	3.75	3.75	30

MIC, minimum inhibitory concentration; MFC, minimum fungicidal concentration; ND, not done.

The activity of the essential oil from the root of *E. acris* may be due to polyacetylenic compounds, *i.e.* matricaria and lachnophyllum esters that are the main components. Interestingly, the oil from the herb of the plant with the lowest content of polyacetylenes did not have such an activity. The same effect was observed in extracts from herb and root of *E. annuus*. Significant antiproliferative and cytotoxic activity was only shown by lipophilic extracts from roots in which polyacetylenic compounds can also be present, but the composition of these extracts unfortunately was not established (Réthy *et al.*, 2007).

The composition of essential oils is unique due to the variety of chemical structures of their constituents which are deciding their wide activity. The predominant one is strong antimicrobial activity. The mechanism of antifungal action is complex and can involve inhibition of chitin-producing enzymes, membrane leakage or respiratory chain inhibition (Pauli, 2006). The antimicrobial activity of oils depends on their individual components, but an advantageous synergistic effect of the essential oil constituents has also been observed (Kalemba and Kunicka, 2003). One of the components responsible for the highest activity of the essential oil from the herb of *E. acris* seems to be β-pinene (15.6% of oil), for which considerable antifungal activity has been demonstrated (Hammer *et al.*, 2003). Polyacetylenes also possess antifungal activity. Fungitoxic activity of matricaria and lachnophyllum esters and lactones was demonstrated against a phytopathogenic fungus (Vidari *et al.*, 2006). The essential oils of herb and root of *E. acris* are probably more active against *C. albicans* than tea tree oil, a well known antimicrobial agent (Hammer *et al.*, 2003).

The data presented suggest that essential oils from *E. acris* and *E. annuus* possess antifungal activity against various *Candida* species and antiproliferative activity against breast cancer MCF-7 cells.

Experimental

Plant material

The flowering aerial part of *E. acris* and *E. annuus*, and the root of *E. acris* were collected in July 2008 and 2009 in the vicinity of Białystok, Poland. Voucher specimens (EAC 01007 and EAN 01008) have been deposited in the herbarium of the Department of Pharmacognosy, Medical University of Białystok, Poland.

The essential oil was obtained by hydrodistillation from dry, ground plant material, according to the European Pharmacopoeia Commission (2004) and was stored at 4 °C until the examination. The root of *E. acris*, the herb of *E. acris*, and the herb of *E. annuus* contained 1.0%, 0.3%, and 0.15% of essential oil, respectively (w/v, based on dried plant material).

Antiproliferative activity

Reagents: DMSO (dimethyl sulfoxide) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were provided by Sigma Corp. (St. Louis, USA), as were most other chemicals and buffers used. Dulbecco's minimal essential medium (DMEM) and fetal bovine serum (FBS) used in cell culture were products of Gibco (Grand Island, USA). Glutamine, penicillin, and streptomycin were obtained from Quality Biologicals Inc. (Gaithersburg, USA).

Tissue culture: All studies were performed on normal human skin fibroblasts (CRL-1474), purchased from the American Type Culture Collection (Manassas, VA, USA) and on breast adenocarcinoma cells (MCF-7 and MDA-MBA-231), human endometrial adenocarcinoma cells (Ishikawa cell line), and human colon adenocarcinoma cells (DLD-1). The cells were maintained in DMEM supplemented with 10% FBS, 2 mmol/L glutamine, 50 U/mL penicillin, 50 μg/mL streptomycin at 37 °C in a 5% CO_2 incubator. Fibroblasts were counted in a hemocytometer and cultured at $1 \cdot 10^5$ cells/well in 2 mL of growth medium in 6-well plates (Costar, Salt Lake City, USA). Cells reached confluence at day 6, and in most cases such cells were used for assays. Fibroblasts were used from the 8th to the 14th passage. Cancer cell lines were cultured at $1 \cdot 10^5$ cells/well in 2 mL of growth medium until reaching 80% confluence.

Cell viability assay: The assay was performed according to the method of Carmichael *et al.* (1987) using MTT. The cells were cultured for 22 h with various concentrations of essential oil in 6-well plates, washed three times with PBS, and then incubated for 4 h in 1 mL of MTT solution (0.5 mg/mL of PBS) at 37 °C. The medium was removed and 1 mL of 0.1 M HCl in absolute isopropanol was added to the attached cells. Absorbance of the converted dye in living cells was measured at a wavelength of 570 nm. Cell viability in the presence of essential oil was calculated as percent of control cells.

Antifungal activity

The antifungal activity of essential oils was tested using the method described by Đorđević *et al*. (2007) with minor modifications. Twenty five strains (in equal numbers) belonging to the following species of fungi: *Candida albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. parapsilosis* were randomly chosen for the examination. *C. parapsilosis* ATCC 22019 strain was included as a control strain.

Minimal inhibitory concentration (MIC) of essential oils was determined using the microdilution method in 96-well microtiter plates. Series of solutions of essential oils ranging from 30 to 0.4 μL/mL were prepared. Oil was dissolved in DMSO, and the solution was then diluted with Sabouraud dextrose broth supplemented with Tween 80 (final content of 5 μL/mL). Test strains were suspended in Sabouraud broth to give a final density of $5 \cdot 10^4$ CFU/mL. Dilutions of oils and suspensions of fungi were inoculated on microtiter plates, adding growth control, sterility control, and oil solution controls. Plates were incubated under normal atmospheric conditions at 30 °C for 48 h. Minimal fungicidal concentrations (MFC) were also determined. For this purpose, 5 μL of essential oils were transferred from each well of the microtiter plate onto Sabouraud agar. Plates were incubated in the same conditions as microtiter plates and MFC values were read.

Statistical analysis

Mean values of three independent experiments done in duplicate ± standard deviation (SD) were calculated. The results were submitted to statistical analysis using Student's t-test, accepting $P < 0.05$ as significant.

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