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# EVALUATION OF SELECTED LICHENS FROM ICELAND FOR CANCER CHEMOPREVENTIVE AND CYTOTOXIC ACTIVITY

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*ABSTRACT*

*Cancer chemopreventive effects of organic extracts from 29 species of lichens collected in Iceland were evaluated using a panel of* in vitro *bioassays whereby extracts were tested for potential to induce quinone reductase (QR) and differentiation of human promyelocytic leukemia (HL-60) cells, inhibit cyclooxygenase-1 (COX-1), phorbol ester-induced ornithine decarboxylase (ODC), aromatase and sulfatase, as well as for antioxidant, estrogenic/anti-estrogenic and antiproliferative activity. In addition, the extracts were tested for cytotoxicity against 12 cancer cell lines. The most significant results were exhibited by extracts from* Xanthoria elegans *and* Alectoria nigricans, *which respectively, induced QR activity (concentration to double activity = 4.8 μg/ml) and inhibited phorbol ester-induced ODC activity with mouse 308 cells in culture ($IC_{50}$ = 2.6 μg/ml). Moderate inhibition of [$^3$H]thymidine incorporation with HL-60 cells was exhibited by the* Peltigera leucophlebia *extract. Several extracts prevented estrogen formation from estrogen precursors by inhibiting the enzymatic activities of aromatase* (Sphaerophorus globosus, Cetrariella delisei, Melanelia hepatizon) *and sulfatase* (Cladonia gracilis, Sphaerophorus fragilis, S. globosus). *None of the extracts demonstrated significant cytotoxic effects with selected cell lines.*

*Keywords:* Cancer chemoprevention, lichens, Icelandic plants, ornithine decarboxylase, quinone reductase, HL-60 cell differentiation, aromatase, sulfatase, cytotoxicity.

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## INTRODUCTION

Compared to higher plants, lichens remain a relatively unexplored source of biologically active compounds. Although a number of lichens have been used for medicinal purposes throughout the world, ethnomedical information on the majority of species is scarce. The limited use of lichens is undoubtedly due in part to the difficulties encountered in collecting these small plants in substantial quantities.

The taxonomical significance of secondary metabolites formed in lichens, many of which are unique to the algal/fungal symbiotic association, has long been recognized (Culberson, 1969, 1970). The first reports of lichens being screened for biological activity came in the 1940s following the discovery of penicillin and other antibiotics from fungi. Numerous lichen species were found to contain compounds active against Gram positive bacteria and Mycobacteria (Stoll et al., 1947). A number of depsides and depsidones from lichens were later tested for *in vitro* inhibitory effects on cyclooxygenase isolated from rabbit renal microsomes (Sankawa et al., 1982). Recent investigations of bioactive compounds from lichens include reports of *in vitro* HIV-1 RT inhibition (Pengsuparp et al., 1995), HIV-1 integrase inhibition (Neamati et al., 1997), 5-lipoxygenase inhibition (Ingólfsdóttir et al., 1996, 1997a), inhibition of leukotriene formation in guinea pig smooth muscle (Gissurarson et al., 1997), antimicrobial activity (Lauterwein et al., 1995; Ingólfsdóttir et al., 1997b) and antiproliferative activity (Ögmundsdóttir et al., 1998).

From the limited information available, it seems reasonable to assume that lichens are a worthwhile reser-

voir in which to search for lead compounds for drug development. With this in mind, extracts from 29 species of lichens, belonging to 11 families, were subjected to screening in bioassays developed to identify compounds of potential benefit for cancer chemoprevention (Pezzuto, 1993) as well as for cancer chemotherapy. Cancer chemopreventive effects were evaluated using assays designed to reflect various stages of carcinogenesis (Pezzuto, 1997; Pezzuto et al., 1998): Induction of quinone reductase (QR) and antioxidant activity (initiation); inhibition of cyclooxygenase-1 (COX-1) activity and inhibition of phorbol ester-induced ornithine decarboxylase (ODC) activity (promotion); induction of HL-60 cell differentiation, anti-estrogenic activity, inhibition of aromatase, inhibition of sulfatase and antiproliferative effects (progression). Cytotoxicity against 12 cancer cell lines was also evaluated.

## MATERIALS AND METHODS

### Plant Material

Samples of selected lichens were collected during the summer of 1997 in various locations within Iceland, mainly northern Iceland (Table 1). The plants were botanically authenticated by one of us (HK). Voucher specimens of each species are deposited at the Icelandic Institute of Natural History, Akureyri. The plants were dried and carefully cleansed of extraneous material prior to grinding.

### Extraction of Plant Material

Samples (5–15 g) of milled plant material were extracted at room temperature through percolation with methanol (Merck 6009). Following *in vacuo* evaporation (40 °C) of the solvent, the extracts were partitioned three times between water and chloroform (Merck 102431). The combined chloroform phases were taken to dryness through rotary evaporation (<40 °C).

### Biological Assays

Established protocols were used for assessing biological activity of the extracts. The cancer chemopreventive bioassays were performed as follows: Induction of quinone reductase (Gerhäuser et al., 1997); antioxidant activity (Lee et al., 1998); inhibition of cyclooxygenase-1 (Jang & Pezzuto, 1997); inhibition of phorbol ester-induced ornithine decarboxylase activity (Gerhäuser et al., 1995); induction of HL-60 cell differen-

Table 1. Botanical and geographical data for lichens from Iceland selected for biological screening.

| Plant | Family | Collection site |
|---|---|---|
| *Alectoria nigricans* (Ach.) Nyl. | Usneaceae | Moldhaugahals |
| *Alectoria ochroleuca* (Hoffm.) Massal. | Usneaceae | Moldhaugahals |
| *Anaptychia runcinata* (With.) Laundon | Physciaceae | Stödvarfjördur |
| *Cetraria aculeata* (Schreb.) Fr. | Parmeliaceae | Glerardalur |
| *Cetraria islandica* (L.) Ach. | Parmeliaceae | Melrakkasletta |
| *Cetrariella delisei* (Bory ex Schaer.) Kärnefelt & Thell | Parmeliaceae | Fljotsdalsheidi |
| *Cladonia ecmocyna* Leight. | Cladoniaceae | Fljotsheidi |
| *Cladonia furcata* (Huds.) Schrad. | Cladoniaceae | Eyjafjördur |
| *Cladonia gracilis* (L.) Willd. | Cladoniaceae | Hrisey |
| *Cladonia rangiferina* (L.) Web. ex Wigg | Cladoniaceae | Fljotsheidi |
| *Cladonia stricta* (Nyl.) Nyl. | Cladoniaceae | Fljotsdalsheidi |
| *Flavocetraria nivalis* (L.) Kärnefelt & Thell | Parmeliaceae | Moldhaugahals |
| *Melanelia hepatizon* (Ach.) Thell | Parmeliaceae | Moldhaugahals |
| *Nephroma expallidum* (Nyl.) Nyl. | Nephromataceae | Gönguskörd |
| *Parmelia omphalodes* (L.) Ach. | Parmeliaceae | Stödvarfjördur |
| *Parmelia saxatilis* (L.) Ach. | Parmeliaceae | Moldhaugahals |
| *Peltigera canina* (L.) Willd. | Peltigeraceae | Gönguskörd |
| *Peltigera leucophlebia* (Nyl.) Gyeln. | Peltigeraceae | Fljotsheidi |
| *Pertusaria oculata* (Dicks.) Th. Fr. | Pertusariaceae | Hrisey |
| *Solorina crocea* (L.) Ach. | Peltigeraceae | Oddskard |
| *Sphaerophorus fragilis* (L.) Pers. | Sphaerophoraceae | Stödvarfjördur |
| *Sphaerophorus globosus* (Huds.) Vain. | Sphaerophoraceae | Hvalfjördur |
| *Stereocaulon alpinum* Laur. | Stereocaulaceae | Eyjafjördur |
| *Stereocaulon arcticum* Lynge | Stereocaulaceae | Bleikalukvisl |
| *Stereocaulon spathuliferum* Vain. | Stereocaulaceae | Stödvarfjördur |
| *Umbilicaria arctica* (Ach.) Nyl. | Umbilicariaceae | Moldhaugahals |
| *Umbilicaria proboscidea* (L.) Schrad. | Umbilicariaceae | Strandir |
| *Xanthoria elegans* (Link) Th. Fr. | Teloschistaceae | Kaupangssveit |
| *Xanthoria parietina* (L.) Th. Fr. | Teloschistaceae | Hvalfjördur |

Table 2. Screening of lichen extracts for cancer chemopreventive activity: induction of quinone reductase (QR), inhibition of phorbol ester-induced ornithine decarboxylase (ODC), induction of HL-60 cell differentiation. Assays were performed as described in "Materials and Methods."

| Species | QR[1] | ODC[2] | HL-60 differentiation[3] | | | |
|---|---|---|---|---|---|---|
| | | | NBT | NSE | SE | TDR |
| *Alectoria nigricans* | >10 | 2.6 | 1.2 | 2.7 | 0.8 | 14.8 |
| *Alectoria ochroleuca* | >10 | >4 | 4.1 | 4.1 | 0.2 | 9.7 |
| *Anaptychia runcinata* | >10 | >4 | 0.5 | 0.2 | 1.8 | 12.3 |
| *Cetraria aculeata* | >10 | >4 | 2.0 | 0 | 1.0 | 12.6 |
| *Cetraria islandica* | >10 | >4 | 0 | 0 | 0.5 | 0 |
| *Cetrariella delisei* | >10 | >4 | 3.4 | 6.3 | 1.2 | 0 |
| *Cladonia ecmocyna* | >10 | >4 | 3.8 | 10.3 | 1.0 | 12.9 |
| *Cladonia furcata* | >10 | >4 | 2.5 | 0.2 | 3.8 | 2.5 |
| *Cladonia gracilis* | >10 | >4 | 2.2 | 0 | 0.5 | 0 |
| *Cladonia rangiferina* | >10 | >4 | 1.0 | 4.4 | 0.8 | -3.3 |
| *Cladonia stricta* | >10 | >4 | 4.1 | 6.4 | 3.6 | 26.1 |
| *Flavocetraria nivalis* | >10 | >4 | 2.5 | 5.5 | 0 | 15.4 |
| *Melanelia hepatizon* | >10 | >4 | 7.5 | tox | 0 | 21.0 |
| *Nephroma expallidum* | >10 | >4 | 1.2 | 0.5 | 2.2 | 13.8 |
| *Parmelia omphalodes* | >10 | >4 | 7.2 | 6.7 | 0 | 32.9 |
| *Parmelia saxatilis* | >10 | >4 | 4.1 | 4.5 | 1.0 | 19.5 |
| *Peltigera canina* | >10 | >4 | 1.5 | 0 | 2.2 | 31.0 |
| *Peltigera leucophlebia* | >10 | >4 | 5.5 | 10.5 | 1.2 | 57.1 |
| *Pertusaria oculata* | >10 | >4 | 1.5 | 0.5 | 3.2 | 0 |
| *Solorina crocea* | >10 | >4 | 2.0 | 0 | 0.2 | 0 |
| *Sphaerophorus fragilis* | >10 | >4 | 3.0 | 7.4 | 0.5 | 0.5 |
| *Sphaerophorus globosus* | >10 | >4 | 1.8 | 5.3 | 0.8 | 16.9 |
| *Stereocaulon alpinum* | >10 | >4 | 1.5 | 0 | 2.8 | 3.9 |
| *Stereocaulon arcticum* | >10 | >4 | 2.8 | 0.2 | 2.8 | 11.5 |
| *Stereocaulon spathuliferum* | >10 | >4 | 2.8 | 0 | 1.5 | 0 |
| *Umbilicaria arctica* | >10 | >4 | 0.5 | 1.5 | 11.7 | 6.4 |
| *Umbilicaria proboscidea* | >10 | >4 | 0.2 | 0 | 0.5 | 0 |
| *Xanthoria elegans* | 4.8 | >4 | 1.2 | 0 | 5.1 | 0 |
| *Xanthoria parietina* | >10 | >4 | 1.5 | 0.8 | 4.8 | 15.9 |

[1] Induction of quinone reductase; results expressed as CD (concentration to double QR activity; μg/ml); activity criterion limit,10 μg/ml.
[2] Inhibition of TPA-induced ornithine decarboxylase; results expressed as $IC_{50}$ (μg/ml); activity criterion limit,4 μg/ml.
[3] Induction of HL-60 cell differentiation; results (%) expressed as nitroblue tetrazolium (NBT) reduction, non-specific esterase (NSE) activity, specific esterase (SE) activity and [$^3$H]thymidine incorporation (TDR).

tiation (Suh et al., 1995); estrogenic and anti-estrogenic activity (Pisha & Pezzuto, 1997); aromatase inhibition (Thompson & Sitteri, 1972); sulfatase inhibition (MacIndoe et al., 1988); antiproliferative activity against human colon cancer (Col 2) cells (Skehan et al., 1990). Cytotoxic activity against the following cell lines was evaluated as described (Likhitwitayawuid et al., 1993): Rat glioma (ASK), human breast carcinoma (BC 1), human colon carcinoma (Col 2), human epidermoid carcinoma in the mouth (KB), vinblastine-resistant KB tested in the presence (KB-$V^+$) or absence (KB-$V^-$) of 1 μg/ml vinblastine in the media, hormone-dependent human prostate carcinoma (LNCaP), human lung carcinoma (LU 1), human neuroblastoma (SKNSH), human ovarian adenocarcinoma (SW 626), taxol-dependent mutants of Chinese hamster ovary cells (Tax 2-4), and human glioblastoma (U373).

## RESULTS

### Cancer Chemopreventive Activity

The extract from *Xanthoria elegans* showed significant induction of QR activity in an assay using cultured Hepa 1c1c7 hepatoma cells (Gerhäuser et al., 1997). The concentration to double activity (CD) was determined as 4.8 μg/ml (Table 2). All other extracts, including the closely related *X. parietina*, exhibited CD values higher than the activity criterion limit of 10 μg/ml (Table 2).

The *Alectoria nigricans* extract exhibited notable inhibition of ODC activity induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in cultured mouse epidermal 308 cells (Gerhäuser et al., 1995) with an $IC_{50}$ value of 2.6 μg/ml (Table 2). All other extracts, including *A. ochroleuca*, were inactive in this assay, with $IC_{50}$ values >4 μg/ml.

Table 3. Lichen extracts exhibiting significant inhibitory activity on estrogen formation through inhibition of sulfatase and aromatase.

| Species | Assay[a] Sulfatase | Aromatase |
|---|---|---|
| *Cetrariella delisei* | –[b] | 82 |
| *Cladonia gracilis* | 83 | – |
| *Melanelia hepatizon* | – | 73 |
| *Sphaerophorus fragilis* | 95 | – |
| *Sphaerophorus globosus* | 90 | 74 |

[a] Extracts were tested at a concentration of 40 μg/ml, and results are expressed as percent inhibition.
[b] –, inactive.

The extracts were tested for potential to induce the expression of cellular markers associated with cell differentiation (NBT, NSE, SE) using human promyelocytic leukemia (HL-60) cells (Suh et al., 1995). The distribution of response rates is shown in Table 2. None of the extracts met the activity criteria limit of $ED_{50}$ values $<4$ μg/ml based on NBT reduction or NSE activity ($ED_{50}$ data not shown). However, the extract of *Peltigera leucophlebia* caused moderate inhibition of [$^3$H]thymidine incorporation (Table 2).

Three species demonstrated significant inhibition of aromatase: *Sphaerophorus globosus*, *Cetrariella delisei* and *Melanelia hepatizon* (Table 3). Extracts from both *Sphaerophorus species* and *Cladonia gracilis* showed inhibition of estrone sulfatase in intact MCF-7 human breast cancer cells by more than 80% (Table 3).

Antioxidant activity as determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity (Lee et al., 1998) was not significant for any of the extracts, with all $IC_{50}$ values exceeding 200 μg/ml. None of the extracts inhibited formation of cyclooxygenase-1 products in sheep seminal vesicle microsomes in the peroxidase co-substrate oxidation assay (Jang & Pezzuto, 1997), the $IC_{50}$ values in all cases being $>25$ μg/ml. No estrogenic/anti-estrogenic activity was detected for any of the extracts using cultured (human endometrial) Ishikawa cells (Pisha & Pezzuto, 1997). Antiproliferative effects on human colon cancer (Col 2) cells were negligible, as determined in the sulforhodamine B (SRB) assay (Skehan et al., 1990).

### Cytotoxicity

As $ED_{50}$ values for all extracts were $>20$μg/ml, it was concluded that cytotoxic activity (Likhitwitayawuid et al., 1993) was not significant with this test panel of 12 cell lines.

## DISCUSSION

The preliminary results obtained for *Xanthoria elegans* and *Alectoria nigricans* in the QR and ODC assays, respectively, clearly merit further study. In light of the *in vivo* functions of QR and ODC, i.e., to catalyze metabolism of certain potential carcinogens rendering them less harmful (QR), and to catalyze formation of tumor-promoting polyamines from putrescine (ODC), it follows that inducers of QR and inhibitors of ODC would be of interest as potential carcinogenesis inhibitors. Extracts of both lichens are thus currently being evaluated for inhibitory effects on carcinogen-induced preneoplastic lesions in mouse mammary organ cultures.

Suppression of estrogen biosynthesis through inhibition of aromatase, which catalyzes the conversion of androstenedione to estrone and the conversion of testosterone to estradiol, is considered a potential target for chemoprevention as well as for chemotherapy (Kelloff et al., 1998). Estrogen has been strongly associated with the development and growth of breast cancer and has been postulated to play a role in prostate cancer. Another target for impeding estrogen formation is estrone sulfatase, which catalyzes the formation of estrone from estrone sulfate, this being considered the key route of estrogen production in breast tumors (Santner et al., 1984). Bioguided fractionations of extracts showing inhibitory effects on aromatase and sulfatase are currently underway.

As a further follow-up to preliminary results, antiproliferative effects of *Peltigera leucophlebia* constituents on HL-60 cells will be examined in more detail.

It is obvious that lichens in their natural state will never become feasible sources for the provision of chemicals on a large-scale. However, with the advances being made in cultivation techniques, synthetic chemistry, spectroscopic technology, combinatorial chemistry and biotechnology, problems of supply cease to be relevant. The present study, wherein eight extracts out of 29 tested showed activity worth pursuing, provides encouragement for our continued quest for biologically active lead compounds from lichens.

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