# 5 Investigations of Lichen Secondary Metabolites with Potential Anticancer Activity

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**Abstract**

Cancers are among the leading causes of morbidity and mortality worldwide. In the past half-century, natural products have served us well in combating cancer. The main sources of these compounds are microorganisms, plants, and marine organisms. Lichens as chemically significant biota represent a large group of symbiotic organisms of fungi (mycobionts) and algae (photobionts) constituting about 17,000 species and are a source of diverse secondary metabolites. This chapter focuses primarily on the anticancer properties of lichen secondary metabolites. We have reviewed various publications related to anticancer activity emphasizing results about specific lichen compounds. We have shown that various isolated lichen compounds often demonstrate significant inhibitory activity against various cancer cell lines at very low concentrations. Although lichens are a source for excellent anticancer active compounds, only a small number have been tested for their biological significance. Our effort is another attempt to expand and deepen research in this area, especially on compounds that have shown promising results.

## 5.1 Introduction

Throughout history, natural products have afforded a rich source of compounds that have found many applications in the fields of medicine, pharmacy, and biology. Within the sphere of cancer, a number of important new commercialized drugs have been obtained from natural sources, by structural modification of natural compounds, or by the synthesis of new compounds, designed following a

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natural compound as a model. The search for improved cytotoxic agents continues to be important in the discovery of modern anticancer drugs. The huge structural diversity of natural compounds and their bioactivity potential have meant that several products isolated from plants, marine flora, and microorganisms can serve as "lead" compounds for improvement of their therapeutic potential by molecular modification. More than 70% of anticancer compounds are either natural products or substances derived from natural products. On the other hand, conjugation of toxic natural products to monoclonal antibodies or polymeric carriers can lead to more efficacious targeted therapies. Because less than 15% of higher plants have been systematically investigated, the natural products research towards chemotherapy requires further attention and multi-scientific collaboration (Karikas 2010). Also, some herbal compounds have been subjected to clinical trials. This chapter focuses only on those herbal compounds originating from lichens whose anticancer effect has been investigated. The aim of this chapter is to highlight the importance of lichen secondary metabolites with potential anticancer activity.

## 5.2 Anticancer Secondary Metabolites of Lichens

### 5.2.1 Significance of Lichens and Lichen Secondary Metabolites

Lichen metabolites in general can be divided into two groups: primary and secondary. Primary metabolites are proteins, lipids, carbohydrates, and other organic compounds involved in lichen metabolism and structure. Secondary metabolites, also known as lichen substances, are produced mainly by the fungus and secreted onto the surface of the lichen hyphae either in amorphous forms or as crystals. They often accumulate in the upper cortex or in specialized structures such as fruiting bodies (Fahselt 1994; Elix 1996). Lichens synthesize a great variety of secondary metabolites, many of which are unique. Approximately 1050 secondary compounds have been identified to date (Stocker-Worgotter 2008). These compounds are produced by the mycobiont (Elix 1996; Huneck 1999) and accumulate in the cortex (such as atranorin, parietin, usnic acid, fungal melanins) or in the medullary layer (such as physodic acid, physodalic acid, protocetraric acid) as tiny extracellular crystals on the outer surfaces of the hyphae. The photobiont might also have an influence on the secondary metabolism of the mycobiont (Brunauer et al. 2006, 2007; Yamamoto et al. 1993, 1994; Molnár and Farkas 2010).

Lichens had to evolve diverse biosynthetic pathways to produce such complex arrays of secondary metabolites: polyketide, shikimic acid, and mevalonic acid pathways. Most of the lichen substances are phenolic compounds. Polyketide-derived aromatic compounds, the depsides, depsidones, dibenzofurans, xanthones, and naphthaquinones, are of great interest. Compounds from other pathways are esters, terpenes, steroids, terphenylquinones, and pulvinic acid (Fahselt 1994; Cohen and Neil Towers 1995; Elix and Stocker-Worgotter 2008; Muller 2001; Brunauer et al. 2006, 2007; Stocker-Worgotter and Elix 2002; Johnson et al. 2011;

Manojlovic et al. 2012). Thus, many lichens and lichen products have proved to be a source of important secondary metabolites for the food and pharmaceutical industries (Huneck 1999; Oksanen 2006), and still hold considerable interest as alternative treatments in various parts of the world (Richardson 1991). Also, we know more about these substances through experimental studies, but the functions of these compounds in lichen symbioses are still poorly understood (Hager et al. 2008): they may impact the biotic and abiotic interactions of lichens with their environment. In addition, the compounds may help to protect the thalli against herbivores, pathogens, competitors, and external abiotic factors, such as high UV irradiation. A wide spectrum of biological potential is shown by the lichens, but they have been long neglected because of their slow-growing nature and difficulties in their artificial cultivation, and have scarcely been studied from a biochemical perspective (Crittenden and Porter 1991; Yamamoto et al. 1995; Yamamoto 2000; Behera et al. 2003, 2004). Industrial-scale harvests of lichens are neither ecologically sensible nor sustainable and, for many species, are not feasible. The new molecular biology technologies are coming into sight for direct access to lichen genomes to reveal and eventually to harvest or produce novel secondary metabolites (Miao et al. 2001).

Furthermore, lichen substances exhibit a great diversity of biological effects, including antimicrobial, antiinflammatory, analgesic, antipyretic, and antiproliferative and cytotoxic activities (Boustie and Grube 2005). However, relatively few lichen substances have been screened in detail for biological activity and therapeutic potential, principally because of difficulties in obtaining them in quantities and purity sufficient for structural elucidation and pharmacological testing (Muggia et al. 2009).

### 5.2.2 Lichen Secondary Metabolites as Potential Anticancer Drugs: Some Earlier Studies

As already noted, more than 1050 secondary metabolites have been reported for lichens and aposymbiotically cultured mycobionts (Molnár and Farkas 2010). Among them, a relatively small number of these secondary products (50–60) occur in non-lichenized fungi or higher plants (Elix and Stocker-Worgotter 2008). One example is the anthraquinone parietina, which is present in other fungi such as *Aspergillus* and *Penicillium*, as well as in the vascular plant genera *Rheum*, *Rumex*, and *Ventilago* (Romagni and Dayan 2002). This metabolic diversity is largely the result of the symbiotic relationship between the lichen partners (Lawrey 1986). Lichen secondary products can constitute up to 20% of the dry thallus weight, but in most lichens the amount varies from 5% to 10%. Many lichen secondary metabolites exhibit cytotoxic properties and could be potential sources of pharmaceutically useful chemicals. The purpose of this study was to provide insights regarding the anticancer properties of lichen secondary metabolites and also to provide information regarding the mode of action of lichen compounds against cancer cells. However, so far only a limited number of studies have been published in which the mechanism of action against cancer cell lines was explored (Molnár and

Farkas 2010). The molecular mechanism of cell death by lichen compounds includes cell-cycle arrest, apoptosis, necrosis, and inhibition of angiogenesis (Brisdelli et al. 2013). There is clearly an urgent need for expanding research in this area of study, including studies of those compounds which have shown promising results as well as a strong focus on identifying specific mechanisms of action and extensive clinical trials using the most promising lichen-based drug therapies, followed by large-scale production of the best of those compounds. Although a large number of representatives of this group has already been tested and become the source of pharmaceutically important anticancer drugs, a vast potential reservoir of untapped possibilities still remains.

Among the more promising possibilities are lichenized fungi with their more than 1000 identified secondary chemicals. The use of lichen secondary products as anticancer drugs dates back to the late 1960s when the activity of lichen polysaccharides against tumor cells was initially explored (Fukuoka et al. 1968; Shibata et al. 1968). Similarly, in early studies Kupchan and Kopperman (1975) first reported the tumor inhibitor activity of usnic acid extracted from *Cladonia* sp. against Lewis lung carcinoma: they reported a 35–52% increase in the lifespan of treated mice versus the control group using a dose range of 20–200 mg/kg usnic acid. The butyrolactone, protolichesterinic acid, was also found active as an antiproliferative against leukemia cells K-562 ($IC_{50}$ = 20 mg/ml) and against Ehrlich solid tumor, whereas nephrosteranic acid derivatives have poor activity (Hirayama et al. 1980). Polyporic acid (a terphenylquinone) and derivatives (Cain 1966), a depsidone, physodalic acid (Shibamoto and Wei 1984), and lichen glucans (Nishikawa et al. 1969, 1979; Hirayama et al. 1980; Nishikawa and Ohno 1981), including lichenin derivatives (Demleitner et al. 1992), have also been investigated in this way.

### 5.2.3 Lichen Secondary Metabolites as Potential Anticancer Drugs: Recent Data

In the past few years, the interest of researchers in active metabolites originating in the lichen that show anticancer potential has not diminished. On the contrary, there is an increasing need for natural compounds that can respond to the challenges of modern therapy and be potential anticancer drugs.

In particular, an interesting study is about the anticancer capacities of ramalin, and secondary metabolites from the Antarctic lichen *Ramalina terebrata*, in the human colorectal cancer cell line HCT116. Sung et al. (2017) showed that ralmalin possesses anticancer activity against HCT116 cells, significantly reducing survival and inducing apoptosis. Also, this study has shown that ramalin induces arrest in the $G_2$/M phase of the cell cycle and affects the modulation of the corresponding genes, such as tumor protein p53, cyclin-dependent kinase inhibitor 1A, cyclin-dependent kinase 1, and cyclin B1. Similarly, another metabolite, lobaric acid, also from the Antarctic lichen *Stereocaulon alpinum*, leads to a significant decrease in the survival

of the treated Hela and HCT116 malignant cells (Ju-Mi et al. 2018). Further analysis showed a significant downregulation of the apoptosis and DNA repair regulators.

Similarly, it is important to note that interest in researching metabolites such as olivetoric, physodic, and psoromic acids is not reduced. Namely, in the study by a group from Turkey (Bugrahan et al. 2016), the human brain GBM cell line U87MG, as well as PRCC cultures obtained from six newborn Sprague–Dawley rats, was used to investigate the anticancer activity of these three metabolites of lichens. This research has shown that olivetolic acid and psoromic acid have good cytotoxic activity, which is promising for application in the treatment of glioblastoma multiforme (GBM).

Another group (Cardile et al. 2017) also investigated the cytotoxicity of physodic acid as well as the other two lichen secondary metabolites, atranorine and gyrophoric acid, on the A375 melanoma cancer cell line. The obtained results showed that the depsidone, physodic acid, possessed good cytotoxicity (dose–response relationship in the range of 6.25–50 mM) associated with apoptosis. According to the data of a new study (Rui et al. 2017), lichen secondary metabolites significantly inhibited atranorin tumorigenesis in human lung cancer cells by affecting the AP-1, Wnt, and STAT signaling and RhoGTPase-suppressing activity. We also mention the results of a particularly interesting study on the evaluation of in vitro anticancer activity of vulpinic acid in which Nil et al. (2018) reported that vulpinic acid significantly inhibited the proliferation of some treated cell lines (CaCo2, HepG2, Hep2C, RD, Wehi, Vero, and L929 cell lines). In addition, the $IC_{50}$ value of vulpinic acid altered the mRNA levels of the Bax, Bcl-2, and P53 genes in all examined cancer cells when compared to untreated cells, which is the first evaluation of the apoptotic effect of vulpinic acid on mRNA levels.

Finally, the research group of Serbia (Ristic et al. 2016a, b) examined the anticancer effect of lecanoric acid and 2-*O*-methyl anziaic acid isolated from the lichens *Melanelia subaurifera* and *Melanelia fuliginosa*. Unfortunately, the investigated compounds showed significantly less activity than the extracts. The same authors (Ristic et al. 2016a, b) also examined the anticancer potential of the species *Ramalina fraxinea* and *R. fastigiata*, especially isolated obtusatic acid, which showed a good cytotoxic effect on all tested malignant cell lines.

Finally, it is clear how important is the investigation in this area of study, including the research of those compounds and their mechanisms of action. One of the important goals in the future is certainly clinical studies using the most promising lichen-based drug therapies.

## 5.3 Overview of the Most Investigated Lichen Secondary Metabolites

### 5.3.1 Usnic Acid

Usnic acid, the most extensively studied lichen metabolite since its first isolation in 1844, exhibited an antiproliferative effect on human leukemia cells (K562) and endometrial carcinoma (HEC-50) cells (Cardarelli et al. 1997; Ingólfsdóttir 2002;

Kristmundsdóttir et al. 2002). In addition, it is known that Kupchan and Kopperman (1975) first reported the tumor inhibitor activity of usnic acid extracted from *Cladonia* sp. against Lewis lung carcinoma. They reported a 35% to 52% increase in the lifespan of treated mice versus the control group using a dose range of 20–200 mg/kg usnic acid. Therefore, usnic acid is one of the most interesting lichen metabolites for the study of their antitumor effects. The cytotoxicity, the in vitro antitumor effects, and the mechanism of action of usnic acid need to be investigated in greater detail to reach clinical trials and to allow further applications (Table 5.1).

Usnic acid, and usnic acid-amine derivatives, showed in vitro antiproliferative/cytotoxic activity against a wide variety of murine and human cancer cell lines (Takai et al. 1979; Cardarelli et al. 1997; Bézivin et al. 2004; Mayer et al. 2005; Bazin et al. 2008; Sahu et al. 2011; Bačkorová et al. 2011; Burlando et al. 2009). The toxicity of usnic acid was associated with increased $P_{450}$ activity and oxidative stress in human hepatoblastoma cells (Sahu et al. 2011), with mitochondrial dysfunction in HepG2 cells (Sahu et al. 2011), in the breast cancer T-47D cell line, and in the pancreatic cancer Capan-2 cell line (Einarsdóttir et al. 2010), with apoptotic induction in murine leukemia L1210 cells (Bézivin et al. 2004; Bazin et al. 2008).

(+)-Usnic acid was found to be a strong hepatotoxic agent against monogastric murine hepatocytes (Han et al. 2004). Also, Correché et al. investigated the cytotoxic and apoptotic effects of usnic acid obtained from continental (Chilean) and Antarctic lichens in primary cultures of rat hepatocytes (Correché et al. 2004).

The (−)-enantiomer of usnic acid (isolated from *Cladonia convoluta*) was moderately cytotoxic to various cancer cell lines, such as murine Lewis lung carcinoma, human chronic myelogenous leukemia, human brain, metastasis of a prostate carcinoma, human breast adenocarcinoma, and human glioblastoma (Molnár and Farkas 2010; Bézivin et al. 2004). Usnic acid also decreased the proliferation of human breast cancer cells and human lung cancer cells without any DNA damage (Mayer et al. 2005). Finding cancer therapies that do not have DNA-damaging effects and that do not cause the development of secondary malignancies later in life is of great interest. Accordingly, usnic acid may represent a novel source for a natural non-genotoxic anticancer drug. Usnic acid from the lichen *Usnea barbata* (Rankovic et al. 2012) induced a significant cytotoxic effect on tested human melanoma Fem-x and human colon carcinoma LS174 cell lines that was stronger than the lichen extracts. Then, as shown by numerous data, there is significant antitumor activity by usnic acid in vitro. For example, usnic acid activated programmed cell death in A2780 and HT-29 cell lines, probably through the mitochondrial pathway (Bačkorová et al. 2012).

Lichen compounds showed differential sensitivity to various cancer cells. Usnic acid was highly effective against the whole spectrum of cell lines (HeLa, MCT-7, A2780, HT-29, Jurkat, SK-BR-3, and HCT-116). Similar to cytotoxicity, usnic acid also significantly inhibited the clonogenic ability of all the tested cell lines. Usnic acid also demonstrated strong pro-apoptotic action associated with the altered cell-cycle distribution and accumulation of cells in S-phase (Brisdelli et al. 2013; Bačkorová et al. 2011).

**Table 5.1** Overview of recent literature related to the in vitro anticancer activity of lichen secondary metabolites (according to Shrestha and St. Clair 2013)

| Lichen metabolites/lichen species | Cell lines tested | References |
|---|---|---|
| Usnic acid (commercial)<br>Atranorin (commercial)<br>Parietin (*X. parietina*)<br>Gyrophoric acid (*Umbilicaria hirsuta*) | Human ovarian carcinoma A2780<br>Human colon adenocarcinoma HT-29 | Bačkorová et al. (2012) |
| Diffractaic acid (*Protousnea magellanica*)<br>Vicanicin (*Psoroma pallidum*)<br>Lobaric acid (*Stereocaulon alpium*)<br>Variolaric acid (*Ochrolechia deceptionis*)<br>Protolichesterinic acid (*Cornicularia aculeate*)<br>Usnic acid (*Cladonia lepidophora*) | Human breast adenocarcinoma MCF-7<br>Human colon adenocarcinoma HCT-116<br>Human cervix adenocarcinoma HeLa | Brisdelli et al. (2013) |
| Atranorin (*Bacidia stipata*)<br>Diffractaic acid (*P. magellanica*)<br>Divaricatic acid (*Protousnea malacea*)<br>Vicanicin (*Psoroma dimorphum*)<br>Protolichesterinic acid (*R. melanophthalma*) | Human prostate cancer androgen-responsive (LNCaP)<br>Human prostate cancer androgen-non responsive DU-145 | Russo et al. (2012) |
| Usnic acid (commercial)<br>Atranorin (commercial)<br>Parietin (*X. parietina*)<br>Gyrophoric acid (*U. hirsuta*) | Human ovarian A2780<br>Human breast MCF-7<br>Human colon HT-29<br>Human T cells, Jurkat Human cervix HeLa Human breast SK-BR-3 Human colon wild type p53 HCT-116 p53+/+<br>Human colon p53 null HCT-116 p53−/− | Bačkorová et al. (2012) |
| Lecanoric acid and it's orsellinate derivatives | Larynx carcinoma HEP-2<br>Breast carcinoma MCF-7<br>Kidney carcinoma 786-0<br>Murine melanoma cell B16-F-I0<br>Vero cell | Bogo et al. (2010) |
| (+) Usnic acid (*C. arbuscula*)<br>(−) Usnic acid (*Alectoria ochroleuca*) | Breast cancer cell line T-47D<br>Pancreatic cancer cell line Capan-2 | Einarsdóttir et al. (2010) |
| Retigeric acid A and Retigeric acid B (*Lobaria kurokawae*) | Human Pca LNCaP PC-3, DU 145<br>Human epidermoid KB and vincristine resistant KB (KB/VCR)<br>Human ovarian 3-AO and cisplatin resistant 3-AO (3-AO/CDDP)<br>Human benign prostate epithelial RWPE1<br>Human hTERT-RPEl<br>Human breast MCF-7<br>Human osteosarcoma U20S and Saos2 | Liu et al. (2010) |

(continued)

**Table 5.1** (continued)

| Lichen metabolites/lichen species | Cell lines tested | References |
|---|---|---|
| Depsidones—Vicanicin, Pannarin, 1-chloropannarin, Salazinic acid, Stictic acid, variolaric acid, Psoromic acid, Fumarprotocetraric acid, Lobaric acid<br>Depsides—Atranorin, Sphaerophorin, Divaricatic acid, Diffractaic acid, Gyrophoric acid, Usnic acid | Hepatocytes from rat | Correché et al. (2004) |
| Pannarin, 10-chloro pannarin, Salazinic acid, Psoromic acid, Fumarprotocetaric acid, Lobaric acid, Vicanicin, Stictic acid, Variolaric acid, Atranorin, Sphaerophorin, Divaricatic acid, Diffractaic acid, Gyrophoric acid | Lymphocytes from rat spleens | Correché et al. (2002) |
| (+)-usnic acid<br>Methyl a-orcinolcarboxylate<br>Ethyl hematommate<br>Diffractaic acid<br>Gyrophoric acid<br>(+)-protolichesterinic acid | Human keratinocyte cell line HaCaT | Kumar and Muller (1999) |
| Lobaric acid (*S. alpinum*)<br>Protolichesterinic acid (*C. islandica*) | Breast cancer cell T-47D and ZR-75-1<br>Erythro-leukemia K-563 | Ögmundsdóttir et al. (1998) |
| Usnic acid derivatives | Lewis lung carcinoma L1210 | Takai et al. (1979) |

Somewhat earlier (Einarsdóttir et al. 2010) it was announced that both (+)- and (−)-usnic acids are effective inhibitors of DNA synthesis, with $IC_{50}$ values of 4.2 and 4.0 μg/ml, respectively, against T-47D (a breast cancer cell line), and 5.3 and 5.0 μg/ml against Capan-2 (a pancreatic cancer cell line). There was a reduction in cell size, and both acids inhibit cell entry into the S-phase. Regarding the mechanism of action, staining with the mitochondrial dye JC-1 demonstrated a dose-dependent loss of mitochondrial membrane potential following treatment with usnic acid in both cell lines. Study of the effects of usnic acid on MCF7 (estrogen-dependent, wild-type p53) indicated no morphological changes in microtubules or increase in the mitotic index, suggesting that the antineoplastic activity of usnic acid is not related to alterations in the formation and stabilization of microtubules (O'Neill et al. 2010).

Usnic acid from *Xanthoparmelia somloensis*, Burlando et al. (2009) have investigated its cytotoxic effect towards malignant mesothelioma cells (MM98), vulvar carcinoma cells (A431), and keratinocytes (HaCaT). Usnic acid showed high cytoxicity for all three cell lines. Further, both types of usnic acid showed dose- and time-dependent cytotoxicity against V79 (Chinese hamster lung fibroblast) and A549 (human lung carcinoma) cell lines. Cytotoxicity was more pronounced in A549 than V79, with cell viability more diminished in A549 versus V79

after 2 days of treatment (Koparal et al. 2006). To investigate the mechanism of action of usnic acid, elevated levels of the p53 and p21 proteins following treatment with usnic acid were confirmed, but there was no p53 transcriptional activity, suggesting that the accumulation of p21 was not secondary to p53 transactivation (Mayer et al. 2005). They concluded that usnic acid has antiproliferative activity against wild-type p53 (MCF7) and nonfunctional p53 (MDA-MB-231) breast cancer cells, as well as against the H1299 lung cancer cell line, which is null for p53. Usnic acid is therefore a non-genotoxic anticancer agent that works in an p53-independent manner. In another study (Bézivin et al. 2004), usnic acid also induced L1210 (murine lymphocytic leukemia) in apoptosis in a dose- and time-dependent manner as fluorescence microscopy revealed condensation of nuclear chromatin, nuclear fragmentation, and formation of apoptotic bodies.

### 5.3.2 Depsides and Depsidones

There are several studies about antitumor activity of depsides and depsidones and especially on atranorin. Bačkorová and colleagues reported that antiproliferative/cytotoxic effects of atranorin efficiently induced apoptosis and inhibited cell proliferation in various cancer cell lines tested. Similar to usnic acid, atranorin demonstrated strong pro-apoptotic action. Moreover, the same authors reported on the sensitivity of up to nine human cancer cell lines (A2780, HeLa, MCF-7, SK-BR-3, HT-29, HCT-116, p53 (+/+), HCT-116, p53 (−/−), HL-60, and Jurkat) to the antiproliferative/cytotoxic effects of some typical secondary metabolites of lichens (parietin and gyrophoric acid). Further, the analysis of cell-cycle distribution also revealed an accumulation of cells in S-phase. This study has confirmed a differential sensitivity of cancer cell lines to lichen secondary metabolites (Bačkorová et al. 2011). In addition, atranorin, diffractaic acid, and divaricatic acid were found to be active against prostate cancer cells [human prostate cancer androgen-responsive (LNCaP) and human prostate cancer androgen-nonresponsive DU-145 cells] only in high concentration (Russo et al. 2006, 2012). This study for the first time showed that apoptosis induced by the compounds appeared to be mediated, at least in part, via the inhibition of Hsp70 expression. Also, the depsides atranorin, sphaerophorin, divaricatic acid, diffractaic acid, and gyrophoric acid, and the depsidones vicanicin, pannarin, 1-chloropannarin, salazinic acid, stictic acid, variolaric acid, psoromic acid, fumarprotocetraric acid, and lobaric acid, were evaluated for their cytotoxic activity towards hepatocytes from rat and lymphocytes from rat spleens (Correché et al. 2002, 2004). The research has shown that salazinic acid, stictic acid, and psoromic acid showed apoptosis of hepatocytes in a dose-dependent manner, with stictic acid showing the strongest apoptotic activity. Ogmundsdottir and associates showed that lobaric acid and protolichesterinic acid when used towards breast cancer cells T-47D and ZR-75-1 as erythro-leukemia K-563 cells caused a significant reduction in DNA synthesis. Significant cell deaths in all three cell lines were observed at concentrations of 20 and 30 μg/ml of protolichesterinic acid and lobaric acid, respectively (Ögmundsdóttir et al. 1998). Similarly, Kumar and Muller showed

that gyrophoric acid, usnic acid, and diffractaic acids were reported as potent antiproliferative agents towards the human keratinocyte cell line HaCaT, inhibiting cell growth at $IC_{50}$ values of 1.7, 2.1, and 2.6 μM (Kumar and Muller 1999). Also, results from the work of Pejin and associates suggested a moderate anticancer activity towards malignant HT29 ($IC_{50}$ value, 29.29 μg/ml) and a low growth inhibition on nonmalignant MRC5 cells ($IC_{50}$ value, 2478.40 μg/ml) of stictic acid (Pejin et al. 2013). These findings may indicate that stictic acid can be considered as a promising lead compound for the design of novel human colon adenocarcinoma drugs. Generally, depsidones showed stronger cytotoxic activity than depsides. The strong biological activity of some depsidones may result from the strong hydrogen bond between the aldehyde group at C3 and the hydroxyl group at C4. Similarly, the cytotoxic activity of depsides may result in part from the presence of a COOH group on C1 and an OH group on C2. Similarly, Manojlovic and associates reported the strong cytotoxic effect of the depsidone salazinic acid as well as the phenolic compound protocetraric acid against FEM-x (human melanoma) and LS174 (human colon carcinoma) cell lines (Manojlovic et al. 2012). Pannarin, a depsidone, was shown to inhibit the growth of DU-145 prostate carcinoma and M14 human melanoma cells (Russo et al. 2006; Brandão et al. 2013) Also, for the purpose of identifying novel agents with antigrowth and pro-apoptotic activity on prostate cancer cells, Russo et al. (2012) evaluated the effect of lichen secondary metabolites, the depsides atranorin, diffrattaic, and divaricatic acids, as well as the depsidone vicanicin, on cell growth in androgen-sensitive (LNCaP) and androgen-insensitive (DU 145) human prostate cancer cells. The depsides resulting from decarboxylation of baeomycesic and squamatic acids showed antiproliferative effects on PC-3 prostate cancer cells [50% growth inhibitory concentration ($GI_{50}$), 70.06 and 79.37 μm, respectively] (Guo et al. 2011). Also, olivetoric acid as a di-depside displayed dose-dependent antiangiogenic activities, inhibited cell proliferation, and disrupted endothelial tube formation in rat adipose tissue endothelial cells (Koparal et al. 2010).

Very good cytotoxic activity against malignant FEM-x and LS174 cells was also shown by depsidone physodic acids and depside atranorin, which were identified from the lichen *Hypogymnia physodes* growing in Serbia (Rankovic et al. 2014). Their $IC_{50}$ values were in the range of 17.89–24.63 μg/ml, respectively, somewhat less than the value of the $IC_{50}$ of usnic acid. These authors examined the antitumor activity of evernic acid, which belongs to depsides, and also depsidone physodic acid isolated from the lichens *Evernia prunastri* and *Pseudevernia furfuracea* (Kosanic et al. 2013). The obtained results show that the tested compounds exhibited high cytotoxic activity against the target cells in vitro. The best cytotoxic activity was exhibited by physodic acid. The effect of tested samples on cell-cycle progression was investigated also in FEM-x and LS174 cells. An increase in cells containing sub-G1 amounts of DNA was observed, indicating that the evernic and physodic acids were inducing cell death. Similarly, somewhat earlier, Russo et al. (2008) reported that the depside sphaerophorin (isolated from *Sphaerophorus globosus*) and the depsidone pannarin (isolated from *Psoroma pholidotoides*) inhibited the growth of M14 human melanoma cells, triggering apoptotic cell death. The data obtained

from cell culture show that these lichen metabolites inhibit the growth of melanoma cells, inducing their apoptotic cell death, demonstrated by the fragmentation of genomic DNA and by a significant increase of caspase-3 activity, and correlated, at least in part, with the increase of reactive oxygen species (ROS) generation. The anticancer activities of these lichen metabolites are promising in the treatment of this aggressive, therapy-resistant skin tumor (Molnár and Farkas 2010). However, one new study was done (Brisdelli et al. 2013) of six lichen metabolites (diffractaic acid, lobaric acid, lips acid, vicanicin, variolaric acid, protolichesterinic acid) on proliferation, viability, and ROS level towards three human cancer cell lines: MCF-7 (breast adenocarcinoma), HeLa (cervix adenocarcinoma), and HCT-116 (colon carcinoma). In this comparative study, lichen metabolites showed various cytotoxic effects in a concentration-dependent manner. Moreover, not all tested lichen compounds exhibited free radical scavenging activity. The lichen metabolites did not significantly increase intracellular ROS level. Further, the cytotoxic activities of the depsidone variolaric acid and two other secondary metabolites of lichens, R-alectoronic acid and ergosterol peroxide, were evaluated against the murine B16 melanoma cell line. All the tested compounds showed a significant antitumor activity, especially variolaric acid and alectoronic acid, as compared to cisplatin as a positive control (Millot et al. 2007). Anziaic acid was also found to act as an inhibitor of human topoisomerase II but had little effect on human topoisomerase I (Cheng et al. 2013). This is the first report of a depside with activity as a topoisomerase poison inhibitor and demonstrates the potential of this class of natural products as a source for new antibacterial and anticancer compounds. Protolichesterinic acid showed an inhibitory effect against 12 cell lines, with $IC_{50}$ values of 2.4–18.1 μg/ml (Russo et al. 2012). Also, in recent years (Bogo et al. 2010) it has been shown that lecanoric acid (para-depside), a secondary metabolite of the lichen *Parmotrema tinctorum*, has moderate antitumor activity against some malignant cell lines tested (MCF7 breast carcinoma, 786-0 kidney carcinoma, and B16-F10 murine melanoma). Similarly, some cytotoxic activity in vitro of lecanoric acid, orsellinic acid, methyl ester, orcinol, and usnic acid isolated from the lichen *Parmelia subrudecta* is shown by other authors (Ivanova et al. 2010). The depsides resulting from decarboxylation of baeomycesic and squamatic acids showed antiproliferative effects on PC-3 prostate cancer cells (Haraldsdóttir et al. 2004; Brandão et al. 2013). Also, Brandao and his research group have reported evaluation of some lichen compounds (the depsides atranorin and diffractaic, divaricatic, and perlatolic acids; the depsidones psoromic, protocetraric, and norstictic acids) tested against UACC-62 and B16-F10 melanoma cell lines, and 3 T3 normal fibroblast cells (Brandão et al. 2013).

### 5.3.3 Naphthoquinones

Naphthazarin and its derivates were isolated from *Cetraria islandica*. This naphthoquinone demonstrated, in in vitro experiments, a strong cytotoxic effect to

human epidermal carcinoma cells. The dimer of this naphthoquinone, hybocarpone, was isolated from *Lecanora hybocarpa* (Babula et al. 2009).

### 5.3.4 Anthraquinones

#### 5.3.4.1 Emodin

The anthraquinones are a large family of compounds having diverse biological properties. Emodin (1,3,8-trihydroxy-6-methylanthraquinone) is a naturally occurring anthraquinone present in numerous lichens (Cohen and Neil Towers 1995). Emodin, first assigned to be a specific inhibitor of the protein tyrosine kinase p65lck, has now a number of cellular targets interacting with it. Its inhibitory effect on mammalian cell-cycle modulation in specific oncogene overexpressed cells formed the basis of using this compound as an anticancer agent. Identification of apoptosis as a mechanism of elimination of cells treated with cytotoxic agents initiated new studies deciphering the mechanism of apoptosis induced by emodin. At present, its role in combination chemotherapy with standard drugs to reduce toxicity and to enhance efficacy is being pursued vigorously. Its additional inhibitory effects on angiogenic and metastasis regulatory processes make emodin a sensible candidate as a specific blocker of tumor-associated events (Srinivas et al. 2007).

#### 5.3.4.2 Parietin

Parietin is derived from polyaromatic ring polyketides and is present in lichen of the genera *Xanthoria* and *Teloschites*, in particular in the lichens *Teloschites chrysothalmus*, *Teloschites spinosus*, and *Xanthoria parietina*. Among others, Bačkorová and the authors showed that certain cytotoxic potential of parietin by a series of cancer cell lines (Bačkorová et al. 2011).

### 5.3.5 Xanthones: Lichexanthone

Lichexanthone was one of the xanthones also tested in a recent study (Brandão et al. 2013), together with other secondary metabolites of lichens. This study showed that lichexanthone was the least active substance tested, delineating a very distinct response relative to the other compounds and the standard doxorubicin.

### 5.3.6 Other Compounds

Apart from compounds derived from common pathways, which are found throughout all major lichen groups, there are also some unusual compound classes among these organisms; for example, arthogalin, a cyclic depsipeptide (Huneck and Himmelreich 1995), and other amino acid-derived compounds such as the cytotoxic scabrosin esters isolated from *Xanthoparmelia scabrosa* (Ernst-Russell et al. 1999). Thus, Magaya et al. tested the effects of arthogalin, a secondary metabolite of the

lichen *Caloplaca inclinans,* on the growth of murine malignant prostate sarcoma cells in vitro (Magaya et al. 2013). The results of this study showed that arthogalin is a potent inhibitor of growth of tested cancer cells. They also showed that arthogalin increases sensitivity of cells to radiation, and this effect is significant at a radiation intensity lower than the standard intensity of cancer radiotherapy. On the basis of this study, arthogalin shows promise for combined-modality cancer treatment. Also, earlier studies have shown that scabrosin esters (SEs), which have been isolated from the lichen *Xanthoparmelia scabrosa*, belong to the epipolythiodioxopiperazine (ETP) class of secondary metabolites characterized by possession of a reactive disulfide bond. Colony-forming assays that were used in these studies have shown that these compounds are active against human tumor cell lines at nanomolar concentrations. Colony-forming assays also show that these toxins are active against human tumor cell lines at nanomolar concentrations (Moerman et al. 2003). These authors show that the typical scabrosin ester acetate butyrate induces early mitochondrial membrane hyperpolarization in accompanied by apoptotic cell death. Here, we mention retigeric acid A (RA) and retigeric acid (RB), both pentacyclic triterpenoids from the lichen species *Lobaria kurokawae*. Liu and associates (Liu et al. 2010) showed cytotoxicity towards malignant cells at lower concentrations ($<$100 μM) of these compounds but RB is more potent than RA. Specially, investigation on the effect of RB on PC-3 cells showed that RB caused a dose-dependent accumulation of cells in the S phase accompanied with decreases in cyclin B and in cyclin E and cyclin A. Both caspase-dependent and caspase-independent pathways were responsible for apoptosis in PC-3 cells. It should also be noted that 16-*O*-acetyl-leucotylic acid, a new triterpenic acid, exhibited potent antiproliferative activity against HL-60 with an $EC_{50}$ value of 21 μM although the leucotylic acid, the derivative of 16-*O*-acetly-leucotylic acid has a higher $EC_{50}$ value (72 μM) (Tokiwano et al. 2009).

## 5.4 Overview of Existing In Vivo Studies

Without in vivo evaluation and clinical trials, no real efficacy in cancer therapy can be claimed for any of the potential agents. First, it is known that the most of the antitumor in vivo studies on lichen extracts have been performed by Japanese scientists in the 1970s (Fujikawa et al. 1972; Hirayama et al. 1974). The allogeneic tumor S-180-forming ascites implanted into albino mice is generally used as the basic screening model. Few antitumor assays have been conducted on Ehrlich carcinomas. In each model, samples dissolved in distilled water were administered by i.p. injection for 10 consecutive days, starting 24 h after tumor implantation. After 30 days, the antitumor effect was evaluated through the inhibition ratio (IR), which is linked to the reduction of tumor weight, and the complete regression rate (CR), which is linked to complete recovery of grafted animals. However, with advances in the isolation and characterization of secondary metabolites of lichens, in addition to in vitro testing of their antitumor effects, there is growing interest for the study in vivo (Ribeiro-Costa et al. 2004). Only scarce in vivo assays in mice have been

attempted for some low molecular weight lichen compounds. One of the first lichen acids found to have some activity on L-1210 and S-180 models in mice was polyporic acid, a dihydroxyquinone isolated from *Sticta coronata.* It was given in a dose of 60 mg/kg administered by intraperitoneal (i.p.) injection (Burton and Cain 1959). The well-known (−)-usnic acid, a dibenzofuran, was proved to have a weak, if any, antitumoral effect against Lewis lung carcinoma and P-388 leukemia cells (Kupchan et al. 1975; cited in Takai et al. 1979). A series of 20 lichen compounds have been tested against Ehrlich carcinomas in mice, revealing some potential for the butyrolactones (+)-protolichesterinic acid and nephrosterinic acid, with 50% and 70% tumor growth inhibition, respectively (Hirayama et al. 1980). A significant in vivo antineoplastic activity (murine leukemia P-388, tested/control × 100 (T/C) = 40% at 160 μg/kg) is reported for ambewelamide A, an original diketopiperazine dione (Williams et al. 1998). This scabrosin ester and derivatives isolated from two lichen species have shown potent in vitro cytotoxic activity ($IC_{50}$ within the μM to nM range for P=388, P-815, and MCF-7) (Williams et al. 1998; Ernst-Russell et al. 1999). Another rare compound, hybocarpone, isolated from a mycobiont culture of *Lecanora hybocarpa* ($IC_{50}$ = 0.27 μM) (Ernst-Russell et al. 1999), is also a relevant compound to be investigated further in terms of anticancer studies. In 2004 Ribeiro-Costa and colleagues investigated the in vitro and in vivo properties of usnic acid encapsulated into poly(lactic-co-glycolic acid (PLGA) microspheres. Microparticles will probably have a promising role in the future of chemotherapy. These polymeric delivery systems are capable of maximizing therapeutic activity while reducing side effects of anticancer agents. In this study, PLGA microspheres contained usnic acid from *Cladonia substellata*. The antitumor assay was performed in mice against sarcoma-180 tumor (UA, 15 mg/ kg weight body/ day) during 7 days. Animals were then killed and tumors and organs were excised for histopathological analysis. A maximum release of 92% was achieved at the fifth day. The $IC_{50}$ values for free and encapsulated usnic acid were 12 and 14 μg /ml, respectively. The encapsulation of usnic acid into microspheres promoted an increase of 21% in tumor inhibition as compared with the free usnic acid treatment. In summary, usnic acid was efficiently encapsulated into PLGA microspheres and the microencapsulation improved its antitumor activity (Ribeiro-Costa et al. 2004).

## 5.5 Lichen Secondary Metabolites as Potential Anticancer Drugs: Prospects and Promise

Chemotherapy is still the method most commonly used and most promising in the treatment of cancer patients. Also, chemotherapy holds the most promise for selectively eradicating cancer cells while at the same time minimizing collateral damage to surrounding tissues. Many of these chemical agents owe their origins to natural sources in the environment, whereas other anticancer chemotherapeutics are wholly designed by pharmaceutical scientists based upon current knowledge of cancer-onset mechanisms. Selectivity for cancer cell destruction without harming healthy cells is the central focus of these treatment protocols, and the chemotherapy's well-known

side effects of chemotherapy (hair loss, nausea, immunocompromise, etc.) are a continuing reminder that much room for progress yet remains. Natural products and their derivatives represent more than 50% of all the drugs in clinical use in the world. Almost 60% of drugs approved for cancer treatment are of natural origin (Fakim 2006). Whether the promise of fully selective anticancer medicines will be realized in our lifetime remains unknown, but exciting developments from the investigation of lichen secondary metabolites lend credibility to the proposition that the best is yet to come. Therefore, increasing research on the lichen natural resources may provide good results for exploiting and developing valuable natural products which benefit humans. For the past 20–30 years, some studies with lichen, even with limited screening effort, have indicated the frequent occurrence of metabolites with antitumor properties (Ren et al. 2009; Rankovic et al. 2011, 2014; Kosanic et al. 2012a, b). All this evidence supports efforts for the development of new anticancer drugs with lichen secondary metabolites as a starting point. The potential of lichen secondary metabolites as a possible source of anticancer drugs is certainly large and visible. As we know, the structures of more than 700 lichen substances are available, but because lichens grow slowly, their availability is insufficient in quantity and difficult in large-scale industrial production, so lichens were frequently ignored by pharmaceutical industries. However, the secondary metabolites of lichen that are deposited on the surface of the mycelium were usually produced by fungi; therefore, it became possible that cultured mycobionts could replace the lichen. Although not many of the lichen metabolites are likely to become therapeutics, the information gained from studying them is likely to lead the development and understanding of novel molecular targets and chemical synthesis or chemical modification of natural metabolites, which in turn may lead to the development of new classes of therapeutic agents. On the other hand, powerful new technologies such as combinatorial chemistry, high-throughput screening, bioinformatics, proteomics, and genomics have emerged and are being integrated widely in the field of pharmaceutical discovery research. These technologies have enormous potential to make use of the chemical diversity of natural products (Lahlou 2013). All this, including compound library design, protein 3D structures, nuclear magnetic resonance (NMR)-based screening, 3D quantitative structure–activity relationship (QSAR) in modern drug design, and computer-aided prediction of drug toxicity and metabolism, may help in the development of new agents modeled on the basis of the secondary metabolites of lichens. Finally, a multidisciplinary collaboration among lichenologists, chemists, pharmacologists, and biologists is expected to be critical in the development of potential anticancer drugs from secondary metabolites of lichens.

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