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Lichens: a promising source of antibiotic and anticancer drugs

Gajendra Shrestha · Larry L. St. Clair

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Abstract Lichens are symbiotic associations between fungi and a photosynthetic alga and/or cyanobacteria. Lichenized fungi have been found to produce a wide array of secondary metabolites, most of which are unique to the lichenized condition. These secondary metabolites have shown an impressive range of biological activities including antibiotics, antifungal, anti-HIV, anticancer, anti-protozoan, etc. This review focuses primarily on the antibiotic and anticancer properties of lichen secondary chemicals. We have reviewed various publications related to antibiotic and anticancer drug therapies emphasizing results about specific lichens and/or lichen compounds, which microbes or cancer cells were involved and the main findings of each study. We found that crude lichen extracts and various isolated lichen compounds often demonstrate significant inhibitory activity against various pathogenic bacteria and cancer cell lines at very low concentrations. There were no studies examining the specific mechanism of action against pathogenic bacteria; however, we did find a limited number of studies where the mechanism of action against cancer cell lines had been explored. The molecular mechanism of cell death by lichen compounds includes cell cycle arrest, apoptosis, necrosis, and inhibition of angiogenesis. Although lichens are a reservoir for various biologically active compounds, only a limited number have been tested for their biological significance. There is clearly an urgent need for expanding research in this area of study, including in depth 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.

Keywords Lichens · Biological role · Natural products · Secondary metabolites · Anti-bacteria · Anti-cancer

G. Shrestha (✉) · L. L. St. Clair
Department of Biology and the M. L. Bean Life Science Museum, Brigham Young University, Provo, UT 84602, USA
e-mail: gssm_us@yahoo.com

Introduction

Lichens are obligate symbiotic systems consisting of a filamentous fungus and a photosynthetic partner (eukaryotic algae and/or cyanobacterium), and in some cases non-photosynthetic bacteria (Hodkinson and Lutzoni 2009; Selbmann et al. 2010). Lichens are ecologically diverse and are distributed from the tropics to the polar regions (Brodo et al. 2001). The worldwide lichen flora is estimated to include approximately 18,500 species (Boustie and Grube 2005; Feuerer and Hawksworth 2007) and cover about 8 % of the earth's land surface (Ahmadjian 1995). Lichens are one of the slowest growing symbiotic associations

and according to Coley (1988) slow growing organisms occupying low-resource habitats produce higher levels of defense chemicals in order to defend themselves from various consumers. This is certainly true in the case of lichens since they are known to produce more than a 1,000 different secondary metabolites. The main natural roles of these compounds include: protection against a large spectrum of microbes, animal predators and plant competitors; defense against environmental stress like UV radiation and desiccation; physiological regulation of metabolism, such as the ability to increase algal cell membrane permeability in order to increase the flow of nutrients to the fungal component (Huneck and Yoshimura 1996). As lichens are very capable of protecting themselves from various microbes including bacteria, non-lichenized fungi, and nematodes, the potential value of these metabolites for medicinal purposes is generating increasing research interest.

Lichens produce two different types of metabolites; primary and secondary. Primary lichen substances have structural functions and roles in cellular metabolism, similar to those in other fungi. Primary metabolites are intracellular in origin and are synthesized independently by both symbionts. Primary compounds consists mainly of chitin (in hyphal walls), lichenin, isolichenin, hemicellulose, pectins, disaccharides, polyalcohols, amino acids, enzymes, pigments like algal chromophores: chlorophyll and, β-carotenes, xanthophylls, etc. (Podterob 2008). In contrast, secondary metabolites are produced exclusively by the fungal partner and are exported outside the fungal hyphae and deposited as crystals in different parts of the thallus, often in the upper cortex or in specialized structures such as fruiting bodies (Fahselt 1994). The exclusive fungal origin of secondary metabolites has been confirmed based on the work of Culberson and Armaleo (1992); Hamada et al. (1996); Kon et al. (1997); Stocker-Wörgötter and Elix (2002). Although lichen secondary metabolites are exclusively of fungal origin, the metabolic interaction between the mycobiont and photobiont is essential to the production of these secondary chemicals. This has been documented by studies where the mycobiont grown without the photobiont does not produce the same metabolites as the intact lichen or produces a completely different suite of chemical products (Molina et al. 2003; Fazio et al. 2009). There are some situations where the photobiont, especially cyanobacteria, also produce some secondary metabolites (Cox et al. 2005; Yang et al. 1993).

Over 1,050 secondary metabolites have been reported for lichens and aposymbiotically cultured mycobionts (Molnar 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-Wörgötter 2008). One example is the anthraquinone parietin which is present in other fungi like *Aspergillus* and *Penicillium*, as well as in the vascular plant genera *Rheum*, *Rumex* and *Ventilago* (Romagni & Dayan 2002). This metabolic diversity is due largely to the symbiotic relationship between the lichen partners (Lawrey 1986). Lichen secondary products can comprise up to 20 % of the thallus dry weight, but in most lichens the amount varies from 5 to 10 %.

The aim of this review paper is to provide insights regarding the antibacterial and anticancer properties of lichen chemicals (either as crude extracts or purified compounds) and also to provide information regarding the mode of action of lichen compounds against bacterial and cancer cells. The results of this review concerning the use of lichen compounds as antibacterial and anticancer therapies are based on a thorough examination of the published literature.

Role of lichens in medicine

Lichens have been used as ingredients in folk medicines for centuries and many cultures have used lichens to treat a variety of ailments as part of their traditional medicines (Dayan and Romagni 2001). The medicinal properties of some lichens are mentioned in the Ayurvedic and Unani systems where they are used to treat a broad array of common ailments, including blood and heart diseases, bronchitis, scabies, leprosy, asthma inflammations, stomach disorders, etc. (Shukla et al. 2010). Recent advances in the medical field have resulted in exploration of the biological activity of a limited number of lichen products with some studies suggesting that some lichen chemicals could possible provide a promising source of future drug therapies. Demonstrated medicinal properties based on lichen chemistry include :antibiotics (Balaji et al. 2006; Burkholder et al. 1944; Paudel et al. 2010; Turk et al. 2003), anti-proliferative (Bucar et al. 2004; Burlando et al. 2009; Kumar and Müller 1999), antioxidants

(Bhattarai et al. 2008; Gulluce et al. 2006; Hidalgo et al. 1994), anti-HIV (Nakanishi et al. 1998; Neamati et al. 1997), anti-cancer (Bézivin et al. 2003; Bezivin et al. 2004; Mayer et al. 2005; O'Neill et al. 2010; Ren et al. 2009), and anti-protozoans (De Carvalho et al. 2005; Schmeda-Hirschmann et al. 2008). It should be noted that there have been reports of liver-related toxicity with the use of usnic acid in dietary supplements (Foti et al. 2008; Sanchez et al. 2006). However, other studies (Sahu et al. 2012) indicate that low non-toxic concentrations of usnic acid do not cause damage to the liver. This review paper focuses specifically on two particularly promising biological responses of lichen acids against pathogenic bacteria and cancer cell lines. We will particularly concentrate on which lichens (either as crude extracts or isolated compounds) have been tested against various bacteria and cancer cell lines while also considering the mechanism of cell death caused by the lichen metabolites.

Lichens as a source of antibiotics

The antibiotic properties of lichen metabolites represent one of the better studied biological roles for lichens. Initial testing of the antibiotic activity of lichens was done by Burkholder et al. (1944). They tested aqueous extracts of 42 lichen species against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*. Results from this early study showed that 27 lichens were active against *S. aureus* and/or *B. subtilis* but none of the species showed any activity against *E. coli*. Since Burkholder's preliminary study various lichens (either as crude extracts or isolated compounds) have been screened against a variety of gram positive, gram negative, and mycobacteria with several lichen metabolites showing promise as potential antibiotics drug therapies (Table 1).

The above table (Table 1) shows that lichen substances have been found to be effective against a variety of pathogenic bacteria, especially gram-positive bacteria. Table 1 also shows that among the various lichen compounds, usnic acid, pulvinic acid derivatives—vulpinic acid, lichesterinic acid (an aliphatic acid), and orcinol-type depsides and depsidones demonstrate the most promising antibiotic properties. Lichen compounds have not only shown their potency against sensitive strains of bacteria, but also against various multi-drug resistant bacterial strains (Martins et al. 2010; Kokobun et al. 2007). In addition, steps have also been taken to incorporate lichen metabolites into medical devices to enhance their activity against the formation of bacterial biofilms (Francolini et al. 2004) as well as in combination with other antibiotics for synergistic effect (Safak et al. 2009). Studies have also demonstrated that lichen compounds inhibit bacterial growth at much lower concentrations when compared to other sources of antibiotic therapies (Weckesser et al. 2007; Gordien et al. 2010). Although over 1,050 secondary metabolites have been identified from lichens, relatively small number ($\sim$50 species) have been screened for antibiotic activity. According to Vartia (1973) more than 50 % of the lichens tested show at least some antibiotic activity. These results demonstrate the need for further efforts in screening lichen extracts in general and specific lichen metabolites in particular. North America is home to a diverse assemblage of lichen species. In spite of the increased interest in the ecological and evolutionary role of North American lichens, the biological significance of their secondary metabolites remains largely unexplored. Our lab is presently screening 36 different lichen species collected from various parts of North American against four different bacterial strains—*S. aureus*, methacillin-resistant *S. aureus* COL, *E. coli*, and *P. aeruginosa*. Our preliminary results indicate that except for *Lobaria pulmonaria* all other lichen extracts showed at least some inhibition against all four bacterial strains at test concentrations of 500–7.8 μg/ml. Although there have been several studies showing effective inhibition of different bacterial strains by lichen metabolites, we did not find any studies that have specifically addressed the mechanism of action of these lichen compounds. We are currently exploring possible inhibition mechanisms related to lichen metabolites which cause bacterial cell death (*S. aureus* COL). We are specifically focusing on the effects of several promising lichen acids (e.g., Usnic acid and Vulpinic acid).

Lichens as a potential source of anti-cancer drug therapies

Worldwide, cancer is one of the most common causes of death. Focused efforts on identifying and developing new anticancer drug therapies from various natural sources, including vascular plants, fungi, prokaryotes,

Table 1 A summary of the literature dealing with the antibiotic activity of lichen secondary chemistry

Lichens/lichen metabolites	Bacteria	Finding	References
Combination of usnic acid and 5 different antibiotics namely-clindamycin, erythromycin, gentamicin, levofloxacin, and oxacillin	20 different methecillin resistant clinical isolates of *S. aureus*	Usnic acid inhibited 50 % of growth of all *S. aureus* at 2 μg/ml while 90 % growth inhibition was found at 4 μg/ml. Combination of usnic acid with gentamicin gave synergistic action, while antagonism was observed with levofloxin whereas there was no difference with erythromycin. However, variability was observed with clindamycin and oxacillin	Segatore et al. (2012)
Crude extract of *Cladonia furcata*, *Hypogymnia physodes*, and *Umbilicaria polyphylla* and fumarprotocetraric acid, gyrophoric acid and physodic acid extract respectively from above mentioned lichens	*Bacillus mycoides* *B. subtilis* *S. aureus* *Enterobacter cloaceae* *E. coli* *Klebsiella pneumoniae*	Both the crude extract of lichens and their components showed a relatively strong antimicrobial activity but comparatively the components were more active than crude extract. The acetone and methanolic extract showed relatively strong antimicrobial activity with MIC ranging from 0.78 to 6.25 mg/ml while the aqueous extract showed no activity against any bacteria. The lichen components showed very strong antimicrobial activity with MIC ranging from 0.031 to 1 mg/ml	Kosanic and Rankovic (2011)
Crude extract of *Umbilicaria cylindrica*	*B. subtilis* *S. aureus* *E. coli* *Proteus vulgaris* *P. mirabilis* *K. pneumoniae*	Both methanol and ethyl acetate extracts exhibited significant inhibitory activity against all tested bacteria. The ethyl acetate extract was most potent against *E. coli* with MIC value of 15.62 μg/ml while methanolic extract was active against *B. subtilis* and *S. aureus* with the MIC value 15.62 μg/ml	Manojlovic et al. (2012)
Various depsides, depsidones, xanthones, usnic acid, orsellinic acid esters, salazinic acid derivatives and lichexanthone derivatives extracted from *Parmotrema dilatatum* *Parmotrema tinctorum* *Pseudoparmelia sphaerospora* *Usnea subcavata*	*Mycobacterium tuberculosis*	Several compounds were found to inhibit growth of *M. tuberculosis;* of which the most active was diffractaic acid (MIC = 15.6 μg/ml) followed by norstictic acid (MIC = 62.5 μg/ml), usnic acid (MIC = 62.5 μg/ml), hypostictic acid (MIC = 94.0 μg/ml) and protocetraric acid (MIC = 125 μg/ml)	Honda et al. (2010)
Usnic acid, norstictic acid, salazinic acid, stictic acid, diffractaic acid, barbatic acid, and galbinic acid from *Usnea baileyi* *Ramalina dendriscoides* *Stereocaulon massartianum* *Cladonia gracilis*	*B. subtilis* *S. aureus* *E. coli* *P. aeruginosa*	Crude lichen extracts were very active against *S. aureus* and *B. subtilis* while moderately active against *E. coli* and *P. aeruginosa*. Among the different lichens, *R. dendriscoides* was the most active against *S. aureus* with MIC and MBC values of 156 and 2,500 μg/ml respectively	Santiago et al. (2010)
Barbatic acid from *Cladia aggregata*	Four multi drug-resistant *S. aureus* strains	Barbatic acid either in crude organic form or in purified form inhibited the growth of *S. aureus.* The MIC values for purified barbatic acid against different strains of *S. aureus* was 50 μg/ml, except for the highly resistant IC 404, whose MIC was 100 μg/ml	Martins et al.(2010)
Crude extract and Usnic acid, Usimine A, Usimine B, Usimine C and Ramalin from *Ramalina terebrata*	*B. subtilis* *S. aureus* *E. coli* *P. aeruginosa*	Both crude extracts and individual fractions showed activity against *B. subtilis* with usnic acid having the highest MIC value of 1.2 μg/mL. *S. aureus* was inhibited by crude extracts and usnic acid only but not by other individual fractions. There was no effect of either crude extracts or individual fractions against *E. coli* and *P. aeruginosa*	Paudel et al. (2010)
Crude extract and 6 isolates (+)-usnic acid, divaric acid, 2,4-di-*O*-methyldivaric acid, Divaricatinic acid, and 2-*O*-Methylnordivaricatic acid from *Evernia divaricata*	*B. subtilis* *S. aureus* *E. coli* *P. aeruginosa*	Crude extracts showed a significantly high inhibition zone against three species—*B. subtilis*, *S. aureus* and *P. aeruginosa*. Usnic acid and divaric acid showed very potent inhibitory activity against the above mentioned bacteria. This is first report concerning the anti-bacterial properties of divaric acid	Yuan et al.(2010)

Table 1 continued

Lichens/lichen metabolites	Bacteria	Finding	References
Crude extract of *Anaptychia ciliaris* *Cetrelia olivetorum* *Lecanora muralis* *Peltigera polydactyla* *Peltigera praetextata* *Ramalina farinacea* *Rhizoplaca melanophthalma* *Umbilicaria vellea* *Xanthoria elegans* *Xanthoria parietina* *Xanthoparmelia tinctina*	*B. subtilis* *S. aureus* *E. coli* *P. aeruginosa* *K. pneumoniae* *Staphylococcus epidermidis*	Aqueous extracts of most lichen showed inhibition against *B. subtilis*, *S. aureus* and *E. coli* while ethanol extracts of some lichens were found to be active against *B. subtilis*, *S. aureus* and *S. epidermidis*. Ethanolic extracts of all tested lichens did not show any activity against *E. coli* while both extracts were not able to inhibit growth of *K. pneumoniae* and *P. aeruginosa*	Karagöz et al. (2009)
Physodic acid (*H. physodes*) Usnic acid (*Parmelia caperata*) Atranorin (*Physcia aipolia*) Gyrophoric acid (*U. polyphylla*)	*B. mycoides* *B. subtilis* *Enterobacter cloacae* *E. coli* *K. pneumoniae* *S. aureus*	All the tested lichen substances inhibited growth of all microorganisms with the lowest recorded MIC (0.0037 mg/ml) against *K. pneumoniae* with usnic acid. Physodic acid had the weakest activity level with an MIC value of 1 mg/ml	Ranković et al. (2008)
Evernic acid (*Evernia prunastri*) Salazinic acid (*Flavoparmelia caperata*) Physodic acid (*H. physodes*) 3-hydroxyphysodic acid (*H. physodes*) Usnic acid (*Lecanora albescens*) Hybocarpone (*Lecanora conizaeoides*) Atranorin (*Lepraria lobificans*) Rhizocarpic acid (*Psilolechia lucida*) Lobaric acid (*Sterocaulon dactylophyllum*) Physcion (*X. parietina*)	Methicillin- and Multidrug-Resistant *S. aureus* (SA-1199B) Two hospital isolates of resistant *S. aureus* Norfloxacin - Susceptible strain of *S. aureus* Two epidemic MRSA strain in UK	Most of the lichen compounds showed consistent activity against various strains of *S. aureus*. Among the tested extracts Hybocarpone was the most active compound against all strains with an MIC value of 4–8 µg/ml while Physcion and salazinic acid showed no activity against any of the strains at any concentration and evernic acid and atronorin showed activity against the *S. aureus*-1199B strain only	Kokubun et al. (2007)
Crude extract of *Rhizoplaca chrysoleuca* *R. melanophthalma* *R. peltata*	*E. coli* *Enterococcus faecalis* *P. mirabilis* *S. aureus* *B. subtilis* *B. megaterium* *P. aeruginosa*	Acetone extracts of *R. chrysoleuca* showed high activity against all bacteria except *P. aeruginosa*. While *R. melanophthalma* extracts showed activity against only 3 bacteria. The zone of inhibition of *R. chrysoleuca* was higher than antibiotic tetracyclin in the case of *E. coli*, *P. mirabilis* and *B. megaterium*	Cansaran et al. (2006)
Crude extract of *Parmelia saxatilis* *Platismatia glauca* *Ramalina pollinaria* *Ramalina polymorpha* *Umbilicaria nylanderiana*	35 strains of bacteria	All extracts from five different lichens species demonstrated antibacterial activity against at least some of the bacterial species tested. *R. pollinaria*, inhibited the most (11) bacterial species. The maximum inhibition zone for bacterial strains based on all lichen extracts was <22 mm with MIC values ranging from 6 to 62.5 µl/ml	Gulluce et al. (2006)
Usnic acid	*S. aureus* *P. aeruginosa*	The capacity of usnic acid to control bio-film formation by *S. aureus* or *P. aeruginosa* was tested by loading the acid into modified polyurethane. Usnic acid loaded polymers did not inhibit the initial attachment of *S. aureus*, but inhibited the formation of a bio-film by killing the attached cells where as in *P. aeruginosa* there was no inhibition of bio-film formation but the morphology of the biofilm was altered	Francolini et al. (2004)

Table 1 continued

Lichens/lichen metabolites	Bacteria	Finding	References
Usnic acid (*Cladonia arbuscula*) Atranorin (*Stereocaulon alpinum*) Lobaric acid (*S. alpinum*) Salazinic acid (*P. saxatilis*) (+)-protolichesterinic acid (*Cetraria islandica*)	*Mycobacterium aurum*	Usnic acid is the most active compound with an MIC value of 32 μg/ml while others were inactive at the tested concentration	Ingólfsdóttir et al. (1998)
(+)-Usnic acid (Commercial) (−)-Usnic acid (*Cladonia stellaris*) Vulpinic acid (*Letharia vulpina*)	10 different bacterial strains and some resistant strains of *S. aureus*	Lichen extracts inhibited the growth of *S. aureus*, *E. faecalis*, *E. faecium*, and some anaerobic species (*Bacteroides* and *Clostridium* species) at the concentrations tested. But gram negative bacteria were not susceptible. Vulpinic acid generally was less active than usnic acid. Susceptibility to usnic acid was not impaired in clinical isolates of *S. aureus* resistant to methicillin and/or mupirocin	Lauterwein et al. (1995)
Crude and (−)-usnic acid, physodic acid, 8′-*O*-ethyl-*p*-alectoronic acid and alectosarmentin from *Alectoria sarmentosa*	*E. coli* *S. aureus* *Salmonella gallinarum* *K. pneumoniae* *Mycobacterium smegmatis* *P. aeruginosa*	Crude extracts as well as all isolated acids were active against *S. aureus* and *M. semgmatis* but not *E. coli*, *S. gallinarum*, *K. pneumoniae* and *P. aeruginosa*	Gollapudi et al. (1994)
17 species of lichens and fractions from two lichens	*B. subtilis* *S. aureus* *E. coli* *P. aeruginosa*	Extracts from the majority of the lichen species studied were active against gram-positive organisms and several against the gram-negative as well. Of the different fractions Methyl 1-orsellinate had the lowest MIC value against all microbes	Ingolfsdottir et al. (1985)

marine organisms, etc. are essential. Although representatives of these groups have already been screened and have been the source of pharmaceutically important anticancer drugs, there still remains a vast potential reservoir of untapped possibilities. Among the more promising possibilities are lichenized fungi with their more than 1,000 identified secondary chemicals. The use of lichen secondary products as anti-cancer 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, 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 life span of treated mice versus the control group using a dose range of 20–200 mg/kg of usnic acid. Since these early studies many other lichens compounds, either in crude extract or purified form, have been screened against various malignant cell lines with several showing cytotoxic effects on various cancer cell lines (Bézivin et al. 2003; Bezivin et al. 2004; Kumar and Müller 1999; Zeytinoglu et al. 2008). Table 2 summarizes the results of the various studies examining the anti-cancer role of lichen metabolites.

Based on the information in the above table we can see that lichens are effective against various cancer cell lines both in crude form (Bézivin et al. 2003; Ren et al. 2009) and purified form (Bačkorová et al. 2011; Burlando et al. 2009; Russo et al. 2006). The literature also shows that lichen metabolites are strongly cytotoxic and have the capability of terminating cell proliferation at micro-molar concentrations (Einarsdóttir et al. 2010). Structural modification of lichen compounds has also been shown to enhance the cytotoxic capacity of many lichen compounds (Bazin et al. 2008; Tokiwano et al. 2009). In addition, the position of different functional groups in lichen compounds also affects levels of cytotoxicity (Correche et al. 2002). Regulation of the cell cycle is critical in controlling the growth and development of cancer cells. Various lichen acids have been found to stop cancer cell growth at the sub-G_1 (Ren et al. 2009) or S-phase (Bačkorová et al. 2011; Liu et al. 2010) of the cell cycle. The mechanism of cell death in various cancer cell lines caused by lichen metabolites include apoptosis (Bačkorová et al. 2011; Bezivin et al. 2004;

Table 2 A summary of the literature dealing with the anti-cancer activity of lichen secondary chemistry

Lichen acid/lichen species	Cell lines tested	Major finding	References
H. physodes	Human breast cancer cell lines (MCF-7) Human breast cancer cell lines (MDA-MB-231)	The methanolic extract of *H. physodes* reduced cell viability in dose dependent manner. The IC_{50} values for MCF-7 and MDA-MB-231 were 50 and 44 μg/ml respectively. Apoptosis was observed only in MCF-7 but not in MDA-MB-231 as it was found M30-antigen level increased in MCF-7 but remained unchanged in MDA-MB-231. Nuclear fragmentation was also observed in MCF-7 further confirming apoptosis. It was further found that *H. physodes* was genotoxic at higher concentrations (250 and 500 μg/ml)	Ari et al. (2012)
Usnic acid (commercial) Atranorin (commercial) Parietin (*X. parietina*) Gyrophoric acid (*Umbilicaria hirsuta*)	Human ovarian carcinoma A2780 Human colon adenocarcinoma HT-29	Usnic acid and atranorin induced a massive loss in the mitochondrial membrane potential, along with caspase-3 activation in HT-29 cells but externalization of phosphatidylserine occurred in both tested cell lines. Cytotoxicity is mainly due to induction of both ROS and especially RNS. Usnic acid and atranorin activated programmed cell death in A2780 and HT-29, probably through the mitochondrial pathway	Bačkorová et al. (2012)
Diffractaic acid (*Protousnea magellanica*)[a] Vicanicin (*Psoroma pallidum*) Lobaric acid (*Stereocaulon alpium*) Variolaric acid (*Ochrolechia deceptionis*) Protolichesterinic acid (*Cornicularia aculeate*) Usnic acid (*Cladonia lepidophora*)	Human breast adenocarcinoma MCF-7 Human colon adenocarcinoma HCT-116 Human cervix adenocarcinoma HeLa	Lichen metabolites exhibited different cytotoxic effects with higher susceptibility in HCT-116 and less responsive in MCF-7. Among the 6 compounds, vicanicin did not showed any activity against any of the cell lines in tested concentration while only diffractaic acid and usnic acid were active against all three cancer cell lines. The apoptotic effect of protolichesterinic acid was further evaluated against HeLa cells by analyzing nuclear morphology and measuring the activity of caspase-3. 27 % of apoptotic cells were found after 72 h of treatment and a significant increase of caspase-3 activity was observed compared to control cell confirming apoptosis	Brisdelli et al. (2012)
Atranorin (*Bacidia stipata*) Diffractaic acid (*P. magellanica*)[a] Divaricatic acid (*Protousnea malacea*) Vicanicin (*Psoroma dimorphum*) Protolichesterinic acid (*R. melanophthalma*)	Human prostate cancer androgen-responsive (LNCaP) Human prostate cancer androgen-non responsive DU-145	Atranorin, diffractaic acid and divaricatic acid were found to be active against prostate cancer cells only in high concentration while vicanicin and protolichesterinic acid showed dose—dependent response causing apoptosis in both types of cells. 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	Russo et al. (2012)
Usnic acid (commercial) Atranorin (commercial) Parietin (*X. parietina*) Gyrophoric acid (*U. hirsuta*)	Human ovarian carcinoma A2780 Human breast adenocarcinoma MCF-7 Human colon adenocarcinoma HT-29 Human promyelocytic leukemia Human T cells lymphocyte leukemia, Jurkat Human cervix adenocarcinoma HeLa Human breast adenocarcinoma SK-BR-3 Human colon carcinoma wild type p53 HCT-116 p53+/+ Human colon carcinoma p53 null HCT-116 p53−/−	Lichen compounds showed differential sensitivity to various cancer cells. All the tested compounds showed some cytotoxic activities but usnic acid and atranorin were highly effective against the whole spectrum of cell lines while the other two were less effective. Similar to cytotoxicity, usnic acid and atranorin also significantly inhibited the clonogenic ability of all the tested cell lines while the other two were effective in some cervix and breast tumor cells. All acids except parietin altered cell cycle distribution accumulating cells in S-phase in all tested cell lines, however, the efficiency and spectrum of the affected cells were different. Similarly, usnic acid and atranorin demonstrated strong pro-apoptotic action while in other two acids, the apoptotic index was less pronounced	Bačkorová et al. (2011)

Table 2 continued

Lichen acid/lichen species	Cell lines tested	Major finding	References
Lecanoric acid and it's orsellinate derivatives	Larynx carcinoma HEP-2 Breast carcinoma MCF-7 Kidney carcinoma 786-0 Murine melanoma cell B16-F-10 Vero cell	Structural modification of lecanoric acid increased the cytotoxicity of the compound as orsellinates derivatives have lower IC_{50} values than lecanoric acid. *n*-Butly orsellinate was the most active compound with IC_{50} values ranging from 7.2 to 14.0 μg/ml. The orsellinate activity has been found to increase with chain elongation (from methyl to *n*-butyl), a likely consequence of an increase in lipophilicity	Bogo et al. (2010)
(+) Usnic acid (*C. arbuscula*) (−) Usnic acid (*Alectoria ochroleuca*)	Breast cancer cell line T-47D Pancreatic cancer cell line Capan-2	Both (+) and (−) usnic acids are effective inhibitors of DNA synthesis, with IC_{50}values of 4.2 and 4.0 μg/ml against T-47D and 5.3 and 5.0 μg/ml against Capan-2. There wasa reduction in cell size and both acids inhibited cell entry into the S-phase. No apoptosis was observed but necrosis was seen in Capan-2. Usnic acid also caused loss of mitochondrial membrane potential	Einarsdóttir et al. (2010)
Retigeric acid A and Retigeric acid B (*Lobaria kurokawae*)	Human Pca LNCaP PC-3 DU 145 Human epidermoid cancer KB and vincristine resistant KB (KB/VCR) Human ovarian cancer 3-AO and cisplatin-resistant 3-AO (3-AO/CDDP) Human benign prostate epithelial RWPE1 Human hTERT-RPE1 Human breast cancer MCF-7 Human osteosarcoma U2OS and Saos2	Both Retigeric acid A (RA) and Retigeric acid (RB) showed cytotoxicity at lower concentrations (>100 μM) but RB is more potent than RA. Structural analysis of RA and RB has shown that a methyl side chain of RA is substituted with –COOH in RB, suggesting a possible structure–activity relationship. Further 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 increases in cyclin E and cyclin A. Both caspase dependent and caspase-independent pathways were responsible for apoptosis in PC-3 cells	Liu et al.(2010)
Olivetoric acid (*Pseudevernia furfuracea*)	Rat adipose tissue endothelial cells	Olivetoric acid displayed dose dependent anti-angiogenic activities, inhibited cell proliferation and disrupted endothelial tube formation in adipose tissue. Similarly, olivetoric acid also inhibited the formation of actin stress fibers in a dose dependent manner which may be due to the decrease in tube formation	Koparal et al. (2010)
Usnic acid (commercial)	Breast cancer cell lines MCF7 (estrogen-dependent, wild-type p53) Lung cancer cell line H1299 (null for p53)	No Morphological changes in microtubules or increase in the mitotic index was observed suggesting the antineoplastic activity of usnic acid is not related to alterations in the formation and/or stabilization of microtubules	O'Neill et al. (2010)
(+) usnic acid (*Xanthoparmelia somloensis*) Salazinic acid (*X. somloensis*) Vulpinic acid (*L. vulpina*) Gyrophoric acid (*Lasallia pustulata*) Evernic acid (*E. prunastri*)	Malignant mesothelioma cells MM98 Vulvar carcinoma cells A431 Keratinocytes HaCaT	Usnic acid showed high toxicity for all three cell lines while vulpinic acid was intermediately toxic and salzinic, gyrophoric and evernic acids showed low toxicity. The EC_{50} value of usnic acid was significantly lower than anionic surfactant SDS. Further, usnic acid and gyrophoric acid showed strong wound closure effects on HaCaT cells but the effect was less for the other three acids. A combination of usnic acid and gyrophoric acid further increased wound closure rates	Burlando et al. (2009)

Table 2 continued

Lichen acid/lichen species	Cell lines tested	Major finding	References
Lethariella zahlbruckneri	Human colon cancer cell (HT-29)	Both acetone and methanolic extracts of *L. zahlbruckneri* decreased viable cell number in both a time and dose dependent manner but an acetone extract showed higher toxicity than the methanolic extract. The acetone extract induced cell death by increasing cell population in the sub-G1 phase, as well as the formation of apoptotic bodies and nuclear condensation while such activities were not seen with the methanolic extract. Apoptosis by the acetone extract was induced both in a caspase-dependent and caspase-independent manner. Apoptosis was mitochondria mediated because there is increased level of Bax and decreased level of the Bcl-2 protein	Ren et al. (2009)
16-*O*-Acetyl-leucotylic acid Leucotylic acid (both from *Myelochroa aurulenta*)	Human leukemia cells HL-60	16-*O*-Acetly-leucotylic acid exhibited potent anti-proliferative activity against HL-60 with an EC50 value of 21 μM while the leucotylic acid, derivative of 16-*O*-acetly-leucotylic acid has a higher EC50value (72 μM). The anti-proliferative properties of these two compounds were higher than other similar compounds (betulin and betulinic acid) derived from higher plants. Structural modification has also been found to affect the cytotoxicity of compounds like Leucotylic acid which was less toxic than16-*O*-Acetly-leucotylic acid	Tokiwano et al. (2009)
Crude extract of *E. prunastri* *X. parietina*	Murine myeloma P3X63-Ag8.653	A crude extract of *X. parietina* significantly reduced cell proliferation in a dose-dependent manner while no such activities were seen with *E. prunastri*. The higher activity of *X. parietina* may be due to the higher content of antioxidants such as peroxidases and superoxide dismutase	Triggiani et al. (2009)
Usnic acid and its 9 derivatives	Lymphocytic leukemia L1210 Murine Lewis lung carcinoma 3LL Chronic myeologenous leukemia K-562 Brain metastasis of prostate carcinoma DU 145 Breast adenocarcinoma MCF 7 Glioblastom U251 Hamster cell lines: CHO and CHO-MG	Usnic acid and its nine derivates were tested for cytotoxicity. Four polyamine derivates showed significant cytotoxicity on L1210 with an IC_{50} value significantly less than the parent compound usnic acid. The most active compound (*N*-tert-butoxycarbonyl-1,8-diaminooctane) had an IC_{50} value of 2.7 μM and induced a dose-dependent and time dependent apoptotic event in L1210. The cytotoxicity of usnic acid can be improved by its conjugation to a polyamine chain	Bazin et al. (2008)
Sphaerophorin (*Sphaerophorus globosus*) Pannarin (*Psoroma* spp.)	Human melanoma cells M14	Both sphaerophorin and pannarin showed significant inhibitory effect on M14 cells at a concentration of 12.5–50 μM. They also induced apoptotic cell death substantiated by DNA fragmentation and increased caspase-3 activity	Russo et al. (2008)
Crude extract of *Cetraria aculeata*	Human uterus carcinoma HeLa Human small lung carcinoma A549 c-H-ras transformed -rat embryonic fibroblast 5RP7 Normal rat embryonic fibroblasts F2408	Acrude extract of *C. aculeata*was found to be cytotoxic against HeLa and A549 with an IC_{50} value of 200 and 500 μg/ml respectively. The extract also demonstrated higher cytotoxic activity against F2408 and 5RP7 with IC50 values of 80 and 280 μg/ml respectively	Zeytinoglu et al. (2008)

Table 2 continued

Lichen acid/lichen species	Cell lines tested	Major finding	References
(+) Usnic acid (*R. farinacea*) (−) Usnic acid (*Cladonia foliacea*)	Chinese Hasmster Lung fibroblast V79 Human lung carcinoma A549	Both types of usnic acid showed dose and time dependent cytotoxicity against V79 and A549 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)
Sphaerophorin (*S. globosus*) Pannarin (*Psoroma* spp.) Epiphorellic acid-1 (*Cornicularia epiphorella*)	Human prostrate carcinoma DU 145 Normal human prostatic epithelial cells	All compounds non-toxic to normal human prostatic epithelial cells at aconcentration of 6–50 μmol/l showed significant inhibitory effects on DU-145 cells. Among the three compounds, Pannarin showed the most activity. At lower concentrations (12 and 25 μmol/l) all compounds induced apoptosis but when the concentration was increased to 50 μmol/l cell death was due to necrosis as documented by a significant release of lactic dehydrogenase. Apoptosis was further confirmed by the large amounts of DNA fragmentation in the 12 and 25 μmol/l concentration but not in 50 μmol/l	Russo et al. (2006)
Usnic acid (commercial)	Breast cancer cell lines MCF7 (estrogen-dependent, wild-type p53) Breast cancer cell lines MDA-MB-231 (estrogen independent, mutant p53) Lung cancer cell line H1299	All three cell lines were sensitive to usnic acid with IC_{50} values of 18.9 μM (MCF7) and 22.3 μM (MDA-MB-231 and H1299). There were elevated levels of the p53 and p21 proteins following treatment with usnic acid but there was no p53 transcriptional activity suggesting that the accumulation of p21 was not secondary to p53 transactivation	Mayer et al. (2005)
(−) Usnic acid Fumarprotocetraric acid 9′-(*O*-methly)protocetraric acid	Murine lymphocytic leukemia L1210 Murine Lewis lung carcinoma 3LL Human chronic myelogenous leukemia U251 Human brain metastasis of a prostate carcinoma DU145 Human breast adenocarcinoma MCF 7 Human glioblastoma RCB-0461	Of the three compound tested only usnic acid showed cytotoxic activity at IC_{50} values of 6, 12.1, 15.8, 17.8, 8.2 and 6.8 μg/ml for L1210, 3LL, DU145, MCF7, K-562 and U251 respectively. Usnic acid also induced apoptosis in L1210 in a dose- and time-dependent manner as fluorescence microscopy revealed condensation of nuclear chromatin, nuclear fragmentation, and formation of apoptotic bodies	Bezivin et al. (2004)
Depsidones—Vicanicin, Pannarin, 1-chloropannarin, Salazinic acid, Stictic acid, variolaric acid, Psoromic acid, Fumarprotocetraric acid, Lobaric acid Depsides—Atranorin, Sphaerophorin, Divaricatic acid, diffractaic acid gyrophoric acid Usnic acid	1. Hepatocytes from rat	IC_{10} and IC_{50} values for 15 different lichen compounds have been reported. Among them Usnic acid has the highest cytotoxicity with an IC_{50} value of 21 μg/ml after 24 h which was measured using lactic acid dehydrogenase. Salazinic acid, stictic acid, and psoromic acid showed apoptosis of hepatocytes in a dose-dependent manner with stictic acid showing the strongest apoptotic activity	Correche et al. (2004)

Table 2 continued

Lichen acid/lichen species	Cell lines tested	Major finding	References
Cladonia convoluta *Cladonia rangiformis* *E. prunastri* *P. caperata* *Parmelia perlata* *P. glauca* *Ramalina cuspidata* *Usnea rubicunda*	Murine lymphocytic leukaemia L1210 Murine Lewis lun carcinoma 3LL Human chronic myelogenous leukaemia Human brain metastasis of a prostate carcinoma DU145 Human breast adenocarcinoma MCF 7 Human glioblastoma RCB-0461 African green monkey kidney cell vero	3 different extracts, n-hexane, diethyl ether, and methanol of 8 species were screened for cytotoxicity against 7 cell lines. Significant cytotoxicity ($IC_{50} \leq 20\mu g/ml$) was found on one of the tested cancer cell lines for at least one extract of each lichen species. Crude extracts of some of the lichens (*C. convoluta*, *C. rangiformis*, *P. caperata*, *P. glauca*, and *R. cuspidata* had very high selectivity indices which suggests a potential role as anti-tumor agents	Bézivin et al. (2003)
Pannarin 1′-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 ratspleens	The screening of depsides and depsidones revealed considerable cytotoxic effect for Pannarin, 1′-chloropannarin, and sphaerophorin with stronger effects than colchicine. Generally, depsidones showed stronger cytotoxic activity than depsides. The strong biological activity of some depsidones may be due to the strong hydrogen bond between the aldehyde group at C_3 and the hydroxyl group at C_4. Similarly, the cytotoxic activity of depsides may be in part due to the presence of a COOH group on C'_1 and an OH group on C'_2	Correche et al. (2002)
(+)-usnic acid Methyl â-orcinolcarboxylate Ethyl hematommate Diffractaic acid Gyrophoric acid (+)-protolichesterinic acid	Human keratinocyte cell line HaCaT	Gyrophoric acid, usnic acid, and diffractaic acids were reported as potent anti-proliferative agents which inhibited cell growth at IC_{50} values of 1.7, 2.1, and 2.6 μM. However, the rest of the compounds did not affect cell growth at concentration of even 5 μM. There was no release of lactate dehydrogenase in the culture medium suggesting no damage to the plasma membranes of keratinocyte cells by lichen acids. This further document that the effects of gyrophoric acid, usnic acid, and diffractaic acid are cytostatic rather than cytotoxic	Kumar and Müller (1999)
Lobaric acid (*S. alpinum*) Protolichesterinic acid (*C. islandica*)	Breast cancer cell T-47D and ZR-75-1 Erythro-leukemia K-563	Both test substances 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. But DNA synthesis and proliferation and survival of normal skin fibroblasts were not affected at higher doses	Ogmundsdottir et al. (1998)
Usnic acid derivatives	Lewis lung carcinoma L1210	Eleven usnic acid derivatives where evaluated for cytotoxicity against L1210 and seven derivatives almost completely inhibited cell growth at 1.4×10^{-7} mol/ml while other derivatives showed somewhat lesser cytotoxicity. The lipophilicity and the β-triketone moiety of usnic acid were found to be an important source of cytotoxicity	Takai et al. (1979)

[a] These reports of diffractaic acid for *P. magellanica* may be in error (Calvelo et al. 2005)

Russo et al. 2008), necrosis (Einarsdóttir et al. 2010; Russo et al. 2006), and angiogenesis inhibition (Koparal et al. 2010). Both caspase dependent (Correche et al. 2004; Liu et al. 2010; Russo et al. 2008) and caspase independent (Liu et al. 2010) pathways were found to initiate apoptosis. Caspase activation takes place along two different pathways—the cell membrane mediated death receptor pathway and mitochondria mediated pathways. There are no studies that document caspase activation though the death receptor pathway; however, there are evidences of mitochondria mediated caspase activation (Liu et al. 2010; Ren et al. 2009). Both studies showed that there is increase in the level of the Bax protein with an associated decline in the Bcl-2 protein. It is well documented that an increase in the Bax/Bcl-2 ratio can stimulate the release of cytochrome c from mitochondria into the cytosol, resulting in activation of caspase-3 which serves as an initiator of apoptosis. In addition to lichen secondary compounds, polysaccharides derived from lichens, especially β-glucan and galactomannan, have been shown to be active against several cancer cell lines (Nishikawa and Ohno 1981; Nishikawa et al. 1974; Watanabe et al. 1986). Recently, there has been additional research examining the use of lichen polysaccharides as immunostimulatory compounds and their potential role in fighting cancer (Olafsdottir and Ingolfsdottir 2001; Cordeiro et al. 2008; Karunaratne et al. 2012).

Future directions

In spite of the fact that lichens are one of the more promising reservoirs of low-molecular weight secondary compounds demonstrating some level of biological activity; a very limited number of compounds have been studied (Boustie and Grube 2005). Hence, there is an urgent need for: (1) continued screening of lichen metabolites across their diversity, (2) more in-depth studies of those compounds that have already shown promising activity against pathogenic bacteria and/or various cancer cell lines, (3) clinical trials for those compounds that have shown significant activity, and finally (4) commercial production and implementation of effective drug lines. One of the key issues in the development of therapeutic drugs is to understand how a drug interacts with our innate and adaptive immune system to combat various pathogens and cancer cells. Recently there has been growing interest in the immunostimulant role of lichen compounds especially lichen polysaccharides (Omarsdottir et al. 2007; Choi et al. 2009; Kim et al. 2010; Karunaratne et al. 2012).

One of the main issues related to the limited use of lichens compound in modern medicine is related to their slow growth rate and challenges with in vitro propagation. However, with recent advancements in technology, culturing lichens in the laboratory is achieving greater success (Behera et al. 2006; Stocker-Wörgötter 2001, 2008; Stocker-Wörgötter and Elix 2002; Yamamoto et al. 1985, 1987, 1993). Similarly, Miao et al. (2001) reviewed the possibilities of using molecular genetic techniques as an alternative approach for exploring the diversity of polyketide biosynthetic pathways in lichens. This approach can be extended to examine other pathways which can then be integrated with conventional culture methods. Also according to Miao et al. (2001) lichen genes can be introduced into a surrogate host with good fermentation characteristics and a well characterized endogenous chemical profile like *Aspergillus nudulans*, *Neurospora crassa*, *Saccharomyces cerevisiae*, *E. coli*, *Streptomyces* spp. etc. to produce promising lichen metabolites in larger quantities. Furthermore, researchers have now been able to synthesis usnic acid in the laboratory from commercially available starting materials. The synthesis involves the methylation of phloracetophenone followed by oxidation with horseradish peroxidase (Hawranik et al. 2009). This work also provided the impetus for synthesizing other lichen metabolites. With these recent advancements in technology, the development of cost effective options for growing and harvesting lichen metabolites commercially as a source of effective drugs against pathogenic bacteria and various forms of cancer show real promise.

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