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Lichen Secondary Metabolites and Its Biological Activity

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ABSTRACT

Lichens are organisms which are formed through symbiotic association of fungi and algae or cyanobacteria. Lichen produces a great variety of secondary metabolites with various biological activities including antimicrobial, antiviral, antitumour, antioxidant, antihervivore, insecticidal, allelochemical and allergenic action. These compounds play a major role in providing photoprotection against intense radiation and can be used as an important candidate for antipyretic and analgesic drugs. Lichen metabolites act as major factor in metal homeostasis and pollution tolerance of lichen. This review describes the biological activities of secondary metabolites produced from lichen.

Keywords: Lichen, Secondary Metabolites, medicinal value, drug, antioxidant.

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INTRODUCTION

Secondary metabolites of lichen has long been used by Mankind, About 17000 species and more than 800 lichen metabolites are known to be utilized by human for several purposes eg., for perfumery, monitoring pollution, dying, floral decorations, dietary and medicinal values,[1]. Lichen metabolites show great varieties of biological activities like antimycobacterial, antiviral, anti-inflammatory, antipyretic, analgesic, antiproliferative and cytotoxic properties [2]. Some lichen have been used in tribal medicine for treatment of different types of diseases and the secondary metabolites present in lichens have antibiotic, antimycobacterial, antiviral, antitumour, analgesic and antipyretic properties [3; 4; 5] and also have antiproliferative and antioxidant [6], anti-HIV properties [7]. Lichens are inherently resistant to microbial infection due to the production of large numbers of secondary metabolites [8]. Lichens and their secondary metabolites have reported to possess great potential as antifungal source [9]. Lichen metabolites showed antihervivore activity [10]. Metabolites produced from lichens are poisonous to insects, snails, and nematodes [3, 4, 11]. In folk medicine lichens has been widely used for treatment of various diseases, such as eczema, respiratory diseases, pulmonary diseases and arthritis. They have been used cosmetics as well as food [12]. Lichen produces aromatic substances which strongly absorb UV light and protect the phycobiont from dangerous irradiation [13, 3]. Lichen secondary metabolites are sensitive to heavy metal accumulation and play a general role in metal homeostasis and pollution tolerance [10].

The Lichens

Lichens are symbiotic organisms composed of a fungal partner (mycobiont) in association with one or more photosynthetic partners (photobiont). The photobiont can be green algae, cyanobacteria, or both [14]. Recent studies show lichens comprise about 18500 species [2, 15]. In lichen (98%) of the fungal partners are Ascomycota [16] and the others are Basidiomycota and anamorphic fungi. It has been estimated that 21% of all fungi are able to act as a mycobiont [16]. About 40 genera, 25 algae and 15 cyanobacteria, as photosynthetic partners are involved in the formation of lichens [10]. Lichen can be found in a wide range of habitat: from arctic to tropical regions, from plains to highest mountains [17], and from aquatic to xeric conditions. They show nearly ubiquitous ability to colonize various substrates and can grow on or inside rocks (epilithic or endolithic), on or inside the bark of woody plants as epiphytes, on wood, soil, mosses, leaves of vascular plants (especially in the tropics), on other lichens, as well as on man-made substrates such as concrete, glass, metals and plastics [18]. Molnar and Farkas, [10] reported that most of the lichens are terrestrial, but a few species occur in freshwater streams and others in marine intertidal zones. According to

Jayanti, [19] lichens basically show three types of growth forms i.e crustose, foliose and fructiose. Crustose (or Crustaceous) lichens are encrusting forms which spread over and into the surface of their habitat and cannot be removed from the surface without crumbling away. Foliose lichens are lichens with leafy lobes, which spread out in a horizontal layer over the surface. They are attached by root-like threads and can be easily removed with a knife. Fruticose lichens are shrubby forms with many branches. They can be removed from the surface by hand. In addition to the above three types some other types of growth form exhibited by lichens are squamulose, filamentous, leprose and gelatinous. Squamulose lichens consist of small scale-like structures and have a portion of their thallus lifted off the substrate to form 'squamules'. Filamentous (hairlike) lichens are totally different. They consist of chains of algal cells wrapped around with fungal hyphae. Leprose (powdery) lichens are an odd group of lichens which have never been observed to produce fruiting bodies. In gelatinous lichens, cyanobacteria produce polysaccharides which absorb and retain water.

Lichen secondary metabolites

A great variety of secondary metabolites are synthesized by lichens with distinct biological properties [10]. Turk, [20] reported that these (aliphatic and aromatic) lichen metabolites have relatively low molecular weight. These metabolites are complex, but predominantly small molecules, which comprise up to 20% of lichen's dry weight [21]. Lichen secondary metabolites comprise many classes of compounds including amino acid derivatives, sugar alcohols, aliphatic acids, macrolytic lactones, monocyclic aromatic compounds, quinines, chromones, xanthones, dibenzofurans, depsides, depsidones, depsones, terpenoids, steroids, carotenoids, and diphenyl ethers [8, 22]. Mycobiont produce the secondary metabolites [8], and accumulate these compounds in the cortex (such as atranorin, parietin, usnic acid, fungal melanins) or in the medullary layer (such as physodic acid, physodalic acid, protocetraric acid) in the form of extracellular tiny crystals on the outer surface of the hyphae. The secondary metabolism of the mycobiont also has some influence by the photobiont [23, 24, 25]. Suitable culture conditions (such as nutrient medium, added sugars or polyols, pH, temperature, light, stress) are required for production of specific secondary metabolites [26]. In many cases, lichen "tissue" cultures, can produce secondary substances [27, 24] but the chemistry is different from the secondary metabolite of the corresponding natural lichen [24]. Proksa and Proksova, [28] reported that usnic acid is a natural lichen compound which is used in pharmaceutical preparation. It is active against microorganisms and viruses as well as analgesics and antipyretics. It is used in the treatment of hyperproliferative skin diseases, such as psoriasis [29] as well as parasitic infestations [30]. Pulvinic acid extracted from several lichens and higher fungi

represent bright yellow and orange pigments with antioxidant properties [19]. Boldine is a natural lichen metabolite having antioxidant activity [31], anti-inflammatory effects, as well as photoprotector capacity [32].

Antibacterial and antifungal activity

Rankovic and Misic, [33] reported that some of the lichen metabolites such as atranorin, fumarprotocetraric acid, gyrophoric acid, lecanoric acid, physodic acid protocetraric acid, stictic acid and usnic acid showed relatively strong antimicrobial effects against six bacteria and ten fungi, among which were human, animal and plant pathogens, mycotoxin-producers and food-spoilage organisms. Bacterial growth can be inhibited by numerous lichen substances for example, alectosarmentin (*Staphylococcus aureus*, *Mycobacterium smegmatitis*; [34], emodin and physcion (*Bacillus brevis*; [35, 36], evernic acid (*Staphylococcus aureus, Bacillus subtilis, B. megaterium*; [4], leprapinic acid derivatives (e.g., leprapinic acid glycinamide; gram-positive and gram-negative bacteria; [37]. Candan, [38] reported that acetone, chloroform, diethyl ether, methanol, and petroleum ether extracts of *Parmelia sulcata* and its constituent (salazinic acid) showed antibacterial activity against *Aeromonas hydrophila, Bacillus cereus, Bacillus subtilis, Listeria monocytogenes, Proteus vulgaris, Yersinia enterocolitica, Staphylococcus aureus, Streptococcus faecalis, Candida albicans, Candida glabrata, Aspergillus niger, Aspergillus fumigatus,* and *Penicillium notatum* . According to Paudel, [39] methanol extracts of five lichens from Antarctica (*Caloplaca regalis*, *Caloplaca* sp., *Ramalina terebrata*, *Stereocaulon alpinum*) showed target-specific antibacterial activity, against Gram-positive bacteria. Usnic acid isolated from lichens from south Spain has high antibacterial activity against Gram-positive bacteria [40]. Hirtusneanoside isolated from *Usnea hirta* showed growth inhibitory activities against Gram-positive bacteria [41]. Schmeda-Hirschmann, [42] reported that dichloromethane and methanol extracts of *Protousnea poeppigii* possess strong antifungal effects against the fungal pathogens *Microsporum gypseum*, *Trichophyton mentagrophytes* and *T. rubrum*. as well as against the yeasts *Candida albicans*, *C. tropicalis*, *Saccharomyces cerevisiae* and the filamentous fungi *Aspergillus niger*, *A. flavus* and *A. fumigates*. *Protousnea poeppigii* contains metabolites like isodivaricatic acid, divaricatinic acid and usnic acid also showed antifungal activity against *Microsporum gypseum*, *Trichophyton mentagrophytes* and *T. rubrum* [10]. Ascospore germination of *Sordaria fimicola* was significantly inhibited by evernic and vulpinic acids [43]. Parietin, anthraquinone isolated from methanol extract of *Caloplaca cerina* has been reported to have significant antifungal activity [44].

Antiviral, cytotoxic and antitumour activity

Some of the lichen secondary metabolites show cytotoxic and antiviral properties and could be

used as potential sources of pharmaceutically useful chemicals [10]. Cervical cancer caused by *human papilloma virus* and the adjuvant treatment with usnic acid and zinc sulphate after radiosurgery promotes reepithelization and reduces recurrence[45]. It was found that an ethyl acetate-soluble fraction of the crude methanolic extract of *Ramalina farinacea* can be used as a broad-spectrum antiviral agent against RNA (respiratory syncytal virus and HIV-1) and DNA (adenovirus and herpes simplex virus type 1) viruses [10]. Wood, and [46] Cohen, [47] suggested that some of the lichen compounds such as emodin, 7-chloroemodin, 7-chloro-1-O-methylemodin, and 5, 7-dichloroemodin exhibit antiviral activity. Usnic acid extracted from the aposymbiotic mycobionts of *Ramalina celastri* showed specific antiviral activity against the Junin virus (Arenaviridae), which cause Argentine hemorrhagic fever in humans, and also against Tacaribe virus which is a non-pathogenic arenavirus [48]. Another metabolite, parietin extracted from the aposymbiotic mycobionts of *Teloschistes chrysophthalmus* exhibited virucidal effects against the same viruses. Neamati, [7] tested 17 depsides and depsidones for their inhibitory activity against human immunodeficiency virus integrase and antiviral activity of glucan-lichenan on tobacco plants [49] of glucan-lichenan on tobacco plants. Aliphatic alpha methylene gamma- lactone isolated from lichen, *C. islandica* was a potent inhibitor of DNA polymerase activity of human immunodeficiency virus-1 reverse transcriptase (HIV-1 RT) [50].

According to Mayer, [51] usnic acid decreases proliferation of human breast cancer cells and human lung cancer cells without any DNA damage. This may prove to be a novel source for a natural non-genotoxic anticancer drug. (+) -Usnic acid has the ability to uncouple and inhibit the electron transport chain in mitochondria and induce oxidative stress in cells and can be used as strong hepatotoxic agent against monogastric murine hepatocytes [52]. Bezivin,[53] reported that the (–)-enantiomer of usnic acid extracted from *Cladonia convolute* induced apoptotic cell death in murine lymphocytic leukemia cells and 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. Lichen metabolites such as depside sphaerophorin (isolated from *Sphaerophorus globosus*) and the depsidone pannarin [isolated from *Psoroma pholidotoides* (as *Psoroma reticulatum*), *P. pulchrum*, and *P. pallidum*] inhibited the growth of M14 human melanoma cells, inducing apoptotic cell death [54]. Some of the lichen substances show antitumor activity i.e polysaccharides [55, 56, 57] acetylated pustulanes [58, 59] , barbatic, 4-*O*-demethylbarbatic, diffractaic, evernic, lichesterinic acids, methyl gyrophorate [60], emodin, chrysophanol derivatives [61], (c)-proto-lichesterinic acid [62], and an extract from *Usnea fasciata* [29].

Antioxidant activity

Free radicals (reactive oxygen species, such as the hydroxyl radical, superoxide anion, and hydrogen peroxide, and reactive nitrogen species, such as nitric oxide) perform an important function in many chemical processes in the cells, but they are also associated with unwanted side effects, leading to cell damage. They attack proteins and nucleic acids, and also unsaturated fatty acids present in cell membranes. The damaging effects of free radicals can be reduced by free radical scavengers and chain reaction terminators – enzymes such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase. Natural antioxidants can be a good substitute to the synthetic antioxidants which are carcinogenic by nature. Lichens synthesize a great variety of secondary lichen metabolites which show strong antioxidant activity [10]. Antioxidants are the substances which interfere with the oxidation process by reacting with free radicals, chelating free catalytic metals and also by acting as oxygen scavengers [19]. According to Shahidi and Wanasundara,[63] phenolic antioxidants function as free radical terminators and metal chelators. Methanol extracts of *Xanthoparmelia camtschadalis* and *X. conspersa* and their extracted lichen compounds such as salazinic acid, stictic acid, and usnic acid protected human astrocytes from hydrogen peroxide-induced damage [64]. Astrocytes form the first line of defense in the brain against neurotoxicity of reactive oxygen species, thus salazinic acid, stictic acid and usnic acid are used as antioxidant agents in those neurodegenerative disorders associated with oxidative damage (*e.g*., Alzheimer's disease and Parkinson's disease). Some depsides, such as atranorin (extracted from Placopsis sp.), divaricatic acid (extracted from Protousnea malacea), depsidones, pannarin (extracted from Psoroma pallidum) and 1'-chloropannarin (extracted from *Erioderma chilense*) showed antioxidant activity [6]. Odabasoglu, [65] reported that usnic acid was shown to be a gastroprotective compound, as it reduced oxidative damage and inhibited neutrophil infiltration in indomethacin-induced gastric ulcers in rats. The extreme conditions in Antarctica (such as low temperature, drought, winter darkness, high UV-B and solar irradiation) increase oxidative stress. Accordingly Antarctic lichens larger amounts of antioxidant substances and have higher antioxidant activity than tropical or temperate lichen. Extract of *Umbilicaria antarctica* was found to be the most effective antioxidant in free radical and superoxide anion scavenging, where Lecanoric acid was the main active compound [66].

Antihervivore and insecticidal activity

Herbivores, *e.g.*, insects, mites, snails, slugs, lepidopteran larvae, caribou, and reindeer graze on lichens [10]. According to Lawrey, [3] and Rundel, [13] herbivory on lichens seems to be rare, presumably due to their low nutritional quality, specific structural features (for example, the

gelatinous sheath in Collemataceae, thick cortex), and the production of defense compounds. Presence of secondary compounds might protect lichens from herbivory [67, 68, 69, 70, 71]. According to Lawrey[3, 4] and Ahad [11] lichen metabolites are poisonous to insects, snails, and nematodes. Since natural plant-derived products have a less detrimental impact on the environment as compared to synthetic chemicals, and thus lichen substances could be good candidates for new pesticides [14]. Lichen substances also exhibit harmful effect on vertebrate herbivores. Cook,[72] Dailey,[73] reported poisoning and subsequent death of an estimated 400 – 500 elk (*Cervus canadensis*) in Wyoming during the winter of 2004, due to ingestion of the lichen *Xanthoparmelia chlorochroa.* The clinical signs are red urine, ataxia, and muscular weakness, which rapidly progressed to recumbency and myodegradation. According to Cetin, [74] both enantiomers of usnic acid, exhibited strong larvicidal activity against the third and fourth instar larvae of the house mosquito (*Culex pipiens*), and larval mortality was dose-dependent. Lawrey, [75, 4] reported that caperatic acid and extracts of the lichens *Flavoparmelia baltimorensis* and *Xanthoparmelia cumberlandia* exhibit antiherbivore activities against the snail *Pallifera varia*. Methyl b-orcinolcarboxylate, ethyl hematommate and 5-chlorohematommate are the lichen metabolites that showed nematocidic activity on larvae of *Toxocara canis* [11]. Giez, [76] and Emmerich [77] studied the effect of some lichen substances i.e atranorin, pulvinic acid dilactone, calycin, parietin, evernic, psoromic, physodic, 3-hydroxyphysodic, fumarprotocetraric, stictic, norstictic, salazinic, vulpinic, rhizocarpic, and usnic acids on the growth and development of the polyphageous insect *Spodoptera littoralis* but did not affect their survival.

Allelopathy

Lichen secondary metabolites affect the growth and development of neighboring lichens, mosses, algae and vascular plants, as well as microorganisms and can function as allelopathic agents called allelochemicals [3, 78, 13]. Macias [78] reported that allelopathic compounds are released into the environment and might influence photosynthesis, respiration, transpiration, protein and nucleic acid synthesis, membrane ion transport, and permeability of other organisms. [79] populations of mosses and lichens frequently occur together on rocks, soil, and trees and they compete for light, substrate, nutrients, and water. Armstrong and Welch, [80] reported that lichen thalli compete for space and light on a variety of substrates, and plays important roles in determining the structure of lichen communities and the distribution of individual species and lichen secondary chemistry might play a role in this competition. Vulpinic and evernic acids severely inhibited ascospore germination of the crustose lichens *Graphis scripta* and *Caloplaca citrine* [81]. Schimmer and Lehner [82] reported that (–) Usnic acid inhibits the growth of the green alga *Chlamydomonas*

reinhardii. Growth of mossess *Hedwigia ciliata* and *Anomodon attenuatum* and the liverwort *Porella platyphylla* found to be inhibited by lichen *Porpidia albocaerulescens* [83]. Spore germination and protonemal growth of three common moss species such i.e *Ceratodon purpureus*, *Funaria hygrometrica* and *Mnium cuspidatum* was retarded by 4-O-methylated depsides evernic and squamatic acids [80]. According to Pyatt [84] lichens have also long been known to inhibit or greatly retard the growth of higher plant. Some of the lichen metabolites such as barbatic acid, diffractaic acid, evernic acid, lecanoric acid, b-Orcinolcarboxylic acids, osrsellinic acids and 4-O-Demethylbarbatic acid exhibit strong allelochemical action against higher plants [85]. Usnic acid inhibits mitosis in *Allium* [86]. Twelve lichen substances identified in "Letharal," the phenolic fraction of *Lethariella canariensis* showed allelopathic activity against the seeds of common garden plants, and inhibited the germination process of cabbage, lettuce, pepper, and tomato [87].

Candidates for antipyretic and analgesic drugs

Some lichen substances have been shown to relieve pain effectively or reduce fever and inflammation in various mammals, and it is reasonable to assume that these compounds could also be effective candidates for antipyretic and analgesic drugs [10]. Vijayakumar, [88] reported that (+) - usnic acid, isolated from *Roccella montagnei*, showed significant, dose-dependent anti-inflammatory activity in rats, reducing carrageenin-induced paw edema. Diffractaic and usnic acids have an analgesic effect in mice *in vitro* [89], and usnic acid also act as an antipyretic agent against lipopolysaccharide-induced fever.

Photoprotection

A number of strategies are used by lichens to protect the light-sensitive algal symbionts against high levels of light and the damaging effects of UV radiation, mainly the xanthophyll cycle in the algal thylakoid membranes, as well as light screening and UV-B protection by lichen compounds [10]. Ertl, [90] formulated the light-screening theory, who found that lichen compounds present in the cortical region, increase the opacity of the upper cortex, and thus decrease high incident irradiance reaching the algal layer. Some of the lichen metabolites like parietin, usnic acid, vulpinic acid act as light-screening pigments, which regulate the solar irradiance reaching the algal layer [91, 92, 13, 93] by absorbing much of the incident light and thus protecting the photosynthetic partner from intense radiation [92]. Galloway, [91], Rundel, [13], Solhaug and Gauslaa [93] reported that many lichen secondary metabolites (including atranorin, calycin, pinastric acid, pulvinic acid, rhizocarpic acid, usnic acid, vulpinic acid) exhibit strong UV absorption abilities and might function as filters for excessive UV-B irradiation. The fluorescence spectrum of the cortical depside atranorin coincides with the absorption spectrum of algal chlorophyll; therefore, the light emitted by atranorin can be used in

photosynthesis [92].

Effect on metal homeostasis and pollution tolerance

Lichen metabolites control metal homeostasis in lichens by promoting the uptake of certain metal cations by reducing the adsorption of others, thus enhancing the tolerance of lichens to heavy metals in polluted areas [10]. According to Jayanthi [19], some secondary metabolites of lichens such as depsides and depsidones produced by the fungal symbiont and accumulated on the outer surface of its hyphae, are supposed to play an important role in the extracellular immoblilization of heavy metals. Lichens accumulate high amounts of heavy metals, (e.g. Cu, Zn, Pb, Cd, Mn) in heavy metal-polluted areas indicating it as a strong metal tolerant [94]. Białonska and Dayan [95] reported that remarkable changes in the levels of secondary compounds were found in lichen *Hypogymnia physodes* transplanted to areas polluted with heavy metals and acidic inorganic sulfur compounds. For example, thalli transplanted to the vicinity of a chemical plant producing chromium, phosphorous and sulfur compounds, showed significant decrease in levels of atranorin, physodic acid and hydroxyphysodic acid. In contrast, the level of physodalic acid was significantly increased, suggesting that this compound might be effective against pollution stress. Hauck and Huneck, [96] used a model system to imitate lichen cell walls, which contain many hydroxy and carboxy groups as binding sites for metal cations and demonstrated the ion-specific increase or decrease of heavy metal adsorption at cation exchange sites (hydroxy groups) on cellulose filters coated with four lichen substances produced by *Hypogymnia physodes* (atranorin, physodic acid, physodalic acid and protocetraric acid). Adsorption of alkali metal ion Na^{+}, the alkaline earth metal ions Ca^{2+} and Mg^{2+}, and the transition metal ions Cu^{2+}, Fe^{2+}, Fe^{3+} and Mn^{2+} were studied. It was concluded that lichen compounds significantly inhibited the adsorption of Na^{+}, Ca^{2+}, Mg^{2+}, Cu^{2+} and Mn^{2+}, whereas they increased the adsorption of Fe^{3+}. The depsidone physodalic acid was found to be the most effective among the above four compounds.

Allergenic activity

Lichens and lichen metabolites can be contact allergens in people who are susceptible. They can cause occupational allergic contact dermatitis in case of forestry and horticultural workers ("woodcutter's eczema"), and in lichen harvesters, as well as cause non-occupational allergic dermatitis during all kinds of outdoor activities, such as cutting and handling firewood, picking berries, hunting, and using cosmetics (perfumes, after-shave lotions, deodorants, and sunscreen products) that contain lichen metabolites [97]. Lichen substances that elicit contact allergy in sensitive persons, include atranorin, barbatic, diffractaic, evernic, fumarprotocetraric, lobaric, perlatolic, physodic, physodalic, protolichesterinic, salazinic, stictic and usnic acids [98, 99, 100].

According to Thune and Solberg [100], some of the lichen compounds (such as atranorin and stictic acid) are able to photosensitize human skin causing photocontact dermatitis, where the exposure to sunlight leads to an aggravation of symptoms.

CONCLUSION

Lichens represent powerful source of secondary metabolites with various biological activities. Structure of more than 800 lichen metabolites available, but even more remain to be characterized in future. Lichens are slow growing organisms, therefore development of improved culture methods and varied growing conditions can positively influence secondary metabolite production in these organisms.

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