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Lichen Secondary Metabolites as Anti-Fungal Agent

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Introduction

Lichens are composite organisms containing algae and fungi. Lichens are a stable and self-supporting symbiosis between fungi (Mycobionts) and algal partners. Lichens are used in traditional medicine, food and various other ethnic uses by cultures across different countries in Asia. Moreover, some lichens are popularly used for lichen produce in ethnic and modern life. Many lichens are very sensitive to environmental disturbances and they can be used to assess air pollution. Lichens distribute on different types of the planet's surface (About 7%); additionally, they are important components of primary producers in a wide range of substrates and habitats, including some of the most extreme conditions on earth [1-11]. Ethnolichenology is a branch of ethnobotany that studies the uses that man makes of lichens traditionally. Lichens are used for many different medicinal purposes, but there are some general categories of use that reoccur across the world. Lichens are often drunk as a decoction to treat ailments relating to either the lungs or the digestive system [12]. About 20,000 species of lichens growing on wide variety of substrates like rocks, walls, exposed soil surfaces and as epiphytes on the bark of trees and leaves have been recorded worldwide [13]. Secondary metabolites of lichen have long been used by Mankind, about 17000 species and more than 800 lichen metabolites are known to be utilized by human for several purposes. Lichen metabolites show great varieties of biological activities like antimycobacterial, antiviral, anti-inflammatory, antipyretic, analgesic, antiproliferative and cytotoxic properties [14].

Some lichens 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 [15] and also have antiproliferative and antioxidant [16], and anti-HIV properties [17]. Lichens and their secondary metabolites have reported to possess great potential as antifungal source [18]. Lichen metabolites showed antihervivore activity [19]. Metabolites produced from lichens are poisonous to insects, snails, and nematodes [20]. 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 [21]. Lichen produces aromatic substances which strongly absorb UV light and protect the photobiont from dangerous irradiation [22]. Lichen secondary metabolites are sensitive to heavy metal accumulation and play a general role in metal homeostasis and pollution tolerance [19].

Secondary metabolites

The simplest definition of secondary metabolites is that they are not generally included in the standard metabolic charts [23]. Secondary metabolites are customarily distinguished from primary metabolites, which are the almost universally distributed compounds of intermediary metabolism. Secondary metabolites are often bioactive, usually of low molecular weight, and are produced as families of related compounds at restricted parts of the life cycle, with production often correlated with a specific stage of morphological differentiation. Secondary metabolites share the enigmatic properties of cellular dispensability - producer organisms can grow without synthesizing these metabolites and restricted taxonomic distribution [24].

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 [25]. The fungus most often represents the Ascomycota division, less frequently the Basidiomycota one [26], while the photobiont belongs to the Chlorophyta or Cyanobacteria division [27]. Recent studies show lichens comprise about 18500 species [28]. In lichen (98%) of the fungal partners are Ascomycota [29], and the others are Basidiomycota and Anamorphic fungi. It has been estimated that 21% of all fungi are able to act as a mycobiont [29]. About 40 genera, 25 algae and 15 cyanobacteria, as photosynthetic partners are involved in the formation of lichens [19]. Lichen can be found in a wide range of habitat: from arctic to tropical regions, from plains to highest mountains [30], and from aquatic to xeric conditions. Also, Molnar and Farkas, [19], reported that most of the lichens are terrestrial, but a few species occur in freshwater streams and others in marine intertidal zones. According to Jayanthi et al., [31], lichens basically show three types of growth forms i. e. crustose, foliose and fruticose. Crustose 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. On the other hand, Fruticose lichens are shrubby forms with many branches and they can be removed from the surface by hand.

To withstand extreme conditions, lichens synthesized metabolites which are valuable sources to develop new biotechnologies [32]. Ability to produce wide range of unique chemical compounds approves usage of lichens from ancient times as sources of colorants, cosmetics and remedies [33]. For example, *Parmelia sulcata* have been used to treat diseases of respiratory system, while *Xanthoria parietina* and *Letharia vulpine* were used against to cure jaundice and gastrointestinal disorders, respectively [34].

Lichen secondary metabolites

A great variety of secondary metabolites are synthesized by lichens with distinct biological properties [19]. These metabolites are complex, but predominantly small molecules, which comprise up to 20 % of lichen's dry weight [35]. The content of secondary metabolites in the lichen thallus varies from 0.1 to 10%, but sometimes can even reach 30% of the dry weight of the thallus [36].

The secondary metabolites in lichens are produced through three major pathways: (1) acetylpolymalonyl, (2) mevalonic acid, and (3) shikimic acid [14]. To date more than 800 secondary metabolites have been identified for lichens. The continuing trends in compounds isolated from lichens approved their importance as a source of new natural products [33]. Long-time lichens were out of attention by pharmaceutical industry reasons of which were their slow-growing nature and difficulties to cultivate in laboratory conditions [33]. For the same reason it is difficult to obtain pure lichen metabolites in needful quantity for checking out their biological activities [33].

Lichen primary metabolism pathways produce basic substances constituting the structure of bionts, such as proteins, pigments, and vitamins [37]. 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 [38].

Lichens promising antimicrobial agents

Ahmed et al., [39], studied different (Successive extraction by acetone, methanol, petroleum ether, and diethyl ether) extracts of *Dirinaria picta, Dirinaria papillulifera*, and *Dirinaria applanata* against Gram-positive and Gram-negative bacteria show solvent dependent inhibition activity. Most of the crude extracts showed far better inhibition activity than commercial streptomycin. As a result, it can be concluded that lichen extracts can be used as an alternative to commercially available antibacterial drugs. Thus, further investigations on purification, characterization, and identification of antimicrobial compound from lichen is needed [39].

Numerous lichen substances inhibit or slow down the growth of many microbial pathogens including bacteria, fungi [40] and viruses [41], as well as cancer cells [42]. Their analgesic, antipyretic [43] and antioxidant [44] properties have also been confirmed. On the other hand, the unusual properties of lichen compounds can be used for pharmacological purposes; hence, the wide interest of modern researchers in this field [45]. Among the widely investigated studies, those on the inhibition of dermatophytes, fungi that degrade keratin and therefore cause fungal infections of skin, hair and nails, are included [46].

Lichens as antifungal agent

The antifungal activity of extracts and secondary

metabolites derived from lichen thalli against several species of dermatophytes was reviewed by a lot of scientists such as *Epidermophyton floccosum* (Harz) responsible for smooth skin mycosis, *Microsporum canis*, *M. audouinii*, *M. nanum*, *M. gypseum* causing mycosis of the scalp and hairy skin, *Trichophyton rubrum* (mycosis of the feet, groin, skin, hands, nails), *T. mentagrophytes* (foot fungus, smoother skin, hair), *T. tonsurans* and *T. violaceum* (hairy and smooth skin mycoses) [47, 48]. Also, the standard methods of culturing and testing the activity of lichen substances against dermatophytes have been provided by the Clinical and Laboratory Standards Institute.

Lichen extracts (Secondary metabolites) used against *Fusarium* sp. moulds by Furmanek et al., [49]. Lichen extracts were obtained from 51 corticolous, 17 terricolous and 18 saxicolous lichen species and 37 secondary compounds were tested against eight fungal species, i.e., *Fusarium acuminatum*, F. avenaceum, *F. culmorum, F. fujikuroi, F. oxysporum, F. roseum*, *F. solani* and *F. udum*. Lichen extracts were obtained by several solvents types. Lichen substances (Single secondary metabolites) were from *Alectoria sarmentosa, Cladonia mitis, C. rangiferina, Flavoparmelia caperata, Hypotrachyna cirrhata, Leucodermia leucomelos, Parmotrema austrosinense, P. reticulatum, Physcia aipolia, Pseudevernia furfuracea, Roccella montagnei* and *Umbilicaria nylanderiana*. Secondary metabolites such as 2-hydroxy-4-methoxy-3,6-dimethylbenzoic acid, atranorin, lecanoric and (+)-usnic acids showed the highest antifungal potential. These activities could compete with the potential of fungicides, such as fucytosine and fuconazole. Different researches data showed that lichen extracts exert significant fungistatic potential [49].

The antifungal activity of secondary lichen metabolites extracted by means of acetone and ethanol from *Cetraria islandica, Cladonia mitis, C. rangiferina, Pseudevernia furfuracea* and *Usnea dasopoga* on the pathogenic fungi *Fusarium oxysporum* and *F. avenacuem* was studied by Tekiela et al., [50]. The most active extracts inhibiting the growth of fungal mycelia contained fumarprotocetraric, salazinic and usnic acids, and atranorin. The growth rate of the two *Fusarium* (*Fusarium oxysporum* and *F. avenacuem*) representatives was strongly inhibited by both alcoholic extracts from *Cladonia mitis* and from *Cetraria rangiferina*, more strongly than by the extracts from the mixed thalli of the two terricolous taxa [50].

Anti- dermatophytes fungi (*Epidermophyton floccosum, Microsporum audouinii, M. canis, M. gypseum, M. nanum, Trichophyton longifusus, T. mentagrophytes, T. rubrum, T. tonsurans* and *T. violaceum*) inhibition by compounds extracted by acetone, ethanol, methanol and water derived from several lichen genera (*Caloplaca, Everniastrum, Heterodermia, Hypotrachyna, Platismatia* and *Ramalina*) were represented by [51]. Lichen secondary compounds, like usnic acid, on the growth rates of these dermatophytes was also represented by Furmanek et al., [51]. The fungicidal activity of water-extracted compounds from Heterodermia leucomela and Hypotrachyna cirrhata and of methanol-extracted compounds from *Evernia divaricata* and *Ramalina pollinaria*, as well as protolichesterinic and 2-hydroxy-4-methoxy-3,6-dimethylbenzoic acids, are significant [51].

Antifungal potential of extracted lichen compounds and individual secondary metabolites against mold species of the genus *Aspergillus* is reported by Furmanek et al., [52]. Crude extracts from 49 epiphytic, 16 epigeic and 22 epilithic species of lichens and 44 secondary metabolites against 10 species, *Aspergillus candidus, A. favus, A. fumigatus, A. nidulans, A. niger, A. ochraceus, A. parasiticus, A. restrictus, A. stellatus* and A. *ustus*, were analysed by Furmanek et al., [52]. Lichen substances were extracted with different solvents (Alcoholic and other organic solvents). Crude extracts from the thalli of the lichens *Cladonia foliacea, Hypotrachyna cirrhata, Leucodermia leucomelos*, *Platismatia glauca* and *Pseudevernia furfuracea* against *Aspergillus favus*, from *Cladonia foliacea*, *Nephroma arcticum* and *Parmelia sulcata* against *Aspergillus* fumigatus and from *Evernia prunastri*, *Hypogymnia physodes, Umbilicaria cylindrica* and *Variospora dolomiticola* against *Aspergillus niger* have the greatest antifungal potential. The lichen secondary metabolites showed a higher inhibitory potential and their secondary metabolites shows that they can compete with commonly used antifungal substances, such as ketoconazole and clotrimazole [52].

Conclusion

Lichens are complex symbiotic associations between fungi and algae which are important constituents of many ecosystems. Lichens are a significant source of secondary metabolites with a wide range of biological functions. The considerable chemical diversity of lichen secondary metabolites makes them a powerful

natural source of nutritional, medicinal, and antimicrobial compounds. The commercialisation of lichens is growing but, in the future, metabolic and biotechnological approaches can be used as an alternative production to overcome the limited availability of biologically active, commercially valuable and medicinally important secondary metabolite components. Further research is required in order to isolate and identify more bioactive compounds responsible for such biological activities.

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