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Secondary metabolites from cetrarioid lichens: Chemotaxonomy, biological activities and pharmaceutical potential

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ABSTRACT

Background: Lichens, as a symbiotic association of photobionts and mycobionts, display an unmatched environmental adaptability and a great chemical diversity. As an important morphological group, cetrarioid lichens are one of the most studied lichen taxa for their phylogeny, secondary chemistry, bioactivities and uses in folk medicines, especially the lichen *Cetraria islandica*. However, insufficient structure elucidation and discrepancy in bioactivity results could be found in a few studies.
Purpose: This review aimed to present a more detailed and updated overview of the knowledge of secondary metabolites from cetrarioid lichens in a critical manner, highlighting their potentials for pharmaceuticals as well as other applications. Here we also highlight the uses of molecular phylogenetics, metabolomics and ChemGPS-NP model for future bioprospecting, taxonomy and drug screening to accelerate applications of those lichen substances.
Chapters: The paper starts with a short introduction in to the studies of lichen secondary metabolites, the biological classification of cetrarioid lichens and the aim. In light of ethnic uses of cetrarioid lichens for therapeutic purposes, molecular phylogeny is proposed as a tool for future bioprospecting of cetrarioid lichens, followed by a brief discussion of the taxonomic value of lichen substances. Then a delicate description of the bioactivities, patents, updated chemical structures and lichen sources is presented, where lichen substances are grouped by their chemical structures and discussed about their bioactivity in comparison with reference compounds. To accelerate the discovery of bioactivities and potential drug targets of lichen substances, the application of the ChemGPS NP model is highlighted. Finally the safety concerns of lichen substances (i.e. toxicity and immunogenicity) and future-prospects in the field are exhibited.
Conclusion: While the ethnic uses of cetrarioid lichens and the pharmaceutical potential of their secondary metabolites have been recognized, the knowledge of a large number of lichen substances with interesting structures is still limited to various *in vitro* assays with insufficient biological annotations, and this area still deserves more research in bioactivity, drug targets and screening. Attention should be paid on the accurate interpretation of their bioactivity for further applications avoiding over-interpretations from various *in vitro* bioassays.

Introduction

Lichens are in fact an ecosystem comprising of a photobiont that can either be a green alga (Chlorophyta) or a cyanobacterium and a mycobiont that in most cases belongs to the Ascomycetes. The nomenclature of a certain lichen taxon stems from its mycobiont partner and they are taxonomically classified within the fungi where majority of lichen-forming fungi belong to Lecanoromycetes (Tehler and Wedin 2008). With a special symbiotic lifestyle, a vast genetic diversity and interactions with various environmental factors, they produce lichen-unique profiles of

Abbreviations: BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; CIOMS, Council for International Organizations of Medical Sciences; COX, cyclooxygenases; DPPH, diphenylpicrylhydrazyl; FAS-II, plasmodial type II fatty acid biosynthesis; ITS, nuclear ribosomal internal transcribed spacer gene region; LC-MS, liquid chromatography-mass spectrometry; LSU, nuclear ribosomal large subunit gene region; MIC, minimum inhibitory concentration; mPGES-1, microsomial prostaglandin E2 synthase-1; PCA, principal component analysis; PKS, polyketide synthase-encoding gene; RATECs, rat adipose tissue endothelial cells; SSU, nuclear ribosomal small subunit gene region; TLC, thin layer chromatography.

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primary and secondary metabolites (i.e. lichen substances) with interesting physiochemical properties, such as lipophilicity and UV filtration (Nguyen et al. 2013), and marked biological activities determined by a series of *in vitro* and *in vivo* assays (Boustie et al. 2011). In particular, antioxidant, antimicrobial, anti-inflammatory and anti-proliferative activities of certain lichen substances are well studied (Fernandez-Moriano et al. 2016; Haraldsdottir et al. 2004; Ingolfsdottir et al, 1997; Ingolfsdottir 2002). Several reviews have recently been published describing the pharmaceutical potential of lichen substances (Gomez-Serranillos et al. 2014; Muller 2001; Shrestha and Clair 2013; Shrestha et al. 2015; Shukla et al. 2010; Zambare and Christopher 2012), their ecological and biotechnological roles (Oksanen 2006), their biosynthetic pathways (Stocker-Worgotter 2008) as well as the methodologies employed in the symbiotic mechanisms of both lichen partners (Eisenreich et al. 2011). Recent interest in lichen substances also tends to expand towards the bioactive compounds produced by lichen-associated bacteria, especially Actinobacteria and Cyanobacteria (Parrot et al. 2015; Suzuki et al. 2016). In particular, the reviewing of lichen substances was pioneered by the prominent monographs, "Chemical and botanical guide to lichen products" by Culberson (1969) and "Identification of lichen substances" by Huneck and Yoshimura (1996) illustrating contemporary analytics and depicting about 700 lichen substances; as well as "Catalogue of standardized chromatographic data and biosynthetic relationships for lichen substances" by Elix (2014) which describes 854 compounds. Furthermore Stocker-Worgotter et al. (2013) has indicated that hitherto the identified lichen substances have outnumbered 1000.

Cetrarioid lichens, one of the most studied groups of lichens in the Parmeliaceae (lichen-forming Ascomycetes) family, are designated by their morphology with "foliose/subfruticose thalli with marginal apothecia and pycnidia", and chemically they contain lichenan, which is a linear β-(1→3, 1→4) homoglycan (Nelsen et al. 2011). Considerable progress has been made in revealing the phylogeny (i.e. evolutionary relationships) of lichenized fungi in cetrarioid lichens using various genetic marker sequences of mycobiont nuclear ribosomal genes, such as group I intron, internal transcribed spacer (ITS), small subunit (SSU) and large subunit (LSU), and even polyketide synthase (PKS) genes (Nelsen et al. 2011; Opanowicz et al. 2006; Thell et al. 2009). Phylogenetic resolution of cetrarioid lichens has further increased and new phylogeny was recently proposed (Nelsen et al. 2011). According to a most recent article on the phylogeny of cetrarioid lichens, this group contains 25 genera and 149 species (Randlane et al. 2013), implying a great diversity of genetic resources and secondary metabolites.

The past two decades have witnessed a mounting number of scientific papers on discovery, biosynthesis and bioactivity of lichen substances, which reflects an increasing research interest in this area. However, accompanying the interest, insufficient structural elucidation (e.g. the absolute configuration is not indicated) and discrepant bioactivity results (e.g. antimicrobial activities of physodic acid as discussed in later section) in some studies may impede the accurate interpretation of bioactivities of lichen substances. In order to improve our understanding and to promote the application of lichen substances, it is urgent to have a critical attitude towards the research findings and a detailed library about the properties of lichen compounds and their bioactivities should be established. Thus, this review aims to present a more detailed and updated overview of the knowledge of secondary metabolites from cetrarioid lichens in a critical manner, highlighting their potentials for pharmaceuticals as well as other applications. Meanwhile, promising tools for bioprospecting, taxonomy and hit screening using molecular phylogenetics, metabolomics and ChemGPS are also proposed.

Ethnic uses of cetrarioid lichens and molecular phylogenetics for future bioprospecting

Crawford (2015) gave a very elaborate record of the ethnic uses of cetrarioid lichens, including *Cetraria islandica, Cetrelia pseudolivetorum, Flavocetraria cucullata, Flavocetraria nivalis, Masonhalea richardsonii, Nephromopsis pallescens, Vulpicida canadensis, Vulpicida juniperinus* and *Vulpicida pinastri*. They were mainly prepared as decoction or herbal tea with a variety of medicinal uses, such as treatment of coughs and inflammation, while *Vulpicida* species has also been used as a poison ingredient for wolves. Particularly, the medicinal lichen *Cetraria islandica*, also known as Iceland Moss, has been included in European Pharmacopoeias from 1600s and traditionally used to treat lung diseases and inflammation of oral and pharyngeal mucosa (Saroya 2011). In Iceland it is also used in folk medicines to treat cold symptoms and other minor ailments as dried and pulverized lichen (sometimes in capsules), as herbal tea or as a traditionally prepared milk soup (lichen boiled in milk) (Johannsdottir 2012). Iceland moss is also sold as polysaccharide-rich mixtures to treat cold and stomach ailments and used as a food ingredient in e.g. bread and soup (Kristinsson 1968).

With an applied perspective, there has been a debate whether the ethnic knowledge of traditional herbal medicines could provide hints for drug discovery. To address this question, recently researchers use molecular phylogeny representing the relationship of plant species and connect that to their ethnic uses, and finally it is found that traditionally medicinal plants tend to cluster in selected groups of plant species, which could be a good direction for future bioprospecting (Saslis-Lagoudakis et al. 2012; Zhu et al. 2011). Therefore, a question could be raised if the molecular phylogeny of lichens could be a powerful tool in predicting the production of bioactive compounds by taking the advantage of the well-established lichen phylogeny using multiple gene locus, such as nr ITS and nr LSU or PKS. Since lichens produce special molecular scaffolds with known bioactivities, such as depsides, depsidones and some special polysaccharides, it can be hypothesized that similar bioactive compounds may cluster in certain group of lichens.

Chemotaxonomy of cetrarioid lichens by secondary and primary metabolites

"A character in itself has no taxonomic value *a priori*, but may have importance when correlated with other independent characters. This, however, can only be evaluated *a posteriori*. Chemical characters do not fundamentally differ from other character sets, their nature, however, leads to an unreflected use when applied schematically."

Above is the conclusion made by Lumbsch (1998) by reviewing the historical opinions on chemical variants and lichen chemotaxonomy. It is hard to define how much value should be given to chemical character of lichens for taxonomic purposes, and actually the value varies from taxa to taxa. At least, chemical character has no independent taxonomic value.

Prior to the advent of modern DNA sequencing techniques, comparative lichen phytochemistry, which is mainly based on lichen secondary metabolites, undeniably is the most useful character accompanying morphology in the species delimitation of lichens (Culberson and Culberson 1970). To this end, a series of standard spot testing and thin layer chromatography (TLC) methods has been used and developed for decades (Culberson and Amman 1979). Even in contemporary lichen taxonomic monographs, one can always find chemical data generated from conventional spot testing and TLC of a certain lichen taxon, as exemplified in the fourth Volume of the Nordic Lichen Flora (Thell and Moberg 2011). From those data, it seems that a certain group of lichen taxa, which were defined by their morphology, tends to have a stable pool of