General aspects regarding the knowledge of secondary metabolites in lichens

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Abstract. The main studies related to lichen secondary metabolites features are presented in a brief review. The significance of secondary metabolites in a vast field of applications, biological effects of lichenic acids, modern techniques to determine their chemical structure and the way of action, the impact of pollution on lichens metabolism are discussed. Secondary metabolites represent a wide domain for the future investigation, being involved in a series of biological processes of interest for the biological research field.

Key Words: lichen substances biosynthesis, lichen thallus, secondary metabolites.

Introduction. Lichens are source of unique metabolites, covering a wide variety of phenolic compounds, most of them with unknown structure and properties produced by the mycobiont by acetyl polymalonyl, shikimic and mavalonic acid pathways, most relevant being aromatic compounds, depsides, depsidones, diphenyl ethers and dibenzofurans (Yousuf et al 2014; Calcott et al 2018; Jeong et al 2021). The complexity of the concept of lichen symbiosis by the asssociation with bacteria resulting in a microbiome architecture (Cernava et al 2015; Aschenbrenner et al 2016) which in time aquired new values (Morillas et al 2022) is correlated with particularities of chemical substances resulted from the interaction of the entire biological system. Lichen phycobionts represented by cyanobacteria produce compounds that are specific for the symbiotic organism (Calcott et al 2018).

Fundamental contributions regarding lichens secondary metabolites related to their structure and biosynthesis in lichenized fungi were previously made by Mosbach (1969) and Culberson et al (1992). Huneck & Yoshimura (1996) classified metabolites and developed a list of chromatographic data for lichen substances. Although are different from a structural point of view, lichen substances have a number of common features: they are crystalline, mostly have an acidic character, and their solubility in water is very poor even in the form of alkaline salts; the color of the mitochondrial cristae varies from transparent to white until reddish yellow; some are optically active (all aliphatic). Lichen substances are stored as microcristals on the outer surface of cell wals (Leuckert et al 1990), confering to the medullary hyphae hydrophobic properties.

Modern techniques have been developed in order to identify the distribution of secondary metabolism products in lichens (Le Pogam et al 2016). According to these findings, usnic acid is distributed above the *Trebouxia* photobiont layer and along internal septae, haemoventosin is located in the red epihymenial layer, while thamnolic acid is located in the hypotecium and upper medula parts of the lichen.

Identification by color reactions are used in order to detect hydrolysable tannins, non hydrolysable tannins, benzofurane derivatives, anthraquinone derivatives and flavonoides; in this frame, the results indicated that most of the identified compounds belong to the polyphenols classes (Voicu et al 2019).

Chemical nature of secondary metabolites. Most commonly applied techniques namely thin-layer chromatography (TLC) including high performance thin-layer chromatography (HPTLC) and high performance liquid chromatography (HPLC) are detailed by Lumbsch (2002).

Mass spectrometry-based methods techniques as Liquid Chromatography–Mass Spectrometry (LC-MS) analytical platforms associating strengths of liquid chromatography with the capabilities of mass spectrometry provide sensitivity and selectivity to analysis of lichen metabolites profiling as multiple reaction monitoring (MRM) approach (Musharraf et al 2017).

Lichens chemistry determination has been achieved for a number of species and each lichen metabolite can reveal unknown therapeutic potential (Huynh et al 2016; Rankovic 2015; Sedrpoushan et al 2022).

Certain lichens as *Lecanora muralis* are source of bioactive antioxidant phytochemicals for green synthesis of nanocomposites; lichen substances can stabilize metalic salts and convert them into metal nanoparticles (Abdullah et al 2020). Lichen metabolites as gyrophoric acid (*Umbilicaria hirsuta*), evernic acid (*Evernia prunastri*), physodic acid, 3-hydroxyphysodic acid, physodalic acid, atranorin (*Hypogymnia physodes*) have radical scavenging activity (Elecko et al 2022). Biological effects of lichens acids cover a wide range (Ernst-Russell et al 1999; Müller 2001; Yamamoto et al 1998). From lichen substances, usnic acid became one of the most studied secondary metabolite and comercially used as antiseptic ointment as „Usno" and „Evosin". Usnic acid has been isolated from some species of *Usnea* (as *Usnea barbata*) and also in other lichen species as *Ramalina reticulata*, *Cetraria islandica*, and *Cladonia alpestris*.

Therefore, some secondary metabolites as montagnetol from the lichen *Roccella montagnei*, synthesized from L-ascorbic acid, are effective against gram negative *Pseudomonas aeruginosa* and the fungal strain *Candida albicans in vitro* (Mallavadhani et al 2018). Antifungal activity of secondary metabolites against fungal species of *Fusarium* (Furmanek et al 2022) has been tested on a wide range of lichens as *Alectoria sarmentosa*, *Cladonia mitis*, *Cladonia rangiferina*, *Flavoparmelia caperata*, *Hypotrachyna cirrhata*, *Leucodermia leucomelos*, *Parmotrema austrosinense*, *Parmelia reticulata*, *Physcia aipolia*, *Pseudevernia furfuracea*, *Roccella montagnei* and *Umbilicaria nylanderiana*. Lichens use secondary metabolites as chelating agents (Purvis et al 1987), as inhibitors of lichenicolous fungi (Lawrey 1984) and to avoid medulla saturation with water (Armaleo et al 2008). They can also represent a way of defence against other organisms. Qualitative determination of secondary metabolites is mentioned in taxonomically keys, being a tool for a right identification of many of lichen species. The knowledge of these allow establishing some phylogenies for some lichen genera, every species having a set of specific secondary metabolites. Therefore, lichen metabolites are used as markers in taxonomy (Olivier-Jimenez et al 2019). Metabolite profiling of lichen secondary metabolites provide information about biosynthetic pathways (Nazem-Bokaee et al 2021).

Early studies of synthesis also underlined the significance of secondary metabolites in lichen taxonomy. There is a corespondence between lichen secondary metabolites abundance in lichens and substrate pH (Paukov et al 2022) or environmental heavy metal accumulation (Elrhzaoui et al 2020; Voicu 2021a). There are metal tolerant lichens as *Xanthoria parietina* and also hyperaccumulator *Diploschistes muscorum*. The mechanism of tolerance of heavy metals consist in complexation of secondary metabolites with heavy metals and synthesis of oxalate which precipitates toxic elements or complexation to carboxylic groups of the fungal cell walls (Sarret et al 1998). In lichen symbioses, fungal secondary metabolites provide UV protection on which lichen algae such as trebouxiophycean green algae - the most prominent group of photobionts in lichen symbioses - sensitively depend (Nguyen et al 2021).

There are a lot of studies concerning the use of lichens as natural dyes and also dye colour extraction from lichens by different methods and correlation of the chemical with the colour (Shukla et al 2014; Rather et al 2018). These studies revealed that lichens contain a rainbow of permanent colors for all types of fibers in textile industry

(Kalra et al 2020; Adeel et al 2020; Yusuf 2020). Considering the slow growth rate of lichens (complex organisms resulted from the symbiosis between one mycobiont and two, rarely more photobionts) in natural conditions, dificulties are encountered in obtaining substantially quantities of lichenic substances necessary for different applications.

Mycobionts *in vitro* culture in order to obtain lichen substances with therapeutic potential open new opportunities of research for using lichen products and also for molecular biology of these. Biosynthesis of secondary metabolites is related to the carbohydrate resulted from photobiont synthesis and transferred to the fungus (Elix & Stocker-Worgotter 2008). Collection of bulk quantities of lichens for useful compounds determined acceleration of the studies on lichens (Boustie & Grube 2005) and also the *in vitro* culture of them.

Beside medicinal plants, lichen mycobionts cultured *in vitro* are reliable sources of new bioactive compounds of secondary metabolism (Tanahashi 2017). Efficient methods measured in reasonable time and effort of culturing lichen fungi as source for secondary metabolites production have been elaborated (Zakeri et al 2022). Photobiont culturing *in vitro* (Voicu 2021b) need further studies about metabolism pathways and compounds synthesized.

Phenol content in lichens can be improved by suitable substances added to *in vitro* composition of culture (Poornima et al 2022). Therefore, beside lichen mycobiont development *in vitro*, as source of secondary metabolites production, enhanced production rate of secondary metabolites in term of total phenol content has been achieved as in mycobiont of *Parmotrema austrosinense* by suitable culture media supplemented with 3.15 g L^{-1} yeast, glucose (4 mg L^{-1}), asparagine (1 mg L^{-1}), $MnSO_4$ $4H_2O$ (20 mg L^{-1}), $ZnSO_4$ $7H_2O$ (20 mg L^{-1}) and H_3BO_3 (4 mg L^{-1}) (Poornima et al 2022). Mycobiont of *Cladonia rangiferina* secondary metabolites biosynthesis depend on the interaction with the co-cultured photobiont *Asterochloris glomerata.* Also, resynthesis events in lichens *in vitro* and photobiont induces biosynthesis of bioactive compounds (Sveshnikova & Piercey-Normore 2021).

Future perspectives. The mechanism of action of secondary metabolites is still of great interest (Adenubi et al 2022) for the future studies as fungal derived metabolites are involved also in lichen environment distribution and protection of the carbon source, namely the photobiont, being speciallised in UV absorbtion (Schweiger et al 2022). Green synthesis of nanoparticles using lichens is also a modern approach (Hamida et al 2021).

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References

Abdullah S. M., Kolo K., Sajadi S. M., 2020 Greener pathway toward the synthesis of lichen-based ZnO@ TiO_2@SiO_2 and Fe_3O_4@SiO_2 nanocomposites and investigation of their biological activities. Food Science and Nutrition 8(8):4044-4054.

Adeel S., Majeed A., Rehman F., Azeem M., Iqbal N., Amin N., 2020 Lichen-derived products as sustainable source of natural dyes. In: Lichen-derived products: extraction and applications. Yusuf M. (ed), Scrivener Publishing LLC, pp. 245-261.

Adenubi O. T., Famuyide I. M., McGaw L. J., Eloff J. N., 2022 Lichens: an update on their ethnopharmacological uses and potential as sources of drug leads. Journal of Ethnopharmacology 298:115657.

Armaleo D., Zhang Y., Cheung S., 2008 Light might regulate divergently depside and depsidone accumulation in the lichen *Parmotrema hypotropum* by affecting thallus temperature and water potential. Mycologia 100(4):565-576.

Aschenbrenner I. A., Cernava T., Berg G., Grube M., 2016 Understanding microbial multi-species symbioses. Frontiers in Microbiology 7:180.

Boustie J., Grube M., 2005 Lichens - a promising source of bioactive secondary metabolites. Plant Genetic Resources 3(2):273-287.

Calcott M. J., Ackerley D. F., Knight A., Keyzers R. A., Owen J. G., 2018 Secondary metabolism in the lichen symbiosis. Chemical Society Reviews 47(5):1730-1760.

Cernava T., Aschenbrenner I. A., Grube M., Liebminger S., Berg G., 2015 A novel assay for the detection of bioactive volatiles evaluated by screening of lichen-associated bacteria. Frontiers in Microbiology 6:398.

Culberson C. F., Culberson W. L., Johnson A., 1992 Characteristic lichen products in cultures of chemotypes of the *Ramalina siliquosa* complex. Mycologia 84(5):705-714.

Elix J. A., Stocker-Worgotter E., 2008 Biochemistry and secondary metabolites. In: Lichen biology. Nash III T. H. (ed), Cambridge University Press, pp. 104-133.

Elrhzaoui G., Divakar P. K., Crespo A., Tahiri H., El Alaoui-Faris F. E., Khellouk R., 2020 Spatial mapping of heavy metals using lichen bioaccumulation capacity to assess air contamination in Morocco. Cryptogam Biodiversity and Assessment 4(1):19-24.

Elečko J., Vilková M., Frenák R., Routray D., Ručová D., Bačkor M., Goga M. A., 2022 A comparative study of isolated secondary metabolites from lichens and their antioxidative properties. Plants 11:1077.

Ernst-Russell M. A., Chai C., Hurne A., Waring P., Hockless D., Elix J., 1999 Structure revision and cytotoxic activity of the scabrosin esters, epidithiopiperazinediones from the lichen *Xanthoparmelia scabrosa*. Australian Journal of Chemistry 52:279-283.

Furmanek L., Czarnota P., Seaward M. R. D., 2022 A review of the potential of lichen substances as antifungal agents: the effects of extracts and lichen secondary metabolites on *Fusarium* fungi. Archives of Microbiology 204:523.

Hamida R. S., Ali M. A., Abdelmeguid N. E., Al-Zaban M. I., Baz L., Bin-Meferij M. M., 2021 Lichens - a potential source for nanoparticles fabrication: a review on nanoparticles biosynthesis and their prospective applications. Journal of Fungi 7(4):291.

Huneck S., Yoshimura I., 1996 Identification of lichen substances. Springer-Verlag, Berlin, Heidelberg, 493 pp.

Huynh B. L. C., Le D. H., Takenaka Y., Tanahashi T., Nguyen K. P. P., 2016 New phenolic compounds from the lichen *Parmotrema praesorediosum* (Nyl.) Hale (Parmeliaceae). Magnetic Resonance in Chemistry 54(1):81-87.

Jeong M. H., Park C. H., Kim J. A., Choi E. D., Kim S., Hur J. S., Park S. Y., 2021 Production and activity of cristazarin in the lichen-forming fungus *Cladonia metacorallifera*. Journal of Fungi 7(8):601.

Kalra R., Conlan X. A., Goel M., 2020 Fungi as a potential source of pigments: harnessing filamentous fungi. Frontiers in Chemistry 8:369.

Lawrey J. D., 1984 Biology of lichenized fungi. New York, Praeger Scientific, 408 pp.

Le Pogam P., Legouin B., Geairon A., Rogniaux H., Lohézic-Le Dévéhat F., Obermayer W., Boustie J., Le Lamer A. C., 2016 Spatial mapping of lichen specialized metabolites using LDI-MSI: chemical ecology issues for *Ophioparma ventosa*. Scientific Reports 6:37807.

Leuckert C., Ahmadjian V., Culberson C. F., Johnson A., 1990 Xanthones and depsidones of the lichen *Lecanora dispersa* in nature and of its mycobiont in culture. Mycologia 82(3):370-378.

Lumbsch H. T., 2002 Analysis of phenolic products in lichens for identification and taxonomy. In: Protocols in lichenology. Kranner I. C., Beckett R. P., Varma A. K. (eds), Springer Lab Manuals, Springer, Berlin, Heidelberg, pp. 281-295.

Mallavadhani U. V., Boddu R., Rathod B. B., Setty P. R., 2018 Stereoselective synthesis of the lichen metabolite, (+) montagnetol and its congeners as antimicrobial agents. Synthetic Communications 48(23):2992-2999.

Morillas L., Roales J., Cruz C., Munzi S., 2022 Lichen as multipartner symbiotic relationships. Encyclopedia 2(3):1421-1431.

Mosbach K., 1969 Biosynthesis of lichen substances, products of a symbiotic association. Angewandte Chemie, International Edition 8(4):240-250.

Musharraf S. G., Siddiqi F., Ali A., Thadhani V. M., 2017 Sensitive analysis of bioactive secondary metabolites in lichen species using liquid chromatography - mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis 146:279-284.

Müller K., 2001 Pharmaceutically relevant metabolites from lichens. Applied Microbiology and Biotechnology 56(1-2):9-16.

Nazem-Bokaee H., Hom E. F. Y., Warden A. C., Mathews S., Gueidan C., 2021 Towards a systems biology approach to understanding the lichen symbiosis: opportunities and challenges of implementing network modelling. Frontiers in Microbiology 12: 667864.

Nguyen T. T. T., Vo T. T. D., Tran Y. H., Truong D. T., Phan D. C., Le P. H., 2021 Photoprotective activity of lichen extracts and isolated compounds in *Parmotrema tinctorum*. Biointerface Research in Applied Chemistry 11(5):12653-12661.

Olivier-Jimenez D., Chollet-Krugler M., Rondeau D., Beniddir M. A., Ferron S., Delhaye T., Allard P. M., Wolfender J. L., Sipman H. J. M., Lücking R., Boustie J., Le Pogam P., 2019 A database of high-resolution MS/MS spectra for lichen metabolites. Scientific Data 6(1):294.

Paukov A., Teptina A., Ermoshin A., Kruglova E., Shabardina L., 2022 The role of secondary metabolites and bark chemistry in shaping diversity and abundance of epiphytic lichens. Frontiers in Forests and Global Change 5:82821.

Poornima S., Rajivgandhi G., Maruthupandy M., Mothana R. A., Noman O. M., Nasr F. A., Ponmurugan P., Moorthy I. G., Sivarajasekar N., 2022 Statistical modeling and optimization for the production of secondary metabolites by lichen mycobiont isolated from *Parmotrema austrosinense*. Physiological and Molecular Plant Pathology 117:101760.

Purvis O. W., Elix J. A., Broomheadj J. A., Jones G. C., 1987 The occurrence of copper-norstictic acid in lichens from cupriferous substrata. The Lichenologist 19(2):193-203.

Rankovic B., 2015 Lichen secondary metabolites: bioactive properties and pharmaceutical potential. Springer International Publishing, 202 pp.

Rather L. J., Jameel S., Ganie S. A., Bhat K. A., 2018 Lichen derived natural colorants: history, extraction, and applications. In: Handbook of renewable materials for coloration and finishing. Yusuf M. (ed), Scrivener Publishing LLC, pp. 103-114.

Sarret G., Manceau A., Cuny D., Van Haluwyn C., Deruelle S., Hazemann J. L., Soldo Y., Eybert-Berard L., Menthonnex J. J., 1998 Mechanisms of lichen resistance to metallic pollution. Environmental Science and Technology 32(21):3325-3330.

Schweiger A. H., Ullmann G. M., Nürk N. M., Triebel D., Schobert R., Rambold G., 2022 Chemical properties of key metabolites determine the global distribution of lichens. Ecology Letters 25(2):416-426.

Sedrpoushan A., Haghi H., Sohrabi M., 2022 A new secondary metabolite profiling of the lichen *Diploschistes diacapsis* using liquid chromatography electrospray ionization tandem mass spectrometry. Inorganic Chemistry Communications 145(13):110006.

Shukla P., Upreti D. K., Nayaka S., Tiwari P., 2014 Natural dyes from Himalayan lichens. Indian Journal of Traditional Knowledge 13(1):195-201.

Sveshnikova N., Piercey-Normore M. D., 2021 Transcriptome comparison of secondary metabolite biosynthesis genes expressed in cultured and lichenized conditions of *Cladonia rangiferina*. Diversity 13(11):529.

Tanahashi T., 2017 [Diversity of secondary metabolites from some medicinal plants and cultivated lichen mycobionts]. Yakugaku Zasshi 137(12):1443-1482. [in Japanese]

Voicu D., Mitoi M. E., Gavriloaie C., Helepciuc F. E., Toma N., 2019 Chemical investigations of lichen biomass in *Usnea barbata*, *Cetraria islandica*, and *Xanthoria parietina* species. ELBA Bioflux 11(1):1-8.

Voicu D. 2021a A comprehensive review on lichens. Muzeul Olteniei Craiova. Oltenia. Studii si comunicari. Stiintele Naturii 37(2):199-207.

Voicu D., 2021b Particularities of the methods used for *in vitro* lichen photobiont culture. ELBA Bioflux 15(2):22-27.

Yamamoto Y., 2000 [Screening of biological activities and isolation of biological-active compounds from lichens]. Shokubutsu no Kagaku Chosetu 35(2):169-179. [in Japanese]
Yousuf S., Choudhary M. I., Atta-ur-Rahman 2014 Lichens: chemistry and biological activities. Studies in Natural Products Chemistry 43:223-259.
Yusuf M. (ed), 2020 Lichen-derived products: extraction and applications. Scrivener Publishing LLC, 304 pp.
Zakeri Z., Junne S., Jäger F., Dostert M., Otte V., Neubauer P., 2022 Lichen cell factories: methods for the isolation of photobiont and mycobiont partners for defined pure and co-cultivation. Microbial Cell Factories 21:80.

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