# Lichen secondary metabolites in *Umbilicaria antarctica* evaluated by acetone rinsing

*Short Communication*

Lora Martic*

*University of Sarajevo, Obala Kulina Bana br. 7/II, 71 000 Sarajevo, Bosnia and Herzegovina*

**Abstract**
Study of the extracts from an Antarctic lichen *Umbilicaria antarctica* was done using a spectrophotometric approach. Secondary compounds were extracted by acetone rinsing from dried thalli of *U. antarctica*. The extracts were dried out, and diluted in ethanol. Then, spectral absorbance of the extracts were measured within the wavelength of 190-700 nm. The spectra of the secondary compounds obtained by acetone rinsing (EAR – re-diluted (ethanol) extract gained during acetone rinsing) were compared with those from untreated thalli (control) and ethanol extract from the thalli of *U. antarctica* that passed acetone rinsing (ART). Spectral absorbance curves of the extracts gained by acetone rinsing were attributed to different prevailing secondary metabolites: usnic acid, lecanoric acid (*U. antarctica*). Spectral absorption curves of control thalli exbibited similar shape as ART spectral curves, however, the absorbance in the range of 230-310 nm reached higher values in control than in ART. Spectral absorbance curves from ART showed that a part of secondary metabolites still remained in the thalli. Photosynthetic pigments (carotenoids and chlorophylls) remained uneffected by acetone rinsing.

***Key words:*** macrolichen, Antarctics, extracts, absorption spectrum

**DOI:** 10.5817/CPR2016-2-17

## Introduction

Lichens produce a large variety of secondary compounds (for review *see* Huneck et Yoshimura 1996). They are mostly weak phenolic acid derivatives that commonly make up 1–5% of the lichen biomass. Majority of lichen secondary compounds are UV screens that provide an effective absorption of incident radiaton, UV-B (280–315 nm), and UV-A (315–400 nm) in particular. These compounds, reviewed recent-

*Received* December 27, 2016, *accepted* February 6, 2017.
*Corresponding author: Lora Martic <martic.lora@gmail.com>
*Acknowledgements*: I am thankful to the members of the Extreme Environments Life laboratory (Department of Experimental Biology, Masaryk University, Brno) for their help and suggestions. I also express my thanks to CzechPolar2 infrastructure that helped me do the experiments.

ly by Huang et al. (2013), represent a mechanism protecting lichens against the damage caused by UV-A and UV-B radiation.

These compounds can be greatly and non-destructively extracted from dry lichens through the use of acetone by the method called acetone rinsing (Solhaug et Gauslaa 1996, 2001). Using this method, several ecophysiological studies showed that some secondary compounds had an important role in deterring lichenivores (see *e.g.* Gauslaa 2005, Nybakken et al. 2010). Acetone rinsing also proved a protective role of secondary lichen compounds in the photoprotection of lichen photobionts from excess light (Solhaug et Gauslaa 2012). Last but not least, lichen secondary compounds extracted by acetone rinsing have been tested for their antimicrobial activity (*e.g.* Lawrey 2009). Acetone rinsing used in dry lichen thalli does not bring any change of the process of rehydration as shown by (Souza-Egipsy et al. 2000) who reported no modification of surface water film formation in *Neofuscelia pokornyi* and *N. pulla*. In this study, *Umbilicaria antarctica* was used to evaluate the constitutive amount of intrathalline secondary metabolites and the amount of metabolites extracted by acetone rinsing. The aim of the study was to evaluate quantity of UV screens that is extracted by acetone rinsing.

## Material and Methods

### *Sample collection and handling before experiments*

Thalli of *U. antarctica*, were collected from rock walls at Galindez Island (Argentine Islands, Antarctica). Collection site was located close to the Akademik Vernadsky station (65° 14´44´´ S, 64° 15´20´´ W). The thalli of *U. antarctica* were collected by Czech participants of the Ukrainian Antarctic expedition. After collection, the thalli were dried under natural outdoor conditions. After drying the thalli were transfered to a laboratory (EEL laboratory, Department of Experimental Biology, Masaryk University, Brno, Czech Republic) where stored in a refrigerator.

For the experiments, the thalli were divided into two groups: (1) control thalli and those thalli selected for (2) acetone rinsing. Control thalli were dried in a lyophilizator, then homogenized to a fine powder in a stainless steel ball mill (Retsch, Germany). Equal weight (300 mg) of samples were used for extracts in ethanol. Then, the absorbances of the extracts were measured by a UV-VIS spectrophotometer (Specord 205, Analytik Jena, Germany) within the wavelength range of 190-700 nm.

### *Acetone rinsing*

Lichen substances were removed according to a modification of the method of acetone rinsing (Solhaug et Gauslaa 1996) using dry fragments of *U. antarctica*. Each fragment was rinsed in pure acetone four times for 1 h each time at room temperature. Fragments were then taken out of acetone and left to dry for 24 h in a laboratory hood with a blower at room temperature to ensure vaporization of residual acetone. Dried fragments were then homogenized and ethanol-extracted (*see* the above

Handling before experiments). Absorbance spectra (190-700 nm) were measured as described above. Acetone extracts were put into Petri dishes and left to evaporate under the same conditions. Then the dried extracts were diluted in ethanol and used in the measurements of absorbances as described above.

## Results and Discussion

As shown in Fig. 1, acetone rinsing of dry thalli of *U. antarctica* led to the extraction of secondary metabolites in the wavelengths of 190-350 nm (*see* C in Fig. 1). Carotenoids- and chlorophyll-related peaks (435 nm, and 665 nm) remained more or less unchanged (Fig. 1B) compared to control (Fig. 1A) indicating the fact that photosynthetic pigments are not disturbed by acetone rinsing. Such finding is supported by the fact that no peak is seen in the extract (AR rediluted in ethanol – *see* C in Fig. 1, 665 nm).

The spectra had several peaks in the range of 190-300 nm (UV) thanks to a high amount of UV-B absorbing compounds, such as *e.g.* umbilicaric acid (Huneck et Yoshimura 1996). Moreover, Quilhot et al. (1991) reported phenolic metabolites in thalli of different ages in *U. antarctica.* Specifically, usnic acid and atranorin concentrations were found highest in individuals of the youngest age class and decrease towards the oldest age classes. Moreover, the same author reported also gyrophoric acids. In *U. antarctica*, another UV-B absorbing compound are *e.g.* lecanoric acid (Luo et al. 2009, Seo et al. 2009), and tocopherols (Strzalka et al. 2011). In general, lichens of genus *Umbilicaria* have numerous secondary metabolites (for review see *e.g.* Narui et al. 1996). Majority of UV-B absorbing compounds extracted from *U. antarctica* have strong antioxidative effects (Hara et al. 2011, Molnár et Farkas 2010). Absorption curve recorded for control thalli correspond to the those reported by Medina et Avalos-Chacon (2015). In general, a high amount of UV-B screening compounds in *Umbilicaria antarctica* represents a constitutive mechanism of lichens providing protection against enhanced UV-B radiation in Antarctic terrestrial ecosystems (Singh et al. 2011). Some species of *Umbilicaria* genus from Antarctica showed decrease in UV-B absorbing compounds and phenolics in the field experiment when UV-B radiation was excluded by filters installed for 4 weeks (Singh et Singh 2014 - *Umbilicaria aprina*).

Our data presented in Table 1 suggest that acetone rinsing is effective in the extraction of UV-B absorbing compounds, while chlorophylls and carotenoids remain unchanged by the treatment (*see* also the curves in Fig. 1).

| Treatments | 280 nm | 310 nm | 435 nm | 665 nm |
|---|---|---|---|---|
| Control | 6.418 | 5.974 | 3.155 | 1.765 |
| ART | 3.051 | 2.008 | 3.179 | 1.776 |
| EAR | 6.154 | 5.098 | n. d. | n. d. |

**Table 1.** Absorbance values in the two wavelengths (280 and 310 nm, according to Buffoni-Hall et al. 2002) related to UV-B screens and the wavelengths related to carotenoids (435 nm), and chlorophylls (665 nm). *Abbreviations*: ART – ethanol extract from the thalli of *U. antarctica* that passed acetone rinsing, EAR – re-diluted (ethanol) extract gained during acetone rinsing, n. d. - not determined.

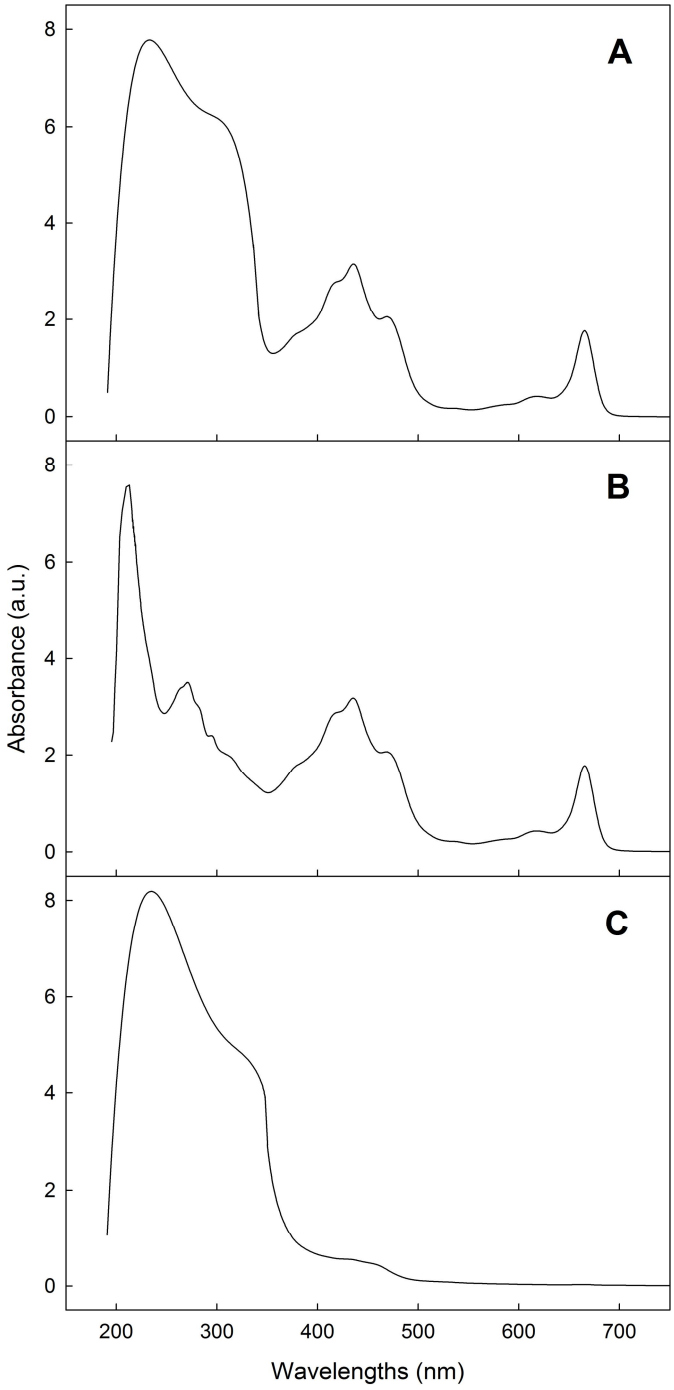


**Fig. 1.** Absorbance spectra of untreated control thalli of *Umbilicaria antarctica* (A), ART – ethanol extract from the thalli of *U. antarctica* that passed acetone rinsing (B), EAR – re-diluted (ethanol) extract gained during acetone rinsing (C).

## References


BUFFONI-HALL, R. S., BORNMAN J. F. and BJÖRN, L. O. (2002): UV-induced changes in pigment content and light penetration in the fruticose lichen *Cladonia arbuscula* ssp. Mitis. *Journal of Photochemistry and Photobiology B: Biology*, 66: 13-20.

GAUSLAA, Y. (2005): Lichen palatability depends on investments in herbivore defence. *Oecologia*, 143: 94-105, doi: 10.1007/s00442-004-1768-z.

HARA, K., ENDO, M., KAWAKAMI, H., KOMINE, M. and YAMAMOTO,Y. (2011): Anti-oxidation activity of ethanol extracts from natural thalli of lichens. *Mycosystema*, 30: 950-954.

HUANG, X., OUYANG, X., YANG, P., LAU, O. S., CHEN, L., WEI. N. and DENG, X.W. (2013): Conversion from CUL4-based COP1-SPA E3 apparatus to UVR8-COP1-SPA complexes underlies a distinct biochemical function of COP1 under UV-B. *Proceedings of the National Academy of Sciences of the United States of America*, 110: 16669-16674, doi: 10.1073/pnas. 1316622110.

HUNECK, S., YOSHIMURA, I. (1996): Identification of lichen substances. Springer, Berlin, 493 p.

LAWREY, J. D. (2009): Chemical defense in lichen symbioses (Chapter 11). *In*: J. F. White Jr., M. S. Torres (eds.): Defensive Mutualism in Microbial Symbiosis. Boca Raton, FL: CRC Press, pp. 167-176, http://dx.doi.org/10.1201/9781420069327.pt3.

LUO, H., YAMAMOTO, Y., KIM, J. A., JUNG, J. S., KOH, Y. S. and HUR, J. S. ( 2009): Lecanoric acid, a secondary lichen substance with antioxidant properties from *Umbilicaria antarctica* in maritime Antarctica (King George Island). *Polar Biology*, 32: 1033e1040, doi: 10.1007/s00300-009-0602-9

MEDINA, M. G., AVALOS-CHACON, R. (2015): Physiological performance of a foliose macrolichen *Umbilicaria antarctica* as affected by supplemental UV-B treatment. *Czech Polar Reports*, 5: 222-229.

MOLNÁR, K., FARKAS, E. (2010): Current results on biological activities of lichen secondary metabolites: a review. *Zeitschrift für Naturforschung*, 65: 157-173.

NARUI, T., CULBERSON, CH. F., CULBERSON, W. L., JOHNSON, A. and SHIBATA, S. (1996): A contribution to the chemistry of the lichen family Umbilicariaceae (Ascomycotina). *The Bryologist*, 99: 199-211.

NYBAKKEN, L., HELMERSEN, A. M., GAUSLAA, Y. and SELAS, V. (2010): Lichen compounds restrain feeding by bank voles (*Myodes glareolus*). *Journal of Chemical Ecology,* 36: 298-304, http://dx.doi.org/ 10.1007/s10886-010-9761-y.

QUILHOT, W., SAGREDO, M.G., CAMPALANS, E., HIDALGO M.E., PEÑA W., FERNANDEZ, E. and PIOVANO, M. (1991): Quantitative variations of phenolic compounds related to thallus age in *Umbilicaria antarctica. Instituto Antártico Chileno* (INACH), *Serie Científica* 41: 91-97.

SEO, C., CHOI, Y. H., AHN, J. S., YIM, J. H., LEE, H. K. and OH, H. (2009): PTP1B inhibitory effects of tridepside and related metabolites isolated from the Antarctic lichen *Umbilicaria antarctica*. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 24: 1133-1137.

SINGH, J., DUBEY, A. K. and SINGH, R. P. (2011): Antarctic terrestrial ecosystem and role of pigments in enhanced UV-B radiations. *Reviews in Environmental Science and Bio/Technology*, 10: 63-77, doi: 10.1007/s11157-010-9226-3.

SINGH, J., SINGH, R. P. (2014): Adverse effects of UV-B radiation on plants growing at Schirmacher oasis, East Antarctica. *Toxicology International*, 21: 101-106.

SOLHAUG, K. A., GAUSLAA, Y. (1996): Parietin, a photoprotective secondary product of the lichen *Xanthoria parietina*. *Oecologia*, 108: 412-418.

SOLHAUG, K. A., GAUSLAA, Y. (2001): Acetone rinsing - A method for testing ecological and physiological roles of secondary compounds in living lichens. *Symbiosis*, 30: 301-315.

SOLHAUG, K. A., GAUSLAA, Y. (2012): Secondary lichen compounds as protection against excess solar radiation and herbivores. *Progress in Botany*, 73: 283-304.

SOUZA-EGIPSY, V., VALLADARES, F. and ASCASO, C. (2000): Water distribution in foliose lichen species: Interactions between method of hydration, lichen substances and thallus anatomy. *Annals of Botany*, 86: 595-601, https://doi.org/10.1006/anbo.2000.1224.

STRZALKA, K., SZYMANSKA, R. and SUWALSKY, M. (2011): Prenyllipids and pigments content in selected antarctic lichens and mosses. *Journal of the Chilean Chemical Society*, 56: 808-811.