INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 15, No. 6, p. 1-15, 2019
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Active metabolites of some lichens growing in Georgia

Badridze Gulnara*, Chkhubianishvili Eva, Rapava Luara, Kikvidze Medea, Chigladze Lali, Tsiklauri Nino, Tsilosani Ketevan, Kupradze Inga, Chanishvili Shota

Department of Plant Physiology, Institute of Botany, Ilia State University, Botanikuri 1, Tbilisi, Georgia

Article published on December 30, 2019

Key words: Active Metabolites, Antioxidants, Lichens, Georgia.

Abstract

The purpose of the presented investigation was to study the content of active metabolites in lichen species: *Anaptychia ciliaris* (L.) A. Massal, *Flavoparmelia caperata* (L.) Hale, *Hypogymnia physodes* (L.) Nyl., *Parmelia sulcata* Taylor, *Peltigera canina* (L.) Willd., *Pseudevernia furfuracea* (L.) Zopf. var. furfuracea, *Ramalina farinacea* (L.) Ach., *Ramalina pollinaria* (Westr.) Ach., *Xanthoparmelia stenophylla* (Ach.) Ahti & D. Hawksw growing in Georgia. The primary and secondary metabolites of photo- and micobiont, in particular photosynthetic pigments, ascorbic acid, anthocyanins, proline, total phenols, soluble carbohydrates, total proteins and total antioxidant activity have been investigated. Spectrophotometrical and titration methods have been used for studies. Remarkably high content of carotenoids was discovered in *Xanthoparmelia stenophylla* compared to other tested species. *Anaptychia ciliaris, Pseudovernia furfuracea* and *Ramalina farinacea* were distinguished by the high content of chlorophylls, carotenoids and anthocyanins among the studied tree-inhabiting species. High content of proline was found in species: *Xanthoparmelia stenophylla, Hypogymnia physodes,* and *Parmelia sulcata*. Especially high content of phenols was determined in *Peltigera canina*. *Ramalina pollinaria, Pseudovernia furfuracea,* and *Flavoparmelia caperata* were distinguished by the high content of soluble carbohydrates, compared to other species. Content of total proteins was high in *Ramalina farinacea, Pseudovernia furfuracea,* and *Flavoparmelia caperata*. *Hypogymnia physodes* was distinguished by the high total antioxidant activity. Influence of the substrate on the quantitative characteristics of studied parameters was revealed. The same species of lichens may reveal different strategies of antioxidant defense according to environmental conditions. Cyanobionts seem to be more resistance to environmental conditions, compared to phycobiont.

***Corresponding Author:** Badridze Gulnara ✉ gbadridze@yahoo.com

Introduction

The active metabolites in lichens may be grouped as primary and secondary ones. Some of the primary metabolites (plastid pigments, anthocyanins, amino acids, proteins, carbohydrates, vitamins), most of which possess antioxidant properties, are characteristic only for photobiont, as for autotrophic photosynthesizer; while others are formed in both symbionts (Kranner *et al.,* 2005; Balarinova *et al*., 2014).

The secondary metabolites of lichens, which except of some exclusion are of micobiont origin, deserve special interest today. These are phenolic substances, fatty acids and polysaccharides united under the name of "lichen substances" (Stocker-Worgotter and Elix 2004; Cox *et al.,* 2005; Akbulut and Yildiz, 2010). The special interest towards them is stipulated by their diverse – antimicrobial, anti-inflammatory, analgetic, anti-carcinogenic, antioxidant etc., properties (Shawuti and Abbas, 2007; Fernandez-Moriano *et al.,* 2016).

Reactive oxygen species are the main reason for damage of a living system under different stresses. Though they play the role of regulators of metabolic and defense systems as well (Foyer and Noctor, 2003). That's why permanent regulation of the level of reactive oxygen species is necessary in a living organism. This is fulfilled by the antioxidant system (Mittler, 2002).

As it was mentioned, various active metabolites of antioxidant nature are synthesized both in photo- and micobiont of lichen, which make the symbiont's united antioxidant system. The last takes an active part in protection against oxidative stress and redox-homeostasis retention, and is essential for existence of lichen as of combined organism (Kranner *et al*., 2005; Gasulla *et al*., 2012; Ranković and Kosanić, 2015). Thanks to active metabolites of both partners, the lichenized organism gains special ability of resistance against different stresses, which would be damaging for a particular partner separately (Kranner *et al*., 2005; Kranner and Birtic, 2005). Accordingly, a great number of original and review papers deal with the qualitative and quantitative characteristics and antioxidant properties of active metabolites, mainly of "lichen substances", of various species lichens from different countries; they deal with the traditional use of lichens in folk medicine and perspectives of their use in classic medicine as well (Muggia *et al*., 2009; White *et al.,* 2014; Plaza *et al.,* 2014; Kumar *et al.,* 2014; Rankovic and Kosanic, 2011; Fernandez-Moriano *et al*., 2016).

The active metabolites of lichens growing in Georgia have not been studied yet. Thus, the purpose of the presented investigation was to study the content of active metabolites in different species of lichens, as well as in same species individuals growing on different substrates. Generally the primary and secondary metabolites of proto- and micobiont, in particular: photosynthetic pigments, ascorbic acid, anthocyanins, proline, total phenols, soluble carbohydrates, total proteins and total antioxidant activity have been investigated. Obtained data may be considered as the basic knowledge about the biochemical characteristics of lichens growing in Georgia and may serve as some kind of reference point for the revealing of interesting species.

Material and methods

Widely spread in Georgia species of lichens - *Anaptychia ciliaris* (L.) A.Massal*, Flavoparmelia caperata* (L.) Hale*, Hypogymnia physodes* (L.) Nyl.*, Parmelia sulcata* Taylor, *Peltigera canina* (L.) Willd.*, Pseudevernia furfuracea* (L.) Zopf. var. furfuracea*, Ramalina farinacea* (L.)Ach., *Ramalina pollinaria* (Westr.)Ach.*, Xanthoparmelia stenophylla* (Ach.) Ahti & D. Hawksw were selected for investigations (Nimis, 2016). Fresh lichen material was used for studying the content of plastid pigments, ascorbic acid, anthocyanins, proline, total phenols, soluble carbohydrates, total proteins and total antioxidant activity; while in other series of experiments for the investigation of substrate influence on tested indices dry material was used (*Anaptychia ciliaris* and *Peltigera canina*). Obtained data are mean values of three biological replicates with standard deviations.

Lichen material was supplied and identified by the specialists of the department of lower plants of the

Institute of Botany of Ilia state University. Lichens were collected at the territory of Algeti National Park (Kvemo Kartli) and in Tianeti region (eastern Georgia) – Sabaduri forest and village Khevsurtsopeli. Voucher material of studied species is stored in the herbarium of the Institute of Botany.

Plastid pigments

Chlorophyls and carotenoids were determined spectrophotometricaly. Fresh leaves (100-200mg) were mashed with sand and $CaCO_3$ and washed with ethanol. Optical density of the filtrate was measured (spectrophotometer SPEKOL 11, KARL ZEISS, Germany). Concentration of chlorophyll a and b, also carotenoids was calculated by the formula of Wintermanns (Gavrilenko *et al.*, 1975).

Ascorbic acid

A titration method was used to measure the content of ascorbic acid. 2g of fresh leaf material was mashed in 15ml of 2% hydrochloric acid and 10ml of 2% metaphosphoric acid, and filtered. Oneml of the filtrate was added to 25ml of distilled water and titrated with a 0.001 M solution of dichlorphenolindophenole (Ermakov, 1987).

Anthocyanins

100mg of grinded leaves were added with 20ml of 96% acidified (with 1% HCl) ethanol (99:1). After 24h retention in dark the optical density at 540nm was measured (spectrophotometer SPEKOL 11, KARL ZEISS, Germany) (Ermakov, 1987).

Proline

0.5g of dry leaves were mashed in 10ml of 3% sulphosalicylic acid and filtered. 2ml of the filtrate was added to 2ml of acid ninhydrin and 2ml of ice acetic acid. After 1 h exposition on a water bath the extract was cooled and added with 4ml of toluene and divided in a separating funnel. Optical density of upper layer was measured on a spectrophotometer (SPEKOL 11, KARL ZEISS, Germany) at 520nm (Bates *et al.*, 1973).

Soluble carbohydrates

Content of soluble carbohydrates was tested with anthrone reagent (Turkina and Sokolova, 1971). To 100mg of air-dry leaf material was added 96° alcohol for extraction (3-fold extraction). The total amount of the obtained extract was evaporated on a water bath and dissolved in 5ml of distilled water. To 0.5ml of the tested water extract was added 2ml of anthrone reagent and heated in a water bath for 10min. After this procedure the test-tubes were placed in a cold water bath and 15min later the optical density of the solution was measured at 620nm with a spectrophotometer (SPECOL 11, KARL ZEISS, Germany).

Total phenols

A 0.5g of fresh leaves was boiled in 80% ethanol for 15 min. After centrifugation the supernatant was saved, and residues of leaves were mashed in 60% ethanol and boiled for 10 min. Obtained extract was added to the first supernatant and evaporated. The sediment was dissolved in distilled water. Oneml of the received solution was added with the Folin-Ciocalteu reagent and optical density was measured at 765nm. The chlorogenic acid served as control (Ferraris *et al.,* 1987).

Total protein assay

Content of proteins was determined after Lowry (1951).

Nitrates

After the water-extraction of 500g of plant material (homogenized for 30min at room temperature), it was filtered. Hydrogene peroxide was added to 10ml of the filtrate and evaporated. disulphophenolic acid was added to the obtained sediment and optical density was determined at 410nm (SPEKOL 11, KARL ZEISS, Germany) (Danilova, 1963; Pleshkov, 1985).

Total antioxidant activity

This index was measured by modified method using diphenyl-picryl-hydrazyl (DPPH) (Koleva *et al.,* 2002). 200 mg of experimental powder was extracted two times with 96 ethanol. The obtained extract was evaporated on a water bath and the remained sediment was dissolved in 10ml of water-alcohol mixture. 0.01ml of the received solution was added

with 4ml of 40µM DPPH solution and after 30 minutes of incubation in the dark the optical density was measured at 515nm by the spectrophotometer (SPEKOL 11, KARL ZEISS, Germany). The percent of inhibition was calculated.

Statistical analysis

One way ANOVA and Tukey's multiple comparison test were used to analyze differences between the means. All calculations were performed using statistical software Sigma Plot 12.5. Mean values and their standard deviations are given on figs.

Results

Plastid pigments

The highest content of chlorophylls and carotenoids was revealed in *Anaptychia ciliaris* among the species taken from tree-substrate. Results for chlorophylls were statistically identical in *Ramalina farinacea*, *Pseudovernia furfuracea* და *Hypogymnia physodes* (p=0.2) and slightly differed from *Anaptychia ciliaris*' index (p=0.003). Statistically similar results were obtained in *Parmelia sulcata* and *Ramalina pollinaria*-ში (p=0.09). The lowest content of chlorophylls was detected in *Flavoparmelia caperata* (Fig. 1).

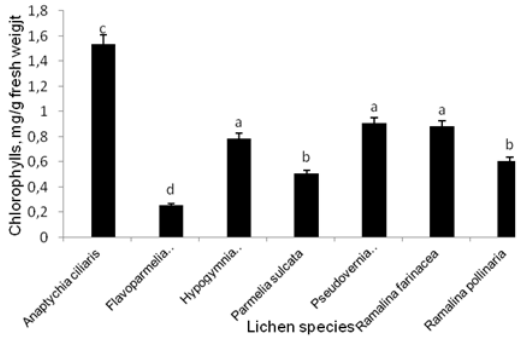

Fig. 1. Content of chlorophylls in some species of lichens growing in Georgia Statistically similar data are marked with same letters.

Content of carotenoids was identical in *Ramalina farinacea, Pseudovernia furfuracea, Hypogymnia physodes* and *Ramalina pollinaria* (p>0.05). The lowest and statistically similar results were revealed in *Parmelia sulcata* and *Flavoparmelia caperata*-ში (p>0.05) (Fig. 2).

Fig. 2. Content of carotenoids in some species of lichens growing in Georgia Statistically similar data are marked with same letters.

Especially high content of carotenoides in *Xanthoparmelia stenophylla* (11-times higher of the maximum), taken from a stone substrate must be mentioned (Fig. 2).

Ascorbic acid

Clear difference between tested species by this index was not detected. Though the results differed statistically. Maximal and minimal meanings differed 1.3-fold. Statistically similar content of ascorbate was detected in *Ramalina farinacea, Pseudovernia furfuracea, Parmelia sulcata* and *Hypogymnia physodes* (p=0.3). The lowest result was obtained in *Xanthoparmelia stenophylla* (Fig. 3).

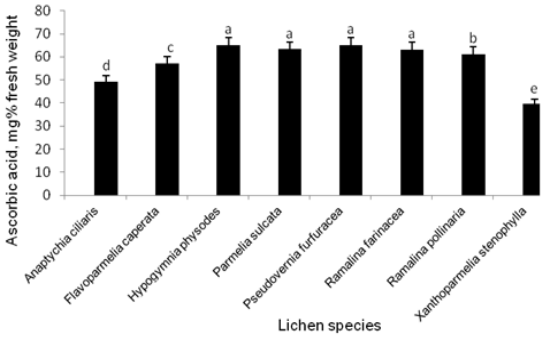

Fig. 3. Content of ascorbic acid in some species of lichens growing in Georgia Statistically similar data are marked with same letters.

Anthocyanins

The highest content of these substances was revealed in *Ramalina farinacea*. Lower and statistically similar data were obtained in *Pseudovernia furfuracea, Anaptychia ciliaris* and *Parmelia sulcata*

(p=0.1). The lowest and statistically identical were results in *Hypogymnia physodes* and *Flavoparmelia caperata*-ðо (p=0.08). Statistically similar were results in *Pseudovernia furfuracea*-ь and *Ramalina pollinaria* (p=0.2) as well (Fig. 4).

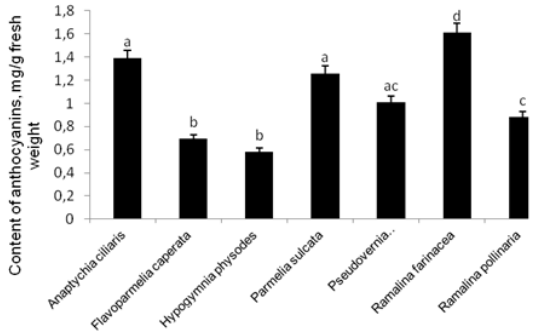

Fig. 4. Content of anthocyanins in some species of lichens growing in Georgia Statistically similar data are marked with same letters.

Proline

The highest content of proline was revealed in *Hypogymnia physodes* and *Xanthoparmelia stenophylla* (p=0.4) among the studied species; slightly low was the index in *Parmelia sulcata* (p=0.002). The lowest result were received in *Ramalina farinacea* and *Ramalina pollinaria* (p=0.001) (Fig. 5).

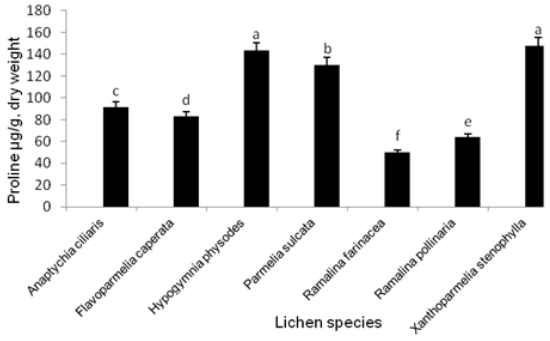

Fig. 5. Content of proline in some species of lichens growing in Georgia Statistically similar data are marked with same letters.

Soluble carbohydrates

The maximal content of soluble carbohydrates was found in *Ramalina pollinaria,* and the minimal – in *Parmelia sulcata* and *Anaptychia ciliaris* (p=0.7; 4.6-times lower of the maximal). Similar was the index in *Pseudovernia furfuracea* and *Flavoparmelia caperata* (p=0.06; 1.9-times lower of the maximal); as well as in *Ramalina farinacea* and *Hypogymnia physodes* (p=0.3; 2.6-lower of the maximal) (Fig.6).

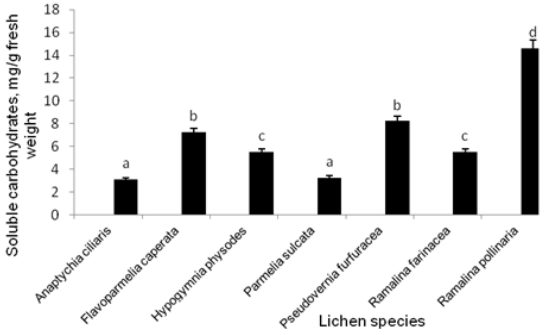

Fig. 6. Content of soluble carbohydrates in some species of lichens growing in Georgia Statistically similar data are marked with same letters.

Soluble phenols

Results with soluble phenols were the highest and statistically similar in *Parmelia sulcata* and *Ramalina pollinaria* (p=0.4); a bit lesser was the index in *Pseudovernia furfuracea* (p<0.05). Statistically similar results were revealed in *Hypogymnia physodes* and *Flavoparmelia caperata* (p=0.2). The lowest content of soluble phenols was discovered in *Ramalina farinacea, Anaptychia ciliaris* ҷь *Xanthoparmelia stenophylla* (p>0.05) (Fig. 7).

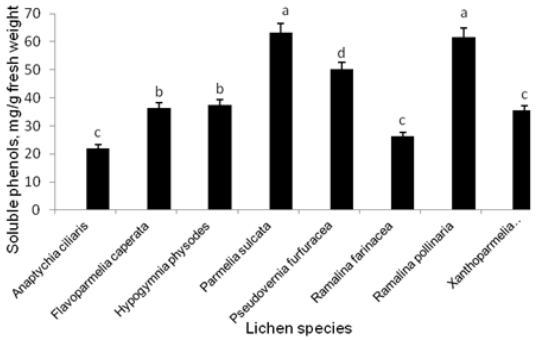

Fig. 7. Content of phenols in some species of lichens growing in Georgia Statistically similar data are marked with same letters.

Total proteins

The highest content of proteins was revealed in *Ramalina farinacea*, the lowest - in *Anaptychia ciliaris* and *Ramalina pollinaria* (p=0.01).

Statistically similar results were obtained in *Pseudovernia furfuracea* and *Flavoparmelia caperata* (p=0.6) (1.4-times lower of the maximal result), as well as in *Parmelia sulcata* and *Hypogymnia physodes* (p=0.07) (1.7-times lower of the maximal result). The minimal results were about 3-4-times lower of the maximal one (Fig.8).

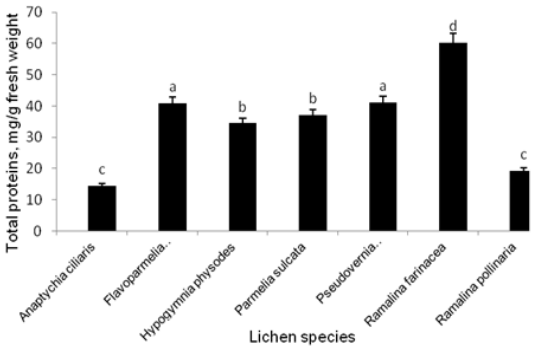

Fig. 8. Content of total proteins in some species of lichens growing in Georgia Statistically similar data are marked with same letters.

Nitrates

Content of nitrates was similar and statistically identical in all investigated species of lichens (p=0.6) (Fig. 9).

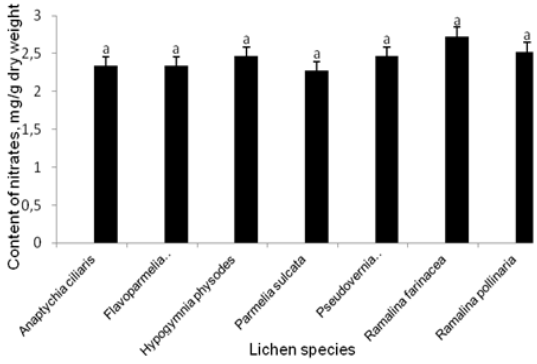

Fig. 9. Content of nitrates in some species of lichens growing in Georgia Statistically similar data are marked with same letters.

Total antioxidant activity

Hypogymnia physodes was distinguished by the highest antioxidant activity. 1.5-time lower index was discovered in *Xanthoparmelia stenophylla*, and 2-times lower – in *Parmelia sulcata*. The total antioxidant activity was about 3-times lower in other studied species (Fig. 10).

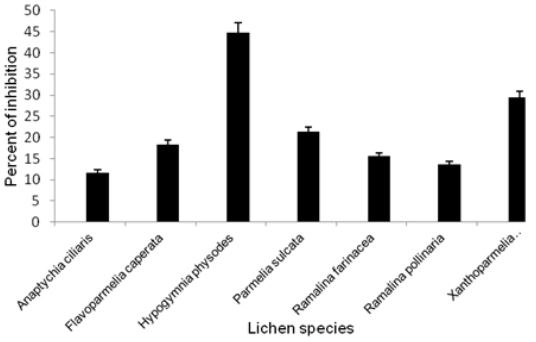

Fig. 10. Total antioxidant activity of some species of lichens growing in Georgia Statistically similar data are marked with same letters.

Observations of one and the same species of lichens living on different substrates

Inhabitation of different species of trees of the same locality by particular species of lichens indicates to the significance of substrate on the composition of lichens community (Favero-Longo and Piervittori, 2010). Tightly grown into the tree bark lichens intensively absorb substances dissolved on the substrate. Thus, they are significantly affected by the bark properties; among them are its texture, chemical composition, water-holding ability and pH, which may vary from acid to neutral on the living tree trunk (Barkman, 1958; Brodo, 1973). Moreover, the epiphytic community is affected by the changes of microclimate along the trunk and by fractions of tree (trunk, branches etc.). The micro-climate of the tree-substrate is formed by the regional climate of the locality as well as by tree architecture and bark properties (Caruso and Thor, 2007).

It is established that the substrate inhabited by lichens is not a simple support for the last. The micobiont, which tightly contacts with the bark, penetrates it by hyphae and reaches the cambial layer and phloem of the host. The fungus has special enzymes to attack the host's bark and degrade cellulose and pectin. Micobiont's hyphae may even grow along the xylem vessels and occupy intercellular space or penetrate cells (Monso´ *et al.*, 1993; Legaz *et al.*, 1988; Laufer *et al.*, 2006).

Thus, lichens establish a deep competitive relation with the substrate as the consortium of organisms

and these two significantly affect each other. The mutual influence of lichens and their tree-substrates is of allelophathic nature. The inhibition of lichen growth by the substances of host tree' bark has been established on the one hand (Koopmann *et al.*, 2007); and abating of the host plant's growth, or seed germination by lichen-released substances – on the other (Peres *et al.,* 2009; Legaz *et al.*, 2004; Sedia and Ehrenfeld, 2003). According to all above mentioned we have studied and compared content of active metabolites in two species of lichens – *Anaptychia ciliaris* and *Peltigera canina*, which were growing on different trees.

Carotenoids

Content of carotenoids was the highest in *Anaptychia* samples growing on ash and fir-trees (p=0.2), and was minimal in samples growing on elder and beech-trees (p=0.3). Results received for other tree-substrates were different as well. (Fig. 11).

In *Peltigera canina* content of carotenoids appeared to be maximal in samples picked in fir-beech forest. Results of other variants were statistically identical (p=0.1). Results of fir-tree for both species were statistically close (p=0.8). Generally by the content of carotenoids *Peltigera canina* exceeded *Anaptychia ciliaris* (Fig. 11).

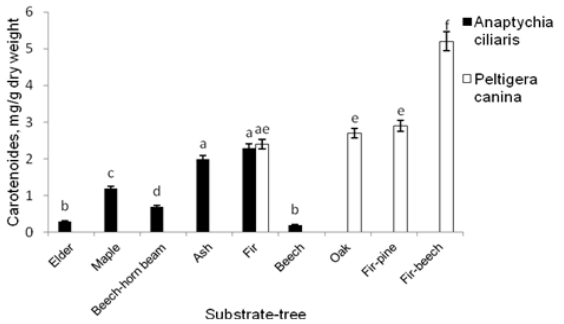

Fig. 11. Content of carotenoids in *Anaptychia ciliaris* and *Peltigera canina* growing on different tree-substrates (statistically similar data are marked with same letters).

Ascorbic acid

The maximal content of ascorbic acid was found in samples of *Anaptychia ciliaris* growing on fir and ash-trees (p=0.13). The content of ascorbate was statistically identical (p=0.06) in individuals growing on beech, beech-hornbeam forest and maple. Minimal results were obtained with variants growing on elder-tree (Fig. 12).

As for *Peltigera canina,* content of ascorbic acid was maximal in oak samples, while minimal – in fir-pine forest samples. Generally, all results obtained for samples from different tree-substrates were statistically different (Fig. 12). *Peltigera canina* exceeded *Anaptychia ciliaris* by this index (Fig. 12).

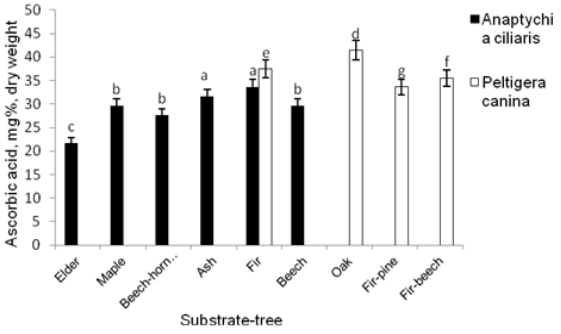

Fig. 12. Content of ascorbic acid in *Anaptychia ciliaris* and *Peltigera canina* growing on different tree-substrates (statistically similar data are marked with same letters).

Proline

The lowest content of proline was discovered in *Anaptychia ciliaris* samples from beech-hornbeam forest. Results were similar in samples taken from elder, maple and ash-tree (p=0.4). Statistically different data were obtained for other variants (Fig. 13).

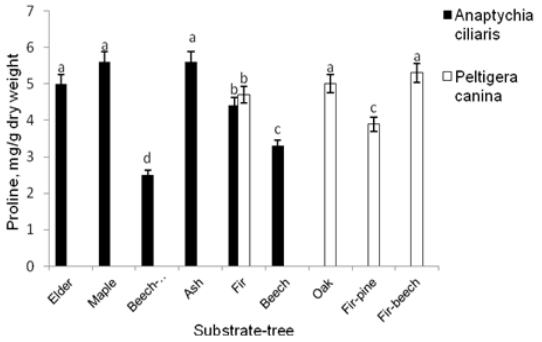

Fig. 13. content of proline in *Anaptychia ciliaris* and *Peltigera canina* growing on different tree-substrates (statistically similar data are marked with same letters).

In *Peltigera canina* the content of proline was high in samples from oak and fir-beech forest (p=0.3), minimal results were obtained in the fir-pine forest material. Statistically identical were data of the variants of both species taken from fir-tree. Generally results by this index were similar in the given species of lichens (p=0.5) (Fig. 13).

Phenols

Significantly high content of soluble phenols was discovered in *Anaptychia ciliaris* sample taken on elder-tree, compared to other samples (2-times and more high). The index was minimal in ash-tree and beech-hornbeam samples (p=0.07). Statistically similar results were revealed in lichens taken from fir and maple trees (p=0.1) (Fig. 14). In *Peltigera canina* the maximal content of phenols was found in samples picked from fir-pine forest, minimal – in individuals growing on oak-tree. Data of all tested variants were statistically different by this index. Generally, by the content of soluble phenols *Peltigera canina* clearly exceeded *Anaptychia ciliaris* (about 6.5-times) (Fig. 14).

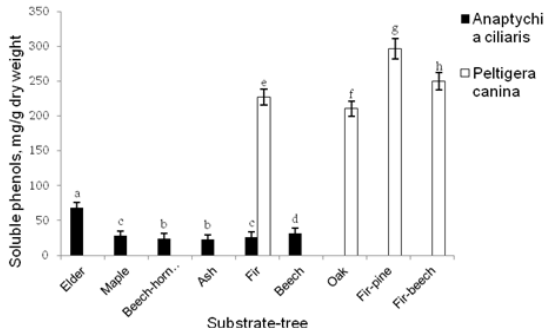

Fig. 14. content of phenols in *Anaptychia ciliaris* and *Peltigera canina* growing on different tree-substrates (statistically similar data are marked with same letters).

Soluble carbohydrates

The lowest content of soluble carbohydrates was established in *Anaptychia ciliaris* samples taken from fir-tree. The index was 2.2times higher in individuals growing on elder and beech-hornbeam forest (p=1), compared to the minimal result. Data of other variants were statistically different as well (Fig. 15).

In *Peltigera canina* content of soluble carbohydrates appeared to be essentially higher in samples of fir-pine forest (2-times and more), compared to other variants with statistically identical results (Fig. 15).

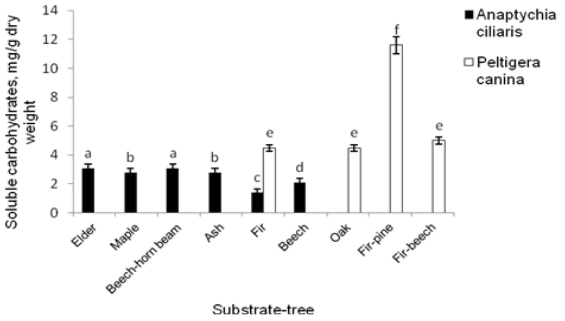

Fig. 15. content of soluble carbohydrates in *Anaptychia ciliaris* and *Peltigera canina* growing on different tree-substrates (statistically similar data are marked with same letters).

Generally by this index Peltigera canina exceeded Anaptychia ciliaris

Total antioxidant activity

The highest total antioxidant activity was established in individuals of *Anaptychia ciliaris* growing on elder-tree; Results of samples from other trees were 1.7 – 2.7 times lower compared to maximal value (Fig. 15). In *Peltigera canina* the maximal result of the total antioxidant activity was obtained in individuals taken in fir-beech forest. Other results were 1.4-1.3times lower (Fig. 15). Almost by all studied indices, among which most were antioxidants, *Peltigera canina* prevailed *Anaptychia ciliaris*. Thus, it is logical that by the total antioxidant activity *Peltigera canina* exceeded *Anaptychia ciliaris*.

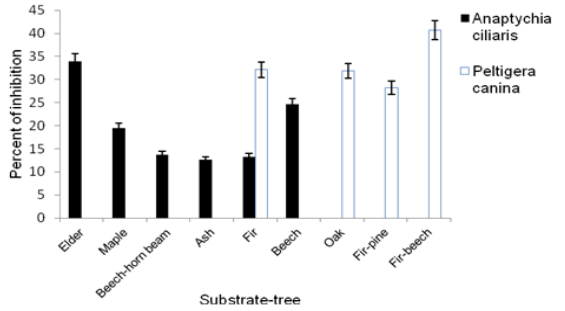

Fig. 16. Total antioxidant activity of *Anaptychia ciliaris* and *Peltigera canina* growing on different tree-substrates (statistically similar data are marked with same letters).

Discussion

Chlorophylls, carotenoids, ascorbic acid

Lichen's photobiont (algae/cyanobacteria) is able to survive under various extreme conditions, retaining photosynthesis and growth and development, which would be impossible under free-living conditions. The thallus of micobiont is a reliable shelter, which protects the photobiont from excess radiation by means of different substances which screen radiation. This fact was confirmed in experiments with separate cultivation of lichenized fungus and its photobiont partner. Content of chlorophylls clearly decreased in the separately cultivated photobiont (Kranner *et al.,* 2005).

Lichen material was fresh-picked in our experiments, and was saved under equal illumination and temperature conditions. Separation of the tested material from its substrate presumably caused partial drying of the material. As it has been established, short-term drying does not affect content of chlorophylls and carotenoids in intact lichens (Kranner *et al.,* 2005); thus, it may be supposed that the indices of photosynthetic pigments of slightly dried material are identical to those under natural conditions. Though, partial dehydration presumably caused increase of active oxygen species in photobiont, the negative effect of which would be neutralized by antioxidant activity of ascorbic acid (Kranner *et al.,* 2002). It is established that ascorbate plays an important role under oxidative stress, caused by different stressors (Calatayud *et al.*, 1997).

The highest chlorophyll content found in *Anaptychia ciliaris* (Fig. 1) among tested lichens, which grow on tree-substrate, may be responsible for the formation of high level of active oxygen species during the photochemical reactions (Gasulla *et al.*, 2012). This may explain the lowest content of ascorbate in this species of lichen, compared to other tree-living ones: more amount of ascorbic acid would be necessary for neutralizing the active oxygen species in this lichen (Fig. 3).

Carotenoids are considered as significant antioxidants as well, which protect chlorophyll molecules from singlet oxygen and excess energy (Mourato *et al.*, 2012). Accordingly, their content will be higher in species with higher chlorophyll content (Fig. 2).

Especially must be mentioned lichen species *Xanthoparmelia stenophylla,* which inhabits stone-substrate. It contained essentially high level of carotenoids (about 11-times higher), and minimal level of ascorbic acid (Fig. 2, 3), compared to other tested lichens. The high content of carotenoids in this lichen may be due to accumulation of carotenoids in micobiont's thallus as well, giving it a yellowish shade. Generally representatives of *Xanthoparmelia* genus are spread in arid and semi-arid regions, inhabiting soil or stones (Rizzi and Giordani, 2012). Stone substrate means more severe stress-conditions, compared to tree from illumination, temperature or other points of view. It may be supposed that one of the stress-resistance mechanisms in *Xanthoparmelia stenophylla* is high content of carotenoids. Experimental results have demonstrated the difference in plastid pigments content among tested species (Fig. 1, 2), that presumably is a specific feature, or may be associated with the age, microclimatic peculiarities, etc. of test-objects (Balarinova *et al.,* 2014).

In spite of the difference in plastid pigments content, the pool of ascorbic acid in tested tree-living lichens slightly differed, that presumably indicates to identical protection of the photosynthetic apparatus from the radicals, which are formed during photochemical reactions (Fig. 3).

Anthocyanins and proline

Anthocyanins are the group of flavonoids, which accumulate in vacuoles and possess strong antioxidant properties (Kahkonen and Heinonen, 2003). Accumulation in vacuoles prevents anthocyanins from the contact with sites of active oxygen species formation. Since, increase of anthocyanins in case of different stresses has been established (Mobin and Khan, 2007).

The specific difference in anthocyanins content has been revealed in tested lichens (Fig. 4). In most experimental species content of anthocyanins was high, presumably playing significant role in adaptability to unfavorable conditions.

Literary data deal with the stress-protective role of proline in plants. Proline, as an osmolyte regulates the cell water retention (Saradhi *et al.*, 1995). Moreover it acts as proteins stabilizer (Anjum *et al.*, 2000), and neutralizes active oxygen species (Matysik *et al.,* 2002). Increase of proline synthesis in chloroplasts supports stabilization of the red-ox balance and homeostasis of cell (Szabados and Savoure, 2010). Though, some authors do not agree with the osmo-regulating role of proline in lichens. According to their data content of proline in water-saturated and fully desiccated lichens was same (Weigel and Jager, 1979).

High content of proline was revealed in experimental lichens on the background of specific differences (Fig. 5). Taking into account the osmolytic properties of this amino acid it may be supposed that lichen species with high proline content: *Hypogymnia physodes Xanthoparmelia stenophylla* and *Parmelia sulcata* would be highly resistant to osmotic stress, compared to other species.

Generally it may be concluded that high content of proline in studied lichens plays protective role against various stresses.

Soluble carbohydrates

Soluble carbohydrates are significant assimilates produced by lichen's photobiont and actively used by micobiont during metabolism (Elshobary, 2016). It was established that the nature of micobiont's secondary metabolites highly depends on the type and amount of soluble carbohydrates (Brunauer *et al.*, 2007).

The specific differences in the content of soluble carbohydrates were revealed in tested lichen; though no regular relation between their content and micobiont's substances has been discovered (Fig. 6).

Soluble phenols

Phenols are considered as the most active secondary metabolites in plants. They neutralize the active oxygen species before the last damage cells (Lovdal *et al.,* 2010). It is considered that phenols in lichens are only of micobiont origin (Stocker-Worgotter and Elix, 2002); though it is evident that photobionts take some part in their synthesis as well. It was demonstrated that cultivation of micobiont without its photobiont-partner caused decrease, or even ceasing of phenols synthesis in micobiont (Molina *et al.*, 2003).

Lichen phenolic substances have different chemical nature than those of vascular plants. They are situated in the form of crystals near the surface of hypae cells. The phenols of micobiont are considered to possess those various activities the "lichens substances" reveal (Shawuti and Abbas, 2007). Evidently the role of phenols is associated with the vital importance of lichen as of united living unit. Phenols protect photobiont from the excess sun radiation, especially ultraviolet (UV) one. They are effective absorbers of UV. The protect lichens from herbivores as well. Generally phenols synthesis in lichens highly dependent on environmental conditions: illumination, temperature, altitude above sea level, seasonality (Watson, 2014).

Different content of phenols was revealed in studied lichens. Presumably specific peculiarities play the role, together with environmental conditions (Fig. 7). *Xanthoparmelia stenophylla* is one of the tested species which had the lowest content of phenols. It may be supposed that phenols are not leading antioxidants in stress-protection of this species.

Total proteins and nitrates

Lichens uptake inorganic nitrogen directly from air in the form of ammonium, nitrates, or air composing nitrogen (in case of cyanolichens) (Hyvarinen and Crittenden, 1998; Ellis *et al.*, 2004). Nitrogen translocation to the photobiont causes its chlorophyll concentration increase and consequently photosynthesis stimulation (Palmqvist *et al.*, 1998; Palmqvist and Dahlman, 2006). Thus, there exists the positive feed-back relation between nitrogen uptake and photosynthesis in lichens in the limits of species nitrogen tolerance. If the concentration of nitrates exceeds this limit, inhibition of the process occurs (Hauck, 2010).

The low and statistically similar concentration of nitrates in tested lichens excludes its influence on studied indices (Fig. 9).

Lichen proteins are considered as potential biologically active substances, with distinguished biochemical properties. Among them were discovered medicinal, UV-protective and untifreezing proteins. These proteins may belong to both - micobiont and photobiont as well (Oksanen, 2006; Anshakova *et al.,* 2011)

By the content of total proteins the specific diversity was revealed among the investigated lichens (Fig. 8).

Total antioxidant activity

Some authors prove the relation between the total antioxidant activity and content of phenols in lichens (Kumar *et al.,* 2014; Rankovic *et al.,* 2011); while others do not agree with it (Stojanovic, 2010; Odabasoglu *et al.,* 2004).

Our observations have not revealed any relation between these two indices as well. In most tested species total antioxidant activity was similar (Fig. 10).

According to experimental data differences by studied indices are clear between the same species of lichens growing on different tree-substrates. (Fig. 11-16).

No evident relation between substrate acidity and studied indices was detected while taking into account the acidity of the bark of those trees where the experimental samples of lichens were taken (Spier *et al.*, 2010). Though the acidity of barks of oak, fir and beech-trees is different, a number of studied indices of *Peltigera canina* growing on these trees were similar.

Determination of the substrate-depended reasons of difference between the studied indices of one and the same species of lichens is difficult. Presumably it is conditioned by several factors (Ihlen *et al.,* 2011); among them is the identity of lichen's photobiont as well. Among the two compared species the photobiont of *Anaptychia ciliaris* is a green algae, while that of *Peltigera canina* is a cyanobacterium. Presumably this was the reason for quantitative differences between the studied active metabolites. Most of the studied indices were higher in *Peltigera canina* that may be indicate to high adaptability of the last to unfavorable conditions.

One is clear: content of studied active metabolites, among which most were antioxidants, varied in same species of lichens according to the species of tree-substrate; e.g. individuals of *Anaptychia ciliaris* growing on fir and ash-trees were distinguished by the high content of carotenoids and ascorbate, compared to individuals of the same species taken from elder-tree, (Fig. 11, 12); while samples of the last prevailed by the content of proline, phenols and soluble carbohydrates (Fig. 13-15). In *Peltigera canina* content of carotenoids, ascorbate and proline appeared to be high in samples taken from oak-tree and fir-beech forest (Fig. 11-13); while in samples picked in fir-pine forest phenols and soluble carbohydrates were higher (Fig. 14, 15).

This indicates that lichen may reveal different antioxidant-defense strategies, following the environmental conditions.

Some authors speak about the key role of tree-substrate' species in lichens colonization; they pay less attention to the bark acidity. These authors mention the adaptation of lichens to changing environment as well, e.g. to increasing pollution of the environment, which is followed by changes of the substrate acidity as well (Spier *et al.*, 2010). We support the ideas of these authors. According to obtained data it may be supposed that lichens posses very flexible mechanisms of adaptation; moreover, cyanobiont seems to be more resistant, compared to phycobiont.

Conclusions

Differences by the studied features have been revealed between the investigated species of lichens according to experimental results. Especially high content of carotenoids was discovered in *Xanthoparmelia stenophylla*.

Anaptychia ciliaris, Pseudovernia furfuracea and *Ramalina farinacea* were distinguished by the high content of chlorophylls, carotenoids and anthocyanins among the tested tree-inhabiting species.

High content of proline was found in species: *Xanthoparmelia stenophylla, Hypogymnia physodes,* and *Parmelia sulcata*.

Especially high content of phenols was determined in *Peltigera canina*. *Parmelia sulcata, Ramalina pollinaria,* and *Pseudovernia furfuracea* were rich in phenols as well.

Ramalina pollinaria, Pseudovernia furfuracea, and *Flavoparmelia caperata* were distinguished by the high content of soluble carbohydrates.

Content of total proteins was high in *Ramalina farinacea, Pseudovernia furfuracea,* and *Flavoparmelia caperata*.

Hypogymnia physodes was distinguished by the high total antioxidant activity.

Influence of the substrate on the quantitative characteristics of studied parameters was revealed. The same species of lichens may reveal different strategies of antioxidant defense according to environmental conditions.

Cyanobionts seem more resistance to environmental conditions, compared to phycobiont.

References

Akbulut G, Yildiz A. 2010. An Overview to Lichens: The Nutrient Composition of Some Species. Kafkas Üniv Fen Bil Enst Derg **3(2),** 79-86.

Anjum F, Rishi V, Ahmed F. 2000. Compatibility of osmolytes with Gibbs energy of stabilization of proteins. Biochim. Biophys. Acta **1476(1),** 75-84.

Anshakova VV, Karataeva EV, Kershengoltc BM. 2011. Bakery products quality improvement by means of the mechanically activated Bioadditives from lichens. Fund Res **8,** 593-6.

Balarinova K, Bartak M, Hazdrova J, Hajek J, Jilkova J. 2014. Changes in photosynthesis, pigment composition and glutathione contents in two Antarctic lichens during a light stress and recovery. Photosynthetica **52(4),** 538-547.

Barkman JJ. 1958. Phytosociology and ecology of cryptogamic epiphytes. Assen, The Netherlands: van Gorcum & Comp. N.V.

Bates LS, Waldren RP, Treare ID. 1973. Rapid determination of free proline for water-stress studies. Plant Soil **39,** 205-207.

Brodo IM. 1973. The lichens. Chapter 12, Substrate ecology. New York:Academic Press 401-441.

Brunauer G, Hager A, Grube M, Turk R, Stocker-Wörgötter E. 2007. Alterations in secondary metabolism of aposymbiotically grown mycobionts of *Xanthoria elegans* and cultured resynthesis stages. Plant Physiol Biochem **45,** 146-151, DOI:10.1016/j.plaphy.2007.01.004.

Calatayud A, Deltoro VI, Barreno E, del Valle Tascon S. 1997. Changes in *in vivo* chlorophyll fluorescence quenching in lichen thalli as a function of water content and suggestion of zeaxanthin associated photoprotection. Physiologia Plantarum **101(1),** 93-102.

Caruso A, Thor G. 2007. Importance of different tree fractions for epiphytic lichen diversity on Picea abies and Populus tremula in mature managed boreonemoral Swedish forests. Scand J Forest Res **22,** 219-230.

Cox PA, Banack SA, Murch SJ, Rasmussen U, Tien G, Bidigare RR, Metcalf JS, Morrison LF, Codd GA, Bergman B. 2005. Diverse taxa of cyanobacteria produce b-N-methylaminoL-alanine, a neurotoxic amino acid. Proceedings of the National Academy of Sciences, USA **102,** 5074-5078.

Danilova NS. 1963. Determination of nitrates in plant material. Fiziologia rasteniy **10(4),** 497-498.(Russian)

Ellis CJ. Crittenden, PD, Scrimgeour ChM. 2004. Soil as a potential source of nitrogen for matforming lichens. Can J Bot. **82,** 145-149.

Elshobary ME. 2016. Algal carbohydrates affect polyketide synthesis of the lichen-forming fungus *Cladonia rangiferina*. Mycologia **108(4),** 646-656.

Ermakov AI, Arasimovich VV, Iarosh VV, Peruanskiy IP, Lugovnikova GA, Ikonnikova MI. 1987. Methods of Plant Biochemical Investigation. Leningrad: Agropromizdat 85-120. (in Russian).

Favero-Longo SE, Piervittori R. 2010. Lichen-plant interactions. Journal of Plant Interactions **5(3),** 163-177.

Fernandez-Moriano C, Gomez-Serranillos MP, Crespo A. 2016. Antioxidant potential of lichen species and their secondary metabolites. A systematic review. Pharm Biol **54(1),** 1-17.
DOI: 10.3109 /13880209.2014.1003354.

Ferraris L, Abbatista-Gentile I, Matta A. 1987. Variations of phenolics concentrations as a consequence of stress that induce resistance to Fusarium wilt of tomato. J Plant Dis Protect **94,** 624-629.

Foyer C, Noctor G. 2003. Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiologia Plantarum **119(3),** 355-364.

Gasulla F, Herrero J, Esteban-Carrasco A, Ros-Barcelo A, Barreno E, Zapata JM, Guera A. 2012. Edvances in Photosynthesis - Fundamental Aspects. In: Najafpour MM, Ed. Photosynthesis in Lichen: Light Reactions and Protective Mechanisms 149-174.

Gavrilenko VF, Ladigina ME, Khandobina LM. 1975. Big practical handbook in plant physiology. Moscow, Visshaia shkola 127-134. (Russian)

Hauck M. 2010. Ammonium and nitrate tolerance in lichens. Environmental Pollution **158(5),** 1127-1133.

Hyvarinen M, Crittenden PD. 1998. Relationships between atmospheric nitrogen inputs and the vertical nitrogen and phosphorus concentration gradients in the lichen Cladonia portentosa. New Phytologist **140(3),** 519-530.

Ihlen PG, Gjerde I, Saetersdal M. 2011. Structural indicators of richness and rarity of epiphytic lichens on *Corylus avellana* in two different forest types within a nature reserve in south-western Norway. Lichenologist **33,** 215-229.

Kahkonen MP, Heinonen M. 2003. Antioxidant activity of anthocyanins and their aglycons. Journal of Agricultural and Food Chemistry **51(3),** 628-633.

Koleva II, van Beek TA, Linssen JP, de Groot A, Evstatieva LN. 2002. Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. Phytochem Anal **13,** 8-17.

Koopmann R, Stevens H, Franzen-Reuter I, Frahm JP, Grote M. 2007. In vitro inhibition of soredial growth in the epiphytic lichen Physcia tenella (Ascomycetes: Lecanorales) by a variety of bark phenols. Lichenologist **39(6),** 567-572.

Kosanic M, Rankovic B. 2011. Lichens as possible sources of antioxidants. Pak. J. Pharm. Sci **24(2),** 165-170.

Kranner I, Beckett RP, Wornik S, Zorn M, Pfeifhofer HW. 2002. Revival of a resurrection plant correlates with its antioxidant status. Plant J. **31(1),** 13-24.

Kranner I, Birtic S. 2005. A modulating role for antioxidants in desiccation tolerance. Integr Comp Biol **45,** 734-740.

Kranner I, Cram WJ, Zorn M, Wornik S, Yoshimura I, Stabentheiner E, Pfeifhofer HW. 2005. Antioxidants and photoprotection in a lichen as compared with its isolated symbiotic partners. Proc. Natl Acad Sci U S A **102(8),** 3141-6.

Kumar J, Dhar P, Tayade AB, Gupta D, Chaurasia OP, Arora R, Srivastava RB, Upreti DK. 2014. Antioxidant Capacities, Phenolic Profile and Cytotoxic Effects of Saxicolous Lichens from Trans-Himalayan Cold Desert of Ladakh. PLoS ONE **9,** 6:e98696. DOI:10.1371/journal.pone.0098696.

Laufer Z, Beckett RP, Minibayeva FV, Luthje S, Bottger M. 2006. Occurrence of laccases in lichenized ascomycetes of the Peltigerinae. Mycol Res **110,** 846-853.

Legaz E, Perez-Urria E, Avalos A, Vicente C. 1988. Epiphytic lichens inhibit the appearance of leaves in Quercus pyrenaica. Biochem System Ecol **16(3),** 253-259.

Legaz ME, Monsor MA, Vicente C. 2004. Harmful effects of epiphytic lichens on trees. Recent Res Devel Agron Hortic **1,** 1-10.

Lovdal T, Olsen KM, Slimestad R, Verheul M, Lillo C. 2010. Synergetic effects of nitrogen depletion, temperature, and light on the content of phenolic compounds and gene expression in leaves of tomato. Phytochemistry **71,** 605-613.

Lowry OH, Rosebrough NT, Farr AL, Randall RJ. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem **139,** 256-275.

Matysik J, Alia A, Bhalu B, Mohanty P. 2002. Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. Current science **82(5),** 525-532.

Mittler R. 2002. Oxidative stress, antioxidants, and stress tolerance. Ternds Plant Sci **7,** 405-410.

Mobin M, Khan NA. 2007. Photosynthetic activity, pigment composition and antioxidative response of two mustard (Brassica juncea) cultivars differing in photosynthetic capacity subjected to cadmium stress. Journal of Plant Physiology **164(5),** 601-610.

Molina MC, Crespo A, Vicente C, Elix JA. 2003. Differences in the composition of phenolics and fatty acids of cultured mycobionts and thallus of Physconia distorta. Plant Physiol. Biochem **41,** 175-180.

Monsor MA, Legaz ME, Vicente C. 1993. A biochemical approach to the hemiparasitic action of the epiphytic lichen Evernia prunastri on Betula pendula. Ann Bot Fenn **30(4),** 299-303.

Mourato M, Reis R, Martins LL. 2012. Characterization of Plant Antioxidative System in Response to Abiotic Stresses: A Focus on Heavy Metal Toxicity. In: Montanaro G and Dichio B Eds Advances in Selected Plant Physiology Aspects. Croatia, Intech. 388p.

Muggia L, Schmitt I, Grube M. 2009. Lichens as treasure chests of natural products. Sim News 85-97. www.simhq.org.

Nimis PL. 2016. The Lichens of Italy. A Second Annotated Catalogue. EUT, Trieste 739 pp.

Odabasoglu F, Aslan A, Cakir A, *et al.* 2004. Comparison of antioxidant activity and phenolic content of three lichen species. Phytother Res **18,** 938-41.

Oksanen I. 2006. Ecological and biotechnological aspects of lichens. Appl Microbiol Biotechnol **73,** 723-34.

Palmqvist K, Dahlman L. 2006. Responses of the green algal foliose lichen *Platismatia glauca* to increased nitrogen supply. New Phytologist **171(2),** 343-56.

Peres MTLP, Mapeli AM, Faccenda O, Gomes AT, Honda NK. 2009. Allelopathic potential of orsellinic acid derivates. Braz Arch Biol Technol **52(4),** 1019-1026.

Plaza CM, Diaz de Torres LE, Lucking RK, Vizcaya M, Medina GE. 2014. Antioxidant activity, total phenols and flavonoids of lichens from Venezuelan Andes. Journal of Pharmacy & Pharmacognosy Research **2(5),** 138-147.

Pleshkov BP. 1985. Practical Handbook of Plant Biochemistry. Moskva: Agropromizdat, 3rd edn 207-208. (in Russian).

Ranković B, Kosanić M. 2015. Lichens as a Potential Source of Bioactive Secondary Metabolites. In: Ranković B. Ed. Lichen Secondary Metabolites. Springer, Cham. DOI 10.1007/978-3-319-13374-4-1

Rankovic BR, Kosanic MM, Stanojkovic TP. 2011. Antioxidant, antimicrobial and anticancer activity of the lichens Cladonia furcata, Lecanora atra and Lecanora muralis. BMC Complementary and Alternative Medicine **11,** 97. http://www.biome dcentral.com/1472-6882/11/97.

Rizzi G, Giordani P. 2012. The ecology of the lichen genus Xanthoparmelia in Italy: An investigation throughout spatial scales. Plant Biosystems **1,** 1-7.

Saradhi PP, Alia Arora S, Prasad KV. 1995. Proline accumulates in plants exposed to UV radiation and protects them against UV induced peroxidation. Biochem. Biophys. Res. Commun **209,** 1-5.

Sedia EG, Ehrenfeld JG. 2003. Lichens and mosses promote alternate stable plant communities in the New Jersey Pineland. Oikos **100,** 447_458.

Shawuti G, Abbas A. 2007. Research progress on biological activities of lichens secondary metabolites. Food Sci J **28,** 624-627.

Spier L, van Dobben H, van Dort K. 2010. Is bark pH more important than tree species in determining the composition of nitrophytic or acidophytic lichen floras? Environ Pollut **158(12),** 3607-11.

Stocker-Worgotter E, Elix JA. 2002. Secondary chemistry of cultured micobiont formation of a complete chemosyndrome by the lichen fungus of Lobaria spathulata. Lichenologist **34(4),** 351-359.

Stocker-Worgotter E, Elix JA. 2004. Experimental studies of lichenized fungi: formation of rare depsides and dibenzofuranes by the culture mycobiont of Bunodophoron patagonicum (Sphaerophoraceae, lichenized Ascomycota). Bibliotheca Lichenologica **88,** 659-669.

Stojanovic G, Stojanovic I, Stankov-JovanovicV, *et al.* 2010. Reducing power and radical scavenging activity of four Parmeliaceae species. Cent Eur J Biol **5,** 808-13.

Szabados L, Savoure A. 2010. Proline: a multifunctional amino acid. Trends Plant Sci **15,** 2, 89-97.

Turkina MV, Sokolova SV. 1971. Methods of determination of mono- and oligosaccharides. In: Pavlinova O.A. ed. Biological methods in plant physiology. Moscow, Nauka, 20-26. (Russian)

Watson R. 2014. Polyphenols in Plants: Isolation, Purification and Extract Preparation. 1st Edition. Academic Press 360.

Weigel HJ, Jager HJ. 1979. Changes in Proline Concentration of the Lichen *Psendevernia furfuracea* during Drought Stress. Phyton (Austria), **19**Fasc. 3-4163-16710. 9.

White PAS, Oliveira RCM, Oliveira AP, Serafini MR, Araujo AAS, Gelain DP, Moreira JCF, Almeida JRGS, Quintans JSS, Quintans-Junior LJ, Santos MRV. 2014. Antioxidant Activity and Mechanisms of Action of Natural Compounds Isolated from Lichens: A Systematic Review. Molecules **19,** 14496-14527.