Comparative analysis of the antioxidant properties of Icelandic and Hawaiian lichens

Kehau Hagiwara[1, *], Patrick R. Wright[2, *], Nicole K. Tabandera[1], Dovi Kelman[1], Rolf Backofen[2, 3], Sesselja Ómarsdóttir[4] and Anthony D. Wright[1, #]

1: Department of Pharmaceutical Sciences, Daniel K. Inouye College of Pharmacy, University of Hawaii at Hilo, 34 Rainbow Drive, Hilo, HI 96720, USA

2: Bioinformatics Group, Department of Computer Science, Albert-Ludwigs-University Freiburg, Georges-Köhler-Allee 106, D-79110 Freiburg, Germany

3: BIOSS Centre for Biological Signaling Studies, University of Freiburg, D-79104 Freiburg, Germany

4: Faculty of Pharmaceutical Sciences, School of Health Sciences, University of Iceland, Hagi, Hofsvallagata 53, IS-107 Reykjavik, Iceland

*: K.H. and P.R.W. contributed equally to this work.

#: To whom correspondence should be addressed; E-Mail: adwright@hawaii.edu; Tel.: +1-808-981-4521; Fax: +1-808-933-2981.

Running title: DPPH and FRAP of Icelandic and Hawaiian lichens

Abstract

Antioxidant activity of symbiotic organisms known as lichens is an intriguing field of research because of its strong contribution to their ability to withstand extremes of physical and biological stress (e.g., desiccation, temperature, UV radiation, and microbial infection). We present a comparative study on the antioxidant activities of 76 Icelandic and 41 Hawaiian lichen samples assessed employing the DPPH and FRAP based antioxidant assays. Utilizing this unprecedented sample size, we show that while highest individual sample activity is

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/1462-2920.12850

present in the Icelandic dataset, the overall antioxidant activity is higher for lichens found in Hawaii. Furthermore, we report that lichens from the genus *Peltigera* that have been described as strong antioxidant producers in studies on Chinese, Russian and Turkish lichens, also show high antioxidant activities in both Icelandic and Hawaiian lichen samples. Finally, we show that opportunistic sampling of lichens in both Iceland and Hawaii will yield high numbers of lichen species that exclusively include green algae as photobiont.

Introduction

Symbioses can be described as biological unions in which two or more organisms coexist and can refer to a variety of interactions, from obligate to facultative and from mutualistic to parasitic. Mutualistic symbiosis is the association of organisms where all members of the relationship benefit. This often occurs in habitats where the individual partners would not be able to survive alone (Kranner *et al*., 2005). Examples of such symbioses include the association of *Rhizobia* with the roots of legumes (Crespi and Gálvez, 2000) and squids that work together with luminous *Vibrio* species (Nyholm and McFall-Ngai, 2004).

Lichens are an association of algae and fungi that was established at least 600 million years ago (Yuan *et al*., 2005) and therefore represent a classic example of a symbiotic relationship. The predominant fungal partner (mycobiont) can be constituted of an ascomycete or a basidiomycete and the algal partner (photobiont) can be exclusively green algae (A), exclusively cyanobacteria (BG), or a mixture of both (ABG) (Ahmadjian, 1993). As a result of this partnership, lichens exhibit a wide diversity of morphologies that are largely influenced

by the mycobiont (Honegger, 1993). In this respect, they are generally classified as foliose (fo), fruticose (fr), crustose (cr) or squamulose (sq) species.

These organisms produce a variety of natural products that assist in maintaining their viability in conditions that may be hazardous or undesirable to many other organisms. This adaption also allows lichens to secure their place in extreme ecological niches (Boustie *et al*., 2011; Kampa *et al*., 2013). These chemical defenses increase their resistance to biological stressors such as herbivores (Kaasalainen *et al*., 2009; Kaasalainen *et al*., 2012), microbes (Burkholder *et al*., 1944) and also against physical stress like extreme temperatures, intense UV radiation, and prolonged drought-desiccation periods (Kranner *et al*., 2008).

An important group of natural products synthesized by lichens and many other organisms are antioxidants (Sato *et al*., 1989; Pietta *et al*., 1998; Andarwulan *et al*., 2010; Chairman *et al*., 2012; Kelman *et al*., 2012). These substances play a central role in lichen viability (Kranner *et al*., 2005; Kranner *et al*., 2008) as rapidly changing conditions, that are frequently present in lichen habitats (Kranner *et al*., 2008), can give rise to reactive oxygen species (ROS) that may play an integral role in severely damaging or altering molecules within living cells (Halliwell, 1987; Kehrer, 1993; França *et al*., 2007) if not appropriately antagonized.

In this study, we surveyed the antioxidant activities of 76 Icelandic and 41 Hawaiian lichens by employing the ferric-reducing antioxidant power (FRAP) (Benzie and Strain, 1996) and free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Brand-Williams *et al*., 1995) methods. These two assays were selected because they; are sensitive, have clearly defined end points, provide reproducible results, are relatively straight forward to perform, are cost effective

and yield information about two distinct modes of oxidation, electron transfer (FRAP) and radical scavenging (DPPH). Both Iceland (arctic) and Hawaii (tropical) are active, isolated volcanic island communities that are populated by a wide diversity of lichen species (Magnusson and Zahlbruckner, 1944; Ingólfsdóttir *et al*., 2000; González *et al*., 2005), and so represent ideal sampling areas for an extensive comparative study of antioxidant activities. Previous work has been conducted on antioxidant activity in diverse lichens from various countries (Ingólfsdóttir *et al*., 2000; Gülçin *et al*., 2002; Behera *et al*., 2003; Odabasoglu *et al*., 2005; Gulluce *et al*., 2006; Bhattarai *et al*., 2008; Paudel *et al*., 2008; Luo *et al*., 2010; Ranković *et al*., 2011; Paudel *et al*., 2014). However, there are no studies with a sample size comparable to that employed here using two distinct antioxidant assays and comparing two locations that are climatically and geographically very different.

Generally, all Icelandic samples were collected at altitudes ranging from 0-500 m. In winter all parts of Iceland experience prolonged periods of temperatures in the range 0 to -10°C, while in summer temperatures vary from 10 to 25°C. In winter there is snow of varying depths and prolonged periods of dark. In contrast, summer has prolonged periods of light and there is rain instead of snow. Hawaii, in this case meaning the Island of Hawaii, is quite different climatically and geographically from Iceland. All samples, except those from Maui, Saddle Road, and Mauna Kea (ca. 1500 m) were collected at altitudes similar to those from Iceland. On Hawaii daylight hours range from 13.5 in summer to 10.8 in winter, with temperatures ranging from 27°C in summer to 19°C in winter. At elevations above 1000 m temperatures drop several degrees per 1000 m. Compared to the Icelandic samples none of the Hawaiian samples were exposed to temperatures approaching freezing point, but they

were all exposed to greater levels of UV radiation and for prolonged periods. Samples from Saddle Road and Mauna Kea were all exposed to extended periods of desiccation.

Results

Classification

The lichen collection from Iceland yielded a total of 76 lichen samples. Of these, 56 were classified as lichens with exclusively green algae photobionts, 11 were classified as lichens with a mixture of algal and cyanobacterial photobionts and nine were classified as lichens with exclusively cyanobacteria as photobionts. The morphological classification of the Icelandic lichens revealed there to be 37 foliose, 37 fruticose and two crustose specimens (Supplementary Table S1). The Hawaiian dataset consisted of 41 lichen samples. Of these, 28 corresponded to algal photobionts, five contained a mixture of algal and cyanobacterial photobionts and eight contained only cyanobacterial photobionts in the symbiosis. Within the Hawaiian dataset, the morphological categorization revealed 18 foliose, 17 fruticose, three crustose, and three squamulose samples (Supplementary Table S2).

*** Figures 1 and 2 could go here ***

Comparison of the antioxidant activity of lichens from Iceland and Hawaii

The highest antioxidant activities from both the FRAP and DPPH assays are from lichen samples collected in Iceland (Figures 1 and 2). However, the mean activity for the FRAP assay is similar for both locations (6.2 µM/µg for Hawaii and 7.0 µM/µg for Iceland). In

contrast, the mean activity in the DPPH assay is markedly higher for the Hawaiian samples (64.7% radical scavenging for Hawaii and 47.5% radical scavenging for Iceland). Statistical comparisons of the Icelandic and Hawaiian data revealed significant differences in the datasets, indicating that Hawaiian lichens generally show higher antioxidant activity even though the samples with the highest overall activities were collected in Iceland ($p = 2.55 \times 10^{-6}$ for FRAP and $p = 49.4 \times 10^{-6}$ for DPPH). The most active Icelandic samples in the FRAP assay are specimens from the genus *Peltigera*, while the most active samples from the sampling in Hawaii are *Parmeliella mariana* and *Sticta weigelii* (Figures 1 and 3). In the DPPH assay highest activities were also exhibited by the lichens from the genus *Peltigera* from Iceland and *S. weigelii* from Hawaii (Figures 2 and 4). All these species have leafy morphology and contain cyanobacteria as photobionts.

*** Figure 3 and 4 could go here ***

Investigation of Peltigera and Peltigera-like specimens in Hawaii

Due to the strong activity observed for the Icelandic *Peltigera* specimens, we attempted to sample *Peltigera* or *Peltigera*-like species in Hawaii. Two fresh specimens collected for this purpose yielded the species *Pseudocyphellaria hawaiiensis* and *P. xanthosticta*. Both are active in the FRAP and DPPH assays (Supplementary Table S2). As no actual *Peltigera* species for screening was found in the wild a museum sample of *Peltigera dolichorhiza* was obtained; originally sampled and stored in 1975. Astonishingly, even after almost 40 years of storage this sample yielded the most active extract in the FRAP assay when compared to the

other Hawaiian specimens. In the DPPH assay *P. dolichorhiza* also showed significant radical scavenging, but did not exhibit peak activity (Supplementary Table S2).

Discussion

In a general sense it seems likely that opportunistic sampling of lichens based on ease of collection, on average, will yield more lichen species that exclusively associate with green algae rather than with cyanobacteria or a mixture of cyanobacteria and green algae as photobiont. Furthermore, our observations indicate that the antioxidant activity measured by either the FRAP or DPPH assay will be comparatively low with some exceptions yielding very high activities. Future studies on lichens aimed at discovering natural products with a significant specific activity, should incorporate a large sample size with high species diversity to increase the probability of identifying leads. This argument is strengthened by the fact that a previous sampling of 29 Icelandic lichens did not yield significant activity in the DPPH assay (Ingólfsdóttir *et al*., 2000), while results in the current study clearly indicate the presence of highly active extracts. Moreover, we report *Peltigera canina* and *Solorina crocea*, both previously reported as inactive (Ingólfsdóttir *et al*., 2000), as species with strong antioxidant activities in both the DPPH and FRAP assays. A direct explanation for this discrepancy in results is difficult to provide as many factors may influence the abundance of antioxidants within lichen samples at any given time. However, this finding serves to illustrate that levels of antioxidants may fluctuate strongly even within lichens from the same species.

The findings for the genus *Peltigera* in Iceland and Hawaii are in agreement with research projects describing antioxidant activity in the DPPH assay for lichens from China, Turkey and Russia (Odabasoglu *et al*., 2005; Luo *et al*., 2010; Paudel *et al*., 2014). All these reports identify species within the *Peltigera* genus in the most active groups. In the dataset from China two of the three most active lichens are *Peltigera* species; the entire dataset consists of 46 lichens. For the most active Russian lichens three out of four are classified as *Peltigera* in a dataset made up of 19 lichens. Furthermore, the most active lichen from the Chinese dataset belongs to the genus *Sticta* (Luo *et al*., 2010); also a very active sample in our Hawaiian dataset. Interestingly, high activity in the DPPH assay does not directly infer the same activity for the FRAP assay and vice versa. However, the samples *Peltigera collina* from Iceland, and *S. weigelii* and *P. dolichorhiza* from Hawaii are active in both the FRAP and the DPPH assays. An activity in both assays may be attributed to either groups of compounds that are active in both assays or compounds that have specific activities and all combinations thereof. At this stage we have made no attempt to quantify the reason for the dual activities. We conclude that strong antioxidant activity in both assays is an indicator of specimens that are worthwhile subjects for further and more detailed research. This notion is reinforced by the fact that lichens belonging to the genus *Peltigera* have been previously described to have a variety of uses in health care (Perez-Llano, 1944).

The initial samples from Hawaii did not contain any lichens from the genus *Peltigera*, even though species from this genus exist in Hawaii (Magnusson and Zahlbruckner, 1944). The follow-up assay of a museum sample of *P. dolichorhiza* from Hawaii placed this species among the most active in the current study. Even though *P. dolichorhiza* was amongst the most active specimens from Hawaii, it did not exhibit peak activity in the DPPH assay. This

could be attributed to the sample being stored for 40 years prior to extraction. A fresh sample of this and other *Peltigera* lichens in Hawaii may show stronger radical scavenging properties and should be targeted in future studies.

Appearance of ROS is partly credited to intense UV radiation (Hideg *et al*., 2013) and rehydration after desiccation (Kranner *et al*., 2008), which makes the presence and abundance of systems that protect lichens against ROS more important in areas that exhibit high UV radiation and strongly fluctuating water availability. On average, it seems reasonable to assume that conditions in Hawaii are more extreme with respect to UV radiation and desiccation and rehydration in comparison to Iceland, thus to some extent explaining the general trend of higher overall activity in the Hawaiian samples when compared to those from Iceland.

Experimental Procedures

Sample collection

Lichen samples were collected based on ease of collection from sites in Iceland (76) and Hawaii (38). The opportunistic collection in Hawaii was followed up by a search specifically for *Peltigera* or *Peltigera*-like species. Two fresh samples (L0058 and L0059) and one museum specimen, collected in 1975 (L0061), were added (Supplementary Tables S1 and S2 for collection areas). Voucher specimens were prepared and sent to the Faculty of Pharmaceutical Sciences, University of Iceland for the Icelandic samples and the Rock Herbarium in Hawaii for the Hawaiian samples.

Extraction

Lichen biomass was dried and exhaustively extracted with methanol (MeOH) over 24 hours at 25°C. All extracts were filtered through sterilized and pre-washed cotton wool and concentrated under reduced pressure at 25°C using a Heidolph Laborota 4000 rotary evaporator. Extracts were stored at 4°C until required for assaying.

Ferric-reducing antioxidant power (FRAP) Assay

Stock solutions for the FRAP assay were prepared as described by Benzie and Strain (Benzie and Strain, 1996) with the following modifications: 300 mM acetate buffer (3.1 g $NaOAc•3H_2O$ and 16 mL glacial acetic acid per liter of deionized water, pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl, and 20 mM $FeCl_3•6H_2O$. The working FRAP reagent was made by combining 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-S-triazine (TPTZ) solution, and 20 mM $FeCl_3•6H_2O$ in a 10:1:1 ratio and heated to 37°C for 30 min prior to use. The standard curve of $FeSO_4$ was prepared from absorbances (595 nm) of $FeSO_4•7H_2O$ solutions of 1000, 500, 200, 150, 100, 75, 50 µM concentrations applied to wells containing FRAP reagent. Working FRAP reagent was added to each well of a 96-well plate in 150 µL volumes. A blank reading was recorded at 595 nm using a Biotek Synergy H1 Hybrid plate reader. Extracts were added in 20 µL volumes to individual wells in triplicate. The assay was incubated at 25°C for 8 min before recording results at 595 nm.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

Radical scavenging abilities of extracts were assessed employing a DPPH assay described by Brand-Williams *et al*. (Brand-Williams *et al*., 1995) that has been modified. The assay was performed under reduced light conditions to limit the possibility of photolytic degradation.

A stock solution was prepared by dissolving 24 mg of DPPH (2, 2-diphenyl-1-picrylhydrazyl) in 100 mL HPLC-grade methanol. The working solution was prepared by making a 1:3 dilution of the stock solution with MeOH in order to obtain an absorbance of 1.1 ± 0.02 units at 490 nm. The standard curve was made using absorbances (490 nm) from vitamin E solutions of 1000, 500, 200, 150, 100, 75, 50, and 25 µM concentrations applied to wells of DPPH reagent. DPPH working solution was added to each well of a 96-well plate in 285 µL volumes. A blank reading was taken at 490 nm using a Biotek Synergy H1 Hybrid plate reader. Extracts and controls were added in 15 µL volumes to individual wells in triplicate. The assay was protected from light and incubated at 25°C. Results were recorded at 490 nm at 2 h.

Statistics

Lichen species that were present more than once in the dataset, were reduced to the most active representative from the DPPH assay for statistical testing. Specifically, the samples that were removed from the dataset prior to statistical testing were I0023 (*Cladonia rangiferina*) from the Icelandic dataset and L0050 (*Cladonia skottsbergii*), L0014 (*Parmeliella mariana*), L0018 (*Parmotrema reticulatum*), L0021 (*Ramalina umbilicata*), L0005 (*Stereocaulon vulcani*), L0003 (*Sticta weigelii*), L0041 (*Teloschistes flavicans*), L0006, L0042 (*Usnea australis*) and L0028 (*Dirinaria applanata*) from the Hawaiian dataset. The three

specimens (L0058, L0059, L0061) from Hawaii that were purposely collected were not used in the statistical comparison.

The Icelandic and Hawaiian datasets were compared to each other employing a Wilcoxon rank-sum test. The test was run in R statistics (R Core Team, 2014) employing the wilcox.test function with standard parameters. The test was performed on the results obtained from the FRAP and DPPH assays individually. P <= 0.01 was set as a significance threshold.

Acknowledgements

We thank C. Smith, Department of Botany, University of Hawaii at Manoa, Manoa, Hawaii, USA and S. Baldursdóttir, DeCode Genetics, Reykjavik, Iceland for their invaluable help in identifying and classifying the lichen species described in this study. We also thank E. Bernstein, Mathematics Department, University of Hawaii at Hilo, Hilo, Hawaii, USA for fruitful discussions concerning the statistical analyses.

References

Ahmadjian, V. (1993) The lichen symbiosis, John Wiley, New York.

Andarwulan, N., Batari, R., Sandrasari, D.A., Bolling, B., and Wijaya, H. (2010) Flavonoid content and antioxidant activity of vegetables from Indonesia. *Food Chemistry* **121**: 1231–1235.

Behera, B.C., Adawadkar, B., and Makhija, U. (2003) Inhibitory activity of xanthine oxidase and superoxide-scavenging activity in some taxa of the lichen family Graphidaceae.

Phytomedicine **10**: 536–543.

Benzie, I.F.F. and Strain, J.J. (1996) The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. *Analytical Biochemistry* **239**: 70–76.

Bhattarai, H.D., Paudel, B., Hong, S.G., Lee, H.K., and Yim, J.H. (2008) Thin layer chromatography analysis of antioxidant constituents of lichens from Antarctica. *Journal of Natural Medicines* **62**: 481–484.

Boustie, J., Tomasi, S., and Grube, M. (2011) Bioactive lichen metabolites: alpine habitats as an untapped source. *Phytochemistry Reviews* **10**: 287–307.

Brand-Williams, W., Cuvelier, M.E., and Berset, C. (1995) Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology* **28**: 25–30.

Burkholder, P.R., Evans, A.W., McVeigh, I., and Thornton, H.K. (1944) Antibiotic Activity of Lichens. *Proceedings of the National Academy of Sciences* **30**: 250–255.

Chairman, K., Singh, A.R., and Alagumuthu, G. (2012) Cytotoxic and antioxidant activity of selected marine sponges. *Asian Pacific Journal of Tropical Disease* **2**: 234–238.

Crespi, M. and Gálvez, S. (2000) Molecular Mechanisms in Root Nodule Development. *J. Plant Growth Regul.* **19**: 155–166.

França, M.B., Panek, A.D., and Eleutherio, E.C.A. (2007) Oxidative stress and its effects during dehydration. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **146**: 621–631.

González, I., Ayuso-Sacido, A., Anderson, A., and Genilloud, O. (2005) Actinomycetes isolated from lichens: Evaluation of their diversity and detection of biosynthetic gene sequences. *FEMS Microbiology Ecology* **54**: 401–415.

Gülçin, İ., Oktay, M., Küfrevioğlu, Ö.İ., and Aslan, A. (2002) Determination of antioxidant

activity of lichen Cetraria islandica (L) Ach. *Journal of Ethnopharmacology* **79**: 325–329.

Gulluce, M., Aslan, A., Sokmen, M., Sahin, F., Adiguzel, A., Agar, G., and Sokmen, A. (2006) Screening the antioxidant and antimicrobial properties of the lichens Parmelia saxatilis, Platismatia glauca, Ramalina pollinaria, Ramalina polymorpha and Umbilicaria nylanderiana. *Phytomedicine* **13**: 515–521.

Halliwell, B. (1987) Oxidative damage, lipid peroxidation and antioxidant protection in chloroplasts. *Chemistry and Physics of Lipids* **44**: 327–340.

Hideg, É., Jansen, M.A.K., and Strid, Å. (2013) UV-B exposure, ROS, and stress: inseparable companions or loosely linked associates? *Trends in Plant Science* **18**: 107–115.

Honegger, R. (1993) Developmental biology of lichens. *New Phytologist*. **125**: 659-677.

Ingólfsdóttir, K., Kook Lee, S., Bhat, K.P.L., Lee, K., Chai, H.-B., Kristinsson, H., Song, L.L., Gills, J., Gudmundsdóttir, J.T., Mata-Greenwood, E., Jang, M.S., and Pezzuto, J. M. (2000) Evaluation Of Selected Lichens From Iceland For Cancer Chemopreventive And Cytotoxic Activity. *Pharmaceutical Biology* **38**: 313–317.

Kaasalainen, U., Fewer, D.P., Jokela, J., Wahlsten, M., Sivonen, K., and Rikkinen, J. (2012) Cyanobacteria produce a high variety of hepatotoxic peptides in lichen symbiosis. *Proceedings of the National Academy of Sciences* **109**: 5886–5891.

Kaasalainen, U., Jokela, J., Fewer, D.P., Sivonen, K., and Rikkinen, J. (2009) Microcystin Production in the Tripartite Cyanolichen *Peltigera leucophlebia*. *Molecular Plant-Microbe Interactions* **22**: 695–702.

Kampa, A., Gagunashvili, A.N., Gulder, T.A.M., Morinaka, B.I., Daolio, C., Godejohann, M., Miao, V.P.W., Piel, J., Andresson, O.S. (2013) Metagenomic natural product discovery in lichen provides evidence for a family of biosynthetic pathways in diverse

symbioses. *Proceedings of the National Academy of Sciences* **110**: E3129–E3137.

Kehrer, J.P. (1993) Free Radicals as Mediators of Tissue Injury and Disease. *Critical Reviews in Toxicology* **23**: 21–48.

Kelman, D., Posner, E.K., McDermid, K.J., Tabandera, N.K., Wright, P.R., and Wright, A.D. (2012) Antioxidant Activity of Hawaiian Marine Algae. *Marine Drugs* **10**: 403–416.

Kranner, I., Beckett, R., Hochman, A., and Nash, T.H. (2008) Desiccation-Tolerance in Lichens: A Review. *The Bryologist* **111**: 576–593.

Kranner, I., Cram, W.J., Zorn, M., Wornik, S., Yoshimura, I., Stabentheiner, E., and Pfeifhofer, H.W. (2005) Antioxidants and photoprotection in a lichen as compared with its isolated symbiotic partners. *Proceedings of the National Academy of Sciences* **102**: 3141–3146.

Luo, H., Yamamoto, Y., Liu, Y., Jung, J.S., Kahng, H., Koh, Y.J., and Hur, J. (2010) The In Vitro Antioxidant Properties of Chinese Highland Lichens. *Journal of Microbiology and Biotechnology* **20**: 1524–1528.

Magnusson, A.H. and Zahlbruckner, A. (1944) Hawaiian Lichens Almqvist & Wiksell.

Nyholm, S.V. and McFall-Ngai, M. (2004) The winnowing: establishing the squid–vibrio symbiosis. *Nature Reviews Microbiology* **2**: 632–642.

Odabasoglu, F., Aslan, A., Cakir, A., Suleyman, H., Karagoz, Y., Bayir, Y., and Halici, M. (2005) Antioxidant activity, reducing power and total phenolic content of some lichen species. *Fitoterapia* **76**: 216–219.

Paudel, B., Bhattarai, H.D., Kim, I.C., Lee, H., Sofronov, R., Ivanova, L., Poryadina, L., and Yim, J.H. (2014) Estimation of antioxidant, antimicrobial activity and brine shrimp toxicity of plants collected from Oymyakon region of the Republic of Sakha (Yakutia), Russia. *Biological Research* **47**: 10. DOI: 10.1186/0717-6287-47-10.

Paudel, B., Bhattarai, H.D., Lee, J.S., Hong, S.G., Shin, H.W., and Yim, J.H. (2008) Antioxidant activity of polar lichens from King George Island (Antarctica). *Polar Biology* **31**: 605–608.

Perez-Llano, G.A. (1944) Lichens their biological and economic significance. *The Botanical Review* **10**: 1–65.

Pietta, P., Simonetti, P., and Mauri, P. (1998) Antioxidant Activity of Selected Medicinal Plants. *Journal of Agricultural and Food Chemistry* **46**: 4487–4490.

Ranković, B.R., Kosanić, M.M., and Stanojković, T.P. (2011) Antioxidant, antimicrobial and anticancer activity of the lichens Cladonia furcata, Lecanora atra and Lecanora muralis. *BMC Complementary and Alternative Medicine* **11**: 97.

Sato, A., Shindo, T., Kasanuki, N., and Hasegawa, K. (1989) Antioxidant Metabolites from the Tunicate Amaroucium Multiplicatum. *Journal of Natural Products* **52**: 975–981.

R Core Team (2014) R: A Language and Environment for Statistical Computing R Foundation for Statistical Computing, Vienna, Austria.

Yuan, X., Xiao, S., and Tylor, T.N. (2005) Lichen-Like Symbiosis 600 Million Years Ago. *Science* **308**: 1017–1020.

Figure legends

Figure 1: Antioxidant activity of 76 lichen samples from Iceland assessed employing the FRAP assay. Color coding: White for lichens associating exclusively with green algae (A); grey for lichens incorporating green algae and cyanobacteria in the symbiosis (ABG); patterned for lichens containing exclusively cyanobacteria as photobionts (BG).

Figure 2: Antioxidant activity of 76 lichen samples from Iceland assessed employing the DPPH assay. Color coding: White for lichens associating exclusively with green algae (A); grey for lichens incorporating green algae and cyanobacteria in the symbiosis (ABG); patterned for lichens containing exclusively cyanobacteria as photobionts (BG).

Figure 3: Antioxidant activity of 41 lichen samples from Hawaii assessed employing the FRAP assay. Color coding: White for lichens associating exclusively with green algae (A); grey for lichens incorporating green algae and cyanobacteria in the symbiosis (ABG); patterned for lichens containing exclusively cyanobacteria as photobionts (BG).

Figure 4: Antioxidant activity of 41 lichen samples from Hawaii assessed employing the DPPH assay. Color coding: White for lichens associating exclusively with green algae (A); grey for lichens incorporating green algae and cyanobacteria in the symbiosis (ABG); patterned for lichens containing exclusively cyanobacteria as photobionts (BG).

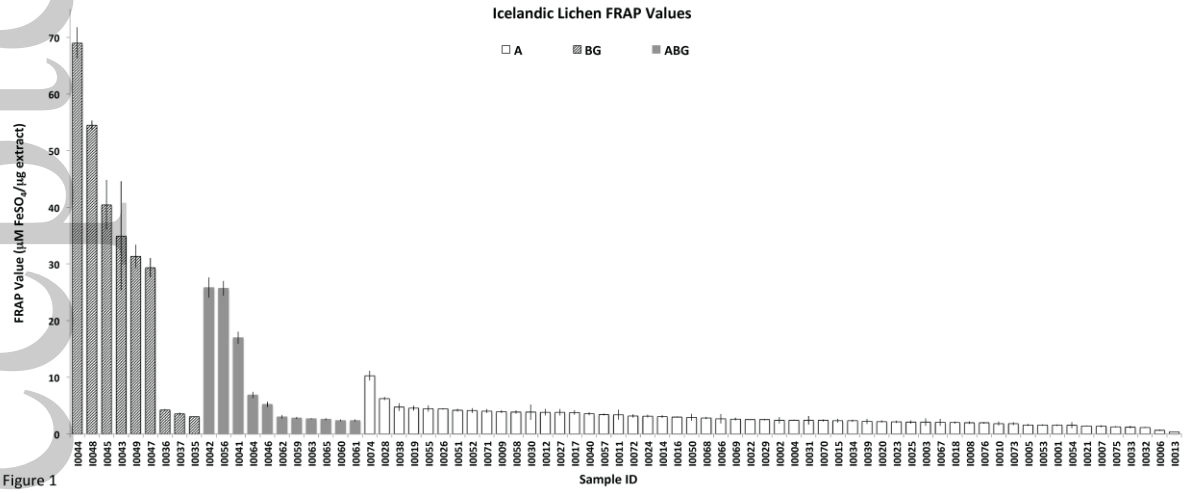

Figure 1

EMI_12850_F1

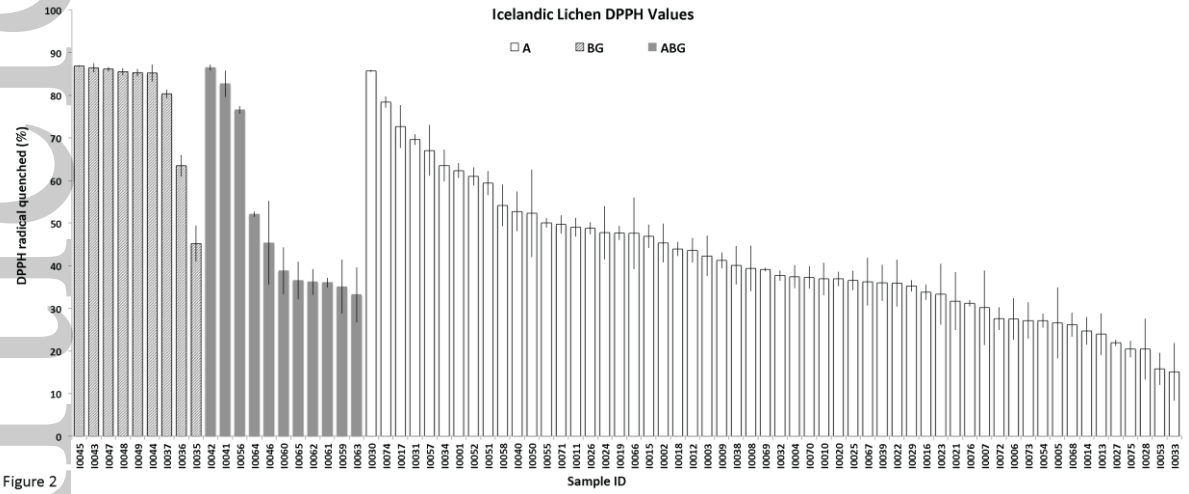

Figure 2

EMI_12850_F2

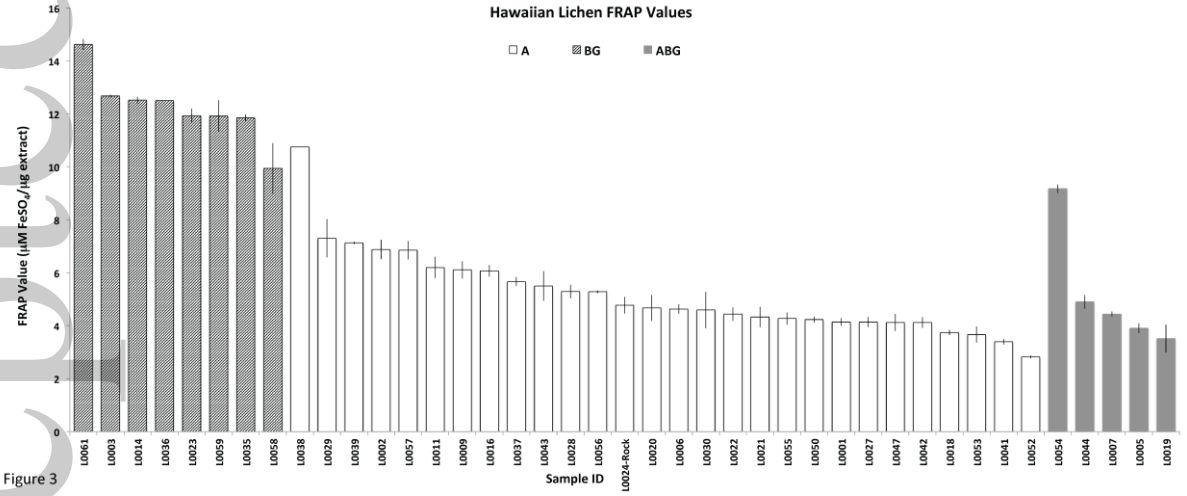

Figure 3

EMI_12850_F3

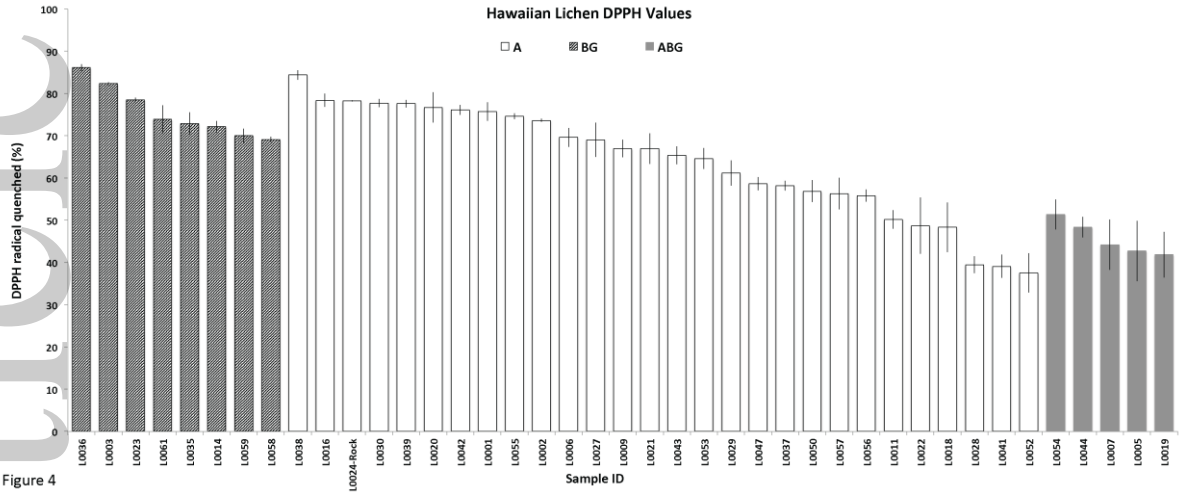

Figure 4

EMI_12850_F4