BIOCELL
2015, 39(1): 25-31

ISSN 1667-5746
ELECTRONIC

Evaluation of the sunscreen lichen substances usnic acid and atranorin

Mehmet VAROL[1,3], Turgay TAY[2], Mehmet CANDAN[1], Ayşen TÜRK[1], Ayşe Tansu KOPARAL[1]

[1] Anadolu University, Department of Biology, Faculty of Science, Yunusemre Campus, Eskisehir TR26470, Turkey
[2] Anadolu University, Department of Chemistry, Faculty of Science, Yunusemre Campus, Eskisehir TR26470, Turkey
[3] Muğla Sıtkı Koçman University, Department of Molecular Biology and Genetics, Faculty of Science, Kotekli Campus, Mugla TR48000, Turkey

Key words: lichen; ultraviolet; photoprotection; photosensitizing.

ABSTRACT: Usnic acid and atranorin are known as both sunscreen substances in natural lichen population and cytotoxic substances on human cell lines. Both substances were isolated from the acetone extracts of *Cladonia foliacea* and *Pseudevernia furfuracea*, respectively. Their photoprotective activities on irradiated human keratinocyte (HaCaT) cells and destructive effects on non-irradiated HaCaT cells were compared by a modified method in order to assess the effects on mitochondrial metabolic activity (MTT assay), membrane integrity (LDH assay), apoptosis (DAPI staining) and cytoskeleton integrity (TRITC-phalloidin staining). This study showed that usnic acid and atranorin have substantial photosensitizing activity, which appears to be linked to their crystallized forms in lichen populations and their decreased amounts in the spring and summer seasons, when ultraviolet B radiation is maximum. However, their cytotoxic, apoptotic and cytoskeleton deleterious activities may be a considerable obstacle for their use in human skin care.

Introduction

Lichens are complex associations between Fungi and photobionts (Cyanobacteria and/or Algae) and their occurrence of lichens is believed to date back 400 million years ago during which time they have adapted to a wide variety of habitats (Chen *et al*., 2000). Lichens are known to synthetize over 800 different compounds produced by the mycobiont partner, and these substances are also named as "lichen acids" cause of their common acidic features (Molnar and Farkas, 2010; Kovacik *et al*., 2011). Studies about lichen acids have shown antimicrobial, antiviral, antiproliferative, anti-inflammatory, antipyretic, analgesic and enzyme inhibitory effects (Koparal *et al*., 2010; Molnar and Farkas, 2010). Also, there has been much interest in the last decade for the use of lichen acids as skin care agents, because of their antioxidant effects and their absorption capacity in the ultraviolet region (Russo *et al*., 2008). It is generally considered that the currently increasing incidence of skin cancers, especially in pale-skinned populations, is a consequence of depletion of the stratospheric ozone layer depletion (Narayanan *et al*., 2010). Indeed, approximately 96% of the all skin malignancies are non-melanoma skin cancers (NMSC) and ultraviolet B radiation has been identified as the primary risk factor for NMSC (Narayanan *et al*., 2010; Ming *et al*., 2011). Thus, development of novel photoprotective agents against ultraviolet B radiation is an urgent need.

Usnic acid and atranorin seem to be the most studied among lichen acids (Cocchietto *et al*., 2002; Ingolfsdottir, 2002; Nybakken and Julkunen-Tiitto, 2006; Guo *et al*., 2008). However, they have been reported as both highly cytotoxic and photo-protective substances (Koparal *et al*., 2006; Engel *et al*., 2007; Burlando *et al*., 2009; Molnar and Farkas, 2010;

*Address correspondence to: Mehmet Varol, mehmetv@anadolu.edu.tr
Received: June 23, 2014. Revised version received: November 23, 2014. Accepted: January 23, 2015.

Backorova *et al.*, 2012). So, there was confusing and missing information about their usage potential against ultraviolet radiation for human skin protection.

Thus, this study reveals the destructive and photo-protective activities of usnic acid and atranorin by means of a modified process from the combination of previously described photo-protective activity determining models (Offord *et al.*, 2002; Tarozzi *et al.*, 2005; Kohlhardt-Floehr *et al.*, 2010; Zanatta *et al.*, 2010). The biological influences of substances were experimented on transformed human keratinocyte cell line (HaCaT) in order to assess the efficacy on mitochondrial metabolic activity (MTT assay), membrane integrity (LDH assay), apoptosis (DAPI staining) and cytoskeleton integrity (TRITC-phalloidin staining). Additionally, this research reveals the effects of these lichen acids and 2.5 J/cm^2 ultraviolet B radiation on cytoskeleton integrity of HaCaT cells for the first time.

Material and Methods

Materials

Culture media, MTT, DAPI, TRITC-phalloidin and the other reagents were purchased from Sigma-Aldrich (St Louis, MO, USA). The tissue culture plates were purchased from TPP (Trasadingen, Switzerland). The round glass coverslip was obtained from Marienfeld (Lauda-Königshofen, Germany). Cytotoxicity detection kit (LDH) was purchased from Roche (Mannheim, Germany). The ELX808IU microplate reader was bought from Biotek (Winooski, USA). El-series 8 watt UV lamp, C-65 chromato-vue UV cabinet and UVX radiometer were purchased from UVP (Upland, CA).

Lichen substances

Usnic acid and atranorin were obtained from the acetone extracts of the lichens *Cladonia foliacea* and *Pseudevernia furfuracea*, respectively. *C. foliacea* samples were gathered in the east position of Mayislar village, Eskisehir Province, Turkey, at 230 m above sea level (40° 02' 10.5" N; 30° 40'0 7.7" E), and those of *P. furfuracea* were collected from the trunks of Pinus nigra pallasiana, in Bozdağ, Eskisehir Province, Turkey at 1,200 m above sea level (39° 54' 40.5" N; 30° 35'5 8.3" E), and *Cedrus libani*, in South of Cukurabanoz village, Anamur, Mersin Province, Turkey at 1,560 m above sea level (36° 17' 46" N; 32° 54' 34" E), as described in previous studies (Yilmaz *et al.*, 2004; Koparal *et al.*, 2006). The characterization of the (-)- usnic acid and atranorin were based on a comparison of the Rf values in the A, C, and G solvent systems with those given in the literature, their melting points and IR spectra (Culberson *et al.*, 1977; Culberson and Ammann, 1979; Edwards *et al.*, 2003; Huneck and Yoshimura, 1996; Orange *et al.*, 2001). Stock solutions of the compounds were initially prepared in DMSO.

Culture conditions

HaCaTs were obtained from ATCC (American Type Culture Collection) and maintained in Dulbecco's Modified Eagle's Media – High glucose (DMEM) supplemented 10% heat-inactivated fetal bovine serum and 1% penicillin-streptomycin mixture. HaCaTs were cultured in a humidified atmosphere containing 5% CO_2 at 37 °C. Before the beginning of the tests, HaCaTs were incubated to reach 70% density in 75 cm^2 flask. After the incubation period, they were raised and seeded into 96 well plates (2x10^4 cells/well) or 12 well plates (2 x10^5 cells/well) which include a round coverslip/well.

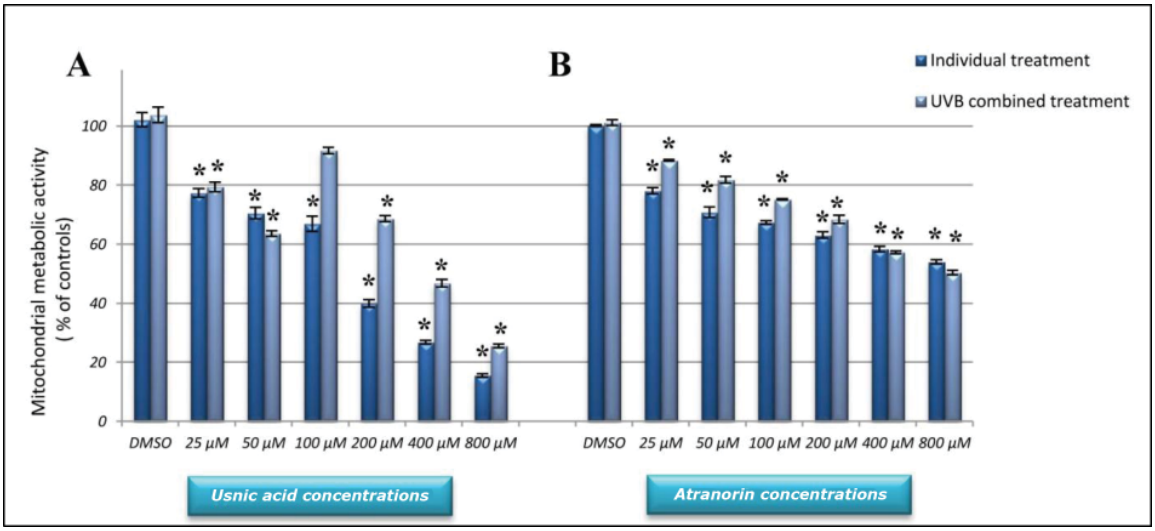

FIGURE 1. Antiproliferative influences of usnic acid and atranorin were measured using mitochondrial metabolic activity. **(A)** Influences of individual and UVB combined treatments of usnic acid. **(B)** Influences of individual and UVB combined treatments of atranorin. The results are expressed as the mean ± SD. *Indicates significant difference from the control group (ANOVA, Tukey test).

Cell treatment and UVB irradiation

We have combined and modified previously used methods (Offord *et al.*, 2002; Tarozzi *et al.*, 2005; Kohlhardt-Floehr *et al.*, 2010; Zanatta *et al.*, 2010). The stock solutions of compounds were diluted in PBS for preparing the applied concentrations. Two previously prepared identical microplates were used for each substance to compare the individual and UVB combined influences of the substances at the same time. Cells in identical microplates were treated with 25, 50, 100, 200, 400 or 800 µM concentrations of the substances in PBS. After a 40 minute incubation period, one of the microplates was irradiated with 2.5 J/cm^2 UVB (40 min. exposure from 15 cm distance) in C65 chromato-vue cabinet by 8 watts El-series UVP lamp while the other was kept in darkness at room temperature. Following the irradiation period, both microplates were incubated again for 40 minutes at 37 °C. After completion of the treatments, PBS was replaced with fresh medium and incubated for 4 hours at 37 °C.

Detection of mitochondrial metabolic activity

The antiproliferative influences of substances were measured by using MTT assay as previously described by Mossmann (1983).

Detection of membrane integrity

The activities of substances on cell membrane entirety were lighted up by utilization of LDH-based cytotoxicity assays, which were performed according to the manufacturer's instructions as previously described (Smith *et al.*, 2011).

Detection of apoptosis by DAPI staining

After treatments and UVB irradiation, HaCaTs grown on the coverslip were fixed with 3.7% paraformaldehyde-PBS and washed twice with sterile PBS solution. Following that, the cells were permeabilized with 0.5% Triton X-100 in PBS for 5 min at 37 °C and stained with 1mg/ml DAPI dissolved in PBS for 30 min at 37 °C. The cells were rinsed twice with PBS and HaCaTs were rinsed with PBS and fluorescent images were viewed in the Olympus BX50 with U-UHK fluorescent attachment microscope.

Detection of cytoskeleton integrity

HaCaTs in coverslips were fixed, rinsed and permeabilized as described above. Afterwards, cells were stained with 5µg/ml TRITC-phalloidin dissolved in PBS for 60 minutes at 37 °C. HaCaTs were rinsed with PBS and fluorescent images were viewed in the Olympus BX50 microscope with U-UHK fluorescent attachment.

Statistical analysis

All experiments were replicated at least three times. The percentages of cell viability and LDH activity were calculated against the untreated control cells. One-way ANOVA followed by the Tukey test was used for comparisons (SPSS, Statistical Package for Social Science). A value of $p<0.05$ was esteemed significant.

Results

Mitochondrial metabolic activity

The half maximal inhibitory energy value (IC50) on HaCaTs was established at 2.5 J/cm^2 ultraviolet B (Zanatta *et al.*, 2010). Both substances caused dose-dependent decreases on

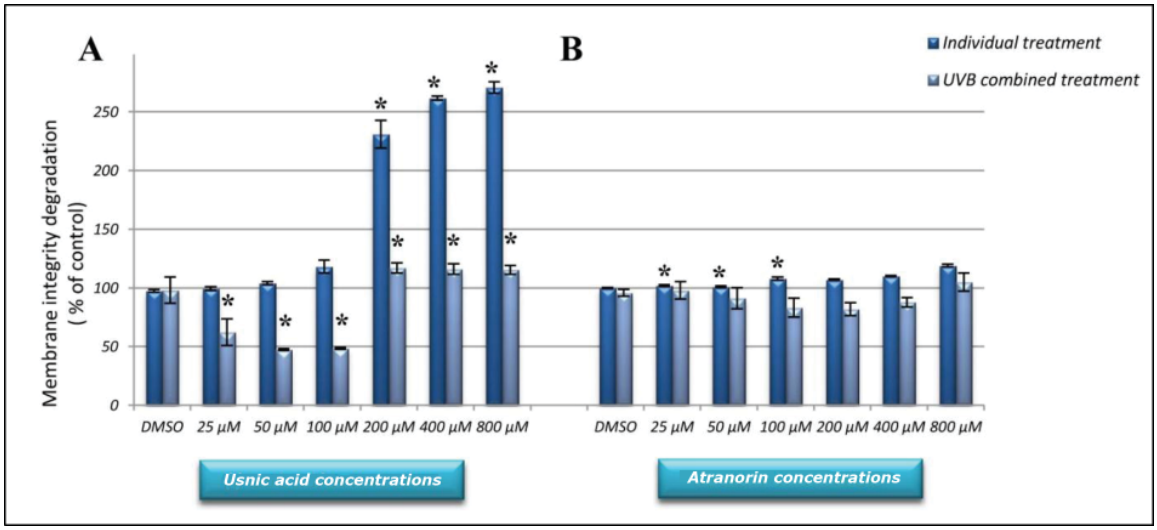

FIGURE 2. Influences of the lichen substances on membrane integrity of cells were evaluated by using LDH cytotoxicity detection kit. **(A)** Influences of individual and UVB combined treatments of usnic acid. **(B)** Influences of individual and UVB combined treatments of atranorin. The results are expressed as the mean ± SD. *Indicates significant difference from the control group (ANOVA, Tukey test).

the mitochondrial metabolic activities (Fig. 1). However, the dose-dependent reduction curve was modified by ultraviolet B irradiation. Usnic acid displayed a clear photosensitizing effect against UVB at the 100 µM concentration, which showed less cytotoxic activity than the 25 and 50 µM, probably because of the electrical transitions within the molecule related to UVB (Fig. 1 A). Similarly, atranorin showed a lesser cytotoxic influence when it was combined with UVB irradiation (Fig.1 B).

Membrane integrity degradation

UVB showed about a 250 % increase in membrane degradation activity on HaCaTs, by the increase according to the non-irradiated control. On the other hand, the obtained data from LDH activity assays was parallel to the mitochondrial activity assays (Fig. 2 A and B).

Detection of apoptosis by DAPI staining

Results showed that UVB irradiation markedly provoked apoptosis and usnic acid induced dose-dependent apoptotic markers as the occurrence of apoptotic bodies, chromatin condensation and nuclear membrane blebbing. On the other hand, apoptosis caused by UVB irradiation was decreased by 25-50-100 µM concentrations of usnic acid. However, this substance also increased the incidence of the apoptotic markers. Atranorin showed a clear apoptotic influence at the only 800 µM and apoptosis induced by UVB irradiation was not impaired by atranorin (Fig. 3).

Cytoskeleton integrity

UBV showed displayed a deleterious effect on cytoskeleton integrity, which was only decreased by the higher applied concentrations of lichen substances (400 and 800 µM). On the other hand, the alteration of the cytoskeleton by irradiation was not diminished by usnic acid and atranorin (Fig. 4).

Discussion

The lichen substances showing a carbonyl group attached to a benzene ring are considered as effective filters to ultraviolet rays, and some of them, such as parietin, rhizocarpic acid, usnic acid, atranorin, and vulpinic acid have been identified as photo-protective factors for the photobiont partner of lichens in natural populations (Fernandez et al., 1998; Hidalgo *et al.*, 2002; Solhaug *et al.*, 2003; Nybakken

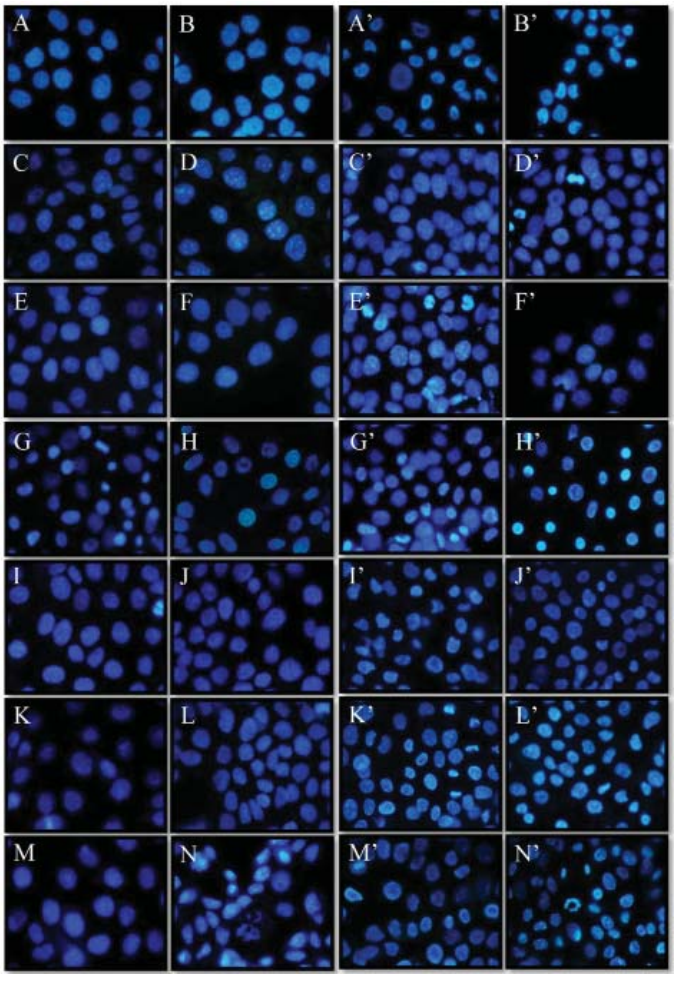

FIGURE 3. Detection of apoptosis by DAPI staining. **(A)** Untreated. **(B)** DMSO. **(C-H)** Cells treated with usnic acid, 25, 50, 100, 200, 400 or 800 µM, respectively. **(I-N)** Cells treated with atranorin, 25, 50, 100, 200, 400 or 800 µM, respectively. **(A'-N')** Irradiated forms of **(A-N)**, respectively.

and Julkunen-Tiitto, 2006). To determine the photo-protective abilities of chemical substances, there are widely different irradiation and treatment methods in the literature (Hernandez-Pigeon *et al*., 2006; Engel *et al*., 2007; Poquet *et al*., 2008; Kohlhardt-Floehr *et al*., 2010; Zanatta *et al*., 2010). Therefore, we decided to develop a modified irradiation and treatment method to compare the photo-protective activity of usnic acid and atranorin on irradiated HaCaTs cells , as well as their deleterious effects on non-irradiated cells, in same experimental conditions.

Usnic acid and atranorin have been indicated as anti-proliferative and allergenic substances (Aalto-Korte *et al.,* 2005; Koparal *et al*., 2006; Backorova *et al*., 2011). Even though they are considered photo-protective agents, it is known that the amount of usnic acid and atranorin in natural lichen populations are decreased in the spring and summer seasons, when UVB radiation is maximum (Begora and Fahselt, 2001). Nguyen *et al.* (2013) reported that a molecule's capability to absorb light energy is due to electronic transitions from the highest occupied molecular orbital (HOMO) to the lowest occupied molecular orbital (LUMO) within the molecule. The absorbed energy can be eliminated by radiation processes (as fluorescence) by non-radiation vibrational relaxation (heat), by transfer to other molecules (sensitization) or via photoreactions. The elimination of the absorbed energy by sensitization and vibrational relaxation are destructive for cells due to the generation of reactive oxygen species (ROS) or heat. Additionally, radiation processes, vibrational relaxation and sensitization cause no changes to the chemical structures of molecules which absorb light, and we think that the reduction in the amounts of usnic acid and atranorin in natural lichen populations when UBV radiation is maximal (Begora and Fahselt, 2001) may have occurred via harmless photoreactions.

It is known that ultraviolet B may cause cell death as a consequence of increasing intracellular ROS, thus provoking oxidative DNA and protein damage (Choi *et al*., 2010; Tsuda, 2012). In this respect, lower concentrations (0.01 and 0.1 μM) of usnic acid have been reported to show an antioxidant effect under 0.1 J/cm^2 ultraviolet B radiation on Jurkat cells) (Kohlhardt-Floehr *et al*., 2010). However, a higher concentration (100 μM) of usnic acid showed an opposite action when combined with a higher intensity of ultraviolet B radiation (5 or 14 J/cm^2) (same study). Additionally, it is known that the actin cytoskeleton may react to a range of extracellular signals including ultraviolet radiation and

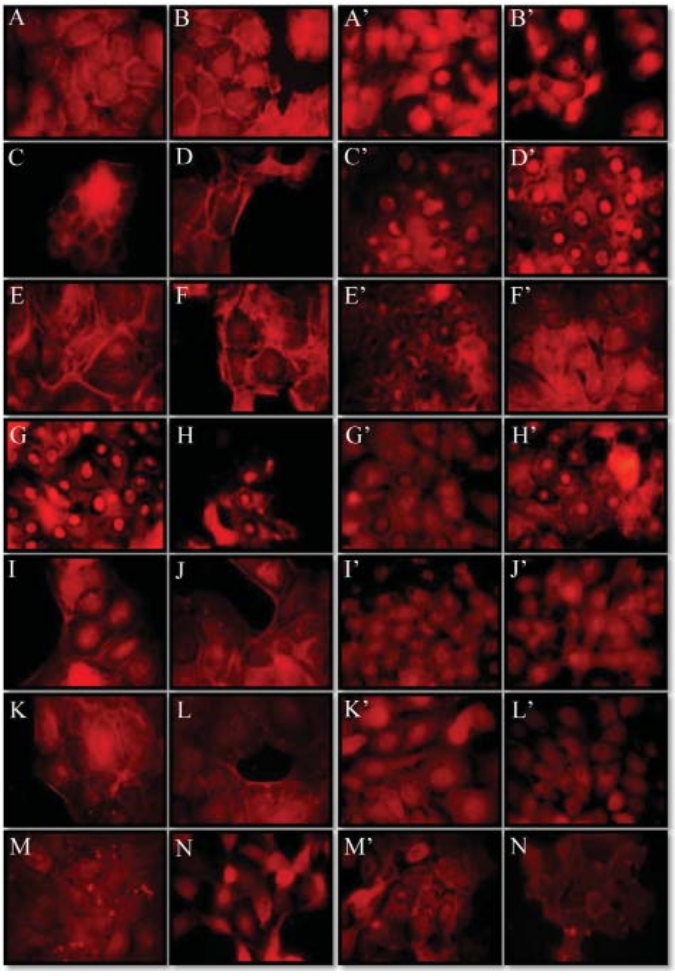

FIGURE 4. The filamentous actin organization was displayed on HaCaTs by staining with TRITC-phalloidin. **(A)** Untreated. **(B)** DMSO. **(C-H)**: Cells treated with usnic acid, 25, 50, 100, 200, 400 or 800 μM. **(I-N)**: Cells treated with atranorin 25, 50, 100, 200, 400 or 800 μM. (A'-N'): Irradiated forms of **(A- N)**, respectively.

therapeutic agents (Visa and Percipalle, 2010; Rock *et al.*, 2011). Our study demonstrated for the first time, 2.5 J/cm^2 ultraviolet B devastates the filamentous actin proteins of HaCaT cells.

In conclusion, usnic acid has shown more deleterious effects than atranorin against HaCaTs cells. Besides that, usnic acid has shown a higher photosensitizing ability as compared with atranorin. The toxic and other detrimental influences of these substances might limit their use in the cosmetic industry (Nguyen *et al.*, 2013). However, pharmacological derivatives or complexes of usnic acid and atranorin would only be useful as sun-screening agents if their deleterious effects are reduced and their photoprotective effects are enhanced. An enhancement of the antimicrobial activity of usnic acid by its incorporation into soluble polyacrylamide complexes has recently been shown (Francolini *et al.*, 2013).

Acknowledgements

This research was supported by the commission of scientific research of Anadolu University, Turkey (Projects #1106F116 and #1206F101).

Reference

Aalto-Korte K, Lauerma A, Alanko K (2005). Occupational allergic contact dermatitis from lichens in present-day Finland. *Contact Dermatitis* **52:** 36-38.

Backorova M, Backor M, Mikes J, Jendzelovsky R, Fedorocko P (2011). Variable responses of different human cancer cells to the lichen compounds parietin, atranorin, usnic acid and gyrophoric acid. *Toxicology In Vitro* **25:** 37-44.

Backorova M, Jendzelovsky R, Kello M, Backor M, Mikes J, Fedorocko P (2012). Lichen secondary metabolites are responsible for induction of apoptosis in HT-29 and A2780 human cancer cell lines. *Toxicology In Vitro* **26:** 462-468.

Begora MD, Fahselt D (2001). Usnic acid and atranorin concentrations in lichens in relation to bands of UV irradiance. *The Bryologist* **104:** 134-140.

Burlando B, Ranzato E, Volante A, Appendino G, Pollastro F, Verotta L (2009). Antiproliferative effects on tumour cells and promotion of keratinocyte wound healing by different lichen compounds. *Planta Medica* **75:** 607-613.

Cocchietto M, Skert N, Nimis PL, Sava G (2002). A review on usnic acid, an interesting natural compound. *Naturwissenschaften* **89:** 137-146.

Chen J, Blume H-P, Beyer L (2000). Weathering of rocks induced by lichen colonization -a review. *Catena* **39:** 121-146.

Choi HK, Kim DH, Kim JW, Ngadiran S, Sarmidi MR, Park CS (2010). Labisia pumila extract protects skin cells from photoaging caused by UVB irradiation. J*ournal of Bioscience and Bioengineering* **109:** 291-296.

Culberson CF, Ammann K (1979). Standardmethode zur Dünnschichtchromatographie von Flechtensubstanzen. *Herzogia* **5:** 1-24.

Culberson CF, Culberson WL, Johnson A (1977). *Second Supplement to 'Chemical and Botanical Guide to Lichen Products'.* American Bryological and Lichenological Society, Missouri Botanical Garden: St. Louis.

Edwards HG, Newton EM, Wynn-williams DD (2003). Molecular structural studies of lichen substances ii: atranorin, gyrophoric acid, fumarprotocetraric acid, rhizocarpic acid, calycin, pulvinic dilactone and usnic acid. *Journal of Molecular Structure* **651:** 27-37.

Engel K, Schmidt U, Reuter J, Weckesser S, Simon-Haarhaus B, Schempp CM (2007). *Usnea barbata* extract prevents ultraviolet-B induced prostaglandin E2 synthesis and COX-2 expression in HaCaT keratinocytes. *Journal of Photochemistry and Photobiology B* **89:** 9-14.

Fernandez E, Reyes A, Hidalgo ME, Quilhot W (1998). Photoprotector capacity of lichen metabolites assessed through the inhibition of the 8-methoxypsoralen photobinding to protein. *Journal of Photochemistry and Photobiology B* **42:** 195-201.

Francolini I, Taresco V, Crisante F, Martinelli A, D'ilario L, Piozzi A (2013). Water soluble usnic acid-polyacrylamide complexes with enhanced antimicrobial activity against Staphylococcus epidermidis. *International journal of molecular sciences* **14:** 7356-7369.

Guo L, Shi Q, Fang JL, Mei N, Ali AA, Lewis SM, Leakey JE, Frankos VH (2008). Review of usnic acid and *Usnea barbata* toxicity. *Journal of Environmental Science and Health, Part C: Environmental Carcinogenesis and Ecotoxicology Reviews* **26:** 317-338.

Hernandez-Pigeon H, Jean C, Charruyer A, Haure MJ, Titeux M, Tonasso L, Quillet-Mary A, Baudouin C, Charveron M, Laurent G (2006). Human keratinocytes acquire cellular cytotoxicity under UV-B irradiation. Implication of granzyme B and perforin. *Journal of Biological Chemistry* **281:** 13525-13532.

Hidalgo ME, Fernandez E, Ponce M, Rubio C, Quilhot W (2002). Photophysical, photochemical, and thermodynamic properties of shikimic acid derivatives: calycin and rhizocarpic acid (lichens). *Journal of Photochemistry and Photobiology B* **66:** 213-217.

Huneck S, Yoshimura I (1996). *Identification of Lichen Substances.* Springer-Verlag: Berlin Heidelberg.

Ingolfsdottir K (2002). Usnic acid. *Phytochemistry* **61:** 729-736.

Kohlhardt-Floehr C, Boehm F, Troppens S, Lademann J, Truscott TG (2010). Prooxidant and antioxidant behaviour of usnic acid from lichens under UVB-light irradiation--studies on human cells. *Journal of Photochemistry and Photobiology B* **101:** 97-102.

Koparal AT, Tuylu BA, Turk H (2006). In vitro cytotoxic activities of (+)-usnic acid and (-)-usnic acid on V79, A549, and human lymphocyte cells and their non-genotoxicity on human lymphocytes. *Natural Product Research* **20:** 1300-1307.

Koparal AT, Ulus G, Zeytinoglu M, Tay T, Turk AO (2010). Angiogenesis inhibition by a lichen compound olivetoric acid. *Phytotherapy Research* **24:** 754-758.

Kovacik J, Klejdus B, Stork F, Malcovska S (2011). Sensitivity of Xanthoria parietina to UV-A: role of metabolic modulators. *Journal of Photochemistry and Photobiology B* **103:** 243-250.

Mcevoy M, Solhaug KA, Gauslaa Y (2007). Solar radiation screening in usnic acid-containing cortices of the lichen *Nephroma arcticum*. *Symbiosis* **43:** 143-150.

Ming M, Feng L, Shea CR, Soltani K, Zhao B, Han W, Smart RC, Trempus CS, He YY (2011). PTEN positively regulates UVB-induced DNA damage repair. *Cancer Research* **71:** 5287-5295.

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

Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods* **65:** 55-63.

Narayanan DL, Saladi RN, Fox JL (2010). Ultraviolet radiation and skin cancer. *International Journal of Dermatology* **49:** 978-986.

Nguyen KH, Chollet-Krugler M, Gouault N, Tomasi S (2013). UV-protectant metabolites from lichens and their symbiotic partners. *Natural Product Reports* **30:** 1490-1508.

Nybakken L, Julkunen-Tiitto R (2006). UV-B induces usnic acid in reindeer lichens. *The Lichenologist* **38:** 477-485.

Offord EA, Gautier JC, Avanti O, Scaletta C, Runge F, Kramer K, Applegate LA (2002). Photoprotective potential of lycopene, beta-carotene, vitamin E, vitamin C and carnosic acid in UVA-irradiated human skin fibroblasts. *Free Radical Biology and Medicine* **32:** 1293-1303.

Orange A, James PW, White FJ (2001). *Microchemical Methods for the Identification of Lichens.* British Lichen Society.

Poquet L, Clifford MN, Williamson G (2008). Effect of dihydrocaffeic acid on UV irradiation of human keratinocyte HaCaT cells. *Archives of Biochemistry and Biophysics* **476:** 196-204.

Rock K, Grandoch M, Majora M, Krutmann J, Fischer JW (2011). Collagen fragments inhibit hyaluronan synthesis in skin fibroblasts in response to ultraviolet B (UVB): new insights into mechanisms of matrix remodeling. *Journal of Biological Chemistry* **286:** 18268-18276.

Russo A, Piovano M, Lombardo L, Garbarino J, Cardile V (2008). Lichen metabolites prevent UV light and nitric oxide-mediated plasmid DNA damage and induce apoptosis in human melanoma cells. *Life Sciences* **83:** 468-474.

Smith SM, Wunder MB, Norris DA, Shellman YG (2011). A simple protocol for using a LDH-based cytotoxicity assay to assess the effects of death and growth inhibition at the same time. *PloS One* **6:** e26908.

Solhaug KA, Gauslaa Y, Nybakken L, Bilger W (2003). UV-induction of sun-screening pigments in lichens. *New Phytologist* **158:** 91-100.

Tarozzi A, Marchesi A, Hrelia S, Angeloni C, Andrisano V, Fiori J, Cantelli-Forti G, Hrelia P (2005). Protective effects of cyanidin-3-O-β-glucopyranoside against UVA-induced oxidative stress in human keratinocytes. *Photochemistry and Photobiology* **81:** 623-629.

Tsuda K (2012). Associations of oxidative stress and inflammation and their role in the regulation of membrane fluidity of red blood cells in hypertensive and normotensive men: An electron spin resonance investigation. *Advances in Bioscience and Biotechnology* **3:** 1020-1027.

Visa N, Percipalle P (2010). Nuclear functions of actin. *Cold Spring Harbor Perspectives in Biology* **2:** a000620.

Yilmaz M, Turk AO, Tay T, Kivanc M (2004). The antimicrobial activity of extracts of the lichen Cladonia foliacea and its (-)-usnic acid, atranorin, and fumarprotocetraric acid constituents. *Zeitschrift für Naturforschung C* **59:** 249-254.

Zanatta CF, Mitjans M, Urgatondo V, Rocha-Filho PA, Vinardell MP (2010). Photoprotective potential of emulsions formulated with Buriti oil *(Mauritia flexuosa)* against UV irradiation on keratinocytes and fibroblasts cell lines. *Food and Chemical Toxicology* **48:** 70-75.