

The pure substance has been formulated in toiletry products such as creams, toothpaste, mouthwash, deodorants and sunscreens, in some cases as an active principle, in others as a preservative (Frankos, 2005). From the second half of the 20th century, UA was extensively researched, encompassing the taxonomic, biological, physiological, ecological, medical and pharmaceutical areas (Cocchietto et al., 2002). The enantiomers have partly different biological effects, although fewer studies have been done on (-) compared to (+) UA.

The biological role of UA in lichen have mainly been understood to serve as UV protection of the photobiont (Cocchietto et al., 2002; Nybakken and Julkunen-Tiito, 2006; McEvoy et al., 2006). More recently, there is evidence for UA controlling the intracellular pH of lichens, through a shift in the usnic acid-usneate equilibrium. This acid tolerance mechanism may affect how lichen colonizes acidic substrata and to how they respond to SO₂ from airborne pollution (Hauch and Jürgens, 2008). Furthermore, the uptake of metal ions as macro- and micronutrients from the substrate appears to be controlled and mediated through complexation with lichen substances, including UA (Hauch et al., 2009).

In addition to the well-documented anti-microbial activity against human and plant pathogens, UA has been shown to exhibit antiviral, antiprotozoal, anti-proliferative, anti-inflammatory and analgesic activities. Moreover, ecological effects such as, growth inhibition, anti-herbivore, anti-insects properties (Cocchietto et al., 2002; Ingólfssdóttir, 2002), in some cases as an ecological bio-indicator or bio-monitor (Bjerke and Dahl, 2002) have been established. During the recent decade, the advancement of UA research has focused on antibiotic and anticancer activities resulting in more than one hundred publications (Shrestha and Clair, 2013). However, a severe limitation to the use of pure UA in commercial formulations is due to its low solubility in water and in some organic solvents. The preparation of derivatives with more favorable solubility is reported to improve formulations of UA particularly for antibiotic activity (Melgarejo et al., 2008).

The antibiotic activity of both (+) and (-) usnic acid has been documented in several reports; see Shrestha and Clair (2013) for a comprehensive review. Gram-negative bacteria appear as the most susceptible to inhibition, but also gram-positives like mycobacteria including *Mycobacterium tuberculosis* are inhibited at low MIC (Ingólfssdóttir et al., 1998; Lucarini et al., 2014). In a number of cancer cell lines that have been tested during the last two decades using either crude extracts or purified forms of lichen substances, both forms of UA demonstrated massive cytotoxic activity by terminating the cell growth and cell proliferation at micro-molar concentration (Bezivin et al. 2003; Einarsdóttir et al., 2010; Bačkorová et al., 2012).

UA is found specifically in the genera *Alectoria*, *Cladonia*, *Evernia*, *Lecanora*, *Parmelia*, *Ramalina* and *Usnea*. Recently, Burkin and co-workers (2012), using an enzyme immunoassay, assessed levels of UA over a broad selection of different lichen families. The highest mean amounts were found in *Cladonia stellaris* (Cladoniaceae) and *Vulpicida pinastri*, (Parmeliaceae). Presently, the genus *Usnea* is used commercially as the main source of natural bioactive UA.

Usnic acid can be prepared by organic synthesis, but then obtained as (±) UA (Ingólfssdóttir, 2002). A dimeric derivative of synthetic UA with spermidine was reported by Tomasi and co-workers (2006) demonstrating antibacterial activity in assays. Chemoenzymatic synthesis of UA was attempted starting from hydroxyacetophenone, but with moderate yields (Hawranik et al., 2009). Biosynthetic UA extracted from lichens represents at present both an available and consistent source of bioactive and enantiomerically pure UA.

Lichens are unique in the world of vegetation and cannot be neatly classified into any of the ordinary categories of plants. Lichens are by definition composite symbiotic organisms, usually composed of a fungal partner, the mycobiont, and one or more photosynthetic partners, the photobiont, which is most often either a green alga or cyanobacterium (Nash, 2008). They are traditionally divided into three main morphological groups; crustose, foliose and fruticose.

C. stellaris is a fruticose (hair-like, star-shaped or shrubby, branched, flat or cylindrical lobes) lichen that forms continuous thick carpets on the ground in boreal (Fig. 2a) and arctic regions of the circumpolar north (boldsystems.org). An extensive study on its biology is given by Yarranton (1975). In Norway, this lichen is known as *Kvitkrull* or Reindeer Moss, *Star-tipped Reindeer Lichen* and *Jaegel* (by Saami people and Laplanders). The lichen is used as winter forage for reindeer and ornamentally in wreaths, floral decorations and architectural models. Its abundance, uniform natural coloring and gorgeous appearance make its collection economically attractive (Kauppi, 1979).

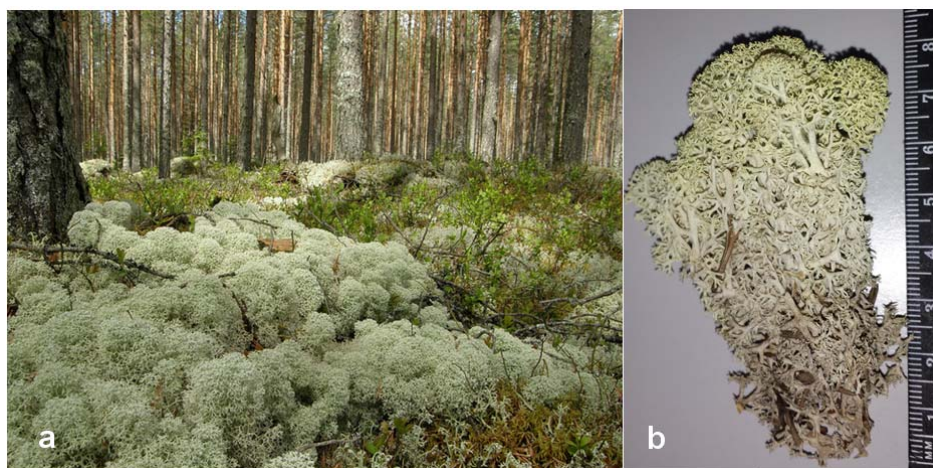


Figure 2. Reindeer lichen (*C. stellaris*), (a) carpet growth in its natural habitat (Cladonia; flickr.com, 2015), and (b) longitudinal view of a dried sample.

C. stellaris is found abundantly in the mountainous regions in Southeastern Norway where in some areas of Hedmark County the lichen has been subject to careful harvesting by skilled hand pickers for almost forty years to sustain regrowth (mose.no). Approximately 60 tons are harvested annually from selected areas on a rotation basis. The upper thallus (20-60 mm) of the lichen has the likely potential as source for sustainable, commercial production of UA. The present study describes the solvent extraction of UA in *C. stellaris* from a number of locations where ornamental harvesting is already in place. The study reports on the possible enantiomer form, identification, purity assessment and quantification of extracted UA compounds.

MATERIALS AND METHODS

Chemicals. All solvent chemicals and acids were of analytical grade and obtained from usual lab suppliers. As a reference compound in this study (+) usnic acid (98%) isolated from *Usnea dasypoga*, from Sigma Aldrich (St. Louis, MS, USA) was used.

Lichen collection, preparation and extraction. Samples of *C. stellaris* were collected from the mountainous forest region in Rendalen municipality, Hedmark, Norway. Samples, ca. (800-1000g) from four locations at different altitudes were named as L01 (61°59'N, 11°24'E), L02 (61°74'N, 11°32'E), L03 (62°07'N, 10°60'E) and L04 (61°69'N, 11°20'E). Fresh samples were stored at +4 °C until used.

Preparation and extraction of lichen samples were carried out essentially according to the method of Smeds and Kytöviita (2010). An aliquot of lichen was carefully cleaned free from substrate debris and dried at 80 °C for 24 hours. The dried lichen thallus (Fig. 2b) was separated ca 5-6 cm from the top in an upper part and a lower part (root), then grinded to powder in a mortar and kept in an airtight container.

Acetone extractions (75 or 150 ml) were carried out with portions of 5 and 10 g powder, but all at 1:15 ratio of mass to solvent. The mixture was kept under stirring for 30 minutes in a covered beaker. The yellowish solution was vacuum filtered (589² white band 70 mm filter; Schleicher & Schuell, Germany) to remove the solids, and further evaporated to dryness at 50 °C (Rotavapor Büchi R-114; Büchi Labortechnik AG, Flawil, Switzerland). Dried material was then re-dissolved in a lesser volume of acetone, and evaporated a second time before the resulting crude extract material was recovered and weighed to calculate the mass yield. Statistical analysis of extraction yields results was carried out using ANOVA with $p < 0.05$.

Recrystallization of crude extract. The crude acetone extract was recrystallized to ensure higher purity of the sample. Material was dissolved in CHCl₃ as suggested by Huneck and Yoshimura (1996) and absolute ethanol was added drop-by-drop while on a magnetic stirrer at 30 °C, until spiky needle-like crystals formed. The crystals were collected and stored at room temperature for further analysis.

Chemical reaction test. A qualitative color test (Huneck and Yoshimura, 1996) was carried out to identify UA and possible trace compounds. Crude extract dissolved in acetone (0.2 mg/ml) was used when testing for UA and olivetoric acid (OA) identification, whereas crude extract dissolved in ethanol (0.1 mg/ml) was used for perlatolic acid (PA). For test of UA 2 % KOH was added to produce a yellow color, and next added NaOCl (0.2 mg/ml) was added to produce a characteristic deep yellow color. OA was tested with NaOCl to first give a red color, followed by purple when FeCl₃ (0.2 mg/ml) was added. PA was tested by adding FeCl₃ to give a violet color. The color test can only provide a visual indication of the presence of these compounds while further analysis and characterization is required for verification.

Melting point and polarimetry. Melting point measurement was carried out using a BÜCHI 530 instrument (Büchi Labortechnik AG, Flawil, Switzerland). Optical rotation was measured using a POLAX-2L polarimeter (ATAGO, Tokyo, Japan), with tetrahydrofuran as solvent and with different concentrations of UA.

Thin layer chromatography (TLC). Thin layer chromatography (TLC) was carried out as one and two-dimensional separations using 5 x 5 cm plates (Silica gel 60 with fluorescent marker, Merck, Darmstadt, Germany). The two solvent systems used were according to Culberson (1972): B, hexane : diethyl ether : formic acid = 130:80:20, and C, toluene : acetic acid = 200:30. For two-dimensional TLC, only one sample was separated per plate, using only the two solvent systems along each axis of migration. Plates were viewed under UV light (254 nm) for dark spots and the migration distance noted for calculation of R_f.

Gas chromatography (GC). GC was carried out with a HP 6890 chromatography system (Agilent Technologies Inc., Palo Alto, CA, USA) fitted with a dimethylpolysiloxane capillary column and flame ionization detector (FID). The selected oven temperature conditions were: 50 °C for 3 min, increase by 20 °C/min to 340 °C, and held at 340 °C for 15 min. The inlet was split-less at 280 °C. Sample and standard were injected (volume 2 µl) manually. The standard UA and the lichen UA samples were dissolved in acetone to a final concentration of 1 ng/µl and stored in capped vials.

Nuclear magnetic resonance (NMR) spectroscopy. NMR spectra of crystals of extracted UA were recorded with a Bruker AWH 400 instrument at 400 MHz (¹H) or at 100 MHz (¹³C) using CDCl₃ as solvent. The analysis was carried out at the Department of Chemistry, University of Oslo.

RESULTS AND DISCUSSION

Yield of usnic acid by acetone extraction. The extractive yields from samples of the upper thallus showed a high degree of similarity (Table 1) varying between 1.5 to 1.9 % of the lichen dry weight. There was no significant difference ($p < 0.05$) in yield between locations, which indicates that the accumulation of UA was not dependent on the altitude in the sampling area. From the root section less than 1 % UA was obtained; this proportion between the upper and lower parts being consistent with findings in *C. stellaris* from the Murmansk region (Tolpysheva, 2014).

Table 1. Mass yields (mg/g dry lichen) from acetone extraction of dried upper thallus (n = 4) and root (*n= 2) of *C. stellaris* obtained from four sampling sites. Different superscript letters indicate significantly different samples (p < 0.05).

Sampling site	Approx. altitude (meter)	Extraction yield (\pm SD)
L01	250 - 350	15.27 \pm 0.59 ^a
L02	500 - 600	18.22 \pm 1.6 ^a
L03	550 - 650	17.68 \pm 3.0 ^a
L04	250	19.29 \pm 1.2 ^a
L01*	250 -350	8.55 \pm 0.91 ^b

Houvinen (1985) reported *C. stellaris* from Northern Norway and Finland to have a content of UA of 2.5% or more, while Smeds & Kytöviita (2010), analyzing both enantiomers in their sample set from Finland, found in average 2.0 % UA. Falk and co-workers (2008) reported that the contents of UA in *C. stellaris* from Alaska sampled between 63° and 67° north, ranged from 0.28 % to 1.7 %. In a Russians study of *C. stellaris*, the range of UA was 1.1-1.4% of lichen dry mass (Polovinka et al., 2012). Hence, the amount of UA obtained from our sample set is representative for this lichen from the northern boreal region, even though extraction methods and solvent systems may vary.

Identification of UA and impurities. The qualitative tests made on the crude extracts indicated that besides UA, both perlatolic acid (PA) and olivetoric acid (OA) were present as observed by their characteristic colors. The analytical determination by Smeds and Kytöviita (2010) listed all three as the dominating compounds in the order UA, PA and OA. In Alaskan samples of *C. stellaris*, PA but not OA, was detected in addition to UA (Falk et al., 2008), and in Siberian samples, UA and PA in addition to a range of other minor secondary metabolites, were detected (Polovinka et al., 2012).

Physical characteristics of the preparations are given in Table 2. In the crude extracts, the average melting point was 178.3 °C, while the recrystallized sample at 180 °C was closer to that of the commercial standard (181.5 °C). The optical rotation of both crude and recrystallized samples gave consistent values for the (-) enantiomer, indicating that *C. stellaris* from the sampled area contains (-) UA as the predominant enantiomer (>97 %).

Extracted amount of UA varies according to different ecological factors such as habitat, growth substrate like soil or rock, sunlight, grazing by reindeer, air pollution, and mountain variation. High UA levels provide tolerance to sunlight radiation, so that such lichen species are found in more light-exposed areas than species with low levels (Bjerke and Dahl, 2002). But *C. stellaris* can also grow under lower light-exposure protected by dwarf shrubs or big leafy pine trees. In addition, UA production also depends on the lichenized fungi in association with their algal partner (Muggia et al., 2009). In nature, *C. stellaris* several metabolic activities has been demonstrated (Nash, 2008; Sadowsky et al., 2012), which theoretically also can explain differences in UA levels and enantiomer variation. Thus, it is likely that the lichen contains predominantly (-) UA or (+) UA, or both. In one study, Kinoshita and co-workers (1997) reported the presence of (-) UA only, and Polovinka and co-workers (2012) also observed that extracted sample only contained (-) UA. However, Smeds and Kytöviita (2010) employing chiral chromatography to separate enantiomers reported proportions from 0.4 up to 10 % of (+) UA in addition to the predominant (-) UA. Comparatively, in *Usnea* species where (+) UA is predominant, its content ranges from 1 – 3% of dry weight (Guo et al., 2008).

Table 2. Melting points (MP) and optical rotation of *C. stellaris* extracts compared to the reference UA and to literature values.

	Crude Sample			Re-crystallized	UA reference	
	L01	L02	L03	L04	Observed	Weast (1986)
MP (°C)	178	176	179	180	181	201
$[\alpha]_D^{26}$ (c= 0.02 g/ml C ₄ H ₈ O)	- 477.4	- 483.6	- 482.3	-486.8	467.1	478 to 498 (c= 0.7 % CHCl ₃)

One-dimensional TLC in two solvent systems of different polarity (Fig. 3a and 3b) showed consistent migration with the UA reference (R_f 0.55 \pm 0.01), both for the samples alone and in combination with the control. When analyzed in solvent system B (Fig. 3a), an impurity in a crude preparation (L01) was evident as a minor, faster migrating spot (R_f 0.64). The same spot was also demonstrated by two-dimensional TLC after separating the sample with both solvent systems (Fig. 3c).

In a single sorbent layer and with two solvent systems of different selectivity, a sample spot should be spread over the whole plate with two truly orthogonal systems (Ciesla and Waksmundzka-Hajnos, 2009). As a result, in the crude sample all the possible spots were clearly visible, including UA and possibly PA or OA as indicated in the color reaction test. The crystallized sample (L04) which as a crude sample showed only one spot in either solvent systems in one-dimensional TLC, consistently also gave only one visible spot after migrating with a diagonal trajectory in two dimensional TLC (Fig. 3d).

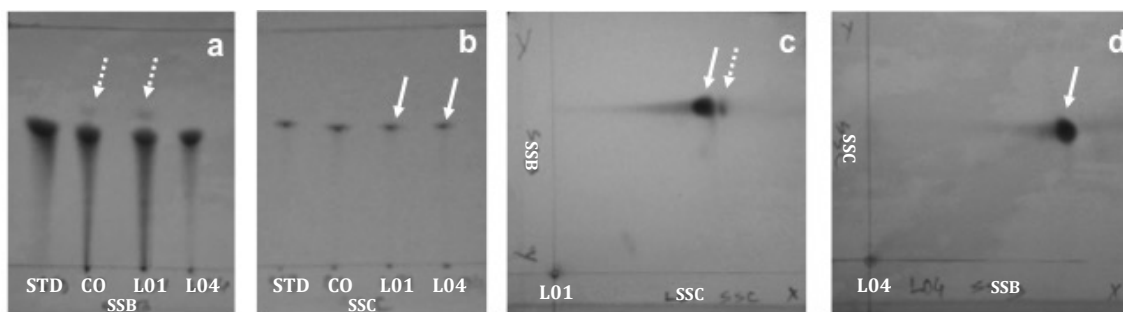


Figure 3. UV-light images of thin layer chromatography of extracts (2 µg/ml) of *C. stellaris*. One-dimensional TLC of samples separated in (a) solvent system B (SSB) and in (b) solvent system C (SSC). Samples applied at the bottom were: STD usnic acid (UA) reference, CO combination of crude acetone extracts L01, L04 and the reference UA, and the individual applications of L01 and L04. Upper lines indicate migration front of the mobile phase. Two-dimensional TLC (solvent systems 1st/2nd dimension = y/x) of (c) crude acetone extract of sample L01 (SSB/SSC); and of (d) recrystallized sample L04 (SSC/SSB). Application was at the lower left corner. Solid arrows point to UA, while hatched arrow is a minor component.

The purity of crude acetone extracts and samples after recrystallization were also obtained by GC-FID analysis (Fig. 4). Retention time of the main peak (14 min) coincided with that of the reference UA (not shown). Impurities in the crude sample (Fig. 4a) were evident as several minor peaks (15-19 min); in the analysis of the recrystallized sample these impurities are largely absent (Fig. 4b). Impurities are likely minor compounds previously reported (i.e. OA, PA) or other unknown compounds. Overall, the acetone extracts were evaluated as enriched with regard to UA. Recrystallization served as a refining step to obtain the pure substance.

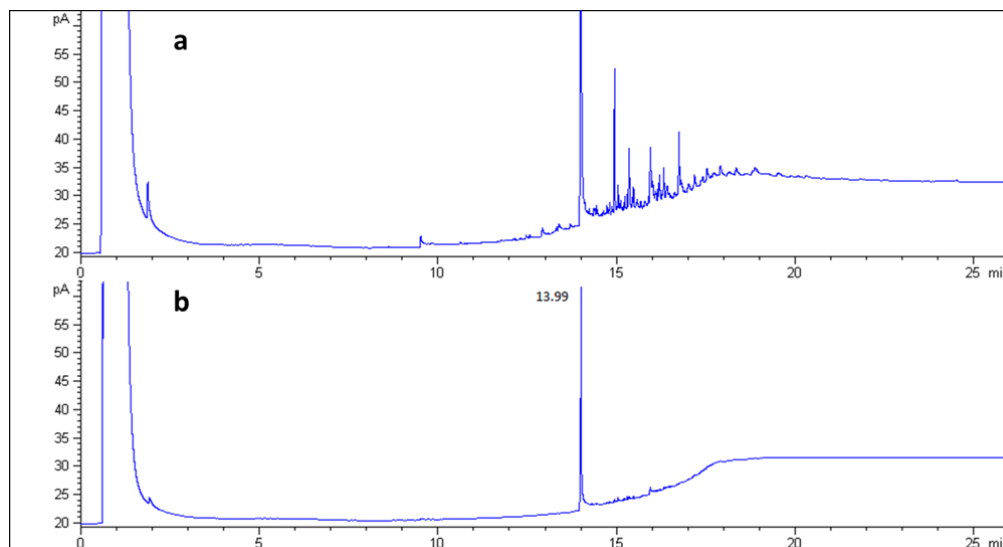


Figure 4. Gas chromatographic analysis of acetone-extracted material (2.3 µg/µl) of *C. stellaris* (a) before, and (b) after recrystallization. The usnic acid peak is at 13.99 min; in panel a, the max peak height was 240 pA, but the FID response axes are at same scale to enhance the detail of minor peaks.

Substance identity and purity a crystallized sample was assessed through NMR spectroscopy. The ^{13}C -spectrum (Fig. 5) demonstrated the presence of an 18-carbon atom structure with shift values consistent with those of UA (Table 3). Moreover, the ^1H NMR also gave shift values consistent with those previously reported by Huneck & Yashimura (1996) and Ataley and co-workers (2011) (Table 3).

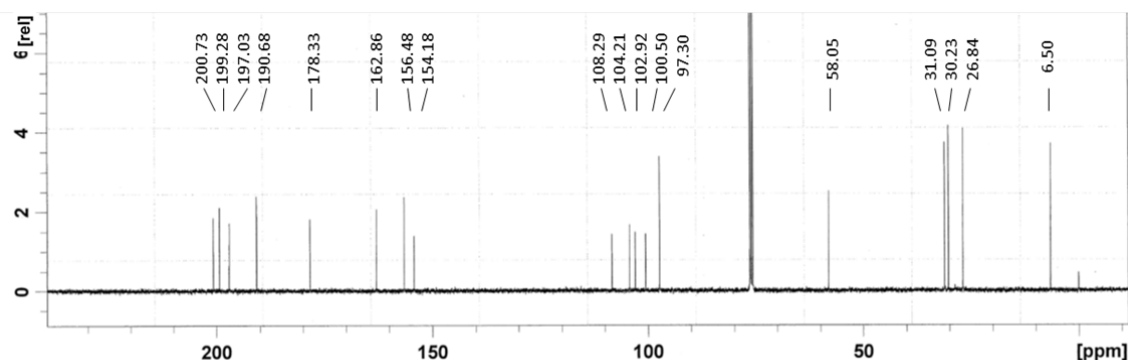


Figure 5. ^{13}C -NMR spectrum of recrystallized usnic acid obtained from an acetone extract of *C. stellaris*.

Table 3. Shift values of ^{13}C and ^1H NMR of a re-crystallized sample from *C. stellaris*, compared to literature data.

Crystallized Sample L04		Huneck & Yoshimura 1996		Atalay et al., 2011	
^{13}C 100MHz	^1H 400MHz	^{13}C 22.63 MHz	^1H 400MHz	^{13}C 100 MHz	^1H 400MHz
200.7	1.68 (3H,s,Me-13)	201.3	1.75 (3H,s, Me-13)	203.7	1.76 (3H,s, Me-13)
199.3	2.02 (3H,s,Me-16)	200.1	2.10 (3H,s,Me-16)	202.3	2.10 (3H,s,Me-16)
197.0	2.58 (3H,s,Me-15)	198.2	2.66 (3H,s,Me-15)	200.0	2.66 (3H,s,Me-15)
190.7	2.60 (3H,s,Me-18)	179.4	2.67 (3H,s,Me-18)	193.7	2.67 (3H,s,Me-18)
178.3	5.90 (1H,s,H-4)	164.1	5.92 (1H,s,H-14)	181.3	5.98 (1H,s,H-14)
162.9	10.94 (1H,s,C-10-OH)	157.6	11.02 (1H,s,C-10-OH)	165.9	11.03 (1H,s,C-10-OH)
156.5	13.22 (1H,s,C-8-OH)	155.1	13.31 (1H,s,C-8=H)	159.5	
154.2		109.5	18.84 (1H,s,C-3-OH)	157.2	
108.3		105.4		111.3	
104.2		104.2		107.2	
103.0		101.7		105.9	
100.5		99.8		103.5	
97.3		98.3		100.3	
58.1		59.2		61.1	
31.1		32.0		34.1	
30.2		30.9		33.2	
26.8		27.4		29.8	
6.5		7.7		9.5	

Although known from long-time use in folk medicine, commercial production and biological role of UA from *C. stellaris* has not yet been realized and presently UA is extracted from other sources (e.g. *Usnea barbata*). Several investigators have reported the possibility to produce UA from *C. stellaris* as an active ingredient or excipient to be used in medicinal and drug-production. In a recent study, Russian scientists described the preparation of Cladosent, which exhibits radio-protective properties based on ground fronds of *C. stellaris* (Polovinka et al., 2012). Lichen extracts containing UA are marketed in Germany with the trade name Omnigran A, Granobil and Usnagren A and T for cosmetics and pharmaceuticals (Frankos, 2005). Some other commercially available selected combination products of UA are Ab-solution®, Zeta N®, LipoKinetix® (withdrawn from the market) (Usnea; health.naturalstandard.com, 2015). UA is known to cause liver damage, therefore until sufficient documentation on safety become available, its use, other than dermatologically, is still limited (Guo et al., 2008).

This study shows that pure (-) UA can be readily extracted from *C. stellaris* growing in areas that are currently under harvesting management for ornamental lichen. The abundant lichen contains UA in levels comparable to current commercial sources of UA from *Usnea*-species. The use of (-) UA in health formulations and as a drug may become more attractive as data on its bioactivity and safety accumulates.

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REFERENCES

- Atalay F, Halici MB, Mavi A, et al. Antioxidant phenolics from *Lobaria pulmonaria* (L.) Hoffm. and *Usnea longissima* Ach. lichen species. *Turkish Journal of Chemistry*. 2011;35(4):647-661.
- Bačkorová M, Jendželovský R, Kello M, Bačkor M, Mikeš J, Fedoročko P. Lichen secondary metabolites are responsible for induction of apoptosis in HT-29 and A2780 human cancer cell lines. *Toxicology in Vitro*. 2012;26(3):462-468.
- Bezivin C, Tomasi S, Lohezic-Le Devehat F, Boustie J. Cytotoxic activity of some lichen extracts on murine and human cancer cell lines. *Phytomedicine*. 2003;10(6):499-503.
- Bjerke JW, Dahl T. Distribution patterns of usnic acid-producing lichens along local radiation gradients in West Greenland. *Nova Hedwigia*. 2002;75(3-4):3-4.
- Burkin A, Kononenko G, Tolpysheva T. Enzyme immunoassay of usnic acid in lichens. *Prikladnaia biokhimiia i mikrobiologiia*. 2012;49(3):322-328.
- Ciešla Ľ, Waksmundzka-Hajnos M. Two-dimensional thin-layer chromatography in the analysis of secondary plant metabolites. *Journal of Chromatography A*. 2009;1216(7):1035-1052.
- Cladonia stellaris*. <https://www.flickr.com/photos/sparkleice/4692623233/>. Accessed 08/01/2015, 2015.
- Cocchietto M, Skert N, Nimis P, Sava G. A review on usnic acid, an interesting natural compound. *Naturwissenschaften*. 2002;89(4):137-146.
- Culberson CF. Improved conditions and new data for identification of lichen products by standardized thin-layer chromatographic method. *Journal of Chromatography A*. 1972;72(1):113-125.
- Einarsdóttir E, Groeneweg J, Björnsdóttir GG, et al. Cellular mechanisms of the anticancer effects of the lichen compound usnic acid. *Planta medica*. 2010;76(10):969-974.
- Falk A, Green TK, Barboza P. Quantitative determination of secondary metabolites in *Cladonia stellaris* and other lichens by micellar electrokinetic chromatography. *Journal of Chromatography A*. 2008;1182(1):141-144.
- Frankos V. NTP nomination for usnic acid and *Usnea barbata* herb. Food and Drug Administration, Division of Dietary Supplement Programs. http://ntp.niehs.nih.gov/ntp/htdocs/ChemBackground/ExSu_mPdf/UsnicAcid.pdf. 2005.
- Guo L, Shi Q, Fang J-L, et al. Review of usnic acid and *Usnea barbata* toxicity. *Journal of Environmental Science and Health, Part C*. 2008;26(4):317-338.
- Hauck M, Jürgens SR. Usnic acid controls the acidity tolerance of lichens. *Environmental Pollution Environmental Pollution*. 2008;156(1):115-122.
- Hauck M, Jürgens SR, Willenbruch K, Huneck S, Leuschner C. Dissociation and metal-binding characteristics of yellow lichen substances suggest a relationship with site preferences of lichens. *Annals of Botany*. 2009;103:13-22.
- Hawranik DJ, Anderson KS, Simmonds R, Sorensen JL. The chemoenzymatic synthesis of usnic acid. *Bioorganic and medicinal chemistry letters*. 2009;19:2383-2385.
- Huneck S, Yoshimura I. *Identification of lichen substances*: Springer; 1996.
- Huovinen K. Variation of lichen acids in *Cladonia stellaris* and *Cladonia rangiferina* in Finland and North Norway. *Acta Pharmaceutica Fennica*. 1985;94:113-123.
- Ingólfssdóttir K, Chung GA, Skúlason VG, Gissurason SR, Vilhelmsdóttir M. Antimycobacterial activity of lichen metabolites in vitro. *European Journal of Pharmaceutical Sciences*. 1998;6(2):141-144.
- Ingólfssdóttir K. Usnic acid. *Phytochemistry*. 2002;61(7):729-736.
- Kauppi M. The exploitation of *Cladonia stellaris* in Finland. *The Lichenologist*. 1979;11(01):85-89.
- Kinoshita Y, Yamamoto Y, Yoshimura I. Distribution of optical isomers of usnic and isousnic acids analyzed by high performance liquid chromatography. *Journal of the Hattori Botanical Laboratory*. 1997:173-178.
- Lucarini R, Tozatti MG, de Oliveira Salloum AI, et al. Antimycobacterial activity of *Usnea steineri* and its major constituent (+)-usnic acid. *African Journal of Biotechnology*. 2014;11(20):4636-4639.
- Mayo DW, Pike RM, Forbes DC. *Microscale organic laboratory: with multistep and multiscale syntheses*: John Wiley & Sons; 2010.
- McEvoy M, Nybakken L, Solhaug KA, Gauslaa Y. UV triggers the synthesis of the widely distributed secondary lichen compound usnic acid. *Mycology Progress*. 2006;5:221-229.
- Melgarejo M, Sterner O, Vila Castro J, Mollinedo P. More investigations in potent activity and relationship structure of the lichen antibiotic (+)-usnic acid and its derivative dibenzoylusnic acid. *Revista Boliviana de Química*. 2008;25:24.
- Moiseeva E. : Biokhimičeskoe svoistva lishainikov i ikh praktičeskoe značenie (Biochemical Properties of Lichens and their Applied Significance), Moscow, Leningrad: USSR Academy of sciences Press; 1961.
- Muggia L, Schmitt I, Grube M. Lichens as treasure chests of natural products. *Sim News*. 2009;59(3):85-97.
- Nash TH. *Lichen biology*, 2nd ed: Cambridge University Press; 2008.
- Nybakken L, Julkunen-Tiitto R. UV-B induces usnic acid in reindeer lichens. *The Lichenologist*. 2006;38(5):477-485.
- Polovinka M, Komarova N, Korchagina D, et al. Secondary metabolites of the lichen *Cladonia stellaris*. *Chemistry of Natural Compounds*. 2012;48(3):392-395.
- Sadowsky A, Hussner A, Ott S. Submersion tolerance in a habitat of *Stereocaulon paschale* (Stereocaulaceae) and *Cladonia stellaris* (Cladoniaceae) from the high mountain region Rondane, Norway. *Nova Hedwigia*. 2012;94(3-4):323-334.
- Shrestha G, Clair LLS. Lichens: a promising source of antibiotic and anticancer drugs. *Phytochemistry Reviews*. 2013;12(1):229-244.
- Smeds AI, Kytöviita M-M. Determination of usnic and perlatolic acids and identification of olivetoric acids in Northern reindeer lichen (*Cladonia stellaris*) extracts. *The Lichenologist*. 2010;42(06):739-749.
- Tolpysheva TY. Mycotoxins and usnic acid and their distribution in lichens belonging to the genera *Cetraria*, *Flavocetraria*, and *Cladonia*. *Moscow University Biological Sciences Bulletin*. 2014;69(3):125-129.
- Tomasi S, Picard S, Lainé C, Babonneau V, Goujeon A, Boustie J, Uriac P. Solid-phase synthesis of polyfunctionalized natural products: Application to usnic acid, a bioactive lichen compound. *Journal of Combinatorial Chemistry*. 2006;8:11-14.
- Usnea*. Monograph for Herbal and Medicine <http://www.health.naturalstandard.com/index-abstract.asp?create-abstract=patient-usnea.asp&title=Usnea>.
- Weast R, Astle M, Beyer W. *CRC Handbook of Chemistry and Physics* CRC, Boca Raton (1986), p. F164.
- Yarranton G. Population growth in *Cladonia stellaris* (Opiz.) Pouz. and Vezda. *New phytologist*. 1975;75(1):99-110.