The Lichenologist (2020), **52**, 397–401
doi:10.1017/S0024282920000316

CAMBRIDGE UNIVERSITY PRESS

Standard Paper

Optimization of usnic acid extraction conditions using fractional factorial design

Agnieszka Galanty[1], Paweł Paśko[2], Irma Podolak[1] and Paweł Zagrodzki[2]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-688 Kraków, Poland and [2]Department of Food Chemistry and Nutrition, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-688 Kraków, Poland

Abstract

Usnic acid is a unique lichen metabolite of industrial importance, widely studied to explore its pharmacological potential and valued especially as an antibacterial agent in cosmetics. Although a vast number of papers describe usnic acid extraction from various lichen species, none has so far provided an unequivocal indication of the best extraction procedure for this compound. Thus, the current study was focused on the direct comparison of three commonly used usnic acid extraction methods (heat reflux, shaking, ultrasound-assisted extractions), which were optimized using fractional factorial design. Heat reflux extraction, shaking extraction and ultrasound-assisted extraction were first optimized in a series of experiments using fractional factorial design, with respect to three parameters: the extraction time, the solvent used and the number of extraction repetitions. HPLC was employed for usnic acid quantitative analysis. The best scores for each extraction method were statistically compared and the optimal conditions were indicated. The optimal set of parameters for usnic acid was established to be a single, 60 min heat reflux extraction with acetone. This extraction scheme provided 4.25 ±0.08 mg g^{-1} d.w. of usnic acid, while for ultrasound-assisted and shaking extractions the amount was two- or even four times lower (2.33 ±0.17 and 0.97 ±0.08 mg g^{-1} d.w., respectively). The optimal procedure for usnic acid extraction described here may be suitable for effective acquisition of this compound for scientific research purposes, but also for applications in the pharmaceutical or cosmetic industries.

Key words: extraction, fractional factorial design, lichen, optimization, usnic acid

(Accepted 17 May 2020)

Introduction

Usnic acid (2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyl-1,3(2H,9bH)-dibenzo-furandione) is a structurally unique, yellow, lipophilic metabolite found selectively in lichens. The biological and pharmacological properties of usnic acid are well documented and comprise antibacterial, antiviral, anti-inflammatory, analgesic, antiprotozoal or insecticidal activities (Galanty *et al.* 2019). The compound has been particularly studied for its anticancer potential and the results seem to be very promising, with a marked activity reported both *in vitro* and *in vivo* (Araújo *et al.* 2015). Moreover, usnic acid has been extensively used by the cosmetic industry, as an antiperspirant or an additive in toothpastes (Ingolfsdottir 2002). Some recent reports also indicate its photoprotective (Varol *et al.* 2015) and wound healing (Pagano *et al.* 2019) properties, when applied externally. Taken together, these attributes of usnic acid, its wide distribution in nature in significant amounts of up to 5% (Galanty *et al.* 2019), and an easy isolation procedure (Ingolfsdottir *et al.* 1998; Lohezic Le Devehat *et al.* 2007; Galanty *et al.* 2017), make this lichen metabolite an attractive subject of research.

Even though there are many reports describing the extraction of usnic acid from natural sources by different methods (König & Wright 1999; Yilmaz *et al.* 2004; Roach *et al.* 2006; Burlando *et al.* 2009; Einarsdottir *et al.* 2010; Honda *et al.* 2010; Brovko *et al.* 2017; Piska *et al.* 2018), they do not provide reliable information on the most effective conditions. This discrepancy and the incompleteness of data concerning the extraction parameters for usnic acid have motivated us to explore this issue in more detail.

Fractional factorial designs are important statistical tools which efficiently allow the influence of several relevant factors to be controlled and varied simultaneously on a planned experiment, in order to obtain the assumed goal in the most effective way. These tools are widely used in monitoring experiments in different research fields, as they can significantly shorten the total time of the experiment, as well as indicate the most effective procedure (Gunst & Mason 2009). Fractional factorial experiments have so far rarely been used for the optimization of lichen metabolite extraction, with the exception of lepraric acid, erythrin (Parrot *et al.* 2015) or diploicine, norstictic and variolaric acids (Bonny *et al.* 2009).

Thus, the aim of the present study was to optimize the extraction conditions for usnic acid using experiments with a fractional factorial design in order to investigate the influence of a number of parameters on the efficacy of the particular extraction method.

For this purpose, we chose *Cladonia arbuscula* (Wallr.) Flot. as a lichen material containing usnic acid and compared different types of extractions: heat reflux extraction in a hot water bath,

Author for correspondence: Agnieszka Galanty. E-mail: agnieszka.galanty@uj.edu.pl
Cite this article: Galanty A, Paśko P, Podolak I and Zagrodzki P (2020) Optimization of usnic acid extraction conditions using fractional factorial design. *Lichenologist* **52**, 397–401. https://doi.org/10.1017/S0024282920000316

dynamic shaking extraction and ultrasound-assisted extraction. For each type of extraction three parameters were selected for comparative purposes: the time of extraction, the solvent used and the number of extraction repetitions. Then, the influence of these parameters was investigated and the most effective conditions for each method were compared with each other to establish the optimal recovery of usnic acid from the lichen.

Material and Methods

Lichen material, chemicals and instrumentation

Cladonia arbuscula subsp. *squarrosa* (Wallr.) Ruoss was collected in July 2015, from northern Poland in dry, non-coastal Scots pine forests; its identity was verified by one of the authors (AG). The voucher specimen was deposited in the herbarium of the Department of Pharmacognosy JU MC (Ref. no. KFg/2015/L3). All samples used during the analysis were prepared from lichen material collected from the one location. The lichen material was dried in the dark at room temperature and cleaned to remove impurities. Before analysis, whole lichen thalli (both upper and lower parts) were ground into small pieces in a mortar to provide uniformity and homogeneity of the tested material. Chloroform, acetone, methanol and water of HPLC grade were obtained from Sigma-Aldrich (Germany) and orthophosphoric acid (98%) from POCh Gliwice (Poland). Usnic acid standard of analytical grade was obtained from Sigma-Aldrich (Germany). A water bath LWT (WSL Poland) and ultrasonic bath Sonic 3 (Polsonic, Poland) were used for heat reflux and ultrasound-assisted extraction, respectively. For dynamic shaking extraction, a laboratory shaker was used (type 3585; ELPAN, Poland).

Usnic acid quantitative determination was performed using a Dionex high-performance liquid chromatography (HPLC) system, with PDA detector, on a C-18 column (5 µm, 250 × 4.6 mm), at 25 °C. The mobile phase consisted of 1% H_3PO_4 A – methanol B, (A:B 15:85, v/v). Detection was carried out at 240 nm. Quantification of usnic acid was achieved by measuring the peak area with regards to the appropriate standard curve. Details of the analysis were described previously (Studzińska-Sroka *et al.* 2019).

Sample preparation and extraction

Aliquots of 0.2 g each of the ground lichen thalli were transferred into glass round-bottomed flasks, tightly closed and kept in darkness until needed for further extraction procedures. For a single extraction, 20 ml of solvent was used.

Heat reflux extraction was conducted on a water bath (90 °C). Dynamic shaking and ultrasonic-assisted extractions were conducted at room temperature. Each combination of the experimental parameters (one of nine) was tested in six replicates. For heat reflux and shaking extractions, three different times were applied, namely 15, 30 and 60 min, while ultrasound-assisted extraction times were 10, 20 and 30 min. Acetone, chloroform and methanol were used as solvents. To investigate the influence of the number of extraction repetitions on its efficacy, a single sample was single-, double- or triple-extracted. This was carried out by treating the sample only once with the solvent (1 × 20 ml) or by repeating the extraction twice (2 × 20 ml) or three times (3 × 20 ml).

After extraction, the obtained extracts were transferred into 10 ml volumetric flasks and the usnic acid content was analyzed by high-performance liquid chromatography (HPLC). Before the analysis, extracts were filtered through the 0.45 µm membrane filters into the HPLC 1.5 ml vials.

Experimental design and statistical approach

To optimize the extraction procedure for usnic acid, the three different extraction methods mentioned above were compared. Within each chosen method three parameters were considered: 1) the time of extraction, 2) the solvent used, 3) the number of extraction repetitions from a single sample. In order to investigate the impact of the experimental parameters on the effectiveness of extraction, an experimental planning method was used (Dobrowolska *et al.* 2016). This was achieved by using the fractional factorial design of experiments: 3^{3-1} in which 1/3 of the full 3^3 design was selected, with 9 different combinations of the chosen factors (parameters), resulting in 9 trials. The scheme of factor coding and the plan of the experiment with the original parameters and their levels are shown in Table 1. We purposefully used different numbers of extraction repetitions from a single sample (1, 2, or 3), treating it as one (potentially) significant factor (i.e. independent parameter on three levels) and according to the plan of the experiment. Similarly, we tested three levels of extraction time, using slightly milder conditions (shorter time periods), for ultrasound-assisted extraction because of the risk of degradation of usnic acid, but the essential scheme of the experiment was always preserved. The amount of usnic acid in each of the tested samples was calculated as mg per gram dry weight (mg g^{-1} d.w.) of the lichen material used, and then the best factorial set was selected for each extraction method.

First, the outlying values of usnic acid concentration, if they appeared in a particular factor (parameter) combination, were removed as outliers. For this purpose, we used the software 'Extreme Outlier' (MP System Co., Chrzanów, Poland), with an algorithm implemented by Shoemaker and based on a robust technique proposed by Tukey (Shoemaker 2018). Altogether we found 10 outliers out of 162 results (6%). Then, the results obtained for each type of extraction were compared using an ANOVA test with a Tukey post-hoc test (using the package STATISTICA v.13; StatSoft, Tulsa, USA) to identify the best (i.e. biggest) two extraction results. The same procedure was applied to ultimately identify the best type of extraction and the best combination of parameters from the six results (two best scores for each tested extraction type) previously obtained. Differences with $P < 0.05$ were considered to be statistically significant.

Results and Discussion

Quantitative analyses of usnic acid content in various lichen species have been performed using several different extraction methods. The results of these studies are difficult to compare because the analytical conditions vary substantially, often depending on the research goal, and giving inaccurate information on the real content of usnic acid in the given lichen material. Thus, the aim of our study was to explore what would be the most effective, optimal extraction conditions for obtaining usnic acid for its further use in pharmacological studies. For this purpose, we compared three different extraction methods, and within each of these methods the influence of the selected three parameters on the extraction efficacy was investigated. A fractional factorial design was applied for the first time to study usnic acid extraction optimization. The results obtained are shown in Table 2.

https://doi.org/10.1017/S0024282920000316 Published online by Cambridge University Press

Table 1. Optimization of usnic acid extraction conditions, from *Cladonia arbuscula*, using experiments with a fractional factorial design: parameter values and coded sets of experimental conditions.

	Time of extraction (min)		Numer of extraction repetitions		Solvent	
Set number	parameter value	code	parameter value	code	parameter value	code
1	HRE, SE: 15 UAE: 10	−1	1	−1	chloroform	−1
2	HRE, SE: 15 UAE: 10	−1	2	0	methanol	0
3	HRE, SE: 15 UAE: 10	−1	3	1	acetone	1
4	HRE, SE: 30 UAE: 20	0	1	−1	methanol	0
5	HRE, SE: 30 UAE: 20	0	2	0	acetone	1
6	HRE, SE: 30 UAE: 20	0	3	1	chloroform	−1
7	HRE, SE: 60 UAE: 30	1	1	−1	acetone	1
8	HRE, SE: 60 UAE: 30	1	2	0	chloroform	−1
9	HRE, SE: 60 UAE: 30	1	3	1	methanol	0

HRE = heat reflux extraction; SE = shaking extraction; UAE = ultrasound assisted extraction

For both tested conventional extractions (heat reflux, shaking) the same set provided the best scores, that is 60 minutes acetone extraction, but the results for heat reflux extraction were four times higher (4.25 ±0.08 mg g^{-1} d.w.) than shaking extraction (0.97 ±0.08 mg g^{-1} d.w.). Such a large variation is probably a consequence of the different temperature conditions for both extractions. This is an additional important observation, emphasizing the influence of temperature on usnic acid extraction. The highest amount of extracted usnic acid was observed for the sets with a single extraction, which means that the samples obtained by different numbers of repetitions did not differ statistically. This result was surprising as it is a common practice, in many pharmaceutical or ecological studies, to carry out two or three consecutive repetitions of the extraction process.

For ultrasound-assisted extraction the best score was achieved for the set with two repetitions of extraction, 20 minutes each, with acetone as a solvent (2.33 ±0.17 mg g^{-1} d.w.); however, the effect did not differ significantly from the set with a single, 10 minute extraction using the same solvent (2.27 ±0.13 mg g^{-1} d.w.). Notably, the amount of usnic acid obtained by ultrasound-assisted extraction was about two times lower (2.33 ±0.17 mg g^{-1} d.w.) when compared to heat reflux extraction (4.25 ±0.08 mg g^{-1} d.w.), $P < 0.05$. The two highest results of each method were finally compared, proving that the optimal conditions were for heat reflux extraction with one repetition and acetone as the solvent ($P < 0.05$).

The methods so far commonly used by various authors for usnic acid extraction have been mainly conventional techniques, with heat reflux (König & Wright 1999; Piska *et al.* 2018), Soxhlet (Einarsdottir *et al.* 2010; Honda *et al.* 2010) or shaking (Yilmaz *et al.* 2004; Roach *et al.* 2006) extractions, while a green technology approach was represented by ultrasound-assisted (Burlando *et al.* 2009; Brovko *et al.* 2017) or supercritical fluid (Brovko *et al.* 2017) extractions. It is interesting to note that these methods are most often used when analytical aspects of usnic acid or its pharmacological activity are of interest, while in the majority of ecologically directed studies, a simple, room-temperature maceration is a common approach (Nybakken *et al.* 2010; Asplund *et al.* 2017). As the extraction times applied by different authors varied to a large extent, from several minutes to hours, in our study we opted for 15, 30 and 60 minutes for heat reflux and shaking extractions, and 10, 20 and 30 minutes for ultrasound-assisted extraction. The latter choice was based on

Table 2. Optimization of usnic acid extraction conditions, from *Cladonia arbuscula*, using experiments with a fractional factorial design: scores for all sets of usnic acid extraction parameters (see Table 1 for parameter values and experimental conditions for each set).

	Usnic acid content (mg g^{-1} dry weight), mean ± SD		
Set number	heat reflux extraction	shaking extraction	ultrasound assisted extraction
1	2.37 ±0.10^{a}	0.66 ±0.02^{a}	1.89 ±0.16^{a}
2	3.23 ±0.05^{b}	0.54 ±0.01^{b}	1.79 ±0.06^{a}
3	3.37 ±0.22^{b}	0.68 ±0.01^{a}	2.27 ±0.13^{b}
4	2.53 ±0.17^{a}	0.56 ±0.07^{b}	1.96 ±1.13^{a}
5	3.69 ±0.04^{c,d}	0.59 ±0.02^{b}	2.33 ±0.17^{b}
6	3.47 ±0.08^{b,c}	0.43 ±0.01	1.83 ±0.14^{a}
7	4.25 ±0.08^{e}	0.97 ±0.08	1.05 ±0.05^{c}
8	4.00 ±0.14^{d,e}	0.66 ±0.02^{a}	1.30 ±0.21^{c}
9	3.46 ±0.16^{b,c}	0.82 ±0.02	0.62 ±0.10

When comparing the results, values with different letters (in superscript) differ significantly for columns only.

https://doi.org/10.1017/S0024282920000316 Published online by Cambridge University Press

our previous preliminary investigation, indicating that exposure to sonication exceeding 30 minutes results in usnic acid degradation (data not shown). As far as the solvents are concerned, data from the literature indicate that hexane, chloroform, acetone or methanol were used most often (Yilmaz *et al.* 2004; Bomfim *et al.* 2009; Behera *et al.* 2012), even though the lipophilic nature of usnic acid together with some experimental data do not support the use of methanol (Stark *et al.* 1950; Podterob 2008). The results of our study also confirmed that methanol is not suitable for effective usnic acid extraction.

The results of our study, indicating a significant advantage of heat reflux extraction over the other two tested methods, do not question the reliability of previously published studies on the content of usnic acid in different lichen species. The discrepancy in usnic acid levels determined in our study and those obtained by other authors may result from many factors, including the differences in preparation of lichen material (degree of grinding) but also the geographical site of its collection. For example, samples of pulverized *C. arbuscula* from Finland, extracted by homogenization with acetone at room temperature, were found to contain 2.1% of usnic acid (Nybakken & Julkunen-Tiitto 2006), while in a consecutive study by the same authors on 'pieces of *C. arbuscula*' collected in Norway, extracted with acetone at room temperature (2 × 20 min), only 0.3% of usnic acid was reported, which is an order of magnitude lower (Nybakken *et al.* 2010). Moreover, in a study by Falk *et al.* (2008) on different samples of *C. arbuscula* collected in Alaska, usnic acid levels ranged from 0.29 to 1.1%; the lichen material was ground in a mortar and extracted by ultrasound-assisted extraction with acetone, followed by 24 hours maceration at room temperature. In our previous unpublished experiments, a direct comparison of usnic acid yield from pulverized and ground *C. arbuscula* indicated significant differences with higher yields from the latter (data not shown).

Conclusions

With the support of fractional factorial design, we have shown that the optimal approach for the most effective extraction of usnic acid from lichen material is a single 60 minute heat reflux method with acetone. This may help substantially in designing the best procedure for the acquisition of usnic acid from lichen material, not only for pharmaceutical research purposes but also for further applications in the cosmetic industry. A simpler, but experimentally optimized, procedure should also be designed in the future for the purposes of ecological experiments. The use of a comparable extraction protocol would undoubtedly help to evaluate the results obtained by various authors within each area of expertise.

However, further studies are needed to examine other factors that might influence the effectiveness of usnic acid extraction, namely the degree of grinding of lichen thalli or the temperature during the process.

Acknowledgements. This work was supported by grants from the Polish Ministry of Science and Higher Education, project N42/DBS/000045.

Author ORCIDs. Agnieszka Galanty, 0000-0001-5636-8646.

References

Araújo AAS, De Melo MGD, Rabelo TK, Nunes PS, Santos SL, Serafini MR, Quintans-Junior LJ and Gelain DP (2015) Review of the biological properties and toxicity of usnic acid. *Natural Product Research* **29**, 2167–2180.

Asplund J, Siegenthaler A and Gauslaa Y (2017) Simulated global warming increases usnic acid but reduces perlatolic acid in the mat-forming terricolous lichen *Cladonia stellaris*. *Lichenologist* **49**, 269–274.

Behera BC, Mahadik N and Morey M (2012) Antioxidative and cardiovascular-protective activities of metabolite usnic acid and psoromic acid produced by lichen species *Usnea complanata* under submerged fermentation. *Pharmaceutical Biology* **50**, 968–979.

Bomfim RR, Araújo AAS, Cuadros-Orellana S, Melo MGD, Quintans-Júnior LJ and Cavalcanti SCH (2009) Larvicidal activity of *Cladonia substellata* extract and usnic acid against *Aedes aegypti* and *Artemia salina*. *Latin American Journal of Pharmacy* **28**, 580–584.

Bonny S, Hitti E, Boustie J, Bernard A and Tomasi S (2009) Optimization of a microwave-assisted extraction of secondary metabolites from crustose lichens with quantitative spectrophotodensitometry analysis. *Journal of Chromatography A* **1216**, 7651–7656.

Brovko OS, Ivakhnov AD, Palamarchuk IA and Boitsova TA (2017) Supercritical fluid extraction of usnic acid from lichen of *Cladonia* genus. *Russian Journal of Physical Chemistry B* **11**, 1306–1311.

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

Dobrowolska J, Zagrodzki P, Woźniakiewicz M, Woźniakiewicz A, Zwolińska M, Winnicka D and Paśko P (2016) Procedure optimization for extracting short-chain fatty acids from human faeces. *Journal of Pharmaceutical and Biomedical Analysis* **124**, 337–340.

Einarsdottir E, Groeneweg J, Björnsdottir GG, Harðardottir G, Omarsdottir S, Ingolfsdottir K and Ögmundsdottir HM (2010) Cellular mechanisms of the anticancer effects of the lichen compound usnic acid. *Planta Medica* **76**, 1–6.

Falk A, Green TK and Barboza P (2008) Quantitative determination of secondary metabolites in *Cladina stellaris* and other lichens by micellar electrokinetic chromatography. *Journal of Chromatography A* **1182**, 141–144.

Galanty A, Koczurkiewicz P, Wnuk D, Paw M, Karnas E, Podolak I, Węgrzyn M, Borusiewicz M, Madeja Z, Czyż J, *et al.* (2017) Usnic acid and atranorin exert selective cytostatic and anti-invasive effects on human prostate and melanoma cancer cells. *Toxicology in Vitro* **40**, 161–169.

Galanty A, Paśko P and Podolak I (2019) Enantioselective activity of usnic acid: a comprehensive review and future perspectives. *Phytochemistry Reviews* **18**, 527–548.

Gunst RF and Mason RL (2009) Fractional factorial design. *WIREs Computational Statistics* **1**, 234–244.

Honda NK, Pavan FR, Coelho RG, de Andrade Leite SR, Micheletti AC, Lopes TIB, Misutsu MY, Beatriz A, Brum RL and Leite CQF (2010) Antimycobacterial activity of lichen substances. *Phytomedicine* **17**, 328–332.

Ingolfsdottir K (2002) Molecules of interest: usnic acid. *Phytochemistry* **61**, 729–736.

Ingolfsdottir K, Chung GAC, Skulason VG, Gissurarson SR and Vilhelmsdottir M (1998) Antimycobacterial activity of lichen substances *in vitro*. *European Journal of Pharmaceutical Sciences* **6**, 141–144.

König GM and Wright AD (1999) ^{1}H and ^{13}C-NMR and biological activity investigations of four lichen-derived compounds. *Phytochemical Analysis* **10**, 279–284.

Lohezic Le Devehat F, Tomasi S, Elix JA, Bernard A, Rouaud I, Uriac P and Boustie J (2007) Stictic acid derivatives from the lichen *Usnea articulata* and their antioxidant activities. *Journal of Natural Products* **70**, 1218–1220.

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

Nybakken L, Helmersen AM, Gauslaa Y and Selås V (2010) Lichen compounds restrain lichen feeding by bank voles (*Myodes glareolus*). *Journal of Chemical Ecology* **36**, 298–304.

Pagano C, Ceccarini MR, Calarco P, Scuota S, Conte C, Primavilla S, Ricci M and Perioli L (2019) Bioadhesive polymeric films based on usnic acid for burn wound treatment: antibacterial and cytotoxicity studies. *Colloids and Surfaces B* **178**, 488–499.

Parrot D, Peresse T, Hitti E, Carrie D, Grube M and Tomasi S (2015) Qualitative and spatial metabolite profiling of lichens by a LC–MS approach combined with optimised extraction. *Phytochemical Analysis* **26**, 23–33.

https://doi.org/10.1017/S0024282920000316 Published online by Cambridge University Press

Piska K, Galanty A, Koczurkiewicz P, Żmudzki P, Potaczek J, Podolak I and Pękala E (2018) Usnic acid reactive metabolites formation in human, rat, and mice microsomes. Implication for hepatotoxicity. *Food and Chemical Toxicology* **120**, 112–118.

Podterob AP (2008) Chemical composition of lichens and their medicinal applications. *Pharmaceutical Chemistry Journal* **42**, 582–588.

Roach JA, Musser SM, Morehouse K and Woo JY (2006) Determination of usnic acid in lichen toxic to elk by liquid chromatography with ultraviolet and tandem mass spectrometry detection. *Journal of Agriculture and Food Chemistry* **54**, 2484–2490.

Shoemaker J (2018) Robust outlier identification using SAS. [WWW document] URL http://www2.sas.com/proceedings/sugi24/Infovis/p161-24.pdf. [Accessed 3 November 2008].

Stark JB, Walter ED and Owens HS (1950) Method of isolation of usnic acid from *Ramalina reticulata*. *Journal of the American Chemical Society* **72**, 1819–1820.

Studzińska-Sroka E, Tomczak H, Malińska N, Wrońska M, Kleszcz R, Galanty A, Cielecka-Piontek J, Latek D and Paluszczak J (2019) *Cladonia uncialis* as a valuable raw material of biosynthetic compounds against clinical strains of bacteria and fungi. *Acta Biochimica Polonica* **66**, 597–603.

Varol M, Tay T, Candan M, Turk A and Koparal AT (2015) Evaluation of the sunscreen lichen substances usnic acid and atranorin. *Biocell* **39**, 25–31.

Yilmaz M, Türk AÖ, Tay T and 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* **59c**, 249–254.

https://doi.org/10.1017/S0024282920000316 Published online by Cambridge University Press