Phytochem Rev (2021) 20:609–630
https://doi.org/10.1007/s11101-020-09717-1

Usnic acid: from an ancient lichen derivative to promising biological and nanotechnology applications

D. C. S. Macedo · F. J. F. Almeida · M. S. O. Wanderley · M. S. Ferraz · N. P. S. Santos · A. M. Q. López · N. S. Santos-Magalhães · M. C. B. Lira-Nogueira

Received: 21 January 2020 / Accepted: 25 August 2020 / Published online: 10 September 2020

Abstract Among the various compounds of natural origin, usnic acid (UA) is one of the best studied. It has several pharmacological activities, standing out as an antimicrobial, antitumor, antiviral, and antiparasitic agent, and despite these relevant properties, it is a toxic molecule. In this context, research has driven the development of innovative alternatives, such as their encapsulation in controlled release systems, an attractive tool for pharmaceutical nanotechnology. These systems allow the active ingredient to be released at the optimal yield speed and reduce the dosing regimen. Consequently, they are able to increase therapeutic efficacy by minimizing side effects. Given the above, this paper presents a review of the literature on chemical and biological properties, analytical methods, mechanism of action and toxicology of UA, and discusses the use of nanotechnology as a tool to overcome the obstacles of its pharmacological application.

Keywords Usnic acid · Chemical and biological properties · Mechanisms of action · Nanotechnology

D. C. S. Macedo · F. J. F. Almeida · M. S. Ferraz · N. P. S. Santos · N. S. Santos-Magalhães (✉) · M. C. B. Lira-Nogueira (✉)
Keizo-Asami Immunopathology Laboratory (LIKA), Center for Biological Sciences (CCB), Federal University of Pernambuco (UFPE), Av. Prof. Moraes Rego, 1235, Cidade Universitária, Recife, PE 50670-901, Brazil
e-mail: nssm@ufpe.br

M. C. B. Lira-Nogueira
e-mail: mariane.lira@ufpe.br

M. S. O. Wanderley
Institute of Biological Sciences (ICB), Pernambuco State University (UPE), Recife, PE, Brazil

N. P. S. Santos · M. C. B. Lira-Nogueira
Academic Center of Vitória (CAV), Federal University of Pernambuco (UFPE), Vitória de Santo Antão, PE, Brazil

A. M. Q. López
Institute of Chemistry and Biotechnology, Federal University of Alagoas (UFAL), Maceió, AL, Brazil

Introduction

Modern science arises interest in the discovery of new molecules with attractive pharmacological applications, whether from natural sources or from chemical modifications from pre-existing molecules. Given the vast biodiversity, these bioactive molecules can be extracted from animals, plants, algae, bacteria and fungi (Xu et al. 2019). In this context, lichens are noteworthy, as they are basic living beings resulting from the symbiosis between fungi and algae and/or cyanobacteria. They have a wide geographical distribution, from the tropics to the poles, inhabiting the surface of rocks, soils and tree trunks. The attention

given to these organisms is due to their ability to produce attractive substances, such as hyphae-synthesized secondary metabolites, which are used as a defense and weather protection mechanism (Müller 2001). Such metabolites are classified into 3 bioenergetically related chemical groups: depsides, depsidones and dibenzofurans (Xavier-Filho et al. 2006), being usnic acid (UA) considered one of the most important biologically active lichen metabolites (Müller 2001).

The first reports of obtaining UA date from 1880 from the species *Usnea barbata*. Interest in the isolation of this molecule has driven its search in several lichen species such as *Usnea deffractas* (Müller 2001), *Lecanora pseudogangalevides* (Lumbsch 1995), *Usnea laevis* (Marcano et al. 1999), *Roccella montagnei* (Vijayakumar et al. 2000), *Sticta weigelii* (Piovano et al. 2000), *Cladonia substellata* (De Carvalho et al. 2005) and *Usnea longissima* (Odabasoglu et al. 2006). Huneck and Yoshimura (1996) published a rich and essential handbook, which contains valuable information about lichens.

According to Ingólfsdóttir et al. (1985), the therapeutic interest of UA was aroused after the use of lichens in folk medicine, since ancient times, in the fight against pulmonary tuberculosis and some skin diseases. Later, other significant biological activities were attributed to it, such as: anti-inflammatory, analgesic, antipyretic (Yamamoto et al. 1995), antifungal (Broska et al. 1996), antimicrobial (Lauterwein et al. 1995), antiparasitic (Fournet et al. 1997), antitumor (Kumar and Müller 1999), antiviral (Campanella et al. 2002), enzymatic inhibition (Huneck 1999) and gastroprotective (Odabasoglu et al. 2006).

Despite being an old molecule, used since the 1950s, (Bustinza 1952), the UA still arises great scientific interest, a fact confirmed by the progressive growth in the number of publications in the past years, as observed in Fig. 1, and until June 2020, 29 articles are already published.

Despite the growing interest in this lichenoid derivative, it is known that UA has significant toxic effects, mainly severe hepatotoxicity (Liu et al. 2012; Piska et al. 2018), but also allergic (Sheu et al. 2006; Pacheco et al. 2012) and teratogenic effects (Silva et al. 2017).

Our research group has expertise with relevant scientific production related to this lichen derivative and the UA encapsulation in nanosystems. In this sense, this paper aimed to present a literature review on this subject, emphasizing the chemical properties, analytical methods, biological properties and toxicological aspects. In addition, for the first time, the use of nanotechnology as a tool to optimize the biological properties of UA will be addressed, overcoming the obstacles of its pharmacological application.

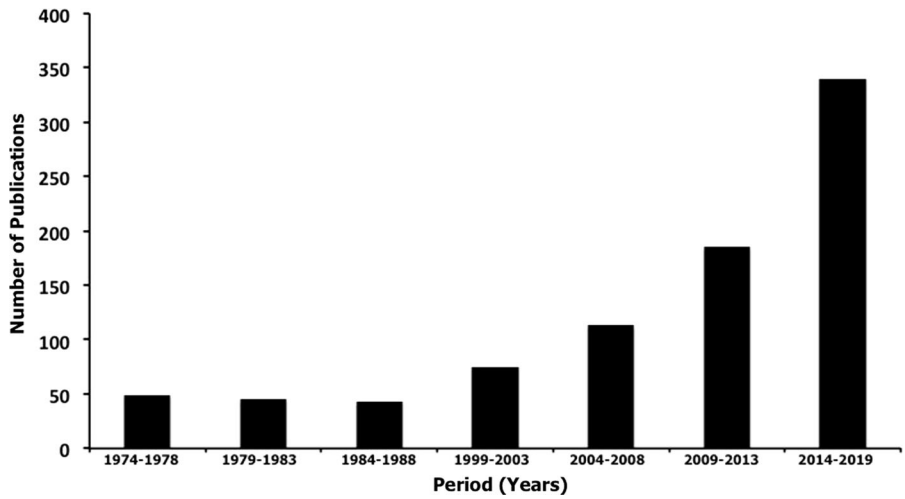

Fig. 1 Number of publications over the years, related to the keyword "usnic acid", based on a survey by the Scopus International Scientific Research Bank (www.scopus.com)

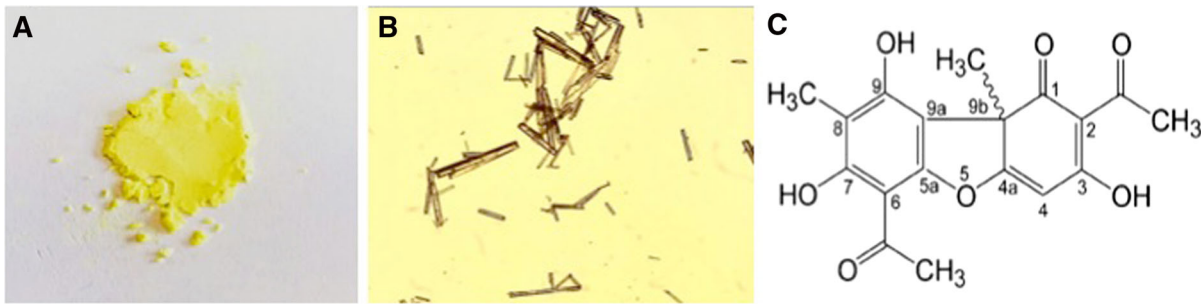

Fig. 2 Macroscopic aspect (**a**), microscopic aspect (optical microscopy 20 ×), and chemical structure of usnic acid (**c**)

Chemical properties of usnic acid

Usnic acid [UA = 2,6-diacetyl-7,9-dihydroxy-8-9b-dimethyl-1,3 (2H, 9b/aH) -dibenzofurandione; $C_{18}H_{16}O_7$, PM = 344.32] (Fig. 2c), is characterized by being a yellow-pigmented substance (Fig. 2a), occurring in two enantiomeric natural forms, (−) and (+), due to the angular projection of the methyl group located at position 9b (Cocchietto et al. 2002; Ingólfsdóttir 2002).

Both UA enantiomers present biological properties, but as reported by Galanty et al. (2019), the predominance of any of the enantiomers is still an open question.

This lichen derivative has a hydrophobic character, with water solubility of less than 10 mg/100 mL at 25 °C (Ingólfsdóttir 2002), being partially soluble in ethanol and easily without hot ether, acetone, benzene, and chloroform. This hydrophobic feature can be explained by the presence of the three-ketone groups, as well as the furan ring that joins the aromatic rings, as well as intramolecular hydrogen bridges (Müller 2001). Its acidity is justified by the presence of the phenolic ring, whose structure is unstable (Shibata 2000). The pKa value for the phenolic hydroxyl group at position 3 is 4.4, caused by the inductive effect of the ketone group at position 1. On the other hand, for the phenolic hydroxyl group at position 9, pKa is 8.8 due to the inductive effect caused by the para-acetyl group located at position 6. For the hydroxyl group located at position 7, pKa is 10.7, probably due to the intramolecular interaction through hydrogen bridges with the acetyl group at position 6 (Sharma and Jannke 1966; Kristmundsdóttir et al. 2002; Han et al. 2004).

Recently, Antonenko et al. (2019) showed that hydroxyls confer protonophoric properties to the UA molecule, so this lichen derivative forms UA-Ca-UA dimers with Ca^{2+} ion, and when complexed with Ca^{2+}, it promotes a dose-dependent response in the induction of electric current through the lipid bilayer. The author also observed that each hydroxyl is important for such a phenomenon, since the removal of any one of them leads to the reduction of the electric current induction.

According to the literature, UA biosynthesis proceeds via the polymalonate acetate pathway (Fig. 3). Initially, through aromatic synthetases, monocyclic phenolic units, which do not have dehydrogenated subunits, are formed, being originated from carboxylic acids derived from acetic acid, i.e. from acetyl-CoA

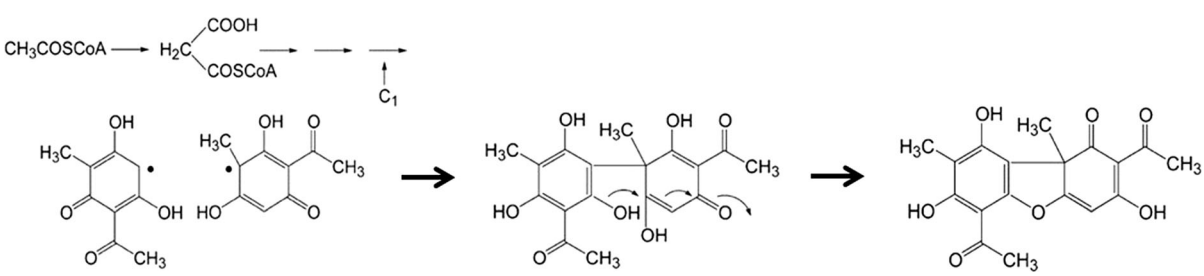

Fig. 3 Usnic acid biosynthesis pathway

and malonyl-CoA. The synthesis of methyl-fluoro-acetophenone (C1), a key intermediate for the formation of usnic acid, is catalyzed by the enzyme polyketidase synthase (Hawranik et al. 2009). Subsequent steps of its biosynthesis involve the stereospecific homologous coupling of two units of methyl-fluoro-acetophenone, thus yielding hydrated UA. Finally, dehydration of the molecule occurs, leading to the formation of the ether bond (Ingólfsdóttir 2002). To date, key methods for obtaining UA consist of lichen extractions using organic solvents and subsequent precipitation, usually using ethanol (Sokolov et al. 2012a).

In addition to the biosynthesis, the characterization of UA is widely described in the literature. Scientific documents describe analyzes using infrared (IR) spectrophotometry, proton nuclear magnetic resonance (H^1 NMR), thermal analysis, among other techniques. Characterization of the molecule by infrared spectrophotometry shows cyclic ketone grouping (1694 cm^{-1}), weak bands at 1716 and 1676 cm^{-1} referring to noncyclic ketone groupings n (C=O) and symmetrical and asymmetric aryl, alkyl bands ether (COC) close to 1288 and 1070 cm^{-1}, respectively (Lira et al. 2009a; Edwards et al. 2003). In this respect, the characterization of UA by proton nuclear magnetic resonance (H^1 NMR) confirmed characteristic peaks of the molecule such as hydroxyls at 13.406 and 11.396 ppm, aromatic ring proton at 6.25 ppm, and methyl group protons at 2.61, 2.04 and 2.50 ppm at positions 14, 15 and 16, respectively (Lira et al. 2009a). Thermal analysis performed by different researchers describe endothermic peak ranging from 200 to 204 °C, corresponding to the peak of fusion of UA, and exothermic peak around 273 °C, corresponding to the beginning of its degradation temperature (Marcano et al. 1999; Lira et al. 2009a).

From a well-characterized molecule, it is possible to make chemical modifications in order to provide an improvement in its characteristics, such as biological activities. Several studies report strategies for obtaining derivatives of UA to potentiate their water solubility as well as its biological activity and minimize its toxic effects, more specifically hepatotoxicity. Changes of UA generally comprise reactions with amine in the carbonyl group and ester formation in hydroxyl groups (Sokolov et al. 2012a). The modification described initially aimed at obtaining the UA salt, with the purpose of finding a water-soluble form, but without losing its biological activity. Na-UA salt was the sodium salt named BINAN, whose antimicrobial activity was like the original molecule (Najdenova et al. 2001).

Following another path, but still aiming to increase the aqueous solubility of UA, Kristmundsdóttir et al. (2002) tested different pH ranges, various co-solvent concentrations, surfactants, and complexing agents such as 2-hydroxypropyl-β- cyclodextrin. The authors observed that the solubility of UA with 2-hydroxypropyl-β-cyclodextrin was 0.68 mg/100 mL. Later, Lira et al. (2009b) developed and characterized inclusion complexes of UA and β-cyclodextrin, using the lyophilization method for this purpose. Chemical interactions between UA and β-cyclodextrin were evaluated by IR, H^1 NMR, X-ray, and thermal analysis. Following the same idea of complexation and the same characterization methods, Nikolic et al. (2013) described the production of UA-inclusion complexes with hydroxypropyl-β-cyclodextrin.

Still aiming to increase the solubility of UA, Lukác et al. (2012) investigated the influence of bisammonium salt (representing a cationic surfactant), dialkylphosphocholine (representing a zwitterionic surfactant) and the mixture of both to increase the solubility of acid in micellar solution. The authors observed that higher solubility was observed for cationic surfactant (20 times) when compared to acid solubility in water.

In addition to chemical modifications to increase aqueous solubility, they are also purposed to potentiate the biological activity of UA. Luzina et al. (2007), for example, investigated condensation of UA with various amino acids such as glycine, β-alanine, L-valine, and L-leucine among others biologically active molecules, to increase their activities. Bazin et al. (2008) also described the synthesis of nine UA-conjugated amino derivatives and evaluated their cytotoxic activity in human and murine cancer cell lines. Sometimes, chemical modifications exhibit greater biological action but they do not yet exhibit synergistic behavior capable of reducing toxic effects. To overcome these disadvantages, an innovative strategy is undoubtedly the encapsulation of UA in controlled drug delivery systems, which will be pioneered described later in this paper.

Despite many studies focusing on physicochemical characterization, many different analytical methods have been reported, describing the identification,

separation, and quantification of UA in lichen extracts (Cansaran et al. 2006; Ivanovic et al. 2013), in plasma (Venkataramana and Krishna 1992) and in pharmaceutical preparations (Ribeiro-Costa et al. 2004; Santos et al. 2005; Siqueira-Moura et al. 2008). Among these, spectrophotometry (Siqueira-Moura et al. 2008), fluorimetry, high-performance liquid chromatography (HPLC) (Venkataramana and Krishna 1992; Ribeiro-Costa et al. 2004; Santos et al. 2005; Ji and Khan 2005), thin layer-chromatography (TLC) (Marcano et al. 1999), zone capillary electrophoresis (CZE) (Kreft and Strukelj 2001), ultra-performance liquid chromatography coupled with electrospray mass spectrometry (UPLC–ESI(-)-MS), as well as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Santos et al. 2015), are described.

Some chromatographic models have been validated, such as the one initially described by Venkataramana and Krisnha (1992), who developed a method using HPLC for determination of UA in human plasma, achieving 98% analyte recovery. The authors used a UV detector (wavelength 280 nm), C18 column, and a mobile phase consisting of methanol—phosphate buffer (pH 7.4) (70:30, v/v). Under other conditions, Ji and Khan (2005) used chromatographic method, with a photodiode array detector (PDA) at 233 nm, to measure the lichen compound in plant materials and market products. The mobile phase was composed of aqueous 0.1% acetic acid and acetonitrile and RP18 column.

In contrast, the capillary zone electrophoresis (CZE) method was developed for the UA-routine analysis in medicinal products containing lichen extract (Kreft and Strukelj 2001). Despite several previously described analytical protocols, only in 2009, Siqueira-Moura et al. proposed and validated a simple ultraviolet (233-290 nm) spectrophotometric method for dosing UA from nanosystems (liposomes) without interference of the formulation.

Biological properties of usnic acid

Although several therapeutic properties have been attributed to usnic acid, since its discovery, most publications refer to its antimicrobial potential and innovate by reporting its action spectrum against increasingly new strains, especially those resistant to traditional antibiotics. At the same time, a progressive increase in publications related to the antitumor property of this lichen derivative has been described, awakening its application against the most different cancer strains. Thus, we will initially describe the antimicrobial and antitumor activities of UA, followed by other biological activities.

Antimicrobial and antiparasitic activities

Antifungal activity

The antifungal activity of UA was also discovered in the 1950s, when inhibition of the fungus *Trichophyton mentagrophytes* was observed after treatment with it (Bustinza 1952). After that, the small number of drugs available for fungal treatments encouraged the search for new chemotherapy agents, so that more studies with UA have been carried out, against different fungi (mold or yeasts). In (1996), for instance, Broska et al. observed growth inhibition of *Penicillium fraquentans* and *Verticillium albo-atrum* following treatment with UA. However, no action of UA against the biofilm formed by the yeasts *Candida orthopsilosis* and *C. parapsilosis* was observed by Pires et al. (2011).

Yu et al. (2016) evaluated the activity of 8 lichen derivatives, including UA, against clinical isolates of the yeast *Candida albicans* and the molds *Trichophyton mentagrophytes, T. rubrum, Aspergillus fumigatus* and *A. flavus*, and only derivatives 7 and 8 designated as usone (whose molecular formula was $C_{20}H_{22}O$) and isousone (an isomer of 7), respectively showed activity against *T. rubrum*, both with a MIC (minimum inhibitory concentration) of 41 μM, while the other compounds presented MIC above of 200 μM. On the other hand, the antifungal activity of UA (2-32 mg/L) and acetone extracts of three fruticose lichens namely, *Cladonia amaurocraea*, *C. rangiferina* and *U. longissima* were investigated against three pathogenic oomycete fungi, which can cause serious fish saprolegniasis: *Saprolegnia parasitica*, *Achlya bisexualis* and *Pythium* sp. (Guo et al. 2017). According to the authors, the MIC of UA for the tested fungi *S. parasitica* and *A. bisexualis* was 2 mg/L and for *Pythium* sp. it was 8 mg/L. UA absolutely inhibited the mycelial growth of *S. parasitica* at 32 mg/L and had better effect than the fungicide nikkomycin Z (ca. 100 mg/L for total inhibition). Mycelial growth of *A. bisexualis* and *Pythium* sp. were totally inhibited by

16 mg/L of UA, and at 2 and 4 mg/L, UA showed growth promoting activities for *Pythium* sp. Among all three oomycetes fungi, the *S. parasitica* growth was inhibited above 50% at concentrations of UA higher than 4 mg/L. Regarding to the three lichen extracts, concentrations higher than 200 mg/L inhibited above 50% of the *S. parasitica* growth, while the growth of the other fungi were inhibited with at least 1600 mg/L of any of the extracts.

Recently, Kumar et al. (2019), using candidiasis and dermatophytosis models, evidenced the antifungal activity of blended cinnamon oil and UA nanoemulsion.

Antibacterial activity

The discovery of UA occurred through the search for new antibiotic compounds (Abrahan and Florey 1949; Bustinza 1952), having their first descriptions against *Streptococcus mutans* (etiological agent of dental caries and other periodontal diseases) and the genera *Escherichia*, *Salmonella,* and *Shigella* (Lauterwein et al. 1995).

Additionally, UA has been used as an alternative against recommended antibiotic resistant strains such as vancomycin resistant Enterococci (VRE) and methicillin resistant *Staphylococcus aureus* (MRSA) (Elo et al. 2007). Still about the activity of UA on such strains, Pompilio et al. (2013), identified its antibiofilm action. In parallel, in order to overcome the resistance of MRSA, Segatore et al. (2012), evaluated the UA combined with other antibiotics, finding its synergistic action with gentamicin, and antagonistic action with levofloxacin, and an indifferent action with clindamycin, erythromycin, gentamycin, levofloxacin, and oxacillin.

Antonenko et al. (2019) found that the hydroxyls present in the UA are intricately linked to its activity against the growth of *Bacillus subtilis*. In addition to UA, three analogs without protons at positions 3, 7 and 9, were tested, being the original UA more effective against *B. subtilis* than its three analogs. So that, the replacement of hydroxyl groups by methoxyl groups reduced the UA-antimicrobial activity.

Still exploring the use of UA to control the growth of *Streptococcus aureus* and *Pseudomonas aeruginosa* biofilms, in despite it does not inhibit the initial fixation of *S. aureus* on artificial polymer surfaces (in situ), it was able to kill cells after adherence, thereby inhibiting biofilm growth. In the case of *P. aeruginosa*, although biofilm formation also occurred, changes in the morphology of the bacteria were observed affecting its aggressiveness (Francoline et al. 2004). This was an important finding, as it has become common in medicine to use artificial devices to repair or replace damaged body parts, which can serve to deposit bacteria and fungi, thus resulting in the formation of biofilms.

Resistance to antibiotic therapy is one of the main problems in the fight against tuberculosis. Therefore, UA was tested against strains susceptible and resistant to isoniazid, streptomycin, and rifampicin - drugs used in current therapy, showing that there was no cross-resistance. This fact suggested that the mechanism of action of usnic acid is different from the drugs currently used in tuberculosis therapy (Ramos and Da Silva 2010). Also, Honda et al. (2010) tested the action of 26 lichen derivatives against *Mycobacterium tuberculosis* (H37Rv), and found that UA was the third most effective metabolite, with a minimum inhibitory concentration (MIC) of 62.5 µg/ml (182 mM), which also evidenced the activity of UA against *M. tuberculosis*.

In addition to the strains listed above, *Helicobacter pylori* was also found susceptible to UA, being this activity dose dependent (Safak et al. 2009). The authors first noted that a specific lichen (*Usnea dasypoga*) was popularly used for the treatment of gastric ulcer, and then, tested the UA and its combination with well know antibiotics against *H. pylori*. It was shown an effective synergism of UA and clarithromycin – this antibiotic, in combination with inhibitors of acid secretion, is a reference drug in the treatment for the eradication of *H. pylori*. In a later study, Luo et al. (2011) evidenced this anti-*H. pylori* activity of UA extracted from *Nephromopsis pallescens* and obtained results comparable to those of the control treatment, ampicillin, and erythromycin.

The mechanism used by *H. pylori* to colonize the gastric mucosa involves the production of extracellular urease, which promotes the increase of the pH in the stomach due to the production of ammonia from urea. Lage et al. (2018), tested the activity of lichen derivatives, including UA, omeprazole (OMP, reference drug), hydroxyurea (HU) and thiourea (TU) (urease inhibitors) against six *H. pylori* clinical isolates, showing that (R)-(+)-UA presented a MIC of 3.8-7.8 times lower than the OMP, and 74-286 times

lower than the MIC of HU or TU. The authors also tested the (S)-(-)-UA, and the results showed that in its S-(-) form, UA had a MIC 2-4 times lower than the MIC of the OMP, 25-99 times lower than the MIC of HU and 39-154 times lower than the MIC of TU.

Given the above information, we summarize in Table 1 some bacterial strains susceptible to UA, evidencing its widespread application against different bacteria.

Antiviral activity

Initially, UA was tested against Epstein–Barr, Human Papillomavirus (HPV), and Rat Polyomavirus (Yamamoto et al. 1995; Scirpa et al. 1999; Campanella et al. 2002). In (1995), Yamamoto et al. tested both enantiomeric forms of UA against Epstein–Barr virus and found that the dextrorotatory form had higher antiviral activity than its levogyre enantiomer. Action against HPV has been observed in the adjuvant treatment of infection associated with zinc sulfate (Scirpa et al. 1999). In this study, it was demonstrated that besides antiviral action, UA favored the reepithelization of the injured tissue. Thus, it enabled adjuvant therapy in the unobstructed surgical treatment and in the HPV injury.

In addition, UA was able to inhibit the proliferation of rat polyomavirus, a fact that occurred by the destruction of viral DNA through inhibition of RNA transcription (Campanella et al. 2002). The activity of UA and its derivatives (synthetically modified) was also evaluated against H1N1 influenza viruses in MDCK cells, and the results suggested that these molecules are anti-influenza substances (Sokolov et al. 2012b). Shtro et al. (2015) demonstrate the antiviral activity of some UA synthetic derivatives against influenza virus in vitro (evaluated by their ability to decrease the virus titer on Madin–Darby Canine Kidney cells) and in vivo (evaluated by decrease of mice mortality and index of protection). One compound, valine enamine-UA, significantly reduced lethality of infected animals and did not give rise to the appearance of resistant strains. Additional studies showed that hepatotoxicity of this compound was reduced comparatively to the natural UA, evidencing that it could be a potential candidate for the development of a new anti-influenza therapy.

Antiparasitic activity

Another activity attributed to UA is its antiparasitic property. The first report focusing on this field was described by Wu et al. (1995), when they detected the (in vitro) activity of UA against *Trichomonas vaginalis.* Then, (+)-UA isolated from Chilean lichen *Protousnea* malacea was tested against the promastigote form of three strains *Leishmania braziliensis, L. amazonenses* and *L. donovani.* In this study, UA

Table 1 Strains of usnic acid susceptible bacteria, according to search in the international scientific database Scopus (www.scopus.com)

Bacteria	MIC of usnic acid (μg/mL)		References
	(+)-Usnic acid	(−)-Usnic acid	
Enterococcus faecalis	4	8	Lauterwein et al. (1995)
Enterococcus faecium	16	16	Lauterwein et al. (1995)
Clostridium perfringens	4	4	Lauterwein et al. (1995)
Bacteroides vulgatus	4	8	Lauterwein et al. (1995)
Pseudomonas aeruginosa	256	ND(*)	Francoline et al. (2004)
Staphylococcus aureus	6	8	Correché et al. (1998)
	8	8	Lauterwein et al. (1995)
	32	ND(*)	Francoline et al. (2004)
	2	ND(*)	Segatore et al. (2012)
	2	ND(*)	Pompilio et al. (2013)
Mycobacterium aurum	32	ND(*)	Ingólfsdóttir et al. (1998)
Mycobacterium tuberculosis	1.56	ND(*)	Ramos and Da Silva (2010)
Helicobacter pylori	0.064	ND(*)	Safak et al. (2009)
	0.012	0.023	Lage et al. (2018)

**ND* non-determined

(25 ml/mL) promoted total lysis of the three strains in the in vitro assays, besides showing a significant reduction of the skin lesion in BALB/c mice infected by *L. amazonenses*, demonstrating its interesting antiparasitic activity (Fournet et al. 1997). The leishmanicidal activity of UA has recently been proven by Derici et al. (2018) against the species *Leishmania major, L. infantum,* and *L. tropica* in their promastigote forms. The IC_{50} of UA was respectively 10.76 μg/mL, 13.34 μg/mL, and 21.06 μg/mL. The authors evaluated the apoptotic mechanism of *Leishmania* caused by UA and concluded that it promoted increase of gene expression of p53, Bax, Casp-3, and Casp-9, thereby reducing cell proliferation of promastigote forms.

Sussmann et al. (2011) stimulated by parasitic resistance to conventional drugs, tested UA against *Plasmodium falciparum*, and found that it has an important action against the parasite as it reversibly inhibited the biosynthesis of vitamin E, an important molecule for parasite development. The in vitro activity of UA-potassium salt against *Schistosoma mansoni* was studied by Araújo et al. (2019), who performed an ultrastructural analysis and suggested that UA-potassium salt could be used for the development of new schistosomicidal agent (Araújo et al. 2020).

Antitumoral activity

Almost four decades ago, the UA-antitumor activity was described for the first in Lewis lung carcinoma (Kupchan and Kopperman 1975). From then on, in vitro studies have shown this action against different cell lines, such as human keratinocytes (HaCaT) (Kumar and Müller 1999; Pereira et al. 1994; Lima et al. 1990; Burlando et al. 2009), cancer cells breast (MCF7 and MDA-MB 231) and lung cancer (H1299 and NCIH 292) (Mayer et al. 2005; Santos et al. 2005). When incorporated into β-cyclodextrin, it has demonstrated its antiproliferative activity against malignant cells of the K-562 (leukemia) lineage (Campanella et al. 2002), T-47-D (breast cancer), Panc1 (pancreatic cancer) and PC-3 (prostate cancer) (Kristmundsdóttir et al. 2005). The UA-antiproliferative activity was observed against model lineage to study the effects of human cancer cytotoxic compounds, vulvar squamous cell carcinoma (A431), and against an aggressive and lethal chemotherapy resistant tumor lineage (malignant mesothelioma (MM98) (Burlando et al. 2009).

The in vitro cell viability of human hepatoblastoma HepG2 has been shown to be compromised with 5 μM concentrations of UA and has a LC_{50} of 30 μM. In the same study, the exposure of these cells to the drug, especially at concentrations above 20 μM, resulted in a significant increase in cytochrome P450 activity, oxidative stress and mitochondrial dysfunction proving the toxicity of UA against these cell lines (Sahu et al. 2011). In (2012), the same researchers, when associating UA with a lipopolysaccharide, observed an increase in toxic effect against HepG2 (Sahu et al. 2011).

In addition, several authors compared the properties of UA with the ones of others lichen derivatives. When it was compared with parietin, atranorin, and gyrophoric acid, UA was found to have a higher antitumor potential against A2780 and HT-29 cancer strains (Backorová et al. 2012). In the work proposed by Brisdelli et al. (2013), UA was the most potent cytotoxic agent for MCF-7, HeLa, and HCT-116 strains when compared with diffractaic acid, lobaric acid, vicanicin, variolaric acid, and protolichesterinic acid. Corroborating the antitumor activity of UA, Brandão et al. (2013) found that it showed cytotoxicity to the UACC-62 melanoma cell line. The anticancer effects of both enantiomeric forms of UA were investigated by Einarsdóttir et al. (2010), who concluded that both forms had an inhibitory effect on cell growth and proliferation of cancerous T-47D (breast cancer) and Capan-2 (pancreas cancer).

Finally, O'Neill et al. (2010), knowing the important role in both cell division and apoptosis cell death mechanisms, investigated whether UA affected the formation and/or stabilization of microtubules as a chemotherapeutic target, but such activity has not been found. Table 2 summarizes some carcinogenic cell lines that showed susceptibility to UA, confirming the high number of citations as an antitumor agent.

Other activities

Given all these reports about antimicrobial and antitumor properties, several authors have attributed other biological activities to usnic acid, as explained below.

Table 2 Usnic acid susceptible cancer cell lines, according to search in the international scientific database Scopus (www.scopus.com)

Cell line	Usnic acid		References
	(+)	(−)	
Epidermoid carcinoma (A431)	**39 $\mu M_{(VC)}$	(ND)	Burlando et al. (2009)
	**72 $\mu M_{(NR)}$		
Malignant mesothelioma (MM98)	**23 $\mu M_{(VC)}$	(ND)	Burlando et al. (2009)
	**64 $\mu M_{(NR)}$		
Human keratinocytes (HaCaT	*2.1 μM	(ND)	Kumar and Müller (1999)
	**35 $\mu M_{(VC)}$	(ND)	Burlando et al. (2009)
	**76 $\mu M_{(NR)}$		
Breast cancer (T-47D)	*12.1 μM	*11.6 μM	Einarsdóttir et al. (2010)
Pancreatic cancer (Capan-2)	*15.3 μM	*14.5 μM	Einarsdóttir et al. (2010)
Human hepatoblastoma (HepG2)	***30 μM	(ND)	Sahu et al. (2011)
Cervix adenocarcinoma (HeLa)	*23.7 μM	(ND)	Brisdelli et al. (2013)
Colon carcinoma (HCT-116)	*17.7 μM	(ND)	Brisdelli et al. (2013)
Breast adenocarcinoma (MCF-7)	*75.7 μM	(ND)	Brisdelli et al. (2013)
Human melanoma (UACC-62)	***534.4 μM	(ND)	Brandão et al. (2013)

*IC_{50}/**EC_{50}/***LC_{50}; *ND* non determined, *VC* violet crystal; *NR* neutral red

Gastroprotective activity

Usnic acid isolated from *Usnea longissima* has been tested in the treatment of indomethacin-induced gastric ulcer in animals. Gastric lesions were reduced at the tested doses (25, 50, 100, and 200 mg/kg body weight), as compared to the reference drug ranitidine (25 mg/kg body weight). This gastroprotective action of usnic acid can be attributed to its reducing effect against oxidative damage and its inhibitory effect on neutrophil infiltration in rat stomach (Odabasoglu et al. 2006), in addition to its ability to inhibit urease, an enzyme synthesized by the bacterium *H. pylori*, which is responsible for the production of ammonia from urea.

Healing, anti-inflammatory and antioxidant activities

The wounded skin generally leads to an increased fluid loss, infection, hypothermia, scarring, compromised immunity, and change in body image, as well as large skin damage can cause mortality (Alemdaroğlu et al. 2006). The wound healing is a process that deserves attention, especially post-surgery and burns. It involves extensive oxidative stress and defense against microbial attack (Francolino et al. 2019a), and the first generally inhibits connective tissue remodeling. Collagen is the major constituent of the connective tissue, and collagen-based film is a potentially useful healing biomaterial that permit controls drug release within target tissues (Gopinath et al. 2004).

In order of the epithelial regeneration to be adequate, a pharmacological therapy that makes the microbial action unfeasible, and simultaneously promotes re-epithelialization, is necessary. Thus, UA has been used in dermatological and cosmetic preparations, such as encapsulated in liposomes, due to its antioxidant and bacteriostatic activities (Francolino et al. 2019b).

Nunes et al. (2011) assessed the effect of collagen-based films containing usnic acid as a wound dressing for dermal burn healing. For that, the second-degree burn wounds were performed in 45 Wistar rats assigned into nine groups, it means, three for treated only with reconstituted bovine type-I collagen-based films (COL), three for treated with collagen films containing empty liposomes (COL-LIPO), and three for the treated with collagen-based films containing COL-UA-LIPO. After 7, 14, and 21 days, the animals were euthanized. The authors found that on the 7th day, the group COL-UA-LIPO presented a moderate infiltration of neutrophils distributed throughout the burn wounds, whereas in the groups COL or COL-LIPO, the severity of the reaction was slighter and still limited to the margins of the burn wounds. On the 14th day, the inflammatory reaction was less intense in tissues treated with COL-UA-LIPO, with remarkable plasma cells infiltration, and on the 21st day, there was

reduction of the inflammation (predominantly composed by plasma cells in all groups, mainly in the tissues treated with COL-UA-LIPO). On the 14th day, it was seen that the use of COL-UA-LIPO provided a more rapid replacement of type-III collagen for type-I, while by the 21st day, the collagenization density had been improved, allowing to conclude that the use of collagen-based films containing UA improved burn healing process in rats.

Bruno et al. (2013), using in vitro and in vivo assays, also evaluated the wound repair property of usnic acid derivatives. Less cytotoxicity to skin cells was observed, as well as better healing performance, suggesting the possibility of using these compounds in wound healing and anti-aging skin preparations.

Furthermore, the healing action of UA is favored by its anti-inflammatory potential. Huang et al. (2011) noted that such property is a consequence of down-regulation of some inflammation mediators such as iNOS (induced-nitric oxide synthase), COX-2 (cycleoxigenase-2), IL-1β (interleukin-1β), IL-6, and TNF-α (Tumor Necrosis Factor-α), and, in addition to overexpression of IL-10 and hemeoxygenase-1. Previously, Kohlhardt-Floehr et al. (2010), showed that UA obtained from *Xanthoparmelia farinosa* showed potent antioxidant and pro-oxidant activity (bifunctional behavior) in human lymphocyte cell lines (Jurkat-cells: E 6-1 acute leukemia) under ultraviolet B (UV-B) irradiation.

It had been also observed before that some lichens, when stimulated by UV light, synthesize metabolites with strong absorption in this spectrum region, generating their own protection against hazardous radiation. According to Rancan et al. (2002), UA has showed a UV protection factor in vivo similar to the synthetic commercial substance used as a reference protector (3.6–5.0), and in vitro a UV protection factor (4.03-4.83) higher than the reference substance (2.66-3.63). This finding together the other known properties of UA, converted it in a potential sunscreen and active compound in different dermatological and cosmetic preparations, since its ability to absorb UV light, plus its antioxidant, anti-inflammatory and healing activities help to protects the skin against the damaging effects of exposure to sunlight or other agents capable of causing injury to the skin.

On the other hand, the UA-antioxidant property is described in other situations. Free radicals are important in the development of atherosclerosis, becoming a risk factor to cardiovascular diseases, so that, the UA-antioxidant property reflected in cardiovascular protection in several trials using this compound extracted from *Usnea complanata* (Behera et al. 2012).

Rabelo et al. (2012) has performed in silico evaluation of UA interactions with genes/proteins and important biomolecules for cellular redox balance and NO pathway, assessing the UA redox properties against different reactive species (RS) generated in vitro. The authors also evaluated the action of this compound on SH-SY5Y neuronal like cells upon hydrogen peroxide (H_2O_2) and found that the total reactive antioxidant potential index (TRAP), a method utilized to estimate the non-enzymatic antioxidant capacity of samples in vitro, based on the quenching of luminol-enhanced chemiluminescence, showed a significant UA-antioxidant capacity at the highest tested concentration, being also effective against hydroxyl radicals and decreasing the NO-formation.

Fernández-Moriano et al. (2017) also evaluated the protective effects of UA against redox impairment (cytotoxicity induced by exogenous H_2O_2) in two models of central nervous system-like cells (U373-MG and SH-SY5Y cell lines). For this, the authors first assessed the radical scavenging activity and the phenolic content (UA) in extracts of the lichen *U. ghattensis*. At the optimal concentrations, pretreatments with UA displayed moderate protection against H_2O_2-induced cytotoxic damage in both models, reversing the alterations in oxidative stress markers (including ROS production, glutathione system, and levels of lipid peroxidation), and cell apoptosis (caspase-3 activity). Such effects were in part mediated by a notable enhancement of the expression of intracellular phase-II antioxidant enzymes; a plausible involvement of the Nrf2 (nuclear factor erythroid-related factor 2) cytoprotective pathway is suggested, as well as the UA deserve further research as a promising antioxidant candidate in the therapy of oxidative stress-related diseases, including the neurodegenerative disorders.

Toxicity of usnic acid and mechanism of action

The scientific community has been endeavored to unravel the biochemical and molecular mechanisms involved in both the biological activities inherent of

UA, as well as in its toxicity, and both are not fully understood yet.

Toxicity of usnic acid to animals

Despite the attractive pharmacological properties of UA, its toxicity has been studied in different assays in vitro (Pramyothin et al. 2004), in vivo (Chitturi and Farrel 2008), and in human patients (Sanchez et al. 2006; Chitturi and Farrel 2008; Foti et al. 2008). Prokopiev et al. (2017) investigated the genotoxic effect of both UA-enantiomers on human peripheral-blood lymphocytes, and found that, at the highest concentrations tested, of 0.15 and 0.30 mM, the effect of (−)-UA was twice as great as that of its (+) enantiomer.

Two years later, the same group observed DNA damage, but there were no significant differences between the genotoxic activities of both UA-isomers in the liver and in the renal cells of rats after oral administration of 100 and 50 mg/kg (Prokopiev et al. (2019). Thus, the scientific literature has shown differences between the biological properties of UA enantiomers, and recently, Galanty et al. (2019) published an excellent general review of these studies, comparing the activity of the two enantiomeric forms. However, in the literature reviewed by these authors or in other works, there is still no definition of which enantiomer is more effective. Depending on the biological activity, one or the other enantiomeric form may present better activity and less side effect.

The hepatotoxicity is the most highlighted toxic effect being handled in literature reviews (Guo et al. 2008; Araujo et al. 2015). The results of the studies of Pramyothin et al. (2004) revealed that (+)-UA could present hepatotoxicity like that of carbon tetrachloride (CCl_4). The authors treated rat primary hepatocytes with 100 or 1000 µM of UA and after 1 h observed a release of hepatic transaminases (AST and ALT). Considering the in vivo data on toxicity, Abo-Khatwa et al. (2015) found that an average dose of 180 mg/kg was lethal for rats that received the (+)-enantiomer of UA. These authors also observed that 2–5 h after the treatment, the symptoms were long chalasia, ponopalmosis or spastic paralysis. Other symptoms such as lethargy, anorexia and abdominal discomfort have been described in domestic sheep, by Dailey et al. (2008), after the administration of (+)-UA, with a toxic dose of 485–647 mg/kg/day, for 7 days.

It is worth mentioning that, in humans, UA hepatotoxicity has been the subject of several reports, causing everything from acute hepatitis to liver failure (Neff et al. 2004; Bunchorntavakul and Reddy 2013). In previous years, hepatocellular damage was reported when individuals who consumed a multi-ingredient supplement called LipoKinetix® experienced acute liver failure. Its composition, in each capsule, was: UA (100 mg) norephedrine hydrochloride (25 mg), 3,5-diiodothyronine (100 µg), yohimbine hydrochloride (3 mg), and caffeine (100 mg) (Neff et al. 2004).

Another relevant study associated with human hepatotoxicity was carried out by Durazo et al. (2004). The authors described an acute liver failure after 2 weeks of UA administration in a dosage of 500 mg/day. Sanchez et al. (2006) also described that two healthy patients developed severe hepatotoxicity, one of them evolved to fulminant hepatic failure (requiring liver transplantation), while consumed 3 times a day, in cycles of 2 weeks, 3 capsules of UCP-1 (BDC Nutrition, Richmond, Ky)—a dietary supplement that contains per capsule: UA (150 mg), L-carnitine (525 mg) and calcium pyruvate (1050 mg).

In addition to several reports mentioning UA-induced liver damage, in vitro assays have shown its neurotoxicity dose-dependent. According to Rabelo et al. (2012), in vitro, at highest concentration of 20 µg/mL for 1 ad 4 h, UA enhanced lipoperoxidation and changed the cellular viability (on SH-SY5Y neuronal like cells), as well as treatment with 2 µg/mL and 20 µg/mL for 24 h, according to MTT (3-(4,5-dimethyl)-2,5-diphenyl tetrazolium bromide) reduction assay. Moreover, the authors found that UA did not display protective effects against H_2O_2-induced cell death in any case. Evaluation of intracellular reactive species (RS) production by the DCFH-DA (2′,7′-dichlorohydrofluorescein diacetate) based assay indicated that the UA was able to induce changes in basal RS production at concentration of 20 µg/mL for 1 h and from 2 ng/mL to 20 µg/mL for 4 h and 24 h, so that it could display variable redox-active properties, according to different system conditions and/or cellular environment.

Mechanism of action of usnic acid

Several study models (in silico, in vitro and in vivo) correlate the mechanism of action of usnic acid to disruption of mitochondrial function, changes in

oxidative stress and inducing cell death (Pramyothin et al. 2004; Han et al. 2004; Liu et al. 2012). As usnic acid is a weak acid of lipophilic characteristic, its diffusion through the mitochondrial membrane is favored, causing, for instance, inhibition of ATP production in the oxidative phosphorylation pathway. This hypothesis also explains the attractive antimicrobial activity of usnic acid since this mechanism does not allow the respirating microorganisms to perform the catabolic and anabolic processes essential to their growth.

The influence of usnic acid on cellular respiration was first cited by Johnson et al. (1950), who found the decoupling of oxidative phosphorylation on rat kidney and liver homogenate, with minimum concentrations of 1.3-2.6 μg/mL of usnic acid. Since then, scientists have been sharing findings that highlighted this hypothesis. Vavasseur et al. (1991) showed the inhibitory potential of usnic acid on aerobic respiratory processes in plant cells of *Cormmelina communis*.

In this context, given that the mitochondrial pathways are important in regulating the stages that synthetize ATP, if they do not work correctly, the consequence for any cell that respirates is death. In this case, the interference in cell respiration and, thus, the reduction in RNA and protein synthesis, are the main suggested mechanisms of usnic acid to reduce the viability of cancer cells (antitumor ability) (Al-Bekairi et al. 1991), without involve DNA damage (Mayer et al. 2005). Also, the antitumor effect of UA may be a consequence of changes in the pH gradient in carcinogenic cells, as shown in T47D and Capan-2 breast and pancreatic cancer strains, respectively, when modifications in the proton transporters of the plasma membrane, generating an electrochemical gradient, were visualized, allowing the accumulation of weak acids inside (Bessadottir et al. 2012).

Abo-Khatwa et al. (1996) had previously reported that rat hepatocytes mitochondria membranes were affected by usnic acid, vulpinic acid and atranorin in the same way as do 2,4-dinitrophenol—the standard uncoupling substance. On the other hand, Fujimoto et al. (2020) described that at concentrations of 10 μM and 30 μM, usnic acid could inhibit mitochondrial ATP production, while Pramyothin et al. (2004) found that (+) usnic acid altered the integrity of the membranes of hepatocytes, allowing the release of hepato-specific enzymes (aspartate aminotransferase and alanine aminotransferase), especially transaminase, and destroying mitochondrial function. Still in (2004), Han et al. used murine models to show 98 and 100% of tissue necrosis (hepatotoxicity) after treating hepatocyte cultures with 5 mM and 10 mM of usnic acid, respectively. In addition, up to 90% reduction in ATP levels and inhibition of mitochondrial respiration were observed. The authors also identified a direct inhibition of mitochondrial function that lead to decreased oxygen uptake by the electron transport chain, and, consequently, to cell death.

Once that decoupling action of oxidative phosphorylation leads to disorders in the cell's energy metabolism, ^{13}C isotopes were used by Sonko et al. (2011) to monitor glucose metabolism in hepatocytes during cytotoxicity induced by low concentrations of usnic acid (1–5 μM), and indicated that increased oxidative phosphorylation may occur as a cellular adaptive response compensating for decreased mitochondrial function. Such influence was readily clarified when Moreira et al. (2013) observed that low concentrations of usnic acid (1–5 μM) stimulated oxygen consumption, reducing mitochondrial NADH/NAD^+ ratio, and strongly inhibited gluconeogenesis, but induces glycolysis, β-oxidation, fructolysis, glycogenolysis, ammoniagenesis and inhibits ureogenesis, causing delay of ketogenesis. In contrast, high concentrations of UA (10 μM) blocked the electron transport chain and the oxidation of medium chain fatty acids. Therefore, these combined deleterious events lead to decreased hepatic glycolysis, brain ketone demand and increased ammonia production.

With the same logic, in silico studies support the hypothesis that exposure to usnic acid causes disturbances in the energy metabolism of amino acids, lipids and nucleotides through oxidative stress (Lu et al. 2011).

Recently, Antonenko et al. (2019) performed a thorough investigation of effects of usnic acid and its analogues on artificial planar bilayer lipid membrane (BLM), rat liver mitochondria and bacteria, and found that all of the three hydroxyl groups of usnic acid appeared to be involved in its proton-shuttling activity on BLM. The authors evidenced the uncoupling activity of UA on mitochondrial respiration, attesting first the chelating properties of usnic acid with calcium (through the metal extraction method). Then, after the formation of complexes of UA-Ca-UA, extracting calcium ions from a hydrophilic medium to a hydrophobic medium, the authors found, for

decoupling studies, that UA suppressed the rat liver mitochondrial membrane potential at a much lower concentration when compared to its analogs (UA molecules with chemical modifications in hydroxyls), and the UA action on mitochondrial membrane potential was suppressed in the presence of the calcium ionophore A23187.

In addition to biochemical and histopathological studies contributing to unravel the mechanism of action of usnic acid, molecular biology tools have been used to obtain data that culminate in the elucidation of the cellular pathways involved with the fascinating biological effects of UA. For this purpose, the genomic analysis showed the expression of genes involved with proton transit against the mitochondrial electron gradient (Bessadottir et al. 2012), electron transport chain complexes I–IV, fatty acid oxidation, the Krebs cycle and apoptosis (Joseph et al. 2009; Bessadottir et al. 2012). Concomitantly, proteins involved with the expression of these genes were identified through proteomics, thus emphasizing that UA-induced hepatotoxicity is associated with oxidative stress (Liu et al. 2012). Piska et al. (2018) described that reactive metabolites formation of UA might explain its hepatotoxicity.

Among the hundreds of proteins related to the UA mechanism of action, the detection of *heat-shock protein* 60, Apo AI, peroxiredoxin proteins (Prx4), and endoplasmic reticulum protein 29 (ERp29) explain the involvement of the lichen molecule in oxidative stress. Mitochondrial disease, in the removal of cholesterol and its consequent slimming effect, and the induction of apoptosis (Liu et al. 2012). Therefore, it is concluded that decoupling action of UA on the oxidative phosphorylation, initially suggested in the 1950s, has been confirmed through different studies (in silico, in vitro and in vivo) and tools (biochemistry, histopathology, genomics and proteomics).

Nanotechnological applications

As discussed in this paper, it is possible to observe the most diverse and promising biological activities of UA. However, limitations to its therapeutic application, such as hepatotoxicity, low water solubility, and consequently reduced efficacy, have driven the development of innovative alternatives, such as its encapsulation in controlled release systems—an attractive tool of pharmaceutical nanotechnology.

Controlled drug release systems allow to modulate the release of the encapsulated active ingredient in concentrations within the therapeutic range, causing a reduction in the dosage regimen and increasing its efficacy by minimizing side effects (Tiwari et al. 2012). In this sense, the UA-encapsulation has become a promising strategy to overcome its physicochemical, toxicological obstacles, and enhance its biological purposes.

In (2004), Ribeiro-Costa et al. described for the first time the UA-encapsulation in microspheres formed by lactic acid and glycolic acid copolymer (PLGA). The authors evaluated the UA antitumor activity in vivo (rats), in both its free and microencapsulated forms. The UA-treated group, in its free form, had a 42% of tumor inhibition compared to the control group, while a 63% of tumor inhibition was obtained for the UA-microsphere treated group, resulting in a significant gain of 21%. Thus, the potentiation of the antitumor activity of encapsulated UA has stimulated further studies on the encapsulation of this molecule in others delivery systems, to explore its various biological activities. Then, PLGA nanocapsules with UA extracted from *Cladonia substella* (1 mg/mL) were developed and again the potentiation of antitumor activity was evidenced against NCI-H 292 cancer cells (Santos et al. 2005). In this study, the UA-nanoencapsulation promoted 68% tumor inhibition, whereas free UA treatment achieves 45% of tumor inhibition.

In (2006), Santos et al. described in vivo activity against Sarcoma-180, and an increase of 25% in the tumor control was found to the nanocapsulated UA form compared to the same dosage (15 mg/kg/day) of the free form. In addition, through biochemical and histopathological analyzes, it was found that there was a reduction in the toxicity of UA-nanocapsulated after the intraperitoneal administration to mice.

To explore antimicrobial activity, liposomes containing UA have been produced and evaluated (Lira et al. 2009a, b; Francolini et al. 2019b). The results showed that the MIC of free and encapsulated UA (UA-LIPO) was 6.5 and 5.8 μg/mL, respectively. Concerning the IC_{50}, the results showed values of 22.5 (± 0.60) and 12.5 (± 0.26) μg/ml for free UA and Lipo-UA, respectively. The results indicated a strong interaction between liposomes and J774 macrophages, facilitating the UA penetration in these defense cells,

as well as the potentiation of its activity against *M. tuberculosis* after nanoencapsulation (Lira et al. 2009b). Ferraz-Carvalho et al. (2016) studied the effect of UA-encapsulation in liposomes (Lipo-UA) in combination with rifampicin (Rif) and isoniazid (INH) against clinical isolates of Multi TB-Resistant Drug (MDR-TB). The MIC found was 31.25 and 0.98 μg/mL for free UA and Lipo-UA, respectively. The results also pointed out a synergism (fractional inhibitory concentration index) between the Rif and UA (FICI = 0.31) and between Rif and LIPO-UA (FICI = 0.28). INH, on the other hand, did not show synergism with free UA or Lipo-UA (FICI: 1.30–2.50). Thus, the authors concluded that UA-loaded liposomes could be used to optimize the antimycobacterial activity of rifampicin, a first-line drug used to treat tuberculosis.

Another approach described in the literature was the UA complexation in cyclodextrins and the encapsulation of liposome inclusion complexes, aiming to develop systems to enhance the UA-antimicrobial property (Lira et al. 2009a). The complexation mechanism of the inclusion between UA and cyclodextrin was investigated by isothermal titration calorimetry (ITC) and phase-solubility diagrams, using pH as a tool for modifying the molecule ionization, as described by Segura-Sanchez et al. (2009).

More recently, other UA-nanosystems have been developed for antimicrobial activity. Examples include magnetic nanoparticles (Taresco et al. 2015), silver nanoparticles (Siddiqi et al. 2018), copper plus silver nanoparticles (Alavi and Karimi 2019), and microparticles with antibacterial activity (Martinelli et al. 2014).

The UA-encapsulation in nanosystems offer new perspectives for its use in the most varied therapeutic approaches, providing significant improvement of its action. In order to summarize the scientific papers approached in the area, a search for scientific papers was performed in the SCOPUS database, using the descriptors "nanoparticle" and "usnic acid", to understand which nanosystems are currently being used to convey usnic acid (Table 3).

Finally, in this review, a survey of the patents that describe the use of nanotechnology to convey UA in its most varied applications was carried out, using the terms "nanoparticles" and "usnic acid" as descriptors in the "United States Patent and Trademark Office Database" (Table 4).

Table 3 Descriptive table of nanosystems, as well as the type of biological activity, studied in the respective papers, based on the search for the keywords "*Usnic acid*" and "*nanoparticles*" in the international scientific index database Scopus (www.scopus.com)

Nanosystem	Biological activity	Main results	References
Microspheres of lactic and glycolic acid copolymer (PLGA)	Antitumoral activity in vivo in mice	Tumor inhibition improvement after treatment with microencapsulated usnic acid	Ribeiro-Costa et al. (2004)
Nanocapsules of PLGA	Antitumoral activity in vitro (cells NCI-H 292)	Usnic acid acts by competing with NCI-H292 cell growth factors or modifying cell adhesion mechanisms	Santos et al. (2005)
Nanocapsules of PLGA	Antitumoral activity in vivo (ascites tumor Sarcoma 180)	Nanoencapsulation increased usnic acid activity by 26.4% when compared to free form and reduced hepatotoxicity	Santos et al. (2006)
Liposome-encapsulated usnic acid (UA) in combination with rifampicin and isoniazid.	Antimicrobial activity against multi drug-resistant clinical isolates of *Mycobacterium tuberculosis* (MDR-TB)	Usnic acid was more efficient in the encapsulated form. Promoting synergism with rifampicin	Ferraz-Carvalho et al. (2016)
Liposomal UA-cyclodextrin inclusion complex.	Development of inclusion complexes, liposome encapsulation and antimicrobial activity	Improvement in aqueous solubility of usnic acid as inclusion complexes with cyclodextrin	Lira et al. (2009a)
Microparticles of poly(L-lactate) carboxylate (CPLLAn-UA)	Antibiofilm activity against *Staphylococcus epidermidis*	Minimum Inhibitory Concentration [MIC]: Free UA: 16 μg/mL $CPLLA_{16}$-UA-: 160 μg/mL. Biofilm reduction (CFU/mm^2): free UA: 1.5-log $CPLLA_{16}$-UA: 2.5-log	Martinelli et al. (2014)

Table 3 continued

Nanosystem	Biological activity	Main results	References
Nanoparticles of zinc oxide (ZnO) with surface modified with sodium stearate or sodium stearate and UA	Antimicrobial properties and antibiofilm against *Salmonella enterica,* subspecies *enterica serovar typhimurium*	Nanoparticles with usnic acid improve the inhibition of biofilm initial formation (24 and 48 h)	Stan et al. (2016)
Nanoparticles of gellan gum modified by heparin	Antitumor properties of HAG-NPs against lung cancer (A549)	Cell cycle block (G2/M phase) at 80 μg/ml concentration when compared to control	Garg et al. (2018)
Liposomes embedded in collagen polymeric films and UA	*In vivo* assessment of wound healing from burns	Modulation of biological events involved in inflammatory response, epithelialization and collagen formation	Nunes et al. (2011)
Metal colloidal nanoformulation (Uh-Au@Nano-CF)	Inhibition of secretion of virulence factors regulated by the *Quorum-sensing* and prevention of biofilm formation by *Streptococcus mutans*	Nanoformulation strongly inhibited the virulence factors (ATPase, enolase, protease, LDH and glucosity) regulated by *Quorum-sensing* of *S. mutans,* as well as biofilm formation, with an inhibition of 94.17%	Singh et al. (2014)
Magnetic nanoparticles	Antimicrobial activity and antibiofilm activity against bacteria GRAM^{+} (*Staphylococcus aureus* and *Enterococcus faecalis*) and GRAM^{-} (*Escherichia coli* and *Pseudomonas aeruginosa*)	*Staphylococcus aureus* inhibition between 15.6 and 1.000 μg/mL; *Enterococcus faecalis* inhibition between 7.8125 and 1.000 μg/mL; *Escherichia coli* inhibition only at 500 and 1.000 μg/mL; *Pseudomonas aeruginosa* inhibition between 3.9 and 1.000 μg/mL	Grumezescu et al. (2013)
Nanoparticles of iron oxide superparamagnetic.	Cytotoxicity studies against MCF-7, HeLa, L929, U87	Usnic acid-free SPION demonstrated toxicity to all cell types	Alpsoy et al. (2018)
Glucosylated liposomes	Bacterial infections	Glucosylated cationic liposomes promotes usnic acid penetration in biofilm matrix	Francolini et al. (2019b)
Liposomes	Antioxidant study	Charge and chain influence liposomes physicochemical properties and the antioxidant effectiveness of usnic acid	Battista et al. (2020)

Conclusion

The scientific community has provided an increasing contribution in the knowledge of usnic acid—a compound originally obtained from lichens. This review aimed to synthesize the state of the art of these studies, providing information on the improvement of methods for extraction, characterization, derivatization and synthesis of usnic acid; on assays of its biological, pharmacological and toxic (for humans) activities; on strategies that minimize their effects toxic; or on the development of biotechnological resources that enable their continuous production with low costs and biomass, generating patents. They are all promising information for the development of new and increasingly diverse studies.

Table 4 Patents on nanosystems filed with the United States Patent And Trademark Office (USPTO)

Pat. no.	Title
US 10,610,104 B2	Gastrointestinal tract detection methods, devices and systems
US 10,161,929 B2	Theragnostic platform and methods of use
US 10,144,759 B2	Nuclear sulfated oxysterol, potent regulator of lipid homeostasis, for therapy of hypercholesterolemia, hypertriglyceridemia, fatty liver diseases, and atherosclerosis
US 9,669,012 B2	Delivery systems
US 9,387,206 B2	Therapeutic approaches for treating Alzheimer's disease
US 9,433,569 B2	Dental care products comprising carbonate-substituted fluorohydroxy-apatite particles
US 9,408,791 B2	Oral care and oral hygiene products having photocatalytic activity comprising inorganic particles superficially functionalized with TiO.sub.2 nanoparticles
US 9,469,616 B2	Cyclic compounds and methods of making and using the same
US 9,211,238 B2	Carrier system for the transport of active substances into the skin
US 8,975,262 B2	Synthetic mimetics of host defense and uses thereof
US 9,127,233 B2	Device and method for solubilizing, separating, removing and reacting carboxylic acids in oils, fats, aqueous or organic solutions by means of micro-or nanoemulsification
US 9,211,259 B2	Antibiotic kit and composition and uses thereof
US 8,809,302 B2	Therapeutic approaches for treating Alzheimer's disease
US 8,734,855 B2	Slow release magnesium composition and uses thereof
US 8,895,561 B2	Compounds and methods for treating candidiasis and aspergillus infections
US 8,563,603 B2	Polycyclic compounds and methods related thereto
US 8,367,043 B2	Biologically active nanoparticles a carbonate-substituted hydroxyapatite, process for their preparation and compositions incorporating the same
US 8,377,473 B2	Slow release magnesium composition and uses thereof
US 8,486,374 B2	Hydrophilic, non-aqueous pharmaceutical carriers and compositions and uses
US 8,278,309 B2	Synthetic mimetics of host defense and uses thereof
US 7,993,630 B2	Protection of skin from UV and peroxide
US 7,635,722 B2	Chemical induced intracellular hyperthermia
US 7,597,879 B2	Sunscreen safety and efficacy enhancement
US 7,351,739 B2	Bioactive compounds and methods of uses thereof
US 7,342,044 B2	Preservative blends containing quaternary ammonium compounds
US 6,946,427 B2	Preservative blends containing iodine containing compound

Acknowledgements The first and last authors thank the Brazilian National Council for Scientific and Technological Development (CNPq), for their Ph.D. scholarship (#141307/2020-0) and research support (Universal #427243/2016-5), respectively. We also thank the reviewers for their valuable suggestions as these helped us to improve the manuscript.

Compliance with ethical standards

Conflict of interest The author declares that he has no conflict of interest.

References

Abo-Khatwa AN, Al-Robai AA, Al-Jawhari DA (1996) Lichen acids as uncouplers of oxidative phosphorylation of mouse-liver mitochondria. Nat Toxins 4:96–102. https://doi.org/10.1002/19960402nt7

Abo-Khatwa AN, Al-Robai AA, Al-Jawhari DA (2015) The uncoupling of oxidative phosphorylation of mouse-liver mitochondria in vivo by usnic acid. JKAU 17:35–45

Abrahan EP, Florey HW (1949) Antimicrobial substances from lichens and algae. Antibiotic 1:566–575

Alavi M, Karimi N (2019) Biosynthesis of Ag and Cu NPs by secondary metabolites of usnic acid and thymol with biological macromolecules aggregation and antibacterial activities against multi drug resistant (MDR) bacteria. Int J

Biol Macromol 1:893–901. https://doi.org/10.1016/j.ijbiomac.2019.01.177

Al-Bekairi AM, Qureshi S, Chaudhry MA, Krishna DR, Shah AH (1991) Mitodepressive, clastogenic and biochemical effect of (+)-usnic acid in mice. J Ethnopharmacol 33:217–220. https://doi.org/10.1016/0378-8741(91)90079-S

Alemdaroğlu C, Değim Z, Celebi N, Zor F, Öztürk S, Erdoğan D (2006) An investigation on burn wound healing in rats with chitosan gel formulation containing epidermal growth factor. Burns 32:319–327. https://doi.org/10.1016/j.burns.2005.10.015

Alpsoy L, Baykal A, Amir M, Ülker Z, Nawaz M (2018) SPION@APTES@FA-PEG@Usnic acid bionanodrug for cancer therapy. J Supercond Nov Magn 31:1395–1401. https://doi.org/10.1007/s10948-017-4333-9

Antonenko YN, Khailova L, Rokitskaya T, Nosikova E, Nazarov P, Luzina O, Salakhutdinov N, Kotova E (2019) Mechanism of action of an old antibiotic revisited: role of calcium ions in protonophoric activity of usnic acid. Biochim Biophys Acta Bioeng 1860(4):310–316. https://doi.org/10.1016/j.bbabio.2019.01.005

Araújo AAS, de Melo MGD, Rabelo TK, Nunes PS, Santos SL, Serafini MR, Santos MRV, Quintans-Júnior LJ, Gelain DP (2015) Review of the biological properties and toxicity of usnic acid. Nat Prod Res. https://doi.org/10.1080/14786419.2015.1007455

Araújo HDA, Aires AL, Soares C, Brito T, Nascimento W, Martins M, Silva T, Brayner FA, Alves LC, Silva NH, Albuquerque MCPA, Lima VLM (2019) Usnic acid potassium salt from *Cladonia substellata* (Lichen): synthesis, cytotoxicity and in vitro anthelmintic activity and ultrastructural analysis against adult worms of *Schistosoma mansoni*. Acta Trop. https://doi.org/10.1016/j.actatropica.2018.12.024

Araújo HDA, Santos VHB, Brayner FA, Alves LC, Silva NH, Albuquerque MCPA, Aires AL, Lima VLM (2020) In vitro activity of usnic acid potassium salt against different developmental stages of *Schistosoma mansoni*: an ultrastructural study. Acta Trop 201:105–159. https://doi.org/10.1016/j.actatropica.2019.105159

Backorová M, Jendzelovsky R, Kello M, BacKor M, Mikeš J, Fedorocko P (2012) Lichen secondary metabolites are responsible for induction of apoptosis in HT-29 and A2780 human cancer cell lines. Toxicol In Vitro 26:462–468. https://doi.org/10.1016/j.tiv.2012.01.017

Battista S, Campitelli P, Galantini L, Köber M, Vargas-Nadal G, Ventosa N, Giansanti L (2020) Use of N-oxide and cationic surfactants to enhance antioxidant properties of (+)-usnic acid loaded liposomes. Coll Surf. https://doi.org/10.1016/j.colsurfa.2019.124154

Bazin MA, Lamer ACL, Delcros JG, Rouaud I, Uriac P, Boustie J, Corbel JC, Tomasi S (2008) Synthesis and cytotoxic activities of usnic acid derivatives. Bioorg Med Chem 16:6860–6866. https://doi.org/10.1016/j.bmc.2008.05.069

Behera BC, Mahadik N, Morey M (2012) Antioxidative and cardiovascular-protective activities of metabolite usnic acid and psoromic acid produced by lichen species *Usnea complanata* under submerged fermentation. Pharm Biol 50:968–979. https://doi.org/10.3109/13880209.2012.654396

Bessadottir M, Egilsson M, Einarsdottir E, Magnusdottir IH, Ogmundsdottir MH, Omarsdottir S, Ogmundsdottir HM (2012) Proton-shuttling lichen compound usnic acid affects mitochondrial and lysosomal function in cancer cells. PLoS ONE 7:1–7. https://doi.org/10.1371/journal.pone.0051296

Brandão LFG, Alcantara GB, Matos MFC, Bogo D, Freitas DS, Oyama NM, Honda NK (2013) Cytotoxic evaluation of phenolic compounds from lichens against melanoma cells. Chem Pharm Bull 61:176–183. https://doi.org/10.1248/cpb.c12-00739

Brisdelli F, Perilli M, Sellitri D, Piovano M, Garbarino JA, Nicoletti M, Bozzi A, Amicosante G, Celenza G (2013) Cytotoxic activity and antioxidant capacity of purified lichen metabolites: an in vitro study. Phytother Res 27:431–437. https://doi.org/10.1002/ptr.4739

Broska B, Sturdikova M, Pronayova NA, Liptaj T (1996) (-)-Usnic acid and its derivatives. Their inhibition of fungal growth and enzyme activity. Pharmazie 51:195–196

Bruno M, Trucchi B, Burlando B, Ranzato E, Martinotti S, Akkol EK, Süntar I, Keles H, Verotta L (2013) (+)-Usnic acid enamines with remarkable cicatrizing properties. Bioorg Med Chem 21:1834–1843. https://doi.org/10.1016/j.bmc.2013.01.045

Bunchorntavakul C, Reddy KR (2013) Review article: herbal and dietary supplement hepatotoxicity. Aliment Pharmacol Ther 37:3–17. https://doi.org/10.1111/apt.12109

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 Med 75:607–613. https://doi.org/10.1055/s-0029-1185329

Bustinza F (1952) Antibacterial substances from lichens. Econ Bot 6:402–406. https://doi.org/10.1007/BF02984888

Campanella L, Delfini M, Ercole P, Iacoangeli A, Risuleo G (2002) Molecular characterization and action of usnic acid: a drug that inhibits proliferations of mouse polyomavirus in vitro and whose main target is RNA transcription. Biochimie 84:329–334. https://doi.org/10.1016/s0300-9084(02)01386-x

Cansaran D, Kahya D, Yurdakulol E, Atakol O (2006) Identification and quantitation of usnic acid from the lichen *Usnea* species of Anatolia and antimicrobial activity. Z Naturforsch C J Biosci 61:773–776. https://doi.org/10.1515/znc-2006-11-1202

Chitturi S, Farrel GC (2008) Hepatotoxic slimming aids and other herbal hepatotoxins. J Gastroenterol Hepatol 23:366–373. https://doi.org/10.1111/j.1440-1746.2008.05310.x

Cocchietto M, Skert N, Nimis PL, Sava G (2002) A review on usnic acid, an interesting natural compound. Naturwissenschaften 89:137–146. https://doi.org/10.1007/s00114-002-0305-3

Correché ER, Carrasco M, Escudero ME, Velázquez L, Guzmán AMS, Giannini F, Enriz RD, Jaúregui EA, Ceñal JP, Giordano OS (1998) Study of cytotoxicity and antimicrobial activities of usnic acid derivatives. Fitoterapia, LXIX 6:493–501

Dailey RN, Montgomery DL, Ingram JT, Siemion R, Vasquez M, Raisbeck MF (2008) Toxicity of the lichen secondary metabolite (+)-usnic acid in domestic sheep. Vet Pathol 45:19–25. https://doi.org/10.1354/vp.45-1-19

De Carvalho EAB, Andrade PP, Silva NH, Pereira EC, Figueiredo RCBQ (2005) Effect of usnic acid from the lichen *Cladonia substellata* on *Trypanosoma cruzi* in vitro: an ultrastructural study. Mícron 36:155–161. https://doi.org/10.1016/j.micron.2004.09.003

Derici MK, Cansaran-Duman D, Taylan-Özkan A (2018) Usnic acid causes apoptotic-like death in *Leishmania major*, *L. infantum* and *L. tropica*. 3 Biotech 8:384. https://doi.org/10.1007/s13205-018-1409-6

Durazo FA, Lassman C, Han SHB, Saab S, Lee NP, Kawano M, Saggi B, Gordon S, Farmer DG, Yersiz H, Goldstein RLI, Ghobrial M, Busuttil RW (2004) Fulminant liver failure due to usnic acid for weight loss. Am J Gastroent 99(5):950–952. https://doi.org/10.1111/j.1572-0241.2004.04165.x

Edwards HGM, 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. J Mol Struct 651:27–37. https://doi.org/10.1016/S0022-2860(02)00626-9

Einarsdóttir E, Groeneweg J, Björnsdóttir GG, Harðardottir G, Omarsdóttir S, Ingólfsdóttir K, Ögmundsdóttir HM (2010) Cellular mechanisms of the anticancer effects of the lichen compound usnic acid. Planta Med 76(969–974):2010. https://doi.org/10.1055/s-0029-1240851

Elo H, Matikainen J, Pelttari E (2007) Potent activity of the lichen antibiotic (+)-usnic acid against clinical isolates of vancomycin-resistant enterococci and methicillin-resistant *Staphylococcus aureus*. Naturwissenschaften 94:465–468. https://doi.org/10.1007/s00114-006-0208-9

Fernández-Moriano C, Divakar PK, Crespo A, Gómez-Serranillos MP (2017) Protective effects of lichen metabolites evernic and usnic acids against redox impairment-mediated cytotoxicity in central nervous system-like cells. Food Chem Toxicol 105:262–277. https://doi.org/10.1016/j.fct.2017.04.030

Ferraz-Carvalho RS, Pereira MA, Linhares LA, Lira-Nogueira MCB, Cavalcanti IMF, Santos-Magalhães NS, Montenegro LML (2016) Effects of the encapsulation of usnic acid into liposomes and interactions with antituberculous agents against multidrug-resistant tuberculosis clinical isolates. Mem Inst Oswaldo Cruz 111:330–334. https://doi.org/10.1590/0074-02760150454

Foti RS, Dickmann LJ, Davis JA, Greene RJ, Hill JJ, Howard ML, Pearson JT, Rock DA, Tay JC, Wahlstrom JL, Slatter JG (2008) Metabolism and related human risk factor for hepatic damage by usnic acid containing nutritional supplements. Xenobiotica 38:264–280. https://doi.org/10.1080/00498250701802514

Fournet A, Ferreira ME, Arias RA, Ortiz TS, Inchausti A, Yaluff G, Quilhot W, Fernandez E, Hidalgo ME (1997) Activity of compounds isolated from chilean lichens against experimental cutaneous leishmaniasis. Comp Biochem Physiol 116:51–54. https://doi.org/10.1016/S0742-8413(96)00127-2

Francoline P, Norris A, Piozzi G, Donelli P, Stoodley P (2004) Usnic acid, a natural antimicrobial agent able to inhibit bacterial biofilm formation on polymer surfaces. Antimicrob Agents Chemother 48:4360–4365. https://doi.org/10.1128/AAC.48.11.4360-4365.200

Francolini I, Piozzi A, Donelli G (2019a) Usnic acid: potential role in management of wound infections. Adv Exp Med Biol 1214:31–41. https://doi.org/10.1007/5584_2018_260

Francolini I, Giansanti L, Piozzi A, Altieri B, Mauceri A, Mancini G (2019b) Glucosylated liposomes as drug delivery systems of usnic acid to address bacterial infections. Colloids Surf B Biointerfaces 1:632–638. https://doi.org/10.1016/j.colsurfb.2019.05.056

Fujimoto K, Kishino H, Hashimoto K, Watanabe K, Yamoto T, Mori K (2020) Biochemical profiles of rat primary cultured hepatocytes following treatment with rotenone, FCCP, or(+)-usnic acid. J Toxicol Sci 45:339–347. https://doi.org/10.2131/jts.45.339

Galanty A, Paśko P, Podolak I (2019) Enantioselective activity of usnic acid: a comprehensive review and future perspectives. Phytochem Rev 18:527–548. https://doi.org/10.1007/s11101-019-09605-3

Garg A, Garg S, Sahu N, Rani S, Gupta U, Yadav A (2018) Heparin appended ADH-anionic polysaccharide nanoparticles for site-specific delivery of usnic acid. Int J Pharm 557:238–253. https://doi.org/10.1016/j.ijpharm.2018.12.049

Gopinath D, Ahmed MR, Gomathi K (2004) Dermal wound healing processes with curcumin induced collagen films. Biomaterials 25:1911–1917. https://doi.org/10.1016/S0142-9612(03)00625-2

Grumezescu A, Cotar A, Andronescu E, Ficai A, Ghitulica C, Grumezescu V, Vasile B, Chifiriuc M (2013) *In vitro* activity of the new water dispersible Fe_3O_4@usnic acid nanostructure against planktonic and sessile bacterial cells. J Nanopart Res 15:1766. https://doi.org/10.1007/s11051-013-1766-3

Guo L, Shi Q, Fang JL, Mei N, Ali AA, Lewis SM, Leakey JEA, Frankos VH (2008) Review of usnic acid and *Usnea barbata* toxicity. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 26(4):317–338. https://doi.org/10.1080/10590500802533392

Guo S-Y, Liu W-X, Han L-F, Chen JZ (2017) Antifungal activity of lichen extracts and usnic acid for controlling the saprolegniasis. Int J Environ Agric Res 3(5):43–47. https://doi.org/10.25125/agriculture-journal-IJOEAR-APR-2017-35

Han D, Matsumaru K, Rettori D, Kaplowitz N (2004) Usnic acid-induced necrosis of cultured mouse hepatocytes: inhibition of mitochondrial function and oxidative stress. Biochem Pharmacol 67:439–451. https://doi.org/10.1016/j.bcp.2003.09.032

Hawranik DJ, Anderson KS, Simmonds R, Sorensen JL (2009) The chemoenzymatic synthesis of usnic acid. Bioorg Med Chem Lett 19:2381–2383. https://doi.org/10.1016/j.bmcl.2009.03.087

Honda NK, Pavan FR, Coelho RG, De Andrade Leite SR, Micheletti AC, Lopes TI, Misutsu MY, Beatriz A, Brum RL, Leite CQ (2010) Antimycobacterial activity of lichen substances. Phytomedicine 17(5):328–332. https://doi.org/10.1016/j.phymed.2009.07.018

Huang Z, Zheng G, Tao J, Ruan J (2011) Anti-inflammatory effects and mechanisms of usnic acid. J Wuhan Univ Tech Mater Sci 26:955–959. https://doi.org/10.1007/s11595-011-0344-8

Huneck S (1999) The significance of lichens and their metabolites. Naturwissenschaften 86(12):559–570. https://doi.org/10.1007/s001140050676

Huneck S, Yoshimura I (1996) Identification of lichen substances. Springer, Berlin, pp 1–493

Ingólfsdóttir K (2002) Usnic acid. Phytochemistry 61:729–736. https://doi.org/10.1016/s0031-9422(02)00383-7

Ingólfsdóttir K, Bloomfield SF, Hylands PJ (1985) In vitro evaluation of antimicrobial activity of lichen metabolites as potential preservatives. Antimicrob Agents Chemother 28:289–292. https://doi.org/10.1128/aac.28.2.289

Ingólfsdóttir K, Chung GAC, Skúlason VG, Gissurarson SR, Vilhelmsdóttir M (1998) Antimycobacterial activity of lichen metabolites in vitro. Eur J Pharm Sci 6:141–144. https://doi.org/10.1016/s0928-0987(97)00078-x

Ivanovic J, Meyer F, Misic D, Asanin J, Jaeger P, Zizovic I, Eggers R (2013) Influence of different pre-treatment methods on isolation of extracts with strong antibacterial activity from lichen *Usnea barbata* using carbon dioxide as a solvent. J Supercond Fluids 76:1–9. https://doi.org/10.1016/j.supflu.2013.01.005

Ji X, Khan IA (2005) Quantitative determination of usnic acid in usnea lichen and its products by reversed-phase liquid chromatography with photodiode array detector. J AOAC Int Gaithersburg 88(5):1265–1268. https://doi.org/10.1093/jaoac/88.5.1265

Johnson RB, Feldot G, Lardy HA (1950) The mode of action of the antibiotic usnic acid. Ach Biochem 28:317–323

Joseph A, Lee T, Moland CL, Branham WS, Fuscoe JC, Leakey JEA, Allaben WT, Lewis SM, Ali AA, Desai VG (2009) Effect of (+)-usnic acid on mitochondrial functions as measured by mitochondria-specific oligonucleotide microarray in liver of B6C3F1 mice. Mitochondrion 9:149–158. https://doi.org/10.1016/j.mito.2009.02.002

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. J Photochem Photobiol B 101:97–102. https://doi.org/10.1016/j.jphotobiol.2010.06.017

Kreft S, Strukelj B (2001) Reversed-polarity capillary zone electrophoretic analysis of usnic acid. Electrophoresis 22:2755–2757. https://doi.org/10.1002/1522-2683(200108)22:13%3c2755:AID-ELPS2755%3e3.0.CO;2-6

Kristmundsdóttir T, Aradóttir HA, Ingólfsdóttir K, Ögmundsdóttir HM (2002) Solubilization of lichen metabolite (+)-usnic acid for testing in tissue culture. J Pharm Pharmacol 54:1447–1452. https://doi.org/10.1211/002235702225

Kristmundsdóttir T, Jónsdóttir E, Ögmundsdóttir HM (2005) Solubilization of poorly soluble lichen metabolites for biological testing on cell lines. Eur J Pharm Sci 24:539–543. https://doi.org/10.1016/j.ejps.2005.01.011

Kumar S, Müller K (1999) Lichen metabolites 2: antiproliferative and cytotoxic activity of gyrophoric, usnic and diffractaic acid on human keratinocyte growth. J Nat Prod 62:821–823. https://doi.org/10.1021/np980378z

Kumar P, Ramteke P, Pandey A, Pandey H (2019) Evaluation of antifungal activity of blended cinnamon oil and usnic acid nanoemulsion using candidiasis and dermatophytosis models. Biocat Agric Biotechnol 18:101062. https://doi.org/10.1016/j.bcab.2019.101062

Kupchan SM, Kopperman HL (1975) l-Usnic acid: tumor inhibitor isolated from lichens. Experientia 31:625. https://doi.org/10.1007/bf01944592

Lage TCA, Maciel T, Mota Y, Sisto F, Sabino J, Carinhanha CSJ, Figueiredo I, Masia C, de Fátima A, Fernandes S, Modolo L (2018) *In vitro* inhibition of *Helicobacter pylori* and interaction studies of lichen natural products with jack bean urease. New J Chem 42:5356–5366. https://doi.org/10.1039/C8NJ00072G

Lauterwein M, Oethinger M, Belsner K, Peter T, Marre R (1995) In vivo activities of the lichen secondary metabolites vulpinic acid, (+) usnic acid, and (-) usnic acid against aerobic and anaerobic microorganisms. Antimicrob Agents Chemother 39:2541–2543. https://doi.org/10.1128/aac.39.11.2541

Lima RMC, Nascimento SC, Pereira EC, Campos-Takaki GM (1990) Atividade citotóxica e antitumoral de extratos liquênicos. Bol Soc Brot 63(339):348

Lira MCB, Ferraz MS, Da Silva DGVC, Cortes ME, Teixeira KI, Caetano NP, Sinisterra RD, Ponchel G, Santos-Magalhães NS (2009a) Inclusion complex of usnic acid with β-cyclodextrin: characterization and nanoencapsulation into liposomes. J Incl Phenom Macrocycl Chem 64:215–224. https://doi.org/10.1007/s10847-009-9554-5

Lira MCB, Siqueira M, Rolim-Santos HML, Galetti FCS, Simioni A, Santos NP, Egito EST, Silva CL, Tedesco AC, Santos-Magalhães NS (2009b) In vitro uptake and antimycobacterial activity of liposomal usnic acid formulation. J Lipo Res 19:49–58. https://doi.org/10.1080/08982100802564628

Liu Q, Zhao X, Lu X, Fan X, Wang Y (2012) Proteomic study on usnic-acid-induced hepatotoxicity in rats. J Agric Food Chem 60:7312–7317. https://doi.org/10.1021/jf2046834

Lu X, Zhao Q, Tian Y, Xiao S, Jin T, Fan X (2011) A Metabonomic characterization of (+)-usnic acid-induced liver injury by gas chromatography-mass spectrometry-based metabolic profiling of the plasma and liver in rat. Int J Toxicol 30:478–491. https://doi.org/10.1177/1091581811414436

Lukáč M, Prokipčák I, Lacko I, Devínsky F (2012) Solubilisation of (+)-usnic acid in aqueous micellar solutions of gemini and heterogemini surfactants and their equimolar mixture. Acta Fac Pharm Univ Comen 2:36–43. https://doi.org/10.2478/v10219-012-0022-8

Lumbsch HT (1995) A new species in the *Lecanora subfusca* group containing usnic acid in addition to atranorin. Lichenologist 27:161–167. https://doi.org/10.1016/S0024-2829(95)80015-8

Luo H, Yamamoto Y, Jeon H, Liu YP, Jung JS, Koh YJ, Hur J (2011) Production of anti-*Helicobacter pylori* metabolite by the lichen-forming fungus *Nephromopsis pallescens*. J Microbiol 49:66–70. https://doi.org/10.1007/s12275-011-0289-9

Luzina OA, Polovinka MP, Salakhutdinov NF, Tolstikov GA (2007) Chemical modification of usnic acid. Russ Chem Bull 56:1249–1251. https://doi.org/10.1007/s11172-007-0189-7

Marcano V, Rodriguez-Alcocer V, Morales M (1999) Occurrence of usnic acid in *Usnea laevis* Nylander (lichenized ascomycetes) from the Venezuelan Andes.

J Ethnopharmacol 66:343–346. https://doi.org/10.1016/s0378-8741(98)00181-0

Martinelli A, Bakry A, D'Ilario L, Francolini I, Piozzi A, Taresco V (2014) Release behavior and antibiofilm activity of usnic acid-loaded carboxylated poly (L-lactide) microparticles. Eur J Pharm Biopharm 88:415–423. https://doi.org/10.1016/j.ejpb.2014.06.002

Mayer M, O'neill MA, Murray KE, Santos-Magalhães NS, Carneiro-Leão AMA, Thompson AM, Appleyard VCL (2005) Usnic acid, a non-genotoxic compound with anticancer properties. Anticancer Drugs 16:805–809. https://doi.org/10.1097/01.cad.0000175588.09070.77

Moreira CT, Oliveira AL, Comar JF, Peralta RM, Bracht A (2013) Harmful effects of usnic acid on hepatic metabolism. Chem Biol Interact 203:502–511. https://doi.org/10.1016/j.cbi.2013.02.001

Müller K (2001) Pharmaceutically relevant metabolites from lichens. Appl Microbiol Biotechnol 56:9–16. https://doi.org/10.1007/s002530100684

Najdenova V, Lisickov K, Zoltán D (2001) Antimicrobial activity and stability of usnic acid and its derivatives in some cosmetic products. Olaj Szappan Kozmetika 50:158–160

Neff GW, Reddy KR, Durazo FA, Meyer D, Marrero R, Kaplowitz N (2004) Severe hepatotoxicity associated with the use of weight loss diet supplements containing ma huang or usnic acid. J Hepatol 41:1062–1064. https://doi.org/10.1016/j.jhep.2004.06.028

Nikolić V, Stankovic M, Nikolić L, Nikolić G, Ilic-Stojanovic S, Popsavin M, Zlatković S, Kundaković T (2013) Inclusion complexes with cyclodextrin and usnic acid. J Incl Phenom Macrocycl Chem 76:1–2. https://doi.org/10.1007/s10847-012-0187-8

Nunes PS, Albuquerque-Júnior RLC, Cavalcante DRR, Dantas MDM, Cardoso JC, Bezerra MS, Souza JCC, Serafini MR, Quitans-Jr LJ, Bonjardim LR, Araújo AAS (2011) Collagen-based films containing liposome-loaded usnic acid as dressing for dermal burn healing. J Biomed Biotechnol. https://doi.org/10.1155/2011/761593

O'Neill MA, Mayer M, Murray KE, Rolim-Santos HML, Santos-Magalhães NS, Thompson AM, Appleyard VCL (2010) Does usnic acid affect microtubules in human cancer cells? Braz J Biol 70:659–664. https://doi.org/10.1590/s1519-69842010005000001

Odabasoglu F, Cakir A, Suleyman H, Aslan A, Bayir Y, Halici M, Kazaz C (2006) Gastroprotective and antioxidant effects of usnic acid on indomethacin-induced gastric ulcer in rats. J Ethnopharmacol 103:59–65. https://doi.org/10.1016/j.jep.2005.06.043

Pacheco D, Travassos AR, Antunes J, Soares De Almeida L, Filipe P, Correia T (2012) Occupational airborne contact dermatitis caused by usnic acid in a domestic worker. Allergol Immunopathol 42:80–82. https://doi.org/10.1016/j.aller.2012.07.017

Pereira EC, Nascimento SC, Lima RMC, Silva NH, Oliveira AFM, Boitard M, Beriel H, Vicente C, Legaz ME (1994) Analysis of *Usnea fasciata* crude extracts with antineoplasic activity. Tokai J Exp Clin Med 19:47–52

Piovano M, Chamy MC, Garbarino JA, Quilhot W (2000) Secondary metabolites in the genus *Sticta* (lichens). Biochem Syst Ecol 28:589–590. https://doi.org/10.1016/S0305-1978(99)00092-7

Pires RH, Lucarini R, Mendes-Gianninia MJS (2011) Effect of usnic acid on *Candida orthopsilosis* and *C. parapsilosis*. Antimicrob Agents Chemother 56:595–597. https://doi.org/10.1128/AAC.05348-1

Piska K, Galanty A, Koczurkiewicz P, Żmudzki P, Potaczek J, Podolak I, Pękala E (2018) Usnic acid reactive metabolites formation in human, rat, and mice microsomes. Implication for hepatotoxicity. Food Chem. Toxicol 120:112–118. https://doi.org/10.1016/j.fct.2018.07.005

Pompilio A, Pomponio S, Vincenzo VD, Crocetta V, Nicoletti M, Piovano M, Garbarino JA, Bonaventura GD (2013) Antimicrobial and antibiofilm activity of secondary metabolites of lichens against methicillin-resistant *Staphylococcus aureus* strains from cystic fibrosis patients. Fut Microbiol 8:281–292. https://doi.org/10.2217/fmb.12.142

Pramyothin P, Janthasoot W, Pongnimitprasert N, Phrukudomc S, Ruangrungsi N (2004) Hepatotoxic effect of (+)-usnic acid from *Usnea siamensis* Wainio in rats, isolated rat hepatocytes and isolated rat liver mitochondria. J Ethnopharmacol 90:381–387. https://doi.org/10.1016/j.jep.2003.10.019

Prokopiev IA, Filippov EV, Filippova GV, Gladkina NP (2017) Genotoxic effect of usnic acid enantiomers in vitro in human peripheral blood lymphocytes. Tsitologiia 59(1):13–18. https://doi.org/10.1134/S1990519X17020031

Prokopiev I, Filippova G, Filippov E, Voronov I, Sleptsov I, Zhanataev A (2019) Genotoxicity of (+)- and (−)-usnic acid in mice. Mutat Res Genet Toxicol Environ Mutagen. https://doi.org/10.1016/j.mrgentox.2019.01.010

Rabelo TK, Zeidán-Chuliá F, Vasques LM, dos Santos JPA, da Rocha RF, Pasquali MAB, Rybarczyk-Filho JL, Araujo AAS, Moreira JCF, Gelain DP (2012) Redox characterization of usnic acid and its cytotoxic effect on human neuron-like cells (SH-SY5Y). Toxicol In Vitro 26:304–314

Ramos DF, Da Silva PEA (2010) Antimycobacterial activity of usnic acid against resistant and susceptible strains of *Mycobacterium tuberculosis* and non-tuberculous mycobacteria. Pharm Biol 48:260–263. https://doi.org/10.3109/13880200903085490

Rancan F, Rosan S, Boehm K, Fernández E, Hidalgo ME, Quilhot W, Rubio C, Boehm F, Piazena H, Oltmanns U (2002) Protection against UVB irradiation by natural filters extracted from lichens. J Photochem Photobiol 68:133–139. https://doi.org/10.1016/s1011-1344(02)00362-7

Ribeiro-Costa RM, Alves AJ, Santos NP, Nascimento SC, Gonçalves ECP, Silva NH, Honda NK, Santos-Magalhães NS (2004) *In vitro* and in vivo properties of usnic acid encapsulated into PLGA-microspheres. J Microencapsul 21:371–384. https://doi.org/10.1080/02652040410001673919

Safak B, Ciftci IH, Ozdemir M, Kiyildi N, Cetinkaya Z, Aktepe OC, Altindis M, Asik G (2009) *In vitro* anti-*Helicobacter pylori* activity of usnic acid. Phytother Res 23:955–957. https://doi.org/10.1002/ptr.2690

Sahu SC, Amankwa-Sakyi M, O'Donnell MW Jr, Sprando RL (2011) Effects of usnic acid exposure on human hepatoblastoma HepG2 cells in culture. J Appl Toxicol 32:722–730. https://doi.org/10.1002/jat.1721

Sahu SC, O'Donnell MW Jr, Sprando RL (2012) Interactive toxicity of usnic acid and lipopolysaccharides in human

liver HepG2 cells. J Appl Toxicol 32:739–748. https://doi.org/10.1002/jat.2768

Sanchez W, Maple JT, Burgart LJ, Kamath PS (2006) Severe hepatotoxicity associated with use of a dietary supplement containing usnic acid. Mayo Clin Proc 81(4):541–544. https://doi.org/10.4065/81.4.541

Santos NP, Nascimento SC, Silva JF, Pereira EC, Silva NH, Honda NK, Santos-Magalhães NS (2005) Usnic acid-loaded nanocapsules: an evaluation of citotoxicity. J Drug Deliv Sci Technol 15(5):355–361. https://doi.org/10.1016/S1773-2247(05)50065-0

Santos NPS, Nascimento SC, Wanderley MSO, Pontes-Filho NT, Silva JF, Castro CMMB, Pereira EC, Silva NH, Honda NK, Santos-Magalhães NS (2006) Nanoencapsulation of usnic acid: an attempt to improve antitumour activity and reduce hepatotoxicity. Eur J Pharm Biopharm 64:154–160. https://doi.org/10.1016/j.ejpb.2006.05.018

Santos L, Caramantin Soriano M, Mirabal-Gallardo Y, Carrasco Sánchez V, Nachtigall F, Pereira I, Pereira E (2015) Chemotaxonomic fingerprinting of Chilean lichens through MALDI and electrospray ionization mass spectrometry. Braz Arch Biol Technol 58(2):244–253. https://doi.org/10.1590/S1516-8913201400185

Scirpa P, Scambia G, Masciullo V, Battaglia F, Foti E, Lopez R, Villa P, Malecoe M, Mancuso S (1999) Terapia adiuvante con um preparato a base di zinco solfato e acido usnico delle lesioni genitali da Human Papilloma Vírus (HPV) dopo trattamento chirurgico distruttivo. Minerva Ginec 51:255–260

Segatore B, Bellio P, Setacci D, Brisdelli F, Piovano M, Garbarino J, Nicoletti M, Amicosante G, Perilli M, Celenza G (2012) *In vitro* interaction of usnic acid in combination with antimicrobial agents against methicillin-resistant *Staphylococcus aureus* clinical isolates determined by FICI and ΔE model methods. Phytomedicine 19:341–347. https://doi.org/10.1016/j.phymed.2011.10.012

Segura-Sanchez F, Bouchemal K, Lebas G, Vauthier C, Santos-Magalhaes NS, Ponchel G (2009) Elucidation of the complexation mechanism between (+)-usnic acid and cyclodextrins studied by isothermal titration calorimetry and phase-solubility diagram experiments. J Mol Recognit 22:232–241. https://doi.org/10.1002/jmr.936

Sharma RK, Jannke PJ (1966) Acidity of usnic acid. Ind J Chem 4:16–18

Sheu M, Simpson EL, Law SV, Storrs FJ (2006) Allergic contact dermatitis from a natural deodorant: A report of 4 cases associated with lichen acid mix allergy. J Am Acad Dermat 55(2):332–337. https://doi.org/10.1016/j.jaad.2004.12.043

Shibata S (2000) Great discoveries in bryology and lichenology. Bryologist 103(4):710–719

Shtro AA, Zarubaev VV, Luzina OA, Sokolov DN, Salakhutdinov NF (2015) Derivatives of usnic acid inhibit broad range of influenza viruses and protect mice from lethal influenza infection. Antiviral Chem Chemother 24(3–4):92–98. https://doi.org/10.1177/2040206616636992

Siddiqi K, Rashid M, Rahman A, Tajuddin T, Husen A, Rehman S (2018) Biogenic fabrication and characterization of silver nanoparticles using aqueous-ethanolic extract of lichen (*Usnea longissima*) and their antimicrobial activity. Biomater Res 22:23. https://doi.org/10.1186/s40824-018-0135-9

Silva CR, Marinho KSN, Silva TDS, Ferreira DKS, Aguiar GM, Martins MCB, Santos KRP, Aguiar Junior FCA, Santos NPS, Pereira EC, Silva NH (2017) Teratogenic effect of usnic acid from *Cladonia substellata* vainio during organogenesis. BioMed Res Int. https://doi.org/10.1155/2017/5948936

Singh B, Prateeksha G, Pandey G, Jadaun V, Singh S, Bajpai R, Nayaka S, Naqvi S, Rawat A, Upreti D, Singh B (2014) Development and characterization of a novel Swarna-based herbo-metallic colloidal nano-formulation-inhibitor of *Streptococcus mutans* quorum sensing. RSC Adv 5:5809–5822. https://doi.org/10.1039/C4RA11939H

Siqueira-Moura MP, Lira MCB, Santos-Magalhães NS (2008) Validation of a UV-spectrophotometric analytical method for the determination of usnic acid in liposomes. Braz J Pharm Sci 44:621–628. https://doi.org/10.1590/S1516-93322008000400008

Sokolov DN, Luzina OA, Salakhutdinov NF (2012a) Usnic acid: preparation, structure, properties, and chemical transformations. Russ Chem Rev 81:747–768. https://doi.org/10.1070/RC2012v081n08ABEH004245

Sokolov DN, Zarubaev VV, Shtro AA, Polovinka MP, Luzina OA, Komarova NI, Salakhutdinov NF, Kiselev OI (2012b) Anti-viral activity of (−)- and (+)-usnic acids and their derivatives against influenza virus A(H1N1). Bioorg Med Chem Lett 22:7060–7064. https://doi.org/10.1016/j.bmcl.2012.09.084

Sonko BJ, Schmitt TC, Guo L, Shi Q, Boros LG, Leakey JEA, Beger RD (2011) Assessment of usnic acid toxicity in rat primary hepatocytes using 13C isotopomer distribution analysis of lactate, glutamate, and glucose. Food Chem Toxicol 49:2968–2974. https://doi.org/10.1016/j.fct.2011.07.047

Stan M, Constanda S, Grumezescu V, Andronescu E, Ene A, Holban A, Vasile B, Mogoantă L, Balseanu A, Mogoşanu G, Socol G, Grumezescu A, Dinischiotu A, Lazar V, Chifiriuc M (2016) Thin coatings based on ZnO@ C18-usnic acid nanoparticles prepared by MAPLE inhibit the development of *Salmonella enterica* early biofilm growth. Appl Surf Sci 374:318–325. https://doi.org/10.1016/j.apsusc.2015.12.063

Sussmann RAC, Angeli CB, Peres VJ, Kimura EA, Katzin AM (2011) Intraerythrocytic stages of *Plasmodium falciparum* biosynthesize vitamin E. FEBS Lett 585:3985–3991. https://doi.org/10.1016/j.febslet.2011.11

Taresco V, Francolini I, Padella F, Bellusci M, Boni A, Innocenti C, Martinelli A, D'Ilario L, Piozzi A (2015) Design and characterization of antimicrobial usnic acid loaded-core/shell magnetic nanoparticles. Sci Eng, Material. https://doi.org/10.1016/j.msec.2015.03.044

Tiwari G, Sriwastawa B, Bhati L, Pandey S, Pandey P, Bannerjee S (2012) Drug delivery systems: an updated review. Int J Pharm Investig 2:2–11. https://doi.org/10.4103/2230-973X.96920

Vavasseur A, Gautier H, Thibaud MC, Lascève G (1991) Effect of usnic acid on the oxygen exchange properties of mesophyll cell protoplast from *Commelina communis* L. J Plant Physiol 139:90–94. https://doi.org/10.1016/S0176-1617(11)80171-9

Venkataramana D, Krishna DR (1992) High-performance liquid of usnic acid in plasma. J Chromatogr 575:167–170. https://doi.org/10.1016/0378-4347(92)80520-z

Vijayakumar CS, Viswanathan S, Reddy MK, Parvathavarthini AB, Kundu AB, Sukumar E (2000) Anti-inflammatory activity of (+)-usnic acid. Fitoterapia 71:564–566. https://doi.org/10.1016/s0367-326x(00)00209-4

Wu J, Zhang M, Ding D, Yan B (1995) Effect of *Cladonia alpestris* on *Trychomonas vaginalis* in vitro. Chin J Parasitol Parasit Dis 13:126–129

Xavier-Filho L, Legaz ME, Cordoba CV, Pereira EC (eds) (2006) Biologia de Liquens. Âmbito Cultural Edições Ltda., Rio de Janeiro, 664 p

Xu H-Y, Zhang Y-Q, Liu Z-M, Chen T, Lv C-Y, Tang S-H, Zhang X-B, Zhang W, Li Z-Y, Zhou R-R, Yang H-J, Wang X-J, Huang L-Q (2019) ETCM: an encyclopaedia of traditional Chinese medicine. Nucleic Acids Res 47(D1):D976–D982. https://doi.org/10.1093/nar/gky987

Yamamoto Y, Miura Y, Kinoshita Y, Higuchi M, Yamada Y, Murakami A, Ohigashi H, Koshimizu K (1995) Screening of tissues cultures and thalli of lichens and some their active constituents for inhibition of tumor promoter-induced Epstein–Barr virus activation. Chem Pharm Bull 43:1388–1390. https://doi.org/10.1248/cpb.43.1388

Yu X, Guo Q, Su G, Yang A, Hu Z, Qu C, Wan Z, Li R, Tu P, Chai X (2016) Usnic acid derivatives with cytotoxic and antifungal activities from the lichen *Usnea longissima*. J Nat Prod 79(5):1373–1380. https://doi.org/10.1021/acs.jnatprod.6b00109