Chemical composition and biological activities of the extracts and secondary metabolites of lichens belonging to the genus *Usnea*, Parmeliaceae

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Lichens represent a promising source of antimicrobial, cytotoxic and antioxidant agents. Their great pharmacological potential lies in the fact that they represent specific symbiotic organisms and thus possess natural roles allowing them to be highly adaptable to different environmental conditions. On the other hand, stated biological activities of lichens with prospective medicinal significance may be connected to their long-term use in the traditional treatment of various ailments. Genus *Usnea* from the Parmeliaceae family is certainly one of the best studied in terms of chemical composition and biological properties of its extracts and/or isolated compounds. In the first part of the study, a detailed review of the literature has been performed yielding a detailed report on the investigations of biological activities of the lichens belonging to this genus. In the second part of the study, the chemical composition of the lichens from the genus was described and, additionally, a survey of the biological properties of the most representative secondary metabolites in these lichens has been reported. It could be concluded that the extracts and/or isolated compounds from the lichens belonging to the genus *Usnea* may be considered a valuable source of prospective drug candidates with potential clinical relevance.

Key words: *Lichens; Usnea; Parmeliaceae; secondary metabolites; biological activities*

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1. INTRODUCTION

Lichens are considered stable, ecologically obligate symbiotic associations between fungi (mycobiont) and one or more photosynthetic partners: eukaryotic alga and/or cyanobacteria (photobiont) or in some cases non-photosynthetic bacteria (Molnár and Farkas, 2010; Shrestha and St. Clair, 2013). Considering that approximately 21% of all fungi are able to act as mycobiont in lichen, it is not surprising that they represent the largest mutualistic group among fungi. Therefore, since 1983, the name of the lichen refers to its mycobiont, that is, they belong to the kingdom of Fungi according to the biological classification (Shrestha and St. Clair, 2013).

Due to the growing development of new methods of chemical analysis enabling the discovery of new substances and their structures, biochemical analysis of lichens went through "exponential" development in past decades. In the past decades, approximately 1050 secondary metabolites of lichens have been identified, including the substances found in the intact thalli of lichen, as well as compounds identified in the culture of lichens (Shrestha and St. Clair, 2013). Unlike primary metabolites that have a structural function and are synthesized independently by both symbionts, secondary metabolites are produced exclusively by mycobionts, after which they are transported outside the hyphae and are deposited in different parts of the thallus, most often in the upper cortex or in the medullary layer, as extracellular small crystals on the outer surfaces of the hyphae. In accordance to their function, secondary metabolites of lichens are limited to certain parts of the thallus, while the patterns of their distribution are taxons-specific, which is widely used in the lichen systematics (Molnár and Farkas, 2010; Shrestha and St. Clair, 2013).

Secondary metabolites of the lichens include chemically distinct aliphatic and aromatic compounds of relatively small molecular weights, most of which are from the acetyl-polymalonyl biosynthetic pathway, while a smaller number originate from mevalonic acid and shikimic acid biosynthetic pathway (Figure 1). Although derived exclusively from the mycobiont, the metabolic interaction between mycobiont and photobiont in lichens is necessary for the production of

secondary metabolites, which is associated with their large metabolic diversity. Therefore, only a very small number of these compounds (50-60) can be found in other organisms, such as non-lichenized fungi or higher plants (Molnár and Farkas, 2010).

In recent years, the biological activities of lichens and their secondary metabolites with potential medicinal significance have been intensively investigated, which is, on the one hand, related to their natural roles allowing them to be highly adaptable to different environmental conditions (defense against UV radiation and protection against predators and pathogenic microorganisms), and on the other to their traditional use in the treatment of various diseases (bronchitis, asthma, blood and heart diseases, leprosy, scabies, stomach disorders, etc.) (Gómez-Serranillos et al., 2014; Shrestha and St. Clair, 2013). These studies implied a large pharmacological potential of lichens and/or their secondary metabolites as a promising source of potential medicines with anticancer, antimicrobial, anti-inflammatory and antioxidant activity (Gómez-Serranillos et al., 2014; White et al., 2014).

In this regard, the genus *Usnea* of the Parmeliaceae family has been extensively studied. It encompasses around 300 species widely distributed from polar zones to tropical regions. Diagnostic characteristics of the genus include shrubby thallus with pale, yellowish-green branches with radial symmetry, a cartilaginous central and the presence of usnic acid in the cortex (Clerc, 1998; Gómez-Serranillos et al., 2014; Ohmura, 2012). Due to their specific appearance, members of this genus are also referred to as 'beard-like' lichens (Clerc, 1998; Gómez-Serranillos et al., 2014). In this paper chemical composition and biological activities of the species of the genus *Usnea* as well as the biological activities of secondary metabolites identified in the species of this genus will be presented (Table 1).

2. BIOLOGICAL ACTIVITIES OF THE SPECIES BELONGING TO THE GENUS *USNEA*

2.1. Antimicrobial activity

As seen in Table 1, one of the most studied activities of the species belonging to the genus *Usnea* is antimicrobial activity (antibacterial, antimycotic, antiprotozoal and antiviral). Generally, it was observed that all species of Parmeliaceae family had a stronger antimicrobial effect compared to the lichens-members of other families, and also that the extracts prepared with organic solvents were more active than the ones prepared using water. This finding is probably connected to poor solubility of lichen secondary metabolites in water (Gómez-Serranillos et al., 2014). Recent study investigating 24 lichens belonging to 6 different families indicated methanol extract of *Usnea* sp. to possess antimicrobial activity against Gram-positive (G(+)) bacteria, while there were no effects against Gram-negative (G(-)) species and also against pathogenic fungus - *Candida albicans* (Paudel et al., 2012). On the other hand, in the research of Rukayadi et al. (2008) screening 23 medicinal plants/lichens from Thailand against six pathogenic *Candida* species, *U. siamensis* was active against *Candida guilliermondii*. A study of Cansaran et al. (2006) involved 6 members of the genus *Usnea* (*U. florida*, *U. barbata*, *U. longissima*, *U. rigida*, *U. hirta* and *U. subflorida*) against various G(+) and G(-) bacteria. *U. subflorida* showed the strongest antimicrobial activity, which was correlated with the highest content of usnic acid in this species. On the other hand, antimicrobial activity was observed in other studies for two out of six investigated lichens (*U. barbata* and *U. florida*). Namely, antibacterial, antimycotic and antiprotozoal activity was confirmed for dichloromethane and methanol extract of *U. florida* (Schmeda-Hirschmann et al., 2008). Also, comparative analysis of acetone, methanol and aqueous extract of *U. barbata* against 10 bacterial and 5 fungal strains implied the strongest activity against G(+) bacterial species i.e. the strongest antimicrobial effect of the acetone extract (Madamombe and Afolayan, 2003). These findings were confirmed in the recent study of Ranković et al. (2012), which detected stronger antimicrobial activity of the acetone extract of *U. barbata* compared to the acetone extract of *Toninia candida* against 5 bacteria and 5 fungi. Similar results were reported in the study of Weckesser et al. (2007) that screened 9 lichen extracts and isolated compounds against bacterial and fungal species with dermatological relevance, singling out an extract of *U. barbata* prepared using supercritical CO_2 as the most active one, especially against and *Malassezia furfur*. Similarly, Zugic et al. (2015) reported supercritical CO_2 extract of *U. barbata* to have the strongest antimicrobial effect against G(+) bacteria compared to the extracts of this lichen prepared using conventional techniques. Relatively strong activity of *U. ghattensis* was observed in a study that investigated antimicrobial activity of this lichen against G(+) and G(-) human pathogenic bacterial species, whereby methanol extract was the only extract active against *Streptococcus faecalis*, ethanolic extract was more effective than acetone and methanol extract against *Bacillus cereus* and *Pseudomonas aeruginosa*, while acetone and methanol extract showed similar activity against *Staphylococcus aureus* (Srivastava et al., 2013). These findings are in line with the results of Behera et al. (2005a) which also demonstrated antimicrobial activity of acetone and methanol extract of *U. ghattensis* against *S. aureus*, and also three *Bacillus species*. When it comes to antiviral activity, in a study that involved 18 medicinal plants and lichens used as folk medicines against infective diseases, significant antiviral activity was exhibited for the Soxhlet extract of *U. complanata* against *Herpes simplex virus* (HSV-1) in a concentration that was non-toxic to Vero cells (African green monkey kidney cell) (Vijayan et al., 2004).

2.2. Cytotoxic and antitumor activity

Since the early studies screening secondary metabolites of lichens as potential anticancer agents, the activity of a vast number of lichen crude extracts and/or isolated compounds has been investigated against various cell lines suggesting their promising antitumor, antimutagenic and cytotoxic effects (Gómez-Serranillos et al., 2014). One of the first studies that involved acetone extracts of 29 tissue culture and thallus extracts of lichens implied the extract of *U. longissima* to possess the strongest inhibitory effect against Epstein Barr virus-induced tumor (Yamamoto et al., 1995). Cytotoxic activity of *U. fasciata* was demonstrated against sarcoma 180 and Ehrlich tumor cells; stated effect was moderate to strong depending on the fraction isolated from the lichen (Periera et al., 1994). Recent research (Ranković et al., 2012) included investigation of cytotoxic activity of acetone extracts of *U. barbata* and *T. candida* against FemX (human melanoma) and LS174 (human colon carcinoma) cell lines. Obtained IC_{50} values for both tested cell lines were lower for the extract of *U. barbata* compared to *T. candida*, while further analysis of the mechanism of cytotoxic action implied a prominent ability of this extract to induce apoptosis in tested cells. Similar results were reported for a supercritical CO_2 extract of this lichen against B16 mouse melanoma and C6 rat glioma. Namely, supercritical CO_2 extract of *U. barbata* revealed stronger cytotoxic effect against tested tumor cells compared to conventional extracts of this lichen and also pure usnic acid. Suggested mechanisms of cytotoxic effect for both supercritical CO_2 extract of *U. barbata* and usnic acid were the induction of apoptosis and/or autophagy in B16 and C6 cells, indicating higher cytotoxicity of the extract to be related to the higher degree of ROS production (Zugic et al., 2016).

Table 1. Chemical composition and biological activities of the species belonging to *Usnea* genus

Species	Chemical composition	Reference	Biological activity	Reference
Usnea articulata (L.) Hoffm.	Usnic acid, barbatic acid, atranorin, methyl-β-orcinolcarboxylate and other β-orcinol derivatives, ergosterol peroxide, fumarprotocetraric acid, protocetraric acid, stictic acid, norstictic acid, peristictic acid, criptostictic acid, menegazziaic acid, constictic acid, 3-O-methyl-consalazinic acid	Lohézic-Le Dévéhat et al. (2007)	Antigenotoxic, antioxidant	Ceker et al. (2015)
Usnea barbata (L.) Mott.	Usnic acid, norstictic acid, atranorin, chloroatranorin, barbatolic acid, lobaric acid, salazinic acid	List and Hörhammer (1979); Ranković et al. (2012)	Anti-inflammatory, UVB protective effects	Engel et al. (2007)
			Antimicrobial (antibacterial, antimycotic)	Cansaran et al. (2006); Madamombe and Afolayan (2003); Ranković et al. (2012); Weckesser et al. (2007)
			Antimicrobial (antiviral)	Vijayan et al. (2004)
			Antioxidative	Ranković et al. (2012)
			Cytotoxic	Ranković et al. (2012)
Usnea complanata (Müll.Arg.) Motyka	Usnic acid, psoromic acid, salazinic acid, norstictic acid, constictic acid, galbinic acid	Behera et al. (2012); Swinscow and Krog (1979)	Antioxidative, cardioprotective	Behera et al. (2012)
Usnea fasciata Torrey	Usnic acid, isolichenin, raffinose	Periera et al. (1994)	Cytotoxic	Periera et al. (1994)
Usnea fillipendula Stirton	Usnic acid, salazinic acid, thamnolic acid	List and Hörhammer (1979)	Antigenotoxic, antioxidative	Ceker et al. (2015)
Usnea florida (L.) Weber ex Wigg.	Usnic acid, lecanoric acid, thamnolic acid, diffractaic acid, squamatic acid, salazinic acid, constictic acid, norstictic acid, psoromic acid, protocetraric acid, fumarprotocetraric acid, conpsoromic acid, caperatic acid, Evans's substance, lobaric acid	Fiscus (1972); List and Hörhammer (1979)	Antimicrobial (antibacterial)	Cansaran et al. (2006); Schmeda-Hirschmann et al. (2008)
			Antioxidative	Odabasoglu et al. (2004)
Usnea ghattensis G. Awasthi	Usnic acid, norstictic acid	Verma et al. (2008)	Antimicrobial (antibacterial)	Behera et al. (2005a); Srivastava et al. (2013)
			Antioxidative	Behera et al. (2006; 2005a); Verma et al. (2008)
			Hepatoprotective	Verma et al. (2008)

Continued from previous page

Species	Chemical composition	Reference	Biological activity	Reference
Usnea longissima Ach.	Usnic acid, barbatic acid, diffractaic acid, glutinol, longissiminone A longissiminone B, salazinic acid, protocetraric acid, evernic acid, 4-O-demethyl-barbatic acid	Cansaran et al. (2006); Choudhary et al. (2005); Jin et al. (2008); List and Hörhammer (1979); Nishitoba et al. (1987); Odabasoglu et al. (2005; 2012); Yamamoto et al. (1995); Zambare and Christopher (2012);	Antioxidative	Ağar et al. (2011); Kim and Cho (2007); Odabasoglu et al. (2004)
			Antigenotoxic	Ağar et al. (2011)
			Antiulcer	Halici et al. (2005)
			Antitumour	Yamamoto et al. (1995)
			Enzyme-inhibitory	Kim and Cho (2007)
			Antiplatelet	Lee and Kim (2005)
Usnea siamenis Vain.	Usnic acid, stictic acid, constictic acid, norstictic acid	Swinscow and Krog (1979)	Antimicrobial (antimycotic)	Rukayadi et al. (2008)

In addition, extracts of *U. filipendula* and *U. articulata* revealed the ability to protect against aflatoxin B1 (AFB1)-induced genotoxic and oxidative damage, whereby it was concluded that antigenotoxic effects of these lichens may be related to their antioxidative potential (Ceker et al., 2015). Similar antigenotoxic effects were reported for methanol extract od *U. longissima* (Ağar et al., 2011).

2.3. Antioxidative activity

Taking into consideration the polyphenolic nature of the main secondary metabolites of lichens, it would be logical to assume that these symbiotic organisms possess antioxidative properties. Indeed, several studies conducted with the aim to investigate this activity revealed promising results. Generally, antioxidative activity was assessed using *in vitro* tests, such as DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity (DPPH assay), nitric oxide (NO) radical scavenging activity, superoxide anion scavenging (SAS) assay, reduction power and lipid peroxidation inhibition (LPO) assay, whereby methanol was used as the most appropriate solvent for the extraction of bioactive compounds with antioxidative activity from lichens (Gómez-Serranillos et al., 2014). When it comes to the genus *Usnea*, it was shown that methanol extracts of *U. articulata* and *U. filipendula* have a protective role against AFB1 in human lymphocytes via regulation of enzyme activity (Ceker et al., 2015). In another study, several extracts of *U. complanata* revealed free radical scavenging activity (DPPH and NO) and LPO inhibition (Behera et al., 2012). In addition, various extracts of *U. ghattensis* demonstrated good antioxidant potential similar to the referent antioxidants (Behera et al., 2006; 2005a;b; Verma et al., 2008). Also, methanol extract of *U. longissima* exerted strong antioxidative capacity *in vitro* (Ağar et al., 2011; Kim and Cho, 2007), while in an *in vivo* study aqueous extract of this lichen was able to activate superoxide dismutase (SOD) and glutathione S-transferase (GST) reduced by indomethacin (Halici et al., 2005). In the study of Odabasoglu et al. (2004) this lichen revealed better antioxidant activity and higher content of total phenols compared to *U. longissima*. Antioxidant activity was also observed for the two members of the genus *Usnea* found in Antarctic (*U. antarctica* and *U. aurantiacoatra*) (Luo et al., 2009). In the recent study of Ranković et al. (2012) antioxidative activity of acetone extract of *U. barbata* was confirmed using three *in vitro* assays. Stated activity, which was in good correlation with total phenolic content, was, however, weaker than the one demonstrated for acetone extract of *T. candida*. A good correlation of antioxidant capacity and total phenolic/usnic acid content in several extracts of *U. barbata* was also confirmed by Zugic et al. (2016).

2.4. Other activities

Beside antimicrobial, cytotoxic and antioxidative activity, some of the members of the genus *Usnea* were shown to possess additional biological activities. In a study of (Engel et al., 2007) that investigated anti-inflammatory activity of a supercritical CO_2 extract of *U. barbata*, inhibition of prostaglandin (PGE2) and cyclooxygenase (COX-2) production in the HaCaT keratinocytes exposed to ultraviolet-B radiation was demonstrated. Beside the enzymes that are mediators of inflammation, some of the lichens belonging to the genus *Usnea* revealed the ability to inhibit other enzymes. For instance, Kim and Cho (2007) observed an inhibitory effect of the methanol extract of *U. longissima* to melanogenesis via tyrosinase inhibition thus lowering the level of melanin in human melanoma cells. In addition, water extract of the same lichen revealed a promising gastroprotective effect mediated by an antioxidative mechanism (Halici et al., 2005). Moreover, Lee and Kim (2005) reported cardioprotective effects of the methanol extract of the same lichen, which also exerted antiplatelet activity. Cardioprotective activity was also described for *U. complanata* (Behera et al., 2012), while *U. ghattensis* was shown to possess hepatoprotective activity mediated by antioxidative mechanisms against ethanol-induced toxicity in mice (Verma et al., 2008).

3. THE CHEMICAL COMPOSITION OF THE LICHENS BELONGING TO THE GENUS *USNEA* AND THEIR BIOLOGICAL ACTIVITIES

3.1. The chemical composition of the lichens belonging to the genus *Usnea*

Table 1 reveals the chemical composition of the lichens belonging to the genus *Usnea*, for which some of the biological activities described in the first part of this paper have been shown. In the detailed survey of the available literature, 35 metabolites were found in stated species of the genus *Usnea* (Table 1). Thereof, usnic acid was identified in all of the described species, which is not surprising given the fact that this compound is characteristic for the species that have a yellow-green upper cortex, including, in addition to the genus *Usnea*, other genera of the Parmeliaceae family, such as *Alectoria*, *Evernia*, and *Flavoparmelia* (Gómez-Serranillos et al., 2014). In addition to usnic acid, following 13 compounds were identified in more than one species of the genus *Usnea*: salazinic acid (in 6 species), norstictic acid (in 4 species), barbatic, constictic, protocetraric, stictic and thamnolic acid (each in 3 species), atranorin, diffractaic, fumarprotocetraric, galbinic, lobaric and psoromic acid (each in 2 species), while other metabolites were found in only one species (Table 1). For some of these metabolites, different biological activities were described in the literature, which will be presented in the following section.

3.2. Biological activities of the secondary metabolites of the lichens belonging to the genus *Usnea*

3.2.1. *Usnic acid*

Usnic acid (Figure 2) is one of the most prevalent and best studied secondary metabolites of the lichens. It is naturally occurring dibenzofuran derivative, isolated not only from the representatives of the genus *Usnea* (Behera et al., 2012; Brandão et al., 2013; Cansaran et al., 2006; Gupta et al., 2012; Honda et al., 2010; Odabasoglu et al., 2006; Ranković et al., 2012; Safak et al., 2009; Schmeda-Hirschmann et al., 2008; Sultana and Afolayan, 2011; Yamamoto et al., 1995), but also from some other genera such as *Parmelia* (Kumar KC and Müller, 1999a;b; Manojlović et al., 2012; Ranković et al., 2008), *Protousnea* (Fournet et al., 1997; Schmeda-Hirschmann et al., 2008), *Alectoria* (Einarsdóttir et al., 2010; Gollapudi et al., 1994), *Xantoparmelia* (Amo de Paz et al., 2010; Ingólfsdóttir et al., 1998), *Lethariella* (Toledo Marante et al., 2003), *Cladonia* (Behera et al., 2012; Bessadottir et al., 2012; Lohézic-Le Dévéhat et al., 2007; Ranković et al., 2012; Santos et al., 2004; Singh et al., 2013) and others. Usnic acid has been recognized as the carrier of various biological activities, such as antimicrobial, antioxidant, antitumor, neuroprotective, gastroprotective, cardioprotective, cytoprotective, immunostimulatory and anti-inflammatory. Recently, the wound healing properties for this compound were confirmed, as well (Burlando et al., 2009). Antimicrobial activity of usnic acid was investigated in many scientific papers and most of them described screening of this dibenzofuran derivative against various bacteria, fungi, and parasites. Its antibacterial activity has been documented for many G(+) bacteria (Gollapudi et al., 1994; Ivanova et al., 2010; Manojlović et al., 2012; Ranković et al., 2012; 2008; Schmeda-Hirschmann et al., 2008; Sultana and Afolayan, 2011; Weckesser et al., 2007; Zugic et al., 2015). In a recently published study dealing with the evaluation of usnic acid antibacterial activity against methicillin-resistant *S. aureus* (MRSA), it

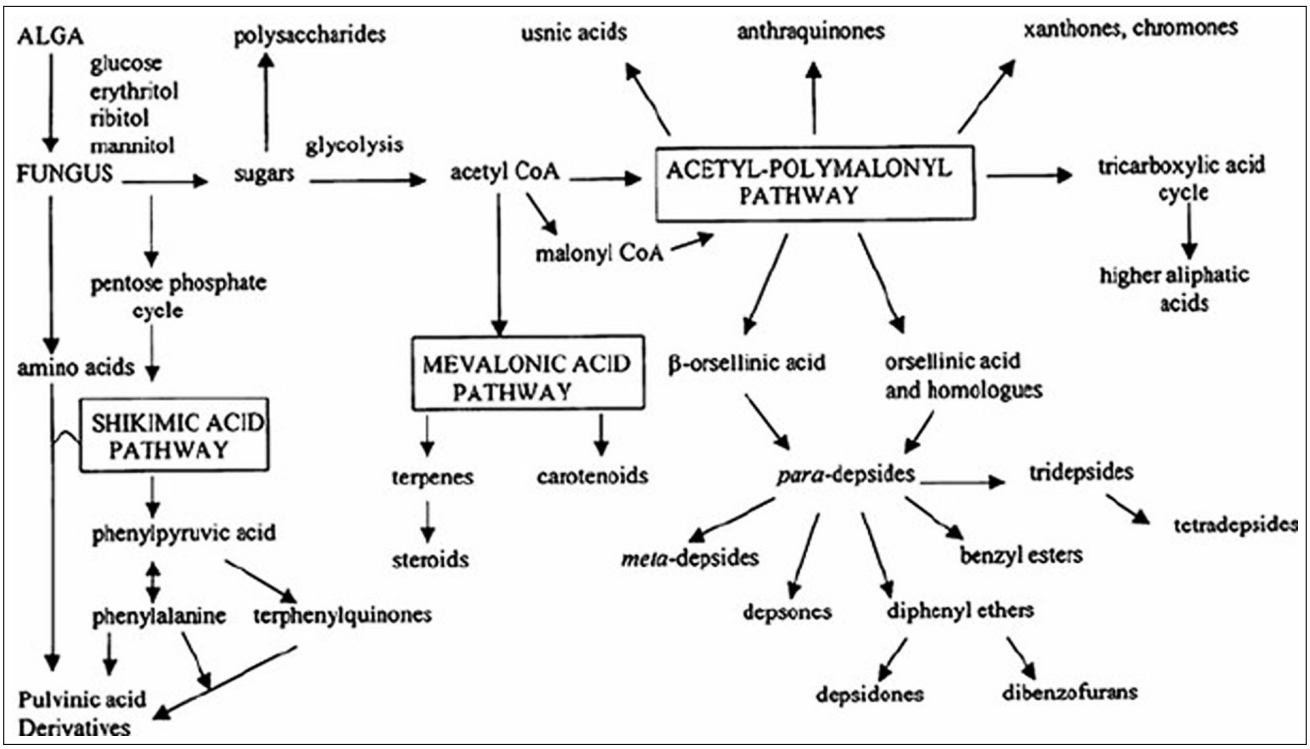

Fig. 1. Biosynthetic pathways of lichens metabolites (taken from Ranković and Kosanić (2015))

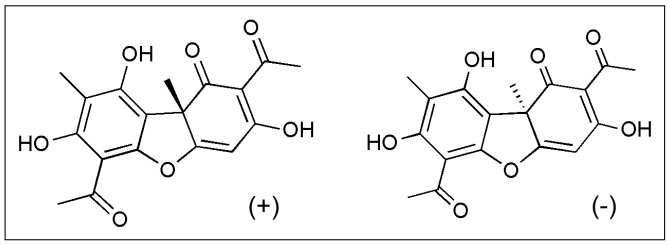

Fig. 2. Chemical structure of (+) and (-) enantiomer of usnic acid

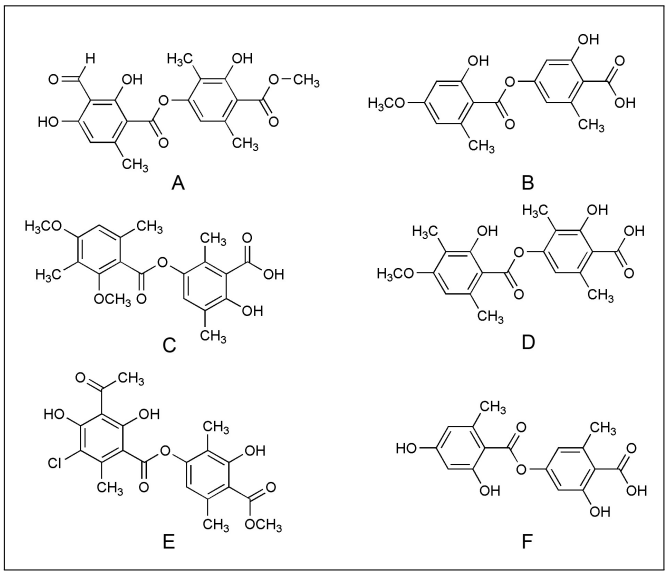

Fig. 3. Chemical structure of metabolites of the *Usnea* lichens with depside structure: atranorin (A), diffractaic acid (B), barbatic acid (C), chloroatranorin (D) evernic acid (E) and lecanoric acid (F)

was found that the mechanism of antistaphylococcal activity was based on its capability to trigger the bacterial cell membrane destruction (Gupta et al., 2012). When it comes to G(-) bacteria and fungi, in the up-to-now available literature, the data are controversial. Namely, some studies pointed to the absence of antibacterial activity of usnic acid against G(-) bacteria (Gollapudi et al., 1994; Weckesser et al., 2007; Zugic et al., 2015) and fungi (Ivanova et al., 2010; Schmeda-Hirschmann et al., 2008; Weckesser et al., 2007). However, in several papers, it was demonstrated that usnic acid exhibited very strong antimicrobial properties against all tested bacteria (including G(+) and G(-)) and fungi, with antibacterial activity being stronger than antimycotic (Manojlović et al., 2012; Ranković et al., 2012; 2008). In addition, antiprotozoal activity against three *Leishmania* species was evaluated *in vitro* as well as *in vivo*, in experimentally induced skin leishmaniasis (Fournet et al., 1997; Schmeda-Hirschmann et al., 2008). Among all the secondary metabolites of the lichens, the best-studied substance for its anticancer effects is definitely usnic acid. Usnic acid exhibited antitumor activity against tumors caused by Epstein Barr virus (Yamamoto et al., 1995). The significant cytotoxic activity was observed in investigations performed on human keratinocytes (Burlando et al., 2009; Kumar KC and Müller, 1999b), malignant mesothelioma cells (MM98), vulvar carcinoma cells (A431 vulvar) (Burlando et al., 2009), mouse fibroblasts cells (L-929), human leukaemia cells (K-562, U937, HL-60, Jurkat) (Bačkorová et al., 2011; Ivanova et al., 2010; Toledo Marante et al., 2003) human cervix carcinoma cells (HeLa) (Brisdelli et al., 2013; Ivanova et al., 2010), human breast adenocarcinoma cells (MCF-7 and SK-BR-3) Bačkorová et al. (2011); Brisdelli et al. (2013), melanoma cells (B16, FemX and UACC-62) (Brandão et al., 2013; Manojlović et al., 2012; Ranković et al., 2012; Zugic et al., 2016), human colon carcinoma (LS174, HCT-116, HT-29) (Bačkorová et al., 2011; Brisdelli et al., 2013; Manojlović et al., 2012; Ranković et al., 2012) and glioma cells (C6) (Zugic et al., 2016).

Taking into account previously performed studies, the proposed mechanisms of usnic acid cytotoxicity might be based on:

(a) antimitotic activity (Cardarelli et al., 1997), causing the inhibition of RNA transcription (Campanella et al., 2002)

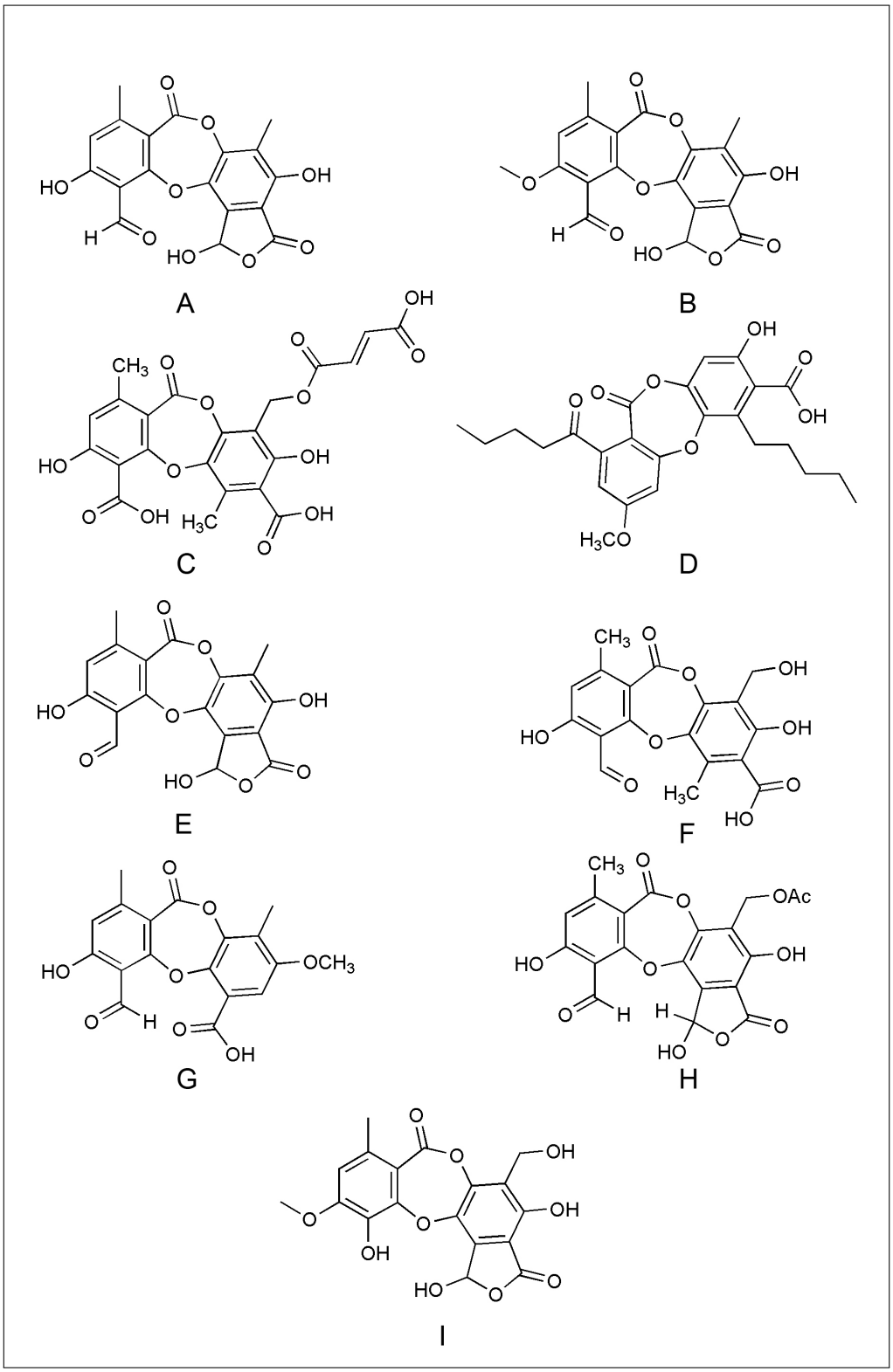

Fig. 4. Chemical structure of metabolites of the *Usnea* lichens with depsidone structure: salazinic acid (A), stictic acid (B), fumaroprotocetric acid (C), lobaric acid (D), norstistic acid (E), protocetraric acid (F), psoromic acid (G), galbinic acid (H) and menegazziaic acid (I)

(b) disruption of internal mitochondrial membrane potential (Einarsdóttir et al., 2010; Han et al., 2004)

(c) induction of apoptosis (Bačkorová et al., 2011; Bézivin et al., 2004; Ranković et al., 2012; Zugic et al., 2016) and autophagy (Bessadottir et al., 2012; Zugic et al., 2016)

Usnic acid specific heterocyclic structure consisting of conjugated dienes and polar OH groups indicates the possible redox potential of this molecule or the ability of its interaction with reactive species involved in oxidative stress. Antioxidant potential of usnic acid, as well as it's biological activities based on antioxidative properties, have recently been described in the review of White et al. (2014). In this respect, the antioxidant activity of this lichen metabolite was detected in numerous *in vitro* assays (Amo de Paz et al., 2010; Behera et al., 2012; Ranković et al., 2012; Singh et al., 2013). However, the results of various biological tests suggested that usnic acid could act both as a prooxidant or antioxidant in different types of cells and tissues, pointing the further research necessity regarding the mechanisms of its activity. Its redox potential might be fully understood only if the further investigations were performed taking into consideration the usnic acid possible effects on modulation of antioxidant enzyme activity and detoxification cell systems (Kohlhardt-Floehr et al., 2010; Polat et al., 2016). In contrast, or better to say in line with the abovementioned, some papers reported the absence of antioxidant activity, as usnic acid did not show the ability to "capture" the free radicals generated in DPPH test (Lohézic-Le Dévéhat et al., 2007; Thadhani et al., 2011; Zugic et al., 2016), what had been explained by the lack of a labile hydrogen atom in the structure of this compound.

When it comes to the biological properties of usnic acid based on its antioxidant potential, this compound exhibited gastroprotective effects against gastric ulcers induced by indomethacin in rats by reduction of oxidative damage (Odabasoglu et al., 2004). Also, usnic acid possessed the protective effects against the damage of human astrocytoma, as the most common type of glioma (U373 MG) caused by hydrogen peroxide (Amo de Paz et al., 2010). Based on the results of this study, the authors suggested that usnic acid could act as an antioxidant agent in neurodegenerative diseases associated with oxidative stress, such as Alzheimer's and Parkinson's diseases. Further, in the study of Santos et al. (2004) usnic acid induced a strong release of NO in peritoneal macrophages, which increased the protection from bacteria and tumors, thereby exhibiting immunostimulatory effect (Santos et al., 2004). In a study investigating the molecular mechanisms responsible for the anti-inflammatory activity of usnic acid, it was found that this substance exhibited a dose-dependent inhibitory effect on the production of tumor necrosis factor-α, TNF-α (Behera et al., 2006). In addition, the cardioprotective activity of usnic acid was demonstrated by Behera et al. (2012). The effect of usnic acid on wound healing, applying the non-toxic dose in the experiment with human keratinocytes (HaCaT cells) was evaluated, as well (Burlando et al., 2009).

3.2.2. Secondary metabolites with depside structure

Atranorin

Atranorin (Figure 3A), one of the most common lichen secondary metabolites after usnic acid, is the best-studied compound within the lichen family Parmeliaceae (White et al., 2014). It represents the main substance of the grey cortex species, such as *Cetrelia*, *Evernia*, *Pseudoevernia*, and *Parmelia*, although it was also found in some species of the *Parmotrema*, *Pseudoevernia*, *Cladina*, *Lethariella*, *Hypotrachyna* and *Usnea* genus (Gómez-Serranillos et al., 2014; White et al., 2014). Several biological activities of atranorin have recently been reviewed by Gómez-Serranillos et al. (2014) and also White et al. (2014). Atranorin exhibited very strong antimicrobial activity against various bacteria and fungi, shown by Kosanić et al. (2014). Accordingly, Toledo Marante et al. (2003) observed moderate antibacterial activity of atranorin against *S. aureus*. However, atranorin did not possess the antimycotic activity against filamentous fungi, as shown in the study of Türk et al. (2006). Cytotoxic activity of atranorin was established in several human cancer cell lines (Bačkorová et al., 2011; Kosanić et al., 2013; Toledo Marante et al., 2003). Antioxidative activity of this compound was confirmed in numerous studies (Jayaprakasha and Rao, 2000; Kosanić et al., 2014; Melo et al., 2011; Papadopoulou et al., 2007; Toledo Marante et al., 2003; Valencia-Islas et al., 2007) and it could contribute to some of its pharmacological effects, such as the ability to reduce skin damage and modulate the wound healing process (Barreto et al., 2013). The study performed by Melo et al. (2011) demonstrated that atranorin, in addition to the antioxidant, exhibited prooxidative activity but only at higher concentrations. However, some researchers observed the absence of the antioxidant activity of atranorin (Thadhani et al., 2011). It was suggested that atranorin might exhibit the anti-inflammatory effect by inhibiting the process of leucotriene B4 (LTB4) synthesis (Kumar KC and Müller, 1999a), or analgesic action by dose-dependent inhibition of cycloxygenase-1 (COX-1) and partial inhibition of COX-2 (Bugni et al., 2009).

Diffractaic acid

Diffractaic acid (Figure 3B) is, apart from several species of the genus *Usnea*, isolated from the species *Parmelia tinctorum* and *Protousnea magellanica*. In up-to-now performed investigations, this compound was shown to possess antioxidant, gastroprotective, immunostimulatory, antitumor, anti-inflammatory and antimicrobial activities (Gómez-Serranillos et al., 2014; White et al., 2014). In specific, Bayir et al. (2006) confirmed that different doses of diffractaic acid induced gastroprotective effects mediated by an antioxidant defense against tissue impairments caused by oxidative stress in indomethacin-induced gastric lesions in the rat. Odabasoglu et al. (2012) demonstrated that diffractaic acid, dissolved in olive oil, after oral administration in rabbits, caused proapoptotic effects in the tissue surrounding the titanium implants, indicating the possible mechanism involved in the protection of the tumor development in various tissues, as a consequence of chemically induced apoptosis. Besides, Santos et al. (2004) noticed a strong influence of diffractaic acid on the release of NO in mouse macrophages, contributing to the immunostimulatory effect. Diffractaic acid also showed cytotoxic activity against two cell lines of melanoma (UACC-62 and B16-F10) (Brandão et al., 2013). In addition, Brisdelli et al. (2013) showed that this compound possessed good antiproliferative activity against human colon carcinoma (HCT-116), breast adenocarcinoma (MCF-7), and cervix carcinoma (HeLa). The antiproliferative activity of diffractaic acid was also demonstrated on human keratinocytes, indicating the potential use of this compound against psoriasis (Kumar KC and Müller, 1999b). The anti-inflammatory activity of diffractaic acid was evaluated, as well (Kumar KC and Müller, 1999b). In addition, the study of Honda et al. (2010) revealed that diffractaic acid, among the examined metabolites of lichens, possessed the best antimicrobial effect against *M. tuberculosis*.

Barbatic acid

Barbatic acid (Figure 3C), another member of the depsides family, except in some species of genus *Usnea*, was also found in the lichens *Cladia aggregates* and *Heterodea muellers* (Molnár and Farkas, 2010; Shrestha and St. Clair, 2013). Barbatic acid has been shown to inhibit the growth of *S. aureus* (Martins et al., 2010). Yamamoto et al. (1995) identified barbatic acid as one of the components of lichen *U. longissima*, which, among

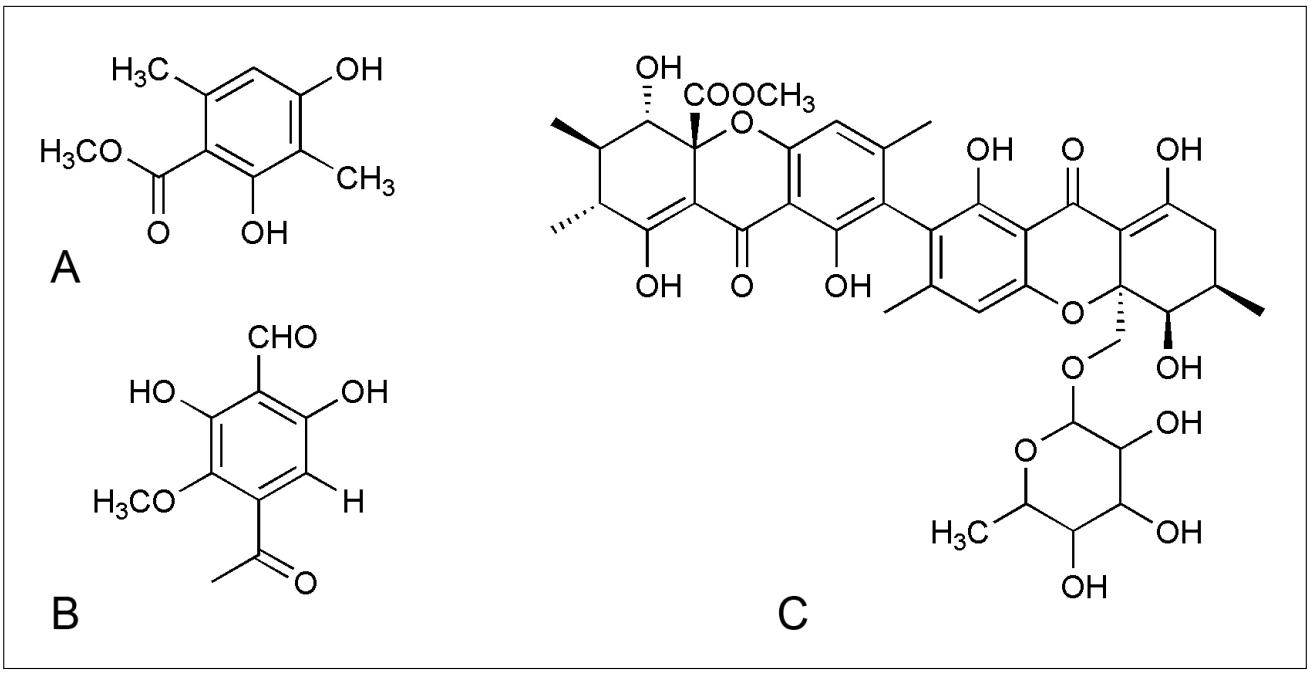

Fig. 5. Chemical structure of other metabolites of the *Usnea* lichens: methyl-β-orsenillate (A), longissiminone A (B) and hirtusneanoside (C)

others, mainly contributed to the inhibitory activity against tumors caused by Epstein Barr virus.

Chloroatranorin

Besides some species of the genus *Usnea*, chloroatranorin (Figure 3D) can also be found in the genus *Parmotrema*, *Pseudevernia* and *Lathariella* (Gómez-Serranillos et al., 2014; White et al., 2014). In the available literature data, the antimicrobial activity of chloroatranorin against various bacteria and fungi was confirmed (Türk et al., 2006). Also, in the study of antioxidant properties of six lichen secondary metabolites, chloroatranorin showed the best ability to scavenge free radicals (Valencia-Islas et al., 2007). In this regard, the study of Toledo Marante et al. (2003) confirmed that chloroatranorin induced the dose-dependent LPO decrease and inhibited the proliferation of monocytic leukemia cell lines (U937 and HL-60). Regarding the chloroatranorin analgesic effects, it was shown this activity was the consequence of partial inhibition of COX-2 (Bugni et al., 2009).

Evernic acid

In the previously performed research, evernic acid (Figure 3E) was isolated from the lichen *E. prunastri* and *U. longissima* (White et al., 2014). Evernic acid exhibited very strong antimicrobial activity against various bacteria and fungi (Kosanić et al., 2013). However, Kokubun et al. (2007) performed an investigation of antimicrobial activity of this compound against several resistant strains of *S. aureus*, revealing that it was active only against one strain. In addition, its antioxidant activity was evaluated (Kosanić et al., 2013). Antitumor activity of evernic acid was established against Epstein Barr virus tumors (Yamamoto et al., 1995), malignant mesothelioma (MM98), vulvar cancer cell lines (A431), keratinocyte (HaCaT) (Burlando et al., 2009), melanoma (FemX) and human colon carcinoma (LS 174) (Kosanić et al., 2013).

Lecanoric acid

Lecanoric acid (Figure 3F), found in several species of genus *Usnea* as well as in the genus *Parmotrema*, revealed antioxidant activity, possibly explained by the existing electron attraction due to two hydrogen bonds between the 2′-OH and the 1′-$COOCH_3$/COOH groups and the 2-OH and the 1-COO^- groups, as well as due to the presence of the COO^- group conjugated to the aromatic ring (Jayaprakasha and Rao, 2000; Thadhani et al., 2011; White et al., 2014).

3.2.3. Secondary metabolites with depsidone structure

Salazinic acid

Salazinic acid (Figure 4A) has been found in some lichen species of *Xanthoparmelia*, *Parmelia*, *Parmotrema*, *Rimelia*, and *Usnea*. For this compound, antimicrobial, antitumor, antioxidant, neuroprotective and immunostimulatory activity was described in two recent review papers (Gómez-Serranillos et al., 2014; White et al., 2014). Interestingly, salazinic acid was found to be a potential agent used against Alzheimer's disease. Namely, having an impact on the reduction of reactive oxygen species (ROS) production in U373MG cells (human astrocytes), it might exhibit the neuroprotective effect through the antioxidant activity or protection against oxidative stress in astrocytes (Amo de Paz et al., 2010). Santos et al. (2004) showed an immunostimulatory effect of salazinic acid. This compound exhibited a strong antioxidant activity (Manojlović et al., 2012; Valencia-Islas et al., 2007). Antimicrobial activity was evaluated against various bacteria (Candan et al., 2007; Manojlović et al., 2012; Sultana and Afolayan, 2011) and fungi (Candan et al., 2007; Manojlović et al., 2012). The cytotoxic activity of this compound was confirmed against cell lines of melanoma (FemX) and colon cancer (LS174) (Manojlović et al., 2012). In addition, Burlando et al. (2009) observed moderate wound healing effect of non-toxic dose of this compound in human keratinocytes (HaCaT cells).

Stictic acid

Stictic acid (Figure 4B) is β-orcinol depsidone, found in lichens *U. articulata*, *Xanthoparmelia conspers*, *X. camtschadalis*, and *Ypotrachyna revolute* (Gómez-Serranillos et al., 2014; White et al., 2014). Several biological activities of stictic acid were established, as outlined by White et al. (2014). For instance, stictic acid was found to possess protective properties in human astrocytes-U373MG by reducing the production of ROS, thereby causing a neuroprotective effect through antioxidant activity (Amo de Paz et al., 2010). Also, significant antioxidative properties of stictic acid were observed (Papadopoulou et al., 2007)), but in the investigation performed by Lohézic-Le Dévéhat et al. (2007) this compound did not exhibit the

antioxidant activity, which was explained by molecular conformation that could affect the ability to "capture" free radicals, especially DPPH.

Fumaroprotocetric acid

Fumaroprotocetric acid (Figure 4C) is the constituent of *Cladonia verticillaris*, *C. rangiferina* and *U. articulata* (White et al., 2014). The performed research about the biological activities of this depsidone was summarized in a recent study (White et al., 2014). Thereby, a strong antioxidant, antimicrobial and antitumor activities against human melanoma cells (FemX) and colon carcinoma (LS174) were established (Kosanić et al., 2014; Lohézic-Le Dévéhat et al., 2007). Also, as demonstrated by Santos et al. (2004) fumaroprotocetric acid exhibited the immunostimulatory effect by stimulating an increase of NO release in macrophages, the cells with an important role in the defense mechanisms of the organism.

Lobaric acid

Lobaric acid (Figure 4D) may be found in some species of the genus *Usnea*. In previously performed studies, it was isolated from several Antarctic lichens such as *Sterocaulon alpinum* and *Cladonia* sp. (White et al., 2014). White et al. (2014) listed several activities of lobaric acid. In particular, this compound showed antimicrobial activity against G(+) *S. aureus* and *B. subtilis* (Bhattarai et al., 2013). Brisdelli et al. (2013) revealed the cytotoxic activity of lobaric acid against human cervix carcinoma (HeLa) and human colon carcinoma (HCT cells) only at high concentrations. In the same study, no antioxidant activity was observed in the DPPH test, what was in contrast to the findings of Brisdelli et al. (2013). Based on different concentration ranges used in these studies, it was concluded that the ability of lobaric acid to scavenge DPPH radicals might be considered dose-dependent.

Norstistic acid

Norstistic acid (Figure 4E) may be found in various species of genus *Usnea*, as well as in other lichens, such as *T. candida* (Gómez-Serranillos et al., 2014; White et al., 2014). The investigation of Ranković et al. (2012) revealed that norstistic acid exhibited antimicrobial activity against various bacteria and fungi. In addition, in the study of lichens secondary metabolites activity against *M. tuberculosis*, it was observed that norstistic acid showed the best activity after diffractaic acid (Honda et al., 2010). This compound also showed an antioxidant activity (Lohézic-Le Dévéhat et al., 2007; Ranković et al., 2012). Additionally, norstistic acid demonstrated antiproliferative activity against human melanoma cells (FemX) and human colon carcinoma (LS174) by inducing the apoptotic death (Ranković et al., 2012).

Protocetraric acid

Protocetraric acid (Figure 4F) was found, aside the genus *Usnea* in other lichens such as *Hypogymnia physodes* and *Parmelia caperata* (Shrestha and St. Clair, 2013; White et al., 2014). Protocetraric acid exhibited strong antimicrobial activity against various bacteria and fungi. In addition, strong antitumor activity against FemX cells and human colon carcinoma (LS174), was observed, with the strong antioxidant activity, as well (Manojlović et al., 2012). Protocetraric acid increased the release of NO and H_2O_2 in macrophages inducing an immunostimulatory effect (Lohézic-Le Dévéhat et al., 2007; Santos et al., 2004).

Other depsidone compounds

Depisdone compound - psoromic acid (Figure 4G), isolated from *Usnea* species showed strong antioxidant activity, possessing the ability to scavenge free radicals (Behera et al., 2012). This compound exhibited cardioprotective activity as well (Behera et al., 2012). Psoromic acid showed the selective cytotoxicity to melanoma cells (UACC-62) (Brandão et al., 2013). For galbinic acid (Figure 4H), another member of the class of depsidones, antimicrobial activity against *B. cereus*, *B. subtilis*, *S. aureus*, and *E. coli* was established. The same study demonstrated antimicrobial activity of menegazziaic acid (Figure 4I) against the same bacteria, with the exception of *S. aureus* (Sultana and Afolayan, 2011).

3.2.4. Other secondary metabolites

Benzoic acid derivative, methyl-β-orcinolcarboxylate or methyl-β-orsenillate (Figure 5A), was shown to possess strong antibacterial activity against *S. aureus* (Toledo Marante et al., 2003) and strong ability to scavenge NO radicals (Thadhani et al., 2011). Research by Choudhary et al. (2005) revealed that the phenol compound, longissiminone A (Figure 5B), isolated from *U. longissima*, exhibited an anti-inflammatory response comparable to aspirin. Deoxyglucoside of dimeric tetrahydroxanthan- hirtusneanoside (Figure 5C), isolated from *U. hirta* leaf, showed antimicrobial activity against G(+) *S.aureus* and *B.subtilis* (Rezanka and Sigler, 2007).

CONCLUSION

A detailed review of the literature regarding chemical composition and biological properties of the genus *Usnea* confirmed lichens belonging to this genus to be a valuable source of compounds with potential medicinal significance. However, further investigations should be performed with the aim of providing evidence of these pharmacological effects *in vivo* as a prerequisite for their prospective clinical confirmation.

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REFERENCES

Ağar, G., Aslan, A., Sarioğlu, E. K., Alpsoy, L. and Çeker, S. (2011). Protective activity of the methanol extract of *Usnea longissima* against oxidative damage and genotoxicity caused by aflatoxin, **41**(6): 1043–1049.

Amo de Paz, G., Raggio, J., Gómez-Serranillos, M., Palomino, O., González-Burgos, E., Carretero, M. and Crespo, A. (2010). HPLC isolation of antioxidant constituents from *Xanthoparmelia* spp., *Journal of Pharmaceutical and Biomedical Analysis* **53**(2): 165–171.

Bačkorová, M., Bačkor, M., Mikeš, J., Jendželovský, R. and Fedoročko, P. (2011). Variable responses of different human cancer cells to the lichen compounds parietin, atranorin, usnic acid and gyrophoric acid, *Toxicology in Vitro* **25**(1): 37–44.

Barreto, R. S., Albuquerque-Júnior, R. L., Pereira-Filho, R. N., Quintans, J. S., Barreto, A. S., DeSantana, J. M., Santana-Filho, V. J., Santos, M. R., Bonjardim, L. R., Araújo, A. A. and Quintans-Júnior, L. J. (2013). Evaluation of wound healing activity of atranorin, a lichen secondary metabolite, on rodents, *Revista Brasileira de Farmacognosia* **23**(2): 310–319.

Bayir, Y., Odabasoglu, F., Cakir, A., Aslan, A., Suleyman, H., Halici, M. and Kazaz, C. (2006). The inhibition of gastric mucosal lesion, oxidative stress and neutrophil-infiltration in rats by the lichen constituent diffractaic acid, *Phytomedicine* **13**(8): 584–590.

Behera, B. C., Mahadik, N. and Morey, M. (2012). Antioxidative and cardiovascular-protective activities of metabolite usnic acid and psoromic acid produced by lichen species *Usnea complanata* under submerged fermentation, *Pharmaceutical Biology* **50**(8): 968–979.

Behera, B. C., Verma, N., Sonone, A. and Makhija, U. (2005b). Evaluation of antioxidant potential of the cultured mycobiont of a lichen *Usnea ghattensis*, *Phytotherapy Research* **19**(1): 58–64.

Behera, B., Verma, N., Sonone, A. and Makhija, U. (2005a). Antioxidant and antibacterial activities of lichen *Usnea ghattensis in vitro*, *Biotechnology Letters* **27**(14): 991–995.

Behera, B., Verma, N., Sonone, A. and Makhija, U. (2006). Determination of antioxidative potential of lichen Usnea ghattensis in vitro, *LWT - Food Science and Technology* **39**(1): 80–85.

Bessadottir, M., Egilsson, M., Einarsdottir, E., Magnusdottir, I. H., Ogmundsdottir, M. H., Omarsdottir, S. and Ogmundsdottir, H. M. (2012). Proton-shuttling lichen compound usnic acid affects mitochondrial and lysosomal function in cancer cells, *PLoS ONE* **7**(12): e51296.

Bhattarai, H. D., Kim, T., Oh, H. and Yim, J. H. (2013). A new pseudodepsidone from the Antarctic lichen *Stereocaulon alpinum* and its antioxidant, antibacterial activity, *The Journal of Antibiotics* **66**(9): 559–561.

Brandão, L. F. G., Alcantara, G. B., Matos, M. d. F. C., Bogo, D., Freitas, D. d. S., Oyama, N. M. and Honda, N. K. (2013). Cytotoxic evaluation of phenolic compounds from lichens against melanoma cells, *Chemical & Pharmaceutical Bulletin* **61**(2): 176–183.

Brisdelli, F., Perilli, M., Sellitri, D., Piovano, M., Garbarino, J. A., Nicoletti, M., Bozzi, A., Amicosante, G. and Celenza, G. (2013). Cytotoxic activity and antioxidant capacity of purified lichen metabolites: An *in vitro* study: Cytotoxicity of lichen metabolites, *Phytotherapy Research* **27**(3): 431–437.

Bugni, T. S., Andjelic, C. D., Pole, A. R., Rai, P., Ireland, C. M. and Barrows, L. R. (2009). Biologically active components of a Papua New Guinea analgesic and anti-inflammatory lichen preparation, *Fitoterapia* **80**(5): 270–273.

Burlando, B., Ranzato, E., Volante, A., Appendino, G., Pollastro, F. and Verotta, L. (2009). Antiproliferative effects on tumour cells and promotion of keratinocyte wound healing by different lichen compounds, *Planta Medica* **75**(06): 607–613.

Bézivin, C., Tomasi, S., Rouaud, I., Delcros, J.-G. and Boustie, J. (2004). Cytotoxic activity of compounds from the lichen: *Cladonia convoluta*, *Planta Medica* **70**(9): 874–877.

Campanella, L., Delfini, M., Ercole, P., Iacoangeli, A. and Risuleo, G. (2002). Molecular characterization and action of usnic acid: a drug that inhibits proliferation of mouse polyomavirus in vitro and whose main target is RNA transcription, *Biochimie* **84**(4): 329–334.

Candan, M., Yılmaz, M., Tay, T., Erdem, M. and Türk, A. z. (2007). Antimicrobial Activity of Extracts of the Lichen *Parmelia sulcata* and its Salazinic Acid Constituent, *Zeitschrift für Naturforschung C* **62**(7-8): 619–621.

Cansaran, D., Kahya, D., Yurdakulol, E. and Atakol, O. (2006). Identification and Quantitation of Usnic Acid from the Lichen *Usnea* Species of Anatolia and Antimicrobial Activity, *Zeitschrift für Naturforschung C* **61**(11-12): 773–776.

Cardarelli, M., Serino, G., Campanella, L., Ercole, P., De Cicco Nardone, F., Alesiani, O. and Rossiello, F. (1997). Antimitotic effects of usnic acid on different biological systems, *Cellular and Molecular Life Sciences (CMLS)* **53**(8): 667–672.

Ceker, S., Orhan, F., Kizil, H. E., Alpsoy, L., Gulluce, M., Aslan, A. and Agar, G. (2015). Genotoxic and antigenotoxic potentials of two *Usnea* species, *Toxicology and Industrial Health* **31**(11): 990–999.

Choudhary, M. I., Azizuddin, Jalil, S. and Atta-ur-Rahman (2005). Bioactive phenolic compounds from a medicinal lichen, *Usnea longissima*, *Phytochemistry* **66**(19): 2346–2350.

Clerc, P. (1998). Species Concepts in the Genus *Usnea* (Lichenized Ascomycetes), *The Lichenologist* **30**(4-5): 321–340.

Einarsdóttir, E., Groeneweg, J., Björnsdóttir, G., Harðardottir, G., Omarsdóttir, S., Ingólfsdóttir, K. and Ögmundsdóttir, H. (2010). Cellular mechanisms of the anticancer effects of the lichen compound usnic acid, *Planta Medica* **76**(10): 969–974.

Engel, K., Schmidt, U., Reuter, J., Weckesser, S., Simon-Haarhaus, B. and Schempp, C. (2007). *Usnea barbata* extract prevents ultraviolet-B induced prostaglandin E2 synthesis and COX-2 expression in HaCaT keratinocytes, *Journal of Photochemistry and Photobiology B: Biology* **89**(1): 9–14.

Fiscus, S. A. (1972). A survey of the chemistry of the *Usnea florida* group in North America, *The Bryologist* **75**(3): 299.

Fournet, A., Ferreira, M.-E., de Arias, A. R., de Ortiz, S. T., Inchausti, A., Yalaff, G., Quilhot, W., Fernandez, E. and Hidalgo, M. E. (1997). Activity of compounds isolated from Chilean lichens against experimental cutaneous leishmaniasis, *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology* **116**(1): 51–54.

Gómez-Serranillos, M. P., Fernández-Moriano, C., González-Burgos, E., Divakar, P. K. and Crespo, A. (2014). Parmeliaceae family: phytochemistry, pharmacological potential and phylogenetic features, *RSC Adv.* **4**(103): 59017–59047.

Gollapudi, S. R., Telikepalli, H., Jampani, H. B., Mirhom, Y. W., Drake, S. D., Bhattiprolu, K. R., Velde, D. V. and Mitscher, L. A. (1994). Alectosarmentin, a new antimicrobial dibenzofuranoid lactol from the lichen, *Alectoria sarmentosa*, *Journal of Natural Products* **57**(7): 934–938.

Gupta, V. K., Verma, S., Gupta, S., Singh, A., Pal, A., Srivastava, S. K., Srivastava, P. K., Singh, S. C. and Darokar, M. P. (2012). Membrane-damaging potential of natural L-(-)-usnic acid in *Staphylococcus aureus*, *European Journal of Clinical Microbiology & Infectious Diseases* **31**(12): 3375–3383.

Halici, M., Odabasoglu, F., Suleyman, H., Cakir, A., Aslan, A. and Bayir, Y. (2005). Effects of water extract of *Usnea longissima* on antioxidant enzyme activity and mucosal damage caused by indomethacin in rats, *Phytomedicine* **12**(9): 656–662.

Han, D., Matsumaru, K., Rettori, D. and Kaplowitz, N. (2004). Usnic acid-induced necrosis of cultured mouse hepatocytes: inhibition of mitochondrial function and oxidative stress, *Biochemical Pharmacology* **67**(3): 439–451.

Honda, N., Pavan, F., Coelho, R., de Andrade Leite, S., Micheletti, A., Lopes, T., Misutsu, M., Beatriz, A., Brum, R. and Leite, C. (2010). Antimycobacterial activity of lichen substances, *Phytomedicine* **17**(5): 328–332.

Ingólfsdóttir, K., Chung, G. A., Skúlason, V. G., Gissurarson, S. R. and Vilhelmsdóttir, M. (1998). Antimycobacterial activity of lichen metabolites *in vitro*, *European Journal of Pharmaceutical Sciences* **6**(2): 141–144.

Ivanova, V., Bačkor, M., Dahse, H.-M. and Graefe, U. (2010). Molecular structural studies of lichen substances with antimicrobial, antiproliferative, and cytotoxic effects from *Parmelia subrudecta*, *Preparative Biochemistry and Biotechnology* **40**(4): 377–388.

Jayaprakasha, G. K. and Rao, L. J. (2000). Phenolic constituents from the lichen *Parmotrema stuppeum* (Nyl.) Hale and their antioxidant activity, *Zeitschrift für Naturforschung C* **55**(11-12): 1018–1022.

Jin, J.-q., Li, C.-q. and He, L.-c. (2008). Down-regulatory effect of usnic acid on nuclear factor- κB-dependent tumor necrosis factor- α and inducible nitric oxide synthase expression in lipopolysaccharide-stimulated macrophages RAW 264.7, *Phytotherapy Research* **22**(12): 1605–1609.

Kim, M.-S. and Cho, H.-B. (2007). Melanogenesis inhibitory effects of methanolic extracts of *Umbilicaria esculenta* and *Usnea longissima*, *Journal of Microbiology (Seoul, Korea)* **45**(6): 578–582.

Kohlhardt-Floehr, C., Boehm, F., Troppens, S., Lademann, J. and Truscott, T. G. (2010). Prooxidant and antioxidant behaviour of usnic acid from lichens under UVB-light irradiation – Studies on human cells, *Journal of Photochemistry and Photobiology B: Biology* **101**(1): 97–102.

Kokubun, T., Shiu, W. and Gibbons, S. (2007). Inhibitory activities of lichen-derived compounds against methicillin- and multidrug-resistant *Staphylococcus aureus*, *Planta Medica* **73**(2): 176–179.

Kosanić, M., Manojlović, N., Janković, S., Stanojković, T. and Ranković, B. (2013). *Evernia prunastri* and *Pseudoevernia furfuraceae* lichens and their major metabolites as antioxidant, antimicrobial and anticancer agents, *Food and Chemical Toxicology* **53**: 112–118.

Kosanić, M., Ranković, B., Stanojković, T., Rančić, A. and Manojlović, N. (2014). *Cladonia* lichens and their major metabolites as possible natural antioxidant, antimicrobial and anticancer agents, *LWT - Food Science and Technology* **59**(1): 518–525.

Kumar KC, S. and Müller, K. (1999a). Lichen metabolites. 1. Inhibitory action against leukotriene B_4 biosynthesis by a non-redox mechanism, *Journal of Natural Products* **62**(6): 817–820.

Kumar KC, S. and Müller, K. (1999b). Lichen metabolites. 2. Antiproliferative and cytotoxic activity of gyrophoric, usnic, and diffractaic acid on human keratinocyte growth, *Journal of Natural Products* **62**(6): 821–823.

Lee, K.-A. and Kim, M.-S. (2005). Antiplatelet and antithrombotic activities of methanol extract of *Usnea longissima*, *Phytotherapy Research* **19**(12): 1061–1064.

List, P. and Hörhammer, L. (eds) (1979). *Hagers handbuch der pharmazeutischen praxis für apotheker, arzneimittelhersteller, arzte und medizinalbeamte*, Springer Verlag, Berlin, Heidelberg, New York.

Lohézic-Le Dévéhat, F., Tomasi, S., Elix, J. A., Bernard, A., Rouaud, I., Uriac, P. and Boustie, J. (2007). Stictic acid derivatives from the lichen *Usnea articulata* and their antioxidant activities, *Journal of Natural Products* **70**(7): 1218–1220.

Luo, H., Yamamoto, Y., A Kim, J., Jung, J. S., Koh, Y. J. and Hur, J.-S. (2009). Lecanoric acid, a secondary lichen substance with antioxidant properties from Umbilicaria antarctica in maritime Antarctica (King George Island), *Polar Biology* **32**(7): 1033–1040.

Madamombe, I. and Afolayan, A. (2003). Evaluation of Antimicrobial Activity of Extracts from South African *Usnea barbata*, *Pharmaceutical Biology* **41**(3): 199–202.

Manojlović, N., Ranković, B., Kosanić, M., Vasiljević, P. and Stanojković, T. (2012). Chemical composition of three *Parmelia* lichens and antioxidant, antimicrobial and cytotoxic activities of some their major metabolites, *Phytomedicine* **19**(13): 1166–1172.

Martins, M. C. B., Lima, M. J. G. d., Silva, F. P., Azevedo-Ximenes, E., Silva, N. H. d. and Pereira, E. C. (2010). *Cladia aggregata* (lichen) from Brazilian northeast: chemical characterization and antimicrobial activity, *Brazilian Archives of Biology and Technology* **53**(1): 115–122.

Melo, M. G. D., dos Santos, J. P. A., Serafini, M. R., Caregnato, F. F., de Bittencourt Pasquali, M. A., Rabelo, T. K., da Rocha, R. F., Quintans, L., de Souza Araújo, A. A., da Silva, F. A., Moreira, J. C. F. and Gelain, D. P. (2011). Redox properties and cytoprotective actions of atranorin, a lichen secondary metabolite, *Toxicology in Vitro* **25**(2): 462–468.

Molnár, K. and Farkas, E. (2010). Current Results on Biological Activities of Lichen Secondary Metabolites: a Review, *Zeitschrift für Naturforschung C* **65**(3-4): 157–173.

Nishitoba, Y., Nishimura, I., Nishiyama, T. and Mizutani, J. (1987). Lichen acids, plant growth inhibitors from *Usnea longissima*, *Phytochemistry* **26**(12): 3181–3185.

Odabasoglu, F., Aslan, A., Cakir, A., Suleyman, H., Karagoz, Y., Bayir, Y. and Halici, M. (2005). Antioxidant activity, reducing power and total phenolic content of some lichen species, *Fitoterapia* **76**(2): 216–219.

Odabasoglu, F., Aslan, A., Cakir, A., Suleyman, H., Karagoz, Y., Halici, M. and Bayir, Y. (2004). Comparison of antioxidant activity and phenolic content of three lichen species, *Phytotherapy Research* **18**(11): 938–941.

Odabasoglu, F., Cakir, A., Suleyman, H., Aslan, A., Bayir, Y., Halici, M. and Kazaz, C. (2006). Gastroprotective and antioxidant effects of usnic acid on indomethacin-induced gastric ulcer in rats, *Journal of Ethnopharmacology* **103**(1): 59–65.

Odabasoglu, F., Yildirim, O. S., Aygun, H., Halici, Z., Halici, M., Erdogan, F., Cadirci, E., Cakir, A., Okumus, Z., Aksakal, B., Aslan, A., Unal, D. and Bayir, Y. (2012). Diffractaic acid, a novel proapoptotic agent, induces with olive oil both apoptosis and antioxidative systems in Ti-implanted rabbits, *European Journal of Pharmacology* **674**(2-3): 171–178.

Ohmura, Y. (2012). A synopsis of the lichen genus *Usnea* (parmeliaceae, ascomycota) in taiwan, *Mem. Natl. Mus. Nat. Sci., Tokyo* **48**: 91–137.

Papadopoulou, P., Tzakou, O., Vagias, C., Kefalas, P. and Roussis, V. (2007). β-Orcinol metabolites from the lichen *Hypotrachyna revoluta*, *Molecules* **12**(5): 997–1005.

Paudel, B., Datta Bhattarai, H., Prasad Pandey, D., Seoun Hur, J., Gyu Hong, S., Kim, I.-C. and Han Yim, J. (2012). Antioxidant, antibacterial activity and brine shrimp toxicity test of some mountainous lichens from Nepal, *Biological Research* **45**(4): 387–391.

Periera, E. C., Nascimento, S. C., Lima, R. C., Silva, N. H., Oliveira, A. F., Bandeira, E., Boitard, M., Beriel, H., Vicente, C. and Legaz, M. E. (1994). Analysis of *Usnea fasciata* crude extracts with antineoplastic activity, *The Tokai Journal of Experimental and Clinical Medicine* **19**(1-2): 47–52.

Polat, Z., Aydın, E., Türkez, H. and Aslan, A. (2016). *In vitro* risk assessment of usnic acid, *Toxicology and Industrial Health* **32**(3): 468–475.

Ranković, B. and Kosanić, M. (2015). Lichens as a potential source of bioactive secondary metabolites, *in* B. Ranković (ed.), *Lichen Secondary Metabolites*, Springer International Publishing, Cham, pp. 1–26.

Ranković, B., Kosanić, M., Stanojković, T., Vasiljević, P. and Manojlović, N. (2012). Biological activities of *Toninia candida* and *Usnea barbata* together with their norstictic acid and usnic acid constituents, *International Journal of Molecular Sciences* **13**(12): 14707–14722.

Ranković, B., Mišić, M. and Sukdolak, S. (2008). The antimicrobial activity of substances derived from the lichens *Physcia aipolia*, *Umbilicaria polyphylla*, *Parmelia caperata* and *Hypogymnia physodes*, *World Journal of Microbiology and Biotechnology* **24**(7): 1239–1242.

Rezanka, T. and Sigler, K. (2007). Hirtusneanoside, an unsymmetrical dimeric tetrahydroxanthone from the lichen *Usnea hirta*, *Journal of Natural Products* **70**(9): 1487–1491.

Rukayadi, Y., Shim, J.-S. and Hwang, J.-K. (2008). Screening of Thai medicinal plants for anticandidal activity, *Mycoses* **51**(4): 308–312.

Safak, B., Ciftci, I. H., Ozdemir, M., Kiyildi, N., Cetinkaya, Z., Aktepe, O. C. and Altindis, M. (2009). *In vitro* anti-*Helicobacter pylori* activity of usnic acid, *Phytotherapy Research* **23**(7): 955–957.

Santos, L., Honda, N., Carlos, I. and Vilegas, W. (2004). Intermediate reactive oxygen and nitrogen from macrophages induced by Brazilian lichens, *Fitoterapia* **75**(5): 473–479.

Schmeda-Hirschmann, G., Tapia, A., Lima, B., Pertino, M., Sortino, M., Zacchino, S., Arias, A. R. d. and Feresin, G. E. (2008). A new antifungal and antiprotozoal depside from the andean lichen *Protousnea poeppigii*, *Phytotherapy Research* **22**(3): 349–355.

Shrestha, G. and St. Clair, L. L. (2013). Lichens: a promising source of antibiotic and anticancer drugs, *Phytochemistry Reviews* **12**(1): 229–244.

Singh, G., Divakar, P. K., Dal Grande, F., Otte, J., Parnmen, S., Wedin, M., Crespo, A., Lumbsch, H. T. and Schmitt, I. (2013). The sister-group relationships of the largest family of lichenized fungi, Parmeliaceae (Lecanorales, Ascomycota), *Fungal Biology* **117**(10): 715–721.

Srivastava, P., Upreti, D. K., Dhole, T. N., Srivastava, A. K. and Nayak, M. T. (2013). Antimicrobial property of extracts of Indian lichen against human pathogenic bacteria, *Interdisciplinary Perspectives on Infectious Diseases* **2013**: 1–6.

Sultana, N. and Afolayan, A. J. (2011). A new depsidone and antibacterial activities of compounds from *Usnea undulata* Stirton, *Journal of Asian Natural Products Research* **13**(12): 1158–1164.

Swinscow, T. D. V. and Krog, H. (1979). The fruticose species of *Usnea* subgenus *Usnea* in East Africa, *The Lichenologist* **11**(03): 207–252.

Thadhani, V. M., Choudhary, M. I., Ali, S., Omar, I., Siddique, H. and Karunaratne, V. (2011). Antioxidant activity of some lichen metabolites, *Natural Product Research* **25**(19): 1827–1837.

Toledo Marante, F. J., García Castellano, A., Estévez Rosas, F., Quintana Aguiar, J. and Bermejo Barrera, J. (2003). Identification and quantitation of allelochemicals from the lichen *Lethariella canariensis*: phytotoxicity and antioxidative activity, *Journal of Chemical Ecology* **29**(9): 2049–2071.

Türk, H., Yılmaz, M., Tay, T., Türk, A. z. and Kıvanç, M. (2006). Antimicrobial activity of extracts of chemical races of the lichen *Pseudevernia furfuracea* and their physodic acid, chloroatranorin, atranorin, and olivetoric acid constituents, *Zeitschrift für Naturforschung C* **61**(7-8): 499–507.

Valencia-Islas, N., Zambrano, A. and Rojas, J. L. (2007). Ozone reactivity and free radical scavenging behavior of phenolic secondary metabolites in lichens exposed to chronic oxidant air pollution from Mexico City, *Journal of Chemical Ecology* **33**(8): 1619–1634.

Verma, N., Behera, B. C. and Makhija, U. (2008). Antioxidant and hepatoprotective activity of a lichen *Usnea ghattensis in vitro*, *Applied Biochemistry and Biotechnology* **151**(2-3): 167–181.

Vijayan, P., Raghu, C., Ashok, G., Dhanaraj, S. A. and Suresh, B. (2004). Antiviral activity of medicinal plants of Nilgiris, *The Indian Journal of Medical Research* **120**(1): 24–29.

Weckesser, S., Engel, K., Simon-Haarhaus, B., Wittmer, A., Pelz, K. and Schempp, C. (2007). Screening of plant extracts for antimicrobial activity against bacteria and yeasts with dermatological relevance, *Phytomedicine* **14**(7-8): 508–516.

White, P., Oliveira, R., Oliveira, A., Serafini, M., Araújo, A., Gelain, D., Moreira, J., Almeida, J., Quintans, J., Quintans-Junior, L. and Santos, M. (2014). Antioxidant activity and mechanisms of action of natural compounds isolated from lichens: A systematic review, *Molecules* **19**(9): 14496–14527.

Yamamoto, Y., Miura, Y., Kinoshita, Y., Higuchi, M., Yamada, Y., Murakami, A., Ohigashi, H. and Koshimizu, K. (1995). Screening of tissue cultures and thalli of lichens and some of their active constituents for inhibition of tumor promoter-induced Epstein-Barr virus activation., *Chemical & Pharmaceutical Bulletin* **43**(8): 1388–1390.

Zambare, V. P. and Christopher, L. P. (2012). Biopharmaceutical potential of lichens, *Pharmaceutical Biology* **50**(6): 778–798.

Zugic, A., Jeremic, I., Isakovic, A., Arsic, I., Savic, S. and Tadic, V. (2016). Evaluation of anticancer and antioxidant activity of a commercially available CO2 supercritical extract of old man's beard (*Usnea barbata*), *PLOS ONE* **11**(1): e0146342.

Zugic, A., Lukic, M., Tasic-Kostov, M., Tadic, V., Arsic, I., Misic, D., Petrovic, S. and Savic, S. (2015). Alkyl polyglucoside-stabilized emulsion as a prospective vehicle for *Usnea barbata* CO_2-supercritical extract: Assessing stability, safety and efficiency of a topical formulation, *Hemijska industrija* **69**(6): 703–712.