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 pharmaco-logical potential of lichens and/or their secondary metabolites as a promising source of potential medicines with anticancer, antimicrobial, anti-inflammatory and antioxidative 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, antifungal, antiprotozoal and antiviral). Generally, it was observed that all species of Parmeliaceae family had a stronger antimicrobial effect compared to the lichen 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 species belonging to the genus Usnea as well as the biological activities of secondary metabolites identified in the species of this genus will be presented (Table 1).

2.2. 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 antimicrobial, antitumor 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 IC50 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 CO2 extract of this lichen against B16 mouse melanoma and C6 rat glioma. Namely, supercritical CO2 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 CO2 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>
<thead>
<tr>
<th>Species</th>
<th>Chemical composition</th>
<th>Reference</th>
<th>Biological activity</th>
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</tr>
</thead>
<tbody>
<tr>
<td><em>Usnea articulata</em> (L.) Hoffm.</td>
<td>Usnic acid, barbatic acid, atranorin, methyl-β- orcinolcarboxylate and other β- orcinol derivatives, ergosterol peroxide, fumarprotocetraric acid, protocetraric acid, stictic acid, norstictic acid, peristentic acid, criptostictic acid, menegazziaic acid, constrictic acid, 3-O-methyl-consalazinic acid</td>
<td>Lohézic-Le Dévèhat et al. (2007)</td>
<td>Antigenotoxic, antioxidant</td>
<td>Ceker et al. (2015)</td>
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<tr>
<td><em>Usnea barbata</em> (L.) Mott.</td>
<td>Usnic acid, norstictic acid, atranorin, chloroatranorin, barbatolic acid, lobaric acid, salazinic acid</td>
<td>List and Hörhammer (1979); Ranković et al. (2012)</td>
<td>Anti-inflammatory, UVB protective effects</td>
<td>Engel et al. (2007)</td>
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<td></td>
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<td>Antimicrobial (antibacterial, antmycotic)</td>
<td>Cansaran et al. (2006); Madamombe and Afolayan (2003); Ranković et al. (2012); Weckesser et al. (2007)</td>
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<td>Antimicrobial (antiviral)</td>
<td>Vijayan et al. (2004)</td>
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<td>Antioxidative</td>
<td>Ranković et al. (2012)</td>
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<td>Cytotoxic</td>
<td>Ranković et al. (2012)</td>
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<tr>
<td><em>Usnea complanata</em> (Müll.Arg.) Motyka</td>
<td>Usnic acid, psoromic acid, salazinic acid, norstictic acid, constrictic acid, galbinic acid</td>
<td>Behera et al. (2012); Swinscow and Krog (1979)</td>
<td>Antioxidative, cardioprotective</td>
<td>Behera et al. (2012)</td>
</tr>
<tr>
<td><em>Usnea fasciata</em> Torrey</td>
<td>Usnic acid, isolichenin, raffinose</td>
<td>Periera et al. (1994)</td>
<td>Cytotoxic</td>
<td>Periera et al. (1994)</td>
</tr>
<tr>
<td><em>Usnea fillipendula</em> Stirton</td>
<td>Usnic acid, salazinic acid, thammolic acid</td>
<td>List and Hörhammer (1979)</td>
<td>Antigenotoxic, antioxidant</td>
<td>Ceker et al. (2015)</td>
</tr>
<tr>
<td><em>Usnea floridita</em> (L.) Weber ex Wigg.</td>
<td>Usnic acid, lecanoric acid, thammolic acid, diffractaic acid, squamatic acid, salazinic acid, constrictic acid, norstictic acid, psromic acid, protocetraric acid, fumaprotocetraric acid, conpsoromic acid, caperatic acid, Evans’s substance, lobaric acid</td>
<td>Fiscus (1972); List and Hörhammer (1979)</td>
<td>Antimicrobial (antibacterial)</td>
<td>Cansaran et al. (2006); Schmeda-Hirschmann et al. (2008)</td>
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<td>Antioxidative</td>
<td>Odabasoglu et al. (2004)</td>
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<td><em>Usnea ghattensis</em> G. Awasthi</td>
<td>Usnic acid, norstictic acid</td>
<td>Verma et al. (2008)</td>
<td>Antimicrobial (antibacterial)</td>
<td>Behera et al. (2005a); Srivastava et al. (2013)</td>
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<td>Antioxidative</td>
<td>Behera et al. (2006; 2005a); Verma et al. (2008)</td>
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<td></td>
<td>Hepatoprotective</td>
<td>Verma et al. (2008)</td>
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<td>Species</td>
<td>Chemical composition</td>
<td>Reference</td>
<td>Biological activity</td>
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<tr>
<td><strong>Usnea longissima Ach.</strong></td>
<td>Usnic acid, barbatic acid, diffractaic acid, glutinol, longissiminone A longissiminone B, salazinic acid, protocetraric acid, evernic acid, 4-O-demethyl-barbatic acid</td>
<td>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);</td>
<td>Antioxidative</td>
<td>Ağar et al. (2011); Kim and Cho (2007); Odabasoglu et al. (2004)</td>
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<td></td>
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<td>Antigenotoxic</td>
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<td>Ağar et al. (2011)</td>
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<td>Antiulcer</td>
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<td>Halici et al. (2005)</td>
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<td>Antitumour</td>
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<td>Yamamoto et al. (1995)</td>
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<td>Enzyme-inhibitory</td>
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<td>Kim and Cho (2007)</td>
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<td></td>
<td></td>
<td>Antiplatelet</td>
<td></td>
<td>Lee and Kim (2005)</td>
</tr>
<tr>
<td><strong>Usnea siamenis Vain.</strong></td>
<td>Usnic acid, stictic acid, constictic acid, norstictic acid</td>
<td>Swinscow and Krog (1979)</td>
<td>Antimicrobial (antimycotic)</td>
<td>Rukayadi et al. (2008)</td>
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</tbody>
</table>
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 of *U. longissima* (Agar 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. glutinosa* demonstrated good antioxidant potential similar to the referent antioxidants (Behera et al., 2006; 2005ab; Verma et al., 2008). Also, methanol extract of *U. longissima* exerted strong antioxidative capacity *in vitro* (Agar 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 antioxidative 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. aurantioatra*) (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 CO2 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 revealed free radical scavenging activity (DPPH AF B1 in human lymphocytes via regulation of enzyme activity and *U. articulata* (2007) observed an inhibitory effect of the methanol extract of *U. ghattensis* (Engel et al., 2011; Kim and Cho, 2007), while in another 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). 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. glutinosa* 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, diffraetica, fumarprotocetraric, galbinic, lobarc 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; Sultan 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* (Fournier 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), *Letharia* (*Toledo Marante* et al., 2003), *Cladonia* (Behera et al., 2012; Besnardottir et al., 2012; Lohéac-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, cytotoxophic, 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; Manojlovic et al., 2012; Ranković et al., 2012; 2008; Schmeda-Hirschmann et al., 2008; Sultan 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
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 antifungal (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 leukemia 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), fumaroprotocetraric 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 its 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 (Kohlhirdt-Floehr et al., 2010; Polat et al., 2016). In contrast, or better to say in line with the above-mentioned, 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 (O dabasoglu 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 (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 Cetraria, Evernia, Pseudocetraria, and Parmelia, although it was also found in some species of the Parmotrema, Pseudocetraria, Cladina, Lethariella, Hypotrachyna and Usnea genus (Gomez-Serranillos et al., 2014; White et al., 2014). Several biological activities of atranorin have recently been reviewed by Gomez-Serranillos et al. (2014) and also White et al. (2014). Atranorin exhibited very strong antimicrobial activity against various bacteria and fungi, shown by Kosanči et al. (2014). Accordingly, Toledo Marante et al. (2003) observed moderate antibacterial activity of atranorin against S. aureus. However, atranorin did not possess the antymycotic 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; Kosanči et al., 2013; Toledo Marante et al., 2003). Antioxidative activity of this compound was confirmed in numerous studies (Jayaprakasha and Rao, 2000; Kosanči et al., 2014; Melo et al., 2011; Papadopoulou et al., 2007; Toledo Marante et al., 2003; Vazquez-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 cyclooxygenase-1 (COX-1) and partial inhibition of COX-2 (Bugini 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 Protosusna magellanica. In up-to-now performed investigations, this compound was shown to possess antioxidant, gastroprotective, immunostimulatory, antitumor, anti-inflammatory and antimicrobial activities (Gomez-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, Briselli 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.

Barbaric acid

Barbaric acid (Figure 3C), another member of the depsides family, except in some species of genus Usnea, was also found in the lichens Cladina aggregates and Heterodera muellers (Molnár and Farkas, 2010; Shrestha and St. Clair, 2013). Barbaric acid has been shown to inhibit the growth of S. aureus (Martins et al., 2010). Yamamoto et al. (1995) identified barbaric 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, Pseudovernia 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 (Kosaníć 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 (Kosaníć 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) (Kosaníć 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'-COOCH₃/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 (Jayaprakash 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 Ypo-trachyna 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.

**Fumaroprotocetraric acid**

Fumaroprotocetraric 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) fumaroprotocetraric 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 Stereocaulon 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 the 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 diffraction 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, and in other lichens such as Stereocaulon alpinum and Cladonia sp. (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 (Manojlovic et al., 2012). Protocetraric acid increased the release of NO and H2O2 in macrophages inducing an immunostimulatory effect (Lohézic-Le Dévéhat et al., 2007; Santos et al., 2004).

**Other depsidone compounds**

Depsidone 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 Afobalay, 2011).

**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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