

## ANTIMICROBIAL ACTIVITY OF THE SECONDARY METABOLITES OF THE GENUS BRYORIA - A REVIEW <sup>†</sup>

UDC 615.281.9 : 582.293.378

**Nina Milovanović<sup>1</sup>, Danijela Kostić<sup>1</sup>, Vesna Milovanović<sup>2</sup>,  
Aleksandra Đorđević<sup>1</sup>, Gordana S. Stojanović<sup>1</sup>**

<sup>1</sup>University of Niš, Department of Chemistry, Faculty of Sciences and Mathematics, Niš, Serbia

<sup>2</sup>Technical High School, Kruševac, Serbia

**Abstract.** *Main secondary metabolites identified in species of genus Bryoria are fumarprotocetraric and confumarprotocetraric acids, followed by stictic and lobaric acids, atranorin, gyrophoric, vulpinic, barbatolic and usnic acids. This review deals with the antimicrobial activity of substances identified from the genus Bryoria. These data may be useful in predicting the activity of extracts if their composition is known.*

**Key words:** *antimicrobial activity, Bryoria, confumarprotocetraric acid, fumarprotocetraric acid, lichens acid*

### 1. INTRODUCTION

For a long time, some lichen species have been used in traditional medicine in the treatment of numerous infectious diseases (Bown, 2001). The use of lichens in medicine is based on the fact that they contain unique and varied biologically active substances. Lichen substances exert a wide variety of biological actions including antibiotic, antimycotic, antiviral, anti-inflammatory, analgesic, antipyretic, antiproliferative and cytotoxic effects (Kosanić et al., 2012; Manojlović et al., 2010; Stojanović et al., 2012).

Metabolites synthesized by lichens are divided into two groups: primary (intracellular) and secondary (extracellular). Primary metabolites are mainly the same substances as in other plants, and they have structural functions and roles in cellular metabolism (Podterob, 2008). Primary metabolites include amino acids, vitamins, enzymes, disaccharides, chitin (in

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Received March 7<sup>th</sup>, 2015; accepted January 20<sup>th</sup>, 2019

<sup>†</sup> Acknowledgement: The authors acknowledge the Ministry of Education, Science and Technological Development of Serbia for the financial support (Grant No 172047).

**Corresponding author:** Nina Milovanović

University of Niš, Department of Chemistry, Faculty of Sciences and Mathematics, Višegradska 33, 18000 Niš, Serbia

E-mail: ninamilovanovic87@yahoo.com

hyphal walls), lichenin, isolichenin, hemicellulose, pectins, polyalcohols, pigments (chlorophylls *a* and *b*,  $\beta$ - and  $\gamma$ -carotenes, xanthophylls), etc. (Podterob, 2008).

The majority of organic compounds found in lichens are secondary metabolites. Lichens may contain substantial amounts of secondary metabolites, usually between 0.1 and 10 % of the dry weight of the thallus, but sometimes up to 30 % (Galun and Shomer-Ilan, 1988; Stocker-Wörgötter, 2008; Solhaug et al., 2009). They are poorly soluble in water and can usually be isolated from lichen by organic solvents (Otzurk et al., 1999). More than one hundred secondary metabolites, mainly monoaromatics, depsides, depsidones, pulvinates, dibenzofurans, anthraquinones and xanthenes, characteristic for lichen, have been detected and isolated (Molnar and Farkaš, 2010). Chemicals structures of these classes of compounds are similar and identification is often very difficult.

Lichen acids, which is a common name for secondary metabolites, are produced primarily by the mycobiont. Secondary metabolites are deposited externally on the hyphae of the cortex and/or medulla (Boustie and Grube, 2005).

Lichen acids are derived from three chemical pathways: acetate-polymalonate pathway, shikimic acid pathway and, mevalonic acid pathway. The acetate-polymalonate pathway includes the most common lichens compounds such as: aliphatic acids, esters, and related derivatives, mononuclear phenolic compounds, depsides, depsidones, depsones, dibenzofuranes, anthraquinones, chromones, naphthoquinones, and xanthenes. The shikimic acid pathway includes pulvinic acid derivatives (yellow pigments) and terphenylquinones. The mevalonic acid pathway includes di-, sester- and triterpenes and steroids.

Apart from compounds derived from these pathways, which are found throughout all major lichen groups, there are also some unusual compounds classes among these organisms, for example, arthogalin, a cyclic depsipeptide (Huneek and Himmelreich, 1995) and other amino acids derived from compound such as the cytotoxic scabrosin esters isolated from *Xanthoparelia scabrosa* (Ernst-Russel et al., 1999). The most common phenolic acid units derived by the acetate-polymalonate pathway and combined to the characteristic lichen substances are of two types: the orcinol type units and  $\beta$ -orcinol type units. While compounds formed from these two types of units are similar in many ways, differences in their structure, especially in their distribution among the lichens, suggest that the usual tendency to consider the ornicol and  $\beta$ -ornicol compounds separately probably has a biosynthetic justification. Derivatives of orcinol and  $\beta$ -orcinol units include depsides, depsidones, depsones, anthraquinones, and xanthenes.

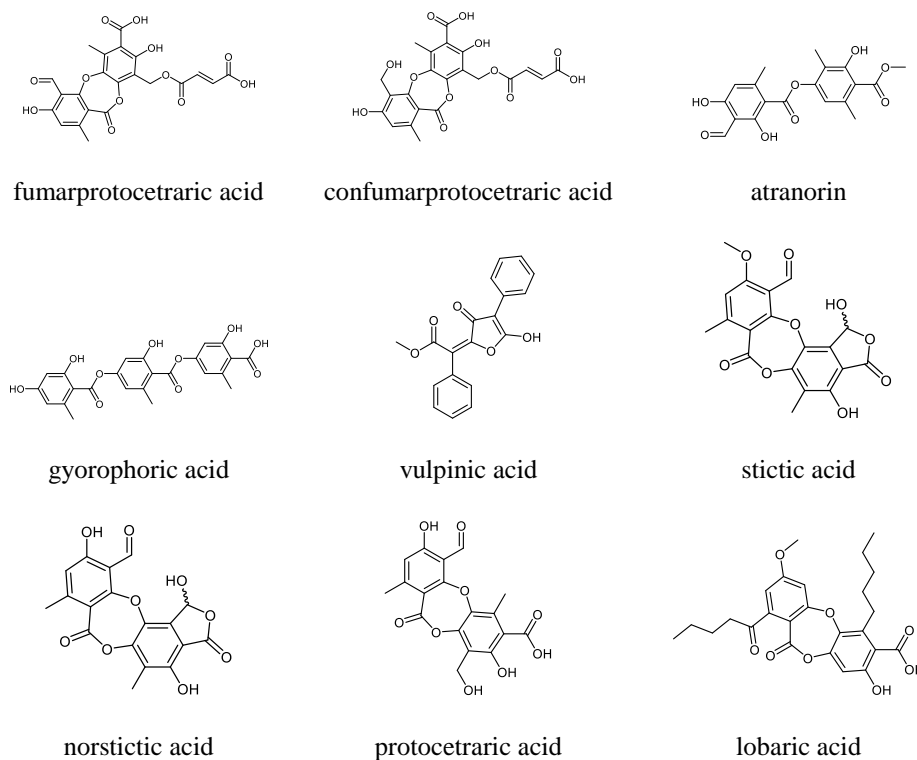
*Bryoria* belongs to the genus of lichen within the Parmeliaceae family, and it contains 51 species. This genus has a widespread distribution, especially in boreal and moderate climatic areas (Kirk et al., 2008). They usually grow on sun-exposed sites, on tree branches and near the water or on branches hanging over brooks (Lukáč, 2009).

Secondary metabolites identified in most of the species of genus *Bryoria* are fumarprotocetraric and confumarprotocetraric acid. Those two lichen acids are considered to be the main components of the species in the genus *Bryoria*. Along with fumarprotocetraric acid and confumarprotocetraric acid, stictic acid, lobaric acid, atranorin, gyrophoric acid, vulpinic acid, barbatolic acid, and usnic acid have been identified (Myllys et al., 2011).

The aim of this work is to present the antimicrobial activity of secondary metabolites identified in species of the genus *Bryoria* in order to predict the antimicrobial potential of extracts of the genus *Bryoria*.

## 2. ANTIMICROBIAL ACTIVITY

The structures and antimicrobial activity of the compounds identified from the species of the genus *Bryoria* are given in Figure 1 and Tables 1 and 2. Fumarprotocetraric acid (sometimes misspelled fumaroprotocetraric acid) is present in almost all species of genus *Bryoria* as the main component. This acid is also specific for *Cladonia* species (Myllys et al., 2011). This acid shows higher antimicrobial activity in comparison with protocetraric acid, stictic acid (Ranković and Mišić, 2008) and atranorin, but considerably weaker than (+)-usnic acid (see Table 1) (Yılmaz et al., 2004). Strong antimicrobial activity of fumarprotocetraric acid isolated from *Cladonia foliacea* is confirmed by Yilmaz et al. (2004). Fumarprotocetraric acid shows better antibacterial than antifungal activity as most of the lichen secondary metabolites (Ranković and Mišić, 2008). There are no data on the antimicrobial activities of confumarprotocetraric acid (according to SciFinder and Google Scholar).



**Fig. 1** Structures of the identified acids from the species of the genus *Bryoria*

**Table 1** Minimal inhibitory concentrations (MIC) of the isolated lichen compounds

TEST ORGANISM	FUM	PRO	STI	GYR	ATR	LOB	NOR	(+)-USN	S	K
<i>B. mycooides</i>	0.062 <sup>a</sup>	0.062 <sup>a</sup> 0.015 <sup>b</sup>	0.5 <sup>a</sup>	0.125 <sup>c</sup>	0.031 <sup>c</sup>	NS	NS	0.0075 <sup>c</sup> 0.0008 <sup>b</sup>	7.81	/
<i>B. subtilis</i>	0.062 <sup>a</sup> 0.0046 <sup>e</sup>	0.062 <sup>a</sup> 0.015 <sup>b</sup>	0.5 <sup>a</sup> -	0.25 <sup>c</sup>	0.031 <sup>c</sup> 0.0156 <sup>e</sup>	NS	1.2506 <sup>f</sup>	0.015 <sup>c</sup> 0.0008 <sup>b</sup> 0.01248 <sup>f</sup>	7.81	/
<i>E. cloaceae</i>	0.062 <sup>a</sup>	0.062 <sup>a</sup>	0.5 <sup>a</sup>	0.25 <sup>c</sup>	0.031 <sup>c</sup>	NS	NS	0.015 <sup>c</sup>	1.95	/
<i>E. coli</i>	0.062 <sup>a</sup>	0.125 <sup>a</sup> 1 <sup>b</sup>	0.5 <sup>a</sup>	0.25 <sup>c</sup>	0.031 <sup>c</sup>	NS	-- <sup>f</sup>	0.031 <sup>c</sup> 0.25 <sup>b</sup> -- <sup>f</sup>	31.25	/
<i>K. pneumoniae</i>	0.031 <sup>a</sup>	0.062 <sup>a</sup> 0.5 <sup>b</sup> -- <sup>f</sup>	0.25 <sup>a</sup> -	0.25 <sup>c</sup>	0.031 <sup>c</sup>	NS	-- <sup>f</sup>	0.0037 <sup>c</sup> 0.0625 <sup>b</sup> -- <sup>f</sup>	1.95	/
<i>S. aureus</i>	0.062 <sup>a</sup> 0.0375 <sup>e</sup>	0.125 <sup>a</sup> 0.015 <sup>b</sup> -- <sup>f</sup>	0.5 <sup>a</sup> -	0.25 <sup>c</sup>	0.031 <sup>c</sup> 0.5 <sup>e</sup>	NS	2.506 <sup>f</sup>	0.015 <sup>c</sup> 0.125 <sup>b</sup> 0.0496 <sup>f</sup>	31.25	/
<i>A. flavus</i>	0.25 <sup>a</sup>	0.125 <sup>a</sup> 1 <sup>b</sup> 1 <sup>a</sup>	0.5 <sup>a</sup>	0.5 <sup>c</sup>	0.5 <sup>c</sup>	NS	NS	0.5 <sup>c</sup> 0.5 <sup>b</sup>	/	3.9
<i>A. fumigatus</i>	0.25 <sup>a</sup>	0.125 <sup>a</sup> 0.25 <sup>b</sup>	1 <sup>a</sup>	0.25 <sup>c</sup>	0.25 <sup>c</sup>	NS	NS	0.5 <sup>c</sup> 0.25 <sup>b</sup>	/	3.9
<i>B. cinerea</i>	0.125 <sup>a</sup>	0.062 <sup>a</sup>	0.5 <sup>a</sup>	0.25 <sup>c</sup>	0.25 <sup>c</sup>	NS	NS	0.25 <sup>c</sup>	/	1.95
<i>C. albicans</i>	0.125 <sup>a</sup> 0.0187 <sup>e</sup>	0.062 <sup>a</sup> 0.25 <sup>b</sup> 0.052 <sup>c</sup> 0.052 <sup>f</sup>	0.5 <sup>a</sup>	0.25 <sup>c</sup>	0.25 <sup>c</sup> 0.5 <sup>e</sup>	NS	0.0386 <sup>f</sup>	0.25 <sup>c</sup> 0.125 <sup>b</sup> 0.0008 <sup>f</sup>	/	1.95
<i>F. oxysporum</i>	0.25 <sup>a</sup>	0.125 <sup>a</sup>	1 <sup>a</sup>	0.25 <sup>c</sup>	0.5 <sup>c</sup>	NS	NS	0.5 <sup>c</sup>	/	3.9
<i>M. mucedo</i>	0.25 <sup>a</sup>	0.125 <sup>a</sup>	1 <sup>a</sup>	0.25 <sup>c</sup>	0.5 <sup>c</sup>	NS	NS	0.5 <sup>c</sup>	/	31.25
<i>P. variotii</i>	0.125 <sup>a</sup>	0.062 <sup>a</sup>	0.5 <sup>a</sup>	0.25 <sup>c</sup>	0.25 <sup>c</sup>	NS	NS	-- <sup>c</sup>	/	1.95
<i>P. purpurescens</i>	0.25 <sup>a</sup>	0.125 <sup>a</sup> 1 <sup>b</sup> 1 <sup>a</sup>	0.5 <sup>a</sup>	0.5 <sup>c</sup>	0.5 <sup>c</sup>	NS	NS	0.25 <sup>c</sup> 0.5 <sup>b</sup>	/	3.9
<i>P. verrucosum</i>	0.25 <sup>a</sup>	0.125 <sup>a</sup> 0.5 <sup>b</sup>	1 <sup>a</sup>	0.5 <sup>c</sup>	0.25 <sup>c</sup>	NS	NS	0.5 <sup>c</sup> 0.5 <sup>b</sup>	/	3.9
<i>T. harsianum</i>	0.25 <sup>a</sup>	0.125 <sup>a</sup>	1 <sup>a</sup>	0.25 <sup>c</sup>	0.25 <sup>c</sup>	NS	NS	0.5 <sup>c</sup>	/	7.81
<i>M. aurum</i>	NS	NS	NS	NS	0.25 <sup>d</sup>	0.125 <sup>d</sup>	NS	0.032 <sup>d</sup>	NS	NS
<i>B. cereus</i>	0.0046 <sup>e</sup>	-- <sup>f</sup>	NS	NS	0.0312 <sup>e</sup>	NS	-- <sup>f</sup>	-- <sup>a</sup> -- <sup>f</sup>	NS	NS
<i>P. vulgaris</i>	0.0375 <sup>e</sup>	-- <sup>f</sup>	NS	NS	0.0625 <sup>e</sup>	NS	0.6253 <sup>f</sup>	0.01248 <sup>f</sup>	NS	NS
<i>A. hydrophila</i>	0.15 <sup>e</sup>	-- <sup>f</sup>	NS	NS	0.0312 <sup>e</sup>	NS	0.312 <sup>f</sup>	-- <sup>f</sup>	NS	NS
<i>S. faecalis</i>	0.15 <sup>e</sup>	-- <sup>f</sup>	NS	NS	0.25 <sup>e</sup>	NS	0.312 <sup>f</sup>	0.0256 <sup>f</sup>	NS	NS
<i>L. monocytogenes</i>	0.0046 <sup>e</sup>	-- <sup>f</sup>	NS	NS	0.156 <sup>e</sup>	NS	0.156 <sup>f</sup>	0.00624 <sup>f</sup>	NS	NS
<i>C. glabrata</i>	0.0187 <sup>e</sup>	0.052 <sup>c</sup> 0.052 <sup>f</sup>	NS	NS	0.5 <sup>e</sup>	NS	0.0386 <sup>f</sup>	0.0008 <sup>f</sup>	NS	NS
<i>P. aeruginosa</i>	NS	-- <sup>c</sup> -- <sup>f</sup>	NS	NS	NS	NS	-- <sup>f</sup>	-- <sup>f</sup>	NS	NS
<i>P. syringae</i>	NS	-- <sup>c</sup> -- <sup>f</sup>	NS	NS	NS	NS	-- <sup>f</sup>	-- <sup>f</sup>	NS	NS
<i>S. typhimurium</i>	NS	-- <sup>c</sup> -- <sup>f</sup>	NS	NS	NS	NS	-- <sup>f</sup>	-- <sup>f</sup>	NS	NS
<i>Y. enterocolitica</i>	NS	-- <sup>c</sup> -- <sup>f</sup>	NS	NS	NS	NS	-- <sup>f</sup>	0.00624 <sup>f</sup>	NS	NS

Values given as mg/mL for lichen compounds and as µg/mL for antibiotics S- Streptomycin, K- Ketoconazole; -- inactive compound; NS- data not shown; FUM- fumarprotocetraric acid; PRO- protocetraric acid; STI- stictic acid; GYR- gyrophoric acid; ATR- atranorin; LOB- lobaric acid; NOR- norstictic acid; (+)USN- usnic acid; <sup>a</sup>lichen compound PRO isolated from *Parmelia caperata*, STI isolated from *Parmelia conspersa*, and FUM isolated from *Cladonia furcata* (Ranković and Mišić, 2008); <sup>b</sup>lichen compound PRO isolated from *Toninia candida* and (+)USN isolated from *Evernia prunastri* (Manojlović et al., 2012); <sup>c</sup>lichen compound GYR isolated from *Umbilica riapolyphylla*, ATR isolated from *Physcia airolia* and (+)USN isolated from *Parmelia caperata* (Ranković et al., 2008); <sup>d</sup>lichen compound LOB isolated from *Stereocaulon alpinum*, (+)USN isolated from *Cladonia arbuscula* (Ingólfssdóttir et al., 1998); <sup>e</sup>lichen compounds isolated from *Cladonia foliacea* (Yilmaza et al., 2004); <sup>f</sup>lichen compounds isolated from *Ramalina farinacea* (Tay et al., 2004).

Atranorin is a para-depside present in the lichens *Bryoria nadvornikiana*, *B. trichodes*, *B. implexa* rarely in lichens *B. capillaris* and *B. lanestris* (Myllys et al., 2011). According to literature data, atranorin, in general, shows weak antimicrobial activity. In comparison with fumarprotocetraric acid, where both of those components are isolated from *C. foliacea*, atranorin shows much weaker antimicrobial activity, even up to 26 times lower for some microorganisms (Yilmaza et al., 2004). Perry et al., (1999) presented inactivity of atranorin against *Bacillus subtilis*, *Trichophyton mentagrophyte* and *Candida albicans* which was not in accordance with the results of Clavalcanti et al., (1983). Saenza et al. (2006) showed atranorin's inactivity to all tested organisms except towards the extremely sensitive bacteria *K. pneumonia* for which atranorin shows weak activity. Atranorin shows similar antimicrobial activity as salazinic acid against *Mycobacterium aurum*. Japanese studies (Shibata and Miura, 1948) were found those components to be inactive against *Mycobacterium tuberculosis* (see Table 1) (Ingólfssdóttir et al., 1998).

Gyrophoric acid is a tridepside which shows antimicrobial activity toward 16 microorganisms tested by Ranković et al., (2008). Gyrophoric acid antibacterial activity is slightly weaker than atranorin and significantly weaker than (+)-usnic acid, while the antifungal activity is very similar for all three components (see Table 1) (Ranković et al., 2008).

Stictic acid is identified only in species *B. nadvornikiana* S 294 (Myllys et al., 2011). Stictic acid shows weaker antimicrobial activity in comparison with fumarprotocetraric acid and protocetraric acid regarding all microorganisms tested by Ranković and Mišić, (2008) and Saenza et al., (2006) (see Table 1). Perry et al., (1999) reported inactivity of stictic acid against all microorganisms which they tested.

Norstictic acid is found in *B. implexa* (Myllys et al., 2011) and shows weak or lack antimicrobial activity (Tay et al., 2004).

Vulpinic acid is diphenyl-butenolide, mostly yellow or orange pigment which protects lichens from UV light. *B. fremontii* is the only one in the genus *Bryoria* producing vulpinic acid (Myllys et al., 2011). Lauterwein et al., (1995) showed that (+)-usnic acid, (-)-usnic acid, and vulpinic acid did not inhibit Gram-negative rods or fungi at concentrations lower than 32 µg/mL but were active against clinical isolates of *Enterococcus faecalis*, *Enterococcus faecium*, and *Staphylococcus aureus*.

Protocetraric acid is present in almost every species of the genus *Bryoria* (except *B. capillaris*, *B. fremontii* and *B. glabra*), but in some species, it is present in small amounts. Protocetraric acid showed similar antibacterial activity as fumarprotocetraric acid and lecanoric acid (Tay et al., 2004; Ranković and Mišić, 2008). Manojlović et al., (2012) presented a similar antimicrobial activity of protocetraric acid and salazinic acid, as well as Yang and Anderson, (1999) and Kosanić et al., (2012).

Lobaric acid is rarely present in the genus *Bryoria*. Myllys et al., (2011) found lobaric acid in *B. perspinosa* S296. Lobaric acid showed stronger antimicrobial activity than atranorin and salazinic acid, but weaker than (+)-usnic acid against bacteria *M. aurum* (see Table 1) (Ingólfssdóttir et al., 1998).

Usnic acid is the most widely used lichen acid in medicine; this is a crystalline substance with a yellow color (Moiseeva et al., 1961). Usnic acid is optically active and both forms are found in lichens from 0.2% (*Cladonia sylvatica*) to 4.0% (*Alectoria achroleuca*). In species of the genus *Bryoria*, usnic acid is not identified, except in *B. hengduanensis* S287 (Myllys et al., 2011). Usnic acid showed the strongest antibacterial

activity from all tested lichen secondary metabolites (see Table 1) (Saenza et al., 2006; Perry et al., 1999; Tay et al., 2004; Manojlović et al., 2012; Ingólfssdóttir et al., 1998; Ranković et al., 2008; Lauterwein et al., 1995).

### 3. CONCLUSIONS

Fumarprotocetraric acid and confumarprotocetraric acid are identified in most of the species of genus *Bryoria* as main components. It has been proven that fumarprotocetraric acid shows antimicrobial activity of medium strength. Other lichen acids identified in the species of genus *Bryoria* (protocetraric acid, stictic acid, lobaric acid, atranorin, gyrophoric acid) show weaker antimicrobial activity in comparison to fumarprotocetraric acids. Secondary metabolites which are present in genus *Bryoria*, show higher antibacterial than antifungal activity. Considering manifested activity of *Bryoria* constituents, one might expect that extracts could exhibit a moderate antimicrobial activity wherein the higher antibacterial than antifungal. This statement is true if there is no synergistic or antagonistic effect among the extracts constituents. Some components that are represented in a small amount may have a high activity and/or synergistic influence. Therefore, it is not enough to identify only the main components for the evaluation of the activity.

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## **ANTIMIKROBNA AKTIVNOST JEDINJENJA IDENTIFIKOVANIH U VRSTAMA RODA *BRYORIA* – PREGLEDNI RAD**

*Glavni sekundarni metaboliti koji su identifikovani u vrstama roda Bryoria su fumarprotocetrarična kiselina i konfumarprotocetrarična kiselina, praćene stiktičnom kiselinom, lobarnom kiselinom, atranorinom, girofornom kiselinom, vulpinskom, barbatoličnom kiselinom i usninskom kiselinom. U ovom preglednom članku data je analiza antimikrobne aktivnosti sastojaka identifikovanih u vrstama roda Bryoria. Ovaj pregledni članak može biti od koristi za predviđanje antimikrobne aktivnost ekstrakata poznatog sastava.*

**Ključne reči:** *antimikrobna aktivnost, Bryoria, konfumarprotocetrarična kiselina, fumarprotocetrarična kiselina, lišajске kiseline*