https://doi.org/10.31925/farmacia.2024.6.16 **ORIGINAL ARTICLE**

# INVESTIGATION OF PHYTOCHEMICAL COMPOSITION AND *IN VITRO* ANTIOXIDANT POTENTIAL OF DIFFERENT EXTRACTS AND GYROPHORIC ACID DERIVED FROM THE LICHEN *UMBILICARIA GRISEA* GROWING IN SERBIA

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## Abstract

This study investigates the chemical composition, total phenolic and flavonoid content and antioxidant activity of acetone, methanol and ethanolic extracts of the poorly researched lichen *Umbilicaria grisea* and its major secondary metabolite, gyrophoric acid. The phytochemical composition was determined by high-performance liquid chromatography (HPLC-UV). In addition to gyrophoric, lecanoric and umbilicaric acid, the examined extracts also contain depsidone norstictic acid and depside atranorin, which were identified for the first time in this species. The study assessed the phenolic and flavonoid content of different solvent extracts from *U. grisea*, revealing that the methanolic extract exhibited the highest phenolic content (67.128 ± 0.222 mg GAE/g Dry Extract), followed by acetonic (56.615 ± 0.385) and ethanol (44.821 ± 0.801) extracts. Total flavonoid content, expressed as rutin and quercetin equivalents, varied significantly among extracts, with acetone demonstrating the highest efficiency for flavonoid extraction from *U. grisea*. The antioxidant activity of tested extracts was estimated spectrophotometrically by determining the ability to scavenge DPPH and ABTS radicals. The acetone extract showed slightly enhanceds antioxidant activity compared to the other two extracts in both tests. Also, gyrophoric acid shows significantly better antioxidant activity compared to all extracts, but weaker compared to ascorbic acid and Trolox. These findings indicate the potential of extracts from *U. grisea* as sources of natural antioxidants.

## Rezumat

Acest studiu evaluează compoziția chimică, conținutul total fenolic și flavonoidic, precum și activitatea antioxidantă a extractelor obținute în acetonă, metanol și etanol din lichenul *Umbilicaria grisea*, și ale metabolitului său secundar principal, acidul giroforic. Compoziția fitochimică a fost determinată prin HPLC-UV. Pe lângă acizii giroforic, lecanoric și ombilicaric, extractele examinate mai conțin acid depsidon-norstictic și depsid-atranorin, care au fost identificate pentru prima dată la această specie. Studiul a evaluat conținutul fenolic și flavonoidic al diferitelor extracte în solvenți diferiți din *U. grisea*, evidențiind că extractul metanolic a prezentat cel mai mare conținut fenolic (67,128 ± 0,222 mg GAE/g extract uscat), urmat de extractul acetonic (56,615 ± 0,385) și cel etanolic (44,821 ± 0,801). Conținutul total de flavonoide, exprimat ca echivalenți de rutină și quercetină, a variat semnificativ între extracte, cel obținut în acetonă demonstrând cea mai mare eficacitate pentru extracția de flavonoide din *U. grisea*. Activitatea antioxidantă a extractelor testate a fost estimată prin determinarea capacității de a chelata radicalii DPPH și ABTS. Extractul acetonic a arătat o activitate antioxidantă superioară celorlalte două extracte, prin ambele teste efectuate. De asemenea, acidul giroforic prezintă o activitate antioxidantă semnificativ mai bună în comparație cu toate extractele, dar redusă comparativ cu acidul ascorbic și Trolox. Aceste rezultate indică potențialul extractelor din *U. grisea* ca surse de antioxidanți naturali.

**Keywords**: *Umbilicaria grisea*, lichen bioactive compounds, antioxidant activity, natural product discovery

## Introduction

Lichens, unique symbiotic organisms consisting of fungus and photosynthetic partners such as algae or cyanobacteria, have garnered considerable attention in recent years due to their remarkable chemical diversity, potential biological activities and potential therapeutic applications. These composite organisms have long been recognised as a rich source of secondary

metabolites with diverse structures and pharmacological properties [1].

Due to more synonyms and misidentifications, the number of species from the lichen genus *Umbilicaria* varies between authors and ranges between 70 and 80 species. [2, 3]. They are widely distributed and mostly grow on rocks at higher altitudes. The thallus of the species is foliose, monophyllous or polyphyllous, with a diameter of 1 - 5 cm, which is attached to the substrate *via* the umbilicus, a short central support. *Umbilicaria grisea* Hoffm. (syn. *Gyrophora grisea*, *Gyrophora murina*, *Umbilicaria murina*) (*U. grisea*), commonly known as "grey umbilicate lichen", is a species found on siliceous, granite, steep surfaces blocks of rock, on slopes with west and east sides in diverse climatic conditions. *U. grisea* is temperately distributed in Europe; occurs in the small area of mountains of Spain, France, Portugal and Greece as well as in Scandinavia and Scotland at altitudes above 1650 m [4-6].

The chemical composition of the *Umbilicaria* lichen species is variable, and it may be correlated with the geographical origin and the morphological characteristics. Gyrophoric acid is the most common tridepside of the *Umbilicaria* genus, as well as the biosynthetic-related depside lecanoric acid, which always occurs as a companion compound of this tridepside, but in a much lower concentration (90:1) [7]. Umbilicaric, hiascic, crustinic and ovoic acids are also available *Umbilicaria* tridepsides as well as depsidone norstictic acid [8]. Lichen extracts also contain various polysaccharides, which possess different biological activities (immunomodulating, antithrombotic and other) [9, 10]. The chemical constituents of lichens are predominantly secondary metabolites produced through the metabolic interplay between the fungal and photosynthetic partners. These secondary metabolites are known to play crucial roles in protecting the lichen from environmental stressors and predation, and they have demonstrated diverse biological activities [11, 12]. Lichens have been used in traditional medicine for centuries in the treatment of various diseases and are a good source of biologically active metabolites. Also, lichens are used in human and animal nutrition, especially in the Arctic region [13]. Lichen crude extracts or their purified metabolites have been confirmed to possess a variety of biological activities, such as cytotoxic, antioxidant, antimicrobial, analgesic, antipyretic and antiproliferative activities [14, 15]. A wide range of biological activities of lichen secondary metabolites is associated with the chemical versatility of these polyaromatic compounds with functional carboxyl and hydroxyl side groups [16].

Despite its wide distribution, *U. grisea* remains relatively understudied, especially regarding its chemical composition and potential bioactivities. Understanding the chemical composition of *U. grisea* and evaluating its antioxidant activities can provide valuable insights into its potential applications in the pharmaceutical and biomedical fields. Serbia, with its diverse ecosystems and climatic conditions, offers a unique habitat for *U. grisea*. However, limited research has been conducted on this lichen species in Serbia, particularly in terms of its chemical profile and biological activity.

This research aimed to identify the main bioactive secondary metabolites of the acetonic, methanolic and ethanolic extracts of the lichen *Umbilicaria grisea* Hoffm. by the HPLC-UV analysis. The total phenolic and flavonoid content and antioxidant (DPPH and ABTS assays) activities of extracts were also investigated. The lichen *U. grisea* species studied in this research can be attractive as a source of bioactive substances with potential health benefits. This research contributes to general knowledge about this lichen species and the genus *Umbilicaria*. Furthermore, this research can provide valuable insights into the potential use of *U. grisea* extracts in the development of new therapeutic agents for combating oxidative stress-related disorders and cancer.

## Materials and Methods

*Sampling*

*U. grisea* was collected by the authors in September 2022 from Mt. Kopaonik, Serbia, from the population growing on granite rocks. The lichen sample was identified according to standard methods and the voucher specimen of the lichen was deposited in the facilities of the Department of Biology and Ecology, Faculty of Science, University of Kragujevac, Serbia.

*Preparation of extracts*

Purified and dried lichen materials (20 g) were ground in a laboratory mill (IKA A11, IKA®-Werke GmbH & Co., Staufen, Germany) and extracted using acetone, methanol and ethanol as solvents (300 mL). Extraction was performed by maceration at room temperature (20 - 22°C) for 5 days. Afterward, extracts were filtered and then evaporated to dryness in a vacuum evaporator (IKA RV10, IKA®-Werke GmbH & Co., Staufen, Germany). Dry extracts were poured into opaque glass bottles and stored in a refrigerator at 2 - 6°C until analysis. The extraction yields were calculated as the percentage of air-dried weight of the lichen thallus sample.

*Isolation of gyrophoric acid*

Following the initial solvent extraction of the lichen *U. grisea*, the solvents (acetone) were removed through vacuum evaporation and then dried at room temperature. The dry acetone extract underwent additional extraction with benzene (3 times). To optimize the yield, the benzene phase underwent centrifugation at 12000 rpm for 10 minutes. The resulting supernatant was combined with the residual precipitate from the benzene extraction. The residue was subsequently dried at room temperature and redissolved in fresh acetone, followed by re-crystallization. Identification was performed by comparing

chromatographic and spectroscopic data with the standard. Standard compounds were previously obtained from diverse sources, and their structures were validated using mass spectrometry, as well as $^{1}H$ and $^{13}C$-NMR data.

*High-performance liquid chromatography (HPLC) analysis*

The extracts were analysed using an Agilent 1200 Series Gradient HPLC System coupled with a photodiode array detector. The separation of extract components was achieved on a reverse-phase chromatographic column (C18; 25 cm × 4.6 mm; particle size 10 µm). The mobile phase consisted of the methanol (pure for HPLC, Merck, Darmstadt, Germany)-water (distilled and purified obtained using the Milli-Q water purification system, Milford, MA, USA)-phosphoric acid solvent system (analytical reagent grade, Sigma Aldrich) in the ratio 80:20:0.9 (*v/v/v*). The time of analysis was 30 min, the temperature of the column thermostat was 22°C, the mobile phase flow was 1.0 mL/min, the injected sample amount was 10 µL and the analytical wavelength was λ = 254 nm [17, 18]. The standards used were obtained from the following sources: norstictic acid was isolated from the lichen *Ramalina furinacea*, lecanoric acid from *Parmotrema tinctorum*, umbilicaric acid from *Umbilicaria polyphylla*, gyrophoric acid from *Umbilicaria crustulosa* and atranorin from lichen *Pseudoevernia furfuraceae*. The standard compounds were isolated in our laboratory and their structures were confirmed by mass spectrometry, $^{1}H$ and $^{13}C$-NMR data.

*Antioxidant activity*

The *in vitro* antioxidant activity of lichen extracts was determined spectrophotometrically (Shimadzu UV-1800 spectrophotometer). All analyses were performed in triplicate, and the results were expressed as mean values ± SD. Reagents used in the measurements were purchased from Sigma-Aldrich, St. Louis, MO, USA.

*DPPH assay*

Spectrophotometric determination of antioxidant activity using the ability to scavenge free DPPH radicals is carried out according to the modified method described in Dorman *et al.* [19]. Methanolic DPPH solution (0.05 mg/mL) was prepared *ex tempore* and stored in a dark and cool place until the experiments were performed. The stock solutions of extracts (1 mg/mL) were prepared in methanol. A two-fold series of dilutions of stock solution was prepared at a concentration ranging from 1000 to 31.25 µg/mL. A volume of 0.2 mL of extract solution was mixed with 2 mL of DPPH solution, vortexed and incubated in a dark place (1 hour), after which the absorbance at 517 nm was measured. The control test was prepared by mixing 1.5 mL of DPPH solution and 0.02 mL of methanol. Ascorbic acid, gallic acid and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Acros Organics, Fair Lawn, NJ, USA) were used as positive controls.

The activity of the tested samples is given as a percentage of inhibition (degree of scavenging of the radical) and is calculated from the Equation 1:

$$\% \, inhibition = 100 * \frac{(A_0 - A)}{A_0}, \qquad \text{(eq. 1)}$$

where, $A_0$ is the absorbance of the control solution and A is the average absorbance value of the test solution containing extracts. The antioxidant capacity of the tested extracts was expressed as an inhibitory concentration, $IC_{50}$. The $IC_{50}$ is the concentration of an extract at which 50% inhibition of free radical activity is observed.

*ABTS assay*

The ability to neutralize free radicals was tested using 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals according to the previously described method with modification [20]. During the preparation of the experiment, the mixture of 7 mM ABTS and 2.45 mM potassium persulfate was incubated at room temperature (r.t.) without the presence of light for 24 hours. This solution was diluted until an absorbance of 0.700 ± 0.02 at 734 nm was achieved. A volume of 300 µL of extract or standard solution was mixed with 600 µL of ABTS solution. This mixture was incubated at room temperature for 30 minutes. Absorbance was measured at 734 nm. Ascorbic acid and Trolox were used as positive controls. The ABTS radical concentration was calculated according to the Equation 2:

$$\% \, inhibition = 100 * \frac{(A_0 - A)}{A_0}, \qquad \text{(eq. 2)}$$

where, $A_0$ denotes the absorbance of the control (which contains all reagents, except the tested extract or standard) and A is the absorbance of the sample. The antioxidant capacity of the tested extracts was expressed as an inhibitory concentration, $IC_{50}$. The $IC_{50}$ is the concentration of an extract at which 50% inhibition of free radical activity is observed.

*Total phenolic content*

The total phenolic content was determined by the Folin-Ciocâlteu method with slight modifications [21]. Gallic acid (GA) was used as a standard and the content of the polyphenols was expressed in mg of GA equivalents *per* gram of dry extract (mg GAE/g dry extract). The test sample was prepared by mixing 0.05 mL of a given extract, 0.15 mL of distilled water and 0.10 mL of Folin-Ciocâlteu reagent in a test tube. After 5 minutes of incubation at room temperature, 0.8 mL of sodium carbonate (7.5%) was added to the mixture, and test tubes were placed in the dark for 2 hours with occasional shaking. The control sample contained all reagents except the extract. The stock solution of GA (1 mg/mL) was diluted, and the following concentrations were obtained (mg/mL): 0.1, 0.5, 0.4, 0.2, 0.1, 0.05 and 0.025. The GA calibration curve of absorbance *versus* concentration was plotted. All measurements were performed at a wavelength λ = 760 nm using quartz cuvettes. The

total content of phenolic compounds in the tested extracts was determined by using an equation that was obtained from a standard GA graph Equation 3:

$$\text{Absorbance} = 0.0026 \times \text{Total phenol} + 0.0808, \quad (R^2 = 0.9978), \quad \text{(eq. 3)}.$$

*Total flavonoid content*

The total flavonoid content was determined by a colorimetric method based on the reaction of flavonoids with $AlCl_3$ [22]. Rutin (RU) and quercetin (QE) were used as standards and the content of the flavonoids expressed in mg of RU or QE equivalents *per* gram of dry extract (mg QE/g or mg RU/g dry extract). The test sample was prepared by mixing an aliquot of 1 mL of sample solution with 0.2 mL solution of $AlCl_3$ (10%) in methanol, 0.2 mL solution $CH_3CO_2K$ (1 mol/L) and 5.6 mL of distilled water. The reagents were mixed and allowed to stand for 30 min at room temperature. The stock solution of RU and QE (1 mg/mL) was diluted, and the following concentrations were obtained (mg/ml): 0.1, 0.5, 0.25, 0.125, 0.0625 and 0.03125. The RU and QE calibration curve of absorbance *versus* concentration was plotted. All measurements were performed at a wavelength $\lambda = 415$ nm using quartz cuvettes. The total content of the flavonoid compounds in the tested extracts was determined by using an equation that was obtained from a standard for QE (Equation 4) and RU (Equation 5):

$$\text{Absorbance} = 0.0018 \times \text{Total flavonoid content} + 0.0571, \quad (R^2 = 0.9986), \quad \text{(eq. 4)};$$

$$\text{Absorbance} = 0.0011 \times \text{Total flavonoid content} + 0.0268, \quad (R^2 = 0.9986), \quad \text{(eq. 5)}.$$

*Statistical analysis*

All data were analysed by using the IBM SPSS Statistics package for social sciences and Microsoft Excel v. 2013. Results are present as a mean ± standard deviation from three measurements.

For measuring the strength and direction of linear correlation between investigated parameters and especially the total amount of phenolic and flavonoid compounds with exposed antioxidant activity Pearson's correlation coefficient (R) has been used. Correlation coefficients were considered significant if the p-value was 0.05, and the strength of correlation and the direction were determined based on Pearson's correlation coefficient value.

## Results and Discussion

The acetone, methanol and ethanol extracts of the lichen *U. grisea* were prepared by the maceration process, and the extraction yield and the presence of secondary metabolites of the extracts are shown in Table I. The extraction yield was expressed as a percentage of crude extract obtained from twenty grams of dried lichen material. The ethanol produced a higher yield of the extract as compared to methanol and acetone as solvents. Being a more polar solvent, methanol may have a higher solubility for certain polar compounds, such as phenolics, which can lead to a higher overall yield of extract.

In addition, the total phenolic and flavonoid contents were determined and presented in Table II. The methanolic extract showed the highest phenolic content (67.128 ± 0.222 mg GAE/g) followed by acetonic (56.615 ± 0.385 mg GAE/g) and ethanol extracts (44.821 ± 0.801 mg GAE/g). Typically, methanol is widely recognised as an effective solvent for extracting phenolic compounds [23]. Consequently, there exists a positive correlation between the yield obtained from the extraction process and the content of phenolic compounds.

**Table I**

Yield of the lichen extracts and the presence of secondary metabolites in the *U. grisea* extracts

| Extracts | Used Eluent | Yield (%) | NOR | LEC | UMB | GYR | ATR |
|---|---|---|---|---|---|---|---|
| UGA | Acetone | 12.37 ± 1.33 | + | + | + | + | + |
| UGM | Methanol | 14.2 ± 2.74 | + | + | + | + | - |
| UGE | Ethanol | 8.75 ± 1.26 | - | + | + | + | - |

UGA – *U. grisea* acetonic extract; UGM – *U. grisea* methanolic extract; UGE – *U. grisea* ethanolic extract; NOR – norstictic acid; LEC – lecanoric acid; UMB – umbilicaric acid; GYR – gyrophoric acid: ATR – atranorin

**Table II**

Total phenolic and flavonoid contents of *U. grisea* extracts (mean ± SD, n = 3)

| Sample | Total Phenolic Content (mg GAE/g Dry Extract) | Total Flavonoid Content (mg QE/g Dry Extract) | Total Flavonoid Content (mg RU/g Dry Extract) |
|---|---|---|---|
| **UGA** | 56.615 ± 0.385 | 62.657 ± 0.160 | 116.303 ± 0.262 |
| **UGM** | 67.128 ± 0.222 | 54.324 ± 0.893 | 102.667 ± 1.461 |
| **UGE** | 44.821 ± 0.801 | 28.213 ± 0.424 | 59.939 ± 0.694 |

GAE – gallic acid equivalent; QE – quercetin equivalent; RU – rutin equivalent; UGA – *U. grisea* acetonic extract; UGM – *U. grisea* methanolic extract; UGE – *U. grisea* ethanolic extract

The content of the total flavonoids was determined by aluminium chloride methods and expressed in rutin or quercetin equivalents *per* gram of dry extract (Table II). The content of the total flavonoids in tested extracts, which was expressed as rutin equivalents (QE), varied from 28.213 ± 0.424 mg QE/g to 62.657 ± 0.160 mg

QE/g. The acetone exhibited the highest efficiency among the tested solvents for the extraction of the flavonoid compounds from *U. grisea.* Previous research findings have demonstrated that the use of acetone as a solvent for extraction leads to extracts with a significantly high flavonoid content [24]. Also, when assessing the total flavonoid content and expressing it in terms of rutin equivalents (RU), the highest concentration of flavonoids was obtained using acetone as the solvent (116.303 ± 0.262 mg RU/g), surpassing both the methanol (102.667 ± 1.461 mg RU/g) and the ethanol (59.939 ± 0.694 mg RU/g). The results indicate that different solvents used for the lichen extraction had varying effects on the flavonoid content. The acetone extraction yielded the highest flavonoid content, followed by the methanol extraction, while the ethanol extraction resulted in the lowest flavonoid content. This suggests that acetone was the most efficient solvent for extracting flavonoids from the *U. grisea*, with a higher ability to dissolve and extract these compounds compared to methanol and ethanol. The differences in polarity and solubility of the solvents likely influenced their effectiveness in extracting the flavonoids from the lichen *U. grisea*. The findings imply that researchers and scientists studying flavonoids in lichens should consider using acetone as the preferred solvent for extracting these compounds, as it demonstrated the highest extraction efficiency in this particular study. Additionally, the results highlight the importance of solvent selection in the lichen extraction protocols to maximize the recovery of specific compounds of interest.

The presence of the secondary metabolites in the acetone, methanol and ethanol extracts was analysed using the HPLC-UV method. Lecanoric acid, umbilicaric acid and gyrophoric acid were identified in all three extracts along with depsidone norstictic acid and the depside atranorin which were reported for the first time in this species.

The compounds were identified based on retention times ($t_R$ values) and UV spectra (200 - 400 nm) compared with standard substances previously isolated from lichens and identified by spectroscopic methods in our laboratory [25]. As can be seen from the HPLC chromatogram of the acetone extract of *U. grisea* as the main metabolites lecanoric acid ($t_R$ = 4.06 ± 0.20 min), umbilicaric acid ($t_R$ = 6.29 ± 0.20 min), gyrophoric acid ($t_R$ = 6.34 ± 0.10 min), norsticic acid ($t_R$ = 3.21 ± 0.10 min) and atranorin ($t_R$ = 18.97 ± 0.10 min) were identified (Table III).

**Table III**

Retention time of the examined lichen substances and their absorbance maxima (nm)

| Secondary metabolite | Substance class | Retention time ($t_R$ ± SD)* (min) | UV spectrum absorbance maxima (nm) |
|---|---|---|---|
| **Norstictic acid (NOR)** | depsidone | 3.21 ± 0.10 | 212, 239, 320 |
| **Lecanoric acid (LEC)** | depside | 4.06 ± 0.20 | 212, 270, 304 |
| **Umbilicaric acid (UMB)** | tridepside | 6.29 ± 0.20 | 210, 254, 292 |
| **Gyrophoric acid (GYR)** | tridepside | 6.34 ± 0.10 | 212, 270, 304 |
| **Atranorin (ATR)** | depside | 18.97 ± 0.10 | 212, 278, $312^m$ |

* Values are the means of three determinations ± SD, m - minor absorbance maximum

Depside lecanoric acid, tridepsides umbilicaric acid and gyrophoric acid have been previously detected in *U. grisea,* while depsidone norstictic acid and depside atranorin were found for the first time in this species [26]. The chromatographic analysis showed that atranorin was not found in the methanol and the ethanol extracts, while norstictic acid was not identified in the ethanol extract. In addition, gyrophoric acid was isolated from the acetone extract of the lichen *U. grisea*. The gyrophoric acid purity was confirmed by HPLC (98.77%). The wavelength of maximum absorbance for gyrophoric acid was detected at 212, 270 and 304 nm. The structures of the identified compounds are shown in Figure 1.

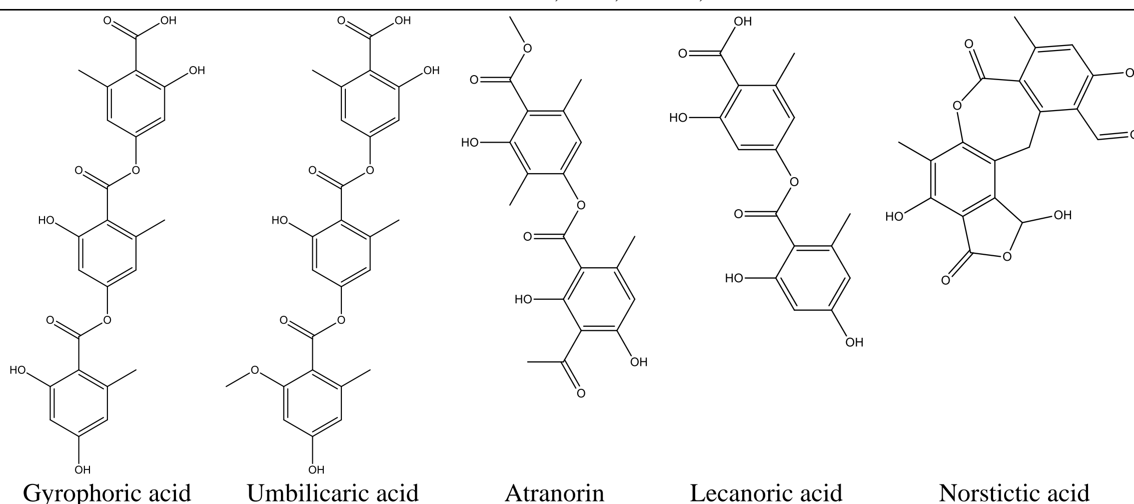
Antioxidant capacities of the tested *U. grisea* extracts were determined spectrophotometrically using DPPH and ABTS methods. Results are shown in Table IV.

**Table IV**

Antioxidant activities of *U. grisea* extracts by using different *in vitro* models (mean ± SD, n = 3)

| Sample | DPPH ($IC_{50}$ µg/mL) | ABTS ($IC_{50}$ µg/mL) |
|---|---|---|
| **UGA** | 3261.00 ± 141.38 | 2441.53 ± 142.71 |
| **UGM** | 3298.00 ± 50.24 | 2103.58 ± 156.69 |
| **UGE** | 3419.33 ± 108.58 | 2689.24 ± 197.78 |
| **GYR** | 354.17 ± 12.15 | 246.99 ± 11.88 |
| **AA** | 10.53 ± 1.57 | 8.28 ± 0.24 |
| **Trolox** | 15.59 ± 3.31 | 12.40 ± 0.40 |

UGA – *U. grisea* acetonic extract; UGM – *U. grisea* methanolic extract; UGE – *U. grisea* ethanolic extract; GYR – gyrophoric acid; AA – ascorbic acid



Gyrophoric acid Umbilicaric acid Atranorin Lecanoric acid Norstictic acid

**Figure 1.**

Structures of the identified secondary metabolites in the lichen *U. grisea*

The acetone extract showed slightly better antioxidant activity compared to the other two extracts in both tests. This may be related to the higher content of the flavonoid compounds in this extract. Also, gyrophoric acid shows significantly better antioxidant activity compared to all extracts, but weaker compared to ascorbic acid and Trolox. On the other hand, it was examined whether there is a correlation in the obtained values of the investigated parameters. It was found that there is a significant strong positive correlation in the values of total flavonoids when quercetin and rutin were used as standards ($R = 0.933$; $p < 0.001$), *i.e.* a significant strong negative correlation in the case of the total content of phenolic compounds and the value of the ability to neutralize ABTS radicals ($R = -0.750$; $p = 0.020$). Correlations among other examined parameters were not statistically significant. The strong negative correlation observed in the case of total phenolic compounds was expected. Since a lower $IC_{50}$ value of ABTS radical neutralization is associated with stronger antioxidant activity, it is expected that compounds with a higher content of phenolic compounds exhibit such activity. On the other hand, total flavonoids are measured using two different standards, so a correlation between these values is expected.

## Conclusions

This is the first detailed examination of the chemical composition of the lichen *Umbilicaria grisea*, a largely unexplored lichen species, using the determination of total phenolic and total flavonoid contents, HPLC-UV analysis, as well as antioxidant activity of tested extracts. In addition, the tridepside gyrophoric acid was isolated from the acetone extract. This gyrophoric acid together with the acetone, methanol and ethanol extracts was used for the determination of the anti-oxidant activity using three methods.

Based on everything presented in this paper, it can be concluded that this is the first detailed lichenochemical analysis and the antioxidant activity of this lichen species that grows in Serbia. Norstictic acid and atranorin were identified for the first time in this species, which has great significance in the phytotaxonomy of the genus *Umbilicaria* and its potential application in medicine and pharmacy. This lichen is also a significant source of gyrophoric acid, umbilicaric acid and lecanoric acid, lichen acids that possess significant biological activity. The results showed that the acetone, methanol and ethanol extracts of this lichen show significant antioxidant activity, which most likely originates precisely from the lichen acids identified in them. These compounds have phenolic groups, which numerous studies have confirmed are responsible for the anti-oxidant activity of lichen compounds [27, 28].

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## Conflict of interest

The authors declare no conflict of interest.

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