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# *RAMALINA* LICHENS AND THEIR MAJOR METABOLITES AS POSSIBLE NATURAL ANTIOXIDANT AND ANTIMICROBIAL AGENTS

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## ABSTRACT

Three lichen species of *Ramalina* (*R. farinacea*, *R. fastigiata* and *R. fraxinea*) were examined. Evernic, fumarprotocetraric, lecanoric, stictic and usnic acid levels were determined by high performance liquid chromatography-diode array detection. Acetone, methanol and ethanol were used to examine the efficiencies of different solvent systems for the extraction of lichen acids. The total phenol contents in the extracts were determined by the Folin–Ciocalteu method. The antioxidant capacities were determined by the ABTS (2,2′-azino-bis[3-ethylbenzothiazoline-6-sulphonic acid]) method. The methanol extracts of the *Ramalina* species showed the highest antioxidant capacities. Broth microdilution testing was performed to determine the minimum inhibitory concentration (MIC) of the methanol extracts of the three *Ramalina* species. The MIC values of all extracts ranged from 64 to 512 μg/mL for all bacterial strains tested in this study.

## PRACTICAL APPLICATIONS

Lichens and their natural products are used worldwide for decorations, brewing and distilling, food, fodder, spice and natural remedies, and in the perfume and dying industries. Lichens produce a large number of phenolic compounds, such as depsides, depsidones and dibenzofurans. Lichens with antioxidant activity have increased abilities to scavenge toxic-free radicals due to their phenolic groups. In recent years, many lichen substances have been found to have several biological activities. This article evaluates the antimicrobial and antioxidant activities and lichen acids of three *Ramalina* species. This is the first study to determine the stictic acid level in a *R. farinacea* extract and fumarprotocetraric acid and lecanoric acid levels in an *R. fastigiata* extract. The results of this study will contribute significantly to current knowledge regarding the utility of antimicrobial and antioxidant materials.

## INTRODUCTION

Lichens and their natural products are used worldwide for decorations, brewing and distilling, food, fodder, spices and natural remedies, and in the perfume and dying industries (Oksanen 2006; Shukla *et al.* 2010). Lichen associations produce a great number of secondary metabolites, most of which are unique. Lichen substances are produced by a mycobiont (a fungal partner of lichen) and accumulate in the cortex or in the medulla as tiny extracellular crystals on the outer surfaces of hyphae (Oksanen 2006; Molnar and Farkas 2010). Using newly developed analytical techniques and experimental methods, more than 1,000 lichen substances have been identified (Molnar and Farkas 2010). In recent years, many of these substances have been found to have several biological activities, including antitumor, antibacterial, antifungal, anti-inflammatory, antimutagenic, antiproliferative, cytotoxic and antioxidant activities (Muller 2001; Oksanen 2006).

Lichens produce a large number of phenolic compounds, such as depsides, depsidones and dibenzofurans (Luo *et al.* 2010). Lichens with antioxidant activity have increased abilities to scavenge toxic-free radicals due to their phenolic groups (Molnar and Farkas 2010). Many studies have proven that some lichens and lichen metabolites have strong antioxidant activities (Hidalgo *et al.* 1994; Gulçin *et al.* 2002; Ozen and Kinalioglu 2008; Kosanić *et al.* 2011). In recent studies, the antimicrobial activities and antioxidant properties of lichen species have been screened against bacteria, and the chemical compositions of lichen species have been determined (Gulluce *et al.* 2006; Çobanoglu *et al.* 2010; Atalay *et al.* 2011; Manojlovic *et al.* 2012a,b).

The genus *Ramalina* belonging to the family Ramalinacea contains over 240 species. Various extracts of the lichen *Ramalina farinacea* have been evaluated to determine its phytochemical contents, and antibacterial, antifungal and cytotoxic properties (Esimone and Adikwu 1999). Tay *et al.* (2004) have shown that (+)–usnic acid, nor stictic acid and protocetraric acid concentrations in *R. farinacea* contribute to its antimicrobial activity. HPLC (high performance liquid chromatography) analysis of *Ramalina* species performed by Cansaran *et al.* (2007) has revealed that usnic acid contributes to their antimicrobial activities. The antioxidant potentials and free radical-scavenging contents of the edible lichen *Ramalina conduplicans* have also been evaluated (Luo *et al.* 2010). Recently, the antibacterial and antioxidant activities of *Ramalina roesleri* have been studied (Sisodia *et al.* 2013). *R. farinacea*, *R. fastigiata* and *R. fraxinea* are common in our study area. On the other hand, there are few detailed studies about these three species in our country (Tay *et al.* 2004; Cansaran *et al.* 2007).

The main objectives of this study were to determine the lichen acids of *R. farinacea*, *R. fastigiata* and *R. fraxinea* by high performance liquid chromatography-diode array detection (HPLC-DAD) and to examine the efficiencies of different solvent systems for the extraction of lichen acids. The total phenol concentrations, antioxidant capacities and antimicrobial activities of *Ramalina* extracts were determined by Folin–Ciocalteu, ABTS and broth microdilution methods, respectively.

## MATERIALS AND METHODS

### Chemicals

Trolox ([±]-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), HPLC-grade methanol, and ethanol and usnic acid were purchased from Sigma-Aldrich (Steinhelm, Germany). Evernic acid, fumarprotocetraric acid, lecanoric acid and stictic acid were purchased from Chromadex (Irvine, CA). Analytical-grade acetone, dimethyl sulfoxide (DMSO), tetrahydrofuran and orthophosphoric acid (89%) were purchased from Merck (Darmstadt, Germany).

### Collection and Identification of Lichen Samples

*R. farinacea* and *R. fraxinea* were collected from the trunks of *Abies* sp., and *R. fastigiata* was collected from the trunks of *Fraxinus* sp. in May 2012 from Uludağ Mountain (Bursa, Turkey). The samples were identified using standard methods according to the literature (Wirth 1995; Smith *et al.* 2009). Voucher specimens have been deposited in the Herbarium of Uludağ University (BULU), Bursa, Turkey.

### Extraction of Lichen Samples

Air-dried and cleaned lichen specimens were milled in a household blender. The lichen samples (1 g) were separately blended with 40 mL of organic solvent (acetone, ethanol and methanol) for 4 h with a magnetic stirrer at room temperature in the dark. The samples (total volumes of 40 mL) were separated from the solid matrix by filtration through sheets of filter paper (Whatman No. 1, Aldrich, Germany). The extracts were used for the determination of the total phenolic contents and total antioxidant capacities by the Folin–Ciocalteu and ABTS methods, respectively. Organic solvent extracts (5 mL) were evaporated to dryness. Finally, residues were dissolved in 2 mL of DMSO. The DMSO extracts were used for HPLC analysis. The standard lichen acids were dissolved in DMSO before HPLC analysis.

### HPLC Analysis

An Agilent 1200 HPLC system (Waldbronn, Germany), consisting of a vacuum degasser, binary pump, auto sampler and diode array detector, was used. Chromatographic separations were carried out using an XBridge C18 (4.6 × 250 mm, 3.5 μm) column from Waters (Ireland). The mobile phase consisted of 0.25% orthophosphoric acid and 1.50% tetrahydrofuran in water (solvent A) and methanol (solvent B). The gradient conditions were as follows: 0–15 min, 30–70% A; 15–30 min, 70–100% A; 30–35 min, 100% A; 35–36 min, 100–30% A; and 36–50 min, 30% A, with a total run time of 50 min. The column was equilibrated for 10 min prior to each analysis. The flow rate was 0.25 mL/min, and the injection volume was 10 μL. Data acquisition and preprocessing were performed with Chemstation for LC (Agilent). The monitoring wavelength was 240 nm.

## Validation of Analytical Method

The linearity of the HPLC-DAD method was investigated for phenolic compounds in the range of 1.2–120 mg/L for fumarprotocetraric acid and lecanoric acid, 1.0–100 mg/L for evernic acid and stictic acid, and 1.4–140 mg/L for usnic acid at nine concentrations. Two calibration plots with correlation coefficients of $R^2 \geq 0.999$ were obtained by reporting the peak areas as a function of the concentration of each phenolic compound (Table 1). The validation of the quantitative determination of the phenolic compounds in the *Ramalina* samples was performed by measuring the limits of detection (LOD, 3 $s/m$), limits of quantification (LOQ, 10 $s/m$), and recoveries (%) of evernic, fumarprotocetraric, lecanoric, stictic and usnic acids (Table 4), where $s$ is the sample standard deviation for the replicates and $m$ is the slope of the calibration curve. LOD values ranged from 0.039 to 0.750 mg/L, and LOQ values ranged from 0.130 to 2.498 mg/L for fumarprotocetraric, lecanoric, evernic, stictic and usnic acids. The extraction efficiencies of the lichen standards of fumarprotocetraric, lecanoric, evernic, stictic and usnic acids were evaluated by spiking the mixture of standards into the samples and performing extractions using acetone, ethanol and methanol (Table 1). All of the other recoveries were in the experimental error range. For the calculation of the final results, the recoveries of the pure phenolic standards were taken into account.

## Spectroscopic Analysis

**Antioxidant Capacity Assay.** Antioxidant capacity was determined by the ABTS method, as described in the literature (Re *et al.* 1999; Sariburun *et al.* 2010). Absorbance was measured by spectrophotometry (Varian Cary 50, Agilent, Victoria, Australia) at 734 nm against a blank after 6 min. The results were expressed as mg Trolox equivalent per 100 g dried weight.

**Folin–Ciocalteu Method.** The detection of total phenol content with Folin–Ciocalteu reagent was carried out according to the procedure reported in the literature (Singleton *et al.* 1999; Sariburun *et al.* 2010). Absorbance was measured by spectrophotometry (Varian Cary 50) at 750 nm. The total phenol content was expressed as mg of gallic acid equivalent (GAE) per 100 g of dried weight.

## Determination of Minimum Inhibitory Concentration

The bacterial strains used in this study were *Escherichia coli* ATCC 25922, *E. coli* O157:H7, *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 33591 and eight FQ-resistant *E. coli* isolates.

Broth microdilution testing was performed to determine the minimum inhibitory concentrations (MICs) of the lichen species according to the guidelines of the Clinical Laboratory Standards Institute (CLSI, 2003). The bacterial cultures were prepared in Mueller-Hinton broth (MHB) at 37C for 16–20 h. All methanol extracts were dissolved in 20:80 methanol/PBS (Phosphate Buffered Saline) (v/v). Freshly prepared stock solutions were sterilized using 0.20 μm single-use filter units (Minisart, Sartorius Stedim Biotech, Germany). Dilutions ranging from 0.008 to 256 mg/L were prepared in MHB, and inocula with densities equivalent to 0.5 McFarland turbidity were added to tubes containing the diluted extracts. After incubation at 37C for 16–20 h, the MIC was defined as the minimum concentration of extract that inhibited the growth of the organism. The optical densities of the cultures were measured at a wavelength of 595 nm (iMark, Bio-Rad, Philadelphia, PA).

# RESULTS AND DISCUSSION

## Identification of Lichen Acids in *Ramalina* Species

The amounts of fumarprotocetraric, lecanoric, evernic, stictic and usnic acids in the acetone, ethanol and methanol extracts from the three *Ramalina* species were determined by HPLC-DAD (Table 2). The identification of these compounds was achieved by comparisons of their retention

**TABLE 1.** VALIDATION PARAMETERS AND RECOVERY OF FUMARPROTOCETRARIC, LECANORIC, EVERNIC, STICTIC AND USNIC ACIDS IN *RAMALINA* EXTRACTS

| Validation parameters | Fumarprotocetraric acid | Lecanoric acid | Evernic acid | Stictic acid | Usnic acid |
|---|---|---|---|---|---|
| LOD (mg/L) | 0.750 | 0.074 | 0.346 | 0.490 | 0.039 |
| LOQ (mg/L) | 2.498 | 0.247 | 0.153 | 1.640 | 0.130 |
| $R^2$ | 0.999 | 0.999 | 0.999 | 0.999 | 0.999 |
| Recovery (%) | | | | | |
| Acetone | 97.28 ± 2.31 | 96.38 ± 1.28 | 94.94 ± 2.04 | 96.37 ± 2.13 | 92.22 ± 1.72 |
| Ethanol | 94.08 ± 1.61 | 95.91 ± 1.76 | 98.16 ± 1.41 | 95.97 ± 1.77 | 94.34 ± 1.56 |
| Methanol | 95.14 ± 1.94 | 98.25 ± 2.47 | 97.23 ± 1.41 | 95.07 ± 1.61 | 95.38 ± 2.38 |

LOD, limits of detection; LOQ, limits of quantification.

**TABLE 2.** THE AMOUNTS OF FUMARPROTOCETRARIC, LECANORIC, EVERNIC, STICTIC AND USNIC ACIDS EXTRACTED FROM *RAMALINA* SPECIES USING DIFFERENT SOLVENTS (MILLIGRAMS PER GRAM DRIED LICHEN)

| | Extraction solvent | Fumarprotocetraric acid | Lecanoric acid | Evernic acid | Stictic acid | Usnic acid |
|---|---|---|---|---|---|---|
| *R. farinacea* | Acetone | – | – | – | 1.08 ± 0.02 | 0.22 ± 0.01 |
| | Ethanol | 2.10 ± 0.03 | – | – | – | 0.24 ± 0.01 |
| | Methanol | 0.94 ± 0.01 | – | – | 0.96 ± 0.01 | 0.34 ± 0.03 |
| *R. fastigiata* | Acetone | 0.37 ± 0.01 | 0.35 ± 0.01 | 9.69 ± 0.07 | – | 0.28 ± 0.01 |
| | Ethanol | – | 0.30 ± 0.01 | 7.67 ± 0.01 | – | 0.22 ± 0.01 |
| | Methanol | – | – | 7.87 ± 0.11 | – | 0.31 ± 0.02 |
| *R. fraxinea* | Acetone | – | – | – | – | 0.14 ± 0.01 |
| | Ethanol | – | – | – | – | 0.15 ± 0.01 |
| | Methanol | – | – | – | – | 0.16 ± 0.01 |

Note: Mean of two determinations ± SD.

times with that of a standard substance purchased from Chromadex and Sigma-Aldrich. The HPLC chromatogram of the acetone extract of *R. fastigiata* is shown in Fig. 1. Lichen acids have been identified in *Ramalina* species at 240 nm. The HPLC signal at 240 nm is not a characteristic wavelength for evernic, fumarprotocetraric, lecanoric, stictic and usnic acids. The wavelengths at 280, 320 and 360 nm may have increased the number of detected compounds, but DMSO solution has a maximum absorbance at 280 nm and also the absorbance of lichen acid at 320 and 360 nm is not enough for HPLC analysis. The quantitative proportions of evernic, fumarprotocetraric, lecanoric and stictic acids in each extract were determined. The fumarprotocetraric acid levels ranged from 0.94 ± 0.01 to 2.10 ± 0.0303 mg/g dried lichen for *R. farinacea*. Fumarprotocetraric acid was detected in the acetone extract of *R. fastigiata* (0.37 ± 0.01 mg/g dried lichen). Lecanoric acid was only detected in the acetone and ethanol extracts of *R. fastigiata*. Fumarprotocetraric and lecanoric acids were reported for the first time in *R. fastigiata* extracts in our study. Our results showed that this extract is a very important source of evernic acid, with levels ranging from 7.67 ± 0.01 to 9.69 ± 0.07 mg/g dried lichen. In our study, stictic acid was found only in the acetone and methanol extracts of *R. farinacea*. This is the first study to assess stictic acid levels in *R. farinacea* extracts. The depsidone stictic acid has apoptotic, cytotoxic and antioxidant activities (Perry *et al.* 1999; Correché *et al.* 2004; Lohézic-Le Dévéhat *et al.* 2007; Amo de Paz *et al.* 2010). Many lichens containing the dibenzofuran usnic acid are used for medicinal perfume and cosmetic applications (Ingólfsdóttir 2002). Our results indicate that the extracts of these three species of *Ramalina* could be used for pharmaceutical purposes because of their levels of usnic acid.

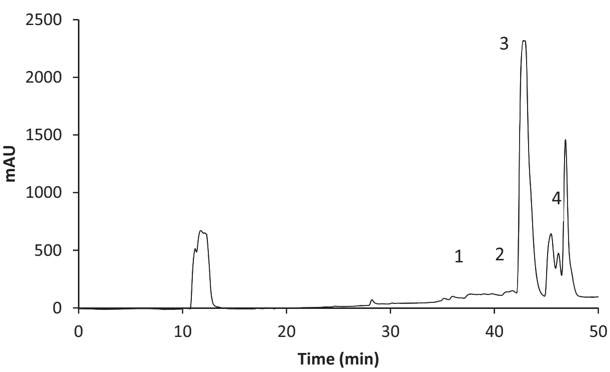


**FIG. 1.** THE HPLC CHROMATOGRAM OF ACETONE EXTRACT OF *RAMALINA FASTIGIATA* AT 240 NM. (1, LECANORIC ACID; 2, FUMARPROTOCETRARIC ACID; 3, EVERNIC ACID; 4, USNIC ACID)

Performing extractions is an essential part of the analysis of herbal samples. The extraction step is very important to their successful identification by chromatographic analysis. The most commonly used solvents are methanol, ethanol, acetone, ethyl acetate and their aqueous mixtures. In this study, acetone was the most efficient solvent for the extraction of lecanoric, evernic and stictic acids from *Ramalina* species. In addition, ethanol was the most efficient solvent for the extraction of fumarprotocetraric acid from *Ramalina* species. The highest amount of usnic acid was found in the methanol extract of *R. farinacea* (0.34 ± 0.03 mg usnic acid/g dried lichen). The usnic acid levels measured by HPLC indicate that the order of solvent efficiency is methanol > ethanol > acetone for *Ramalina* extracts, except for *R. fastigiata*. Solvents with relatively higher polarities were more efficient in general for extracting lichen compounds from the three *Ramalina* species.

## Antioxidant Capacities and Total Phenol Contents of *Ramalina* Species

The antioxidant capacities and total phenol contents were determined in *Ramalina* species using pure solvents of methanol, ethanol and acetone by the ABTS and Folin methods, respectively. The antioxidant capacities and total phenol contents of the three *Ramalina* species are illustrated in Table 3, respectively.

**TABLE 3.** ANTIOXIDANT CAPACITIES (MG TE 100/G DRIED LICHEN) AND TOTAL PHENOL CONTENTS (MG GAE 100/G DRIED LICHEN) OF *RAMALINA* SPECIES USING DIFFERENT SOLVENTS. THE VALUES ARE THE MEAN OF TWO LICHEN EXTRACTS

| | Antioxidant capacity | | | Total phenol content | | |
|---|---|---|---|---|---|---|
| Extraction solvent | *R. farinacea* | *R. fastigiata* | *R. fraxinea* | *R. farinacea* | *R. fastigiata* | *R. fraxinea* |
| Acetone | 90.0 ± 1.3 | 313.8 ± 12.7 | 68.0 ± 5.2 | 118.1 ± 2.8 | 300.9 ± 2.3 | 70.0 ± 0.6 |
| Ethanol | 136.0 ± 6.8 | 267.0 ± 3.9 | 89.0 ± 5.2 | 172.9 ± 10.4 | 326.3 ± 12.3 | 72.4 ± 3.4 |
| Methanol | 133.8 ± 8.3 | 439.3 ± 3.3 | 212.1 ± 1.2 | 182.1 ± 4.3 | 428.3 ± 13.6 | 103.3 ± 0.9 |

The highest antioxidant capacity and total phenol content were established in the methanol extract of *R. fastigiata*. The lowest antioxidant capacity and total phenol content were detected in the acetone extract of *R. fraxinea*. Methanol was the most efficient solvent for the determination of antioxidant capacity in the three *Ramalina* species. The total phenol contents as measured by the Folin method indicate that the order of solvent efficiency is methanol > ethanol > acetone. According to these results, in general, the use of methanol could result in the highest yield in terms of antioxidant capacity and total phenol content. Depsidones in lichens are believed to arise from the oxidation cyclization of depsides. It is known that depsidones are more efficient antioxidants than depsides because of the larger incorporation of depsidones into lipidic microdomains (Hidalgo *et al.* 1994). The antioxidant capacity of the ethanol extract of *R. farinacea* was higher than that of the methanol extract. The reason for this finding may be related to the fact that it contained the highest level of the depsidone fumarprotocetraric acid.

The antioxidant capacity of the 14 lichen species in different solvent extracts has been investigated in the literature (Kumar *et al.* 2014). The 14 lichens are *Dermatocarpon vellereum*, *Umbilicaria vellea*, *Rhizoplaca chrysoleuca*, *Rhizoplaca melanophthalma*, *Pleopsidium flavum*, *Xanthoparmelia mexicana*, *Acarospora badiofusca*, *Xanthoria elegans*, *Lecanora frustulosa*, *Lobothallia alphoplaca*, *Physconia muscigena*, *Melanelia disjuncta*, *Xanthoparmelia stenophylla* and *Peccania coralloides*. The extracts scavenged the ABTS radical in a dose-dependent manner at a concentration of 0.1–0.5 mg/mL for these lichen species. The antioxidant capacities of methanol (11–42% inhibition), acetone (5–31% inhibition) and ethanol (7–25% inhibition) extracts were significantly higher in comparison with 14 lichen extracts. Furthermore, the *Ramalina* extracts scavenged the ABTS radical in a dose-dependent manner at concentration of 0.025 mg/mL in this study. There was no correlation between the antioxidant capacities and total phenol contents of the *Ramalina* extracts. The presence of phenolic groups in lichen metabolites is considered a key factor responsible for their antioxidative effects, but the antagonistic and synergistic effects of the interactions of different chemicals with each other should be considered. Moreover, few studies have considered the possible interactions between phenolics, whereas a potent regeneration of an antioxidant by another antioxidant can increase or decrease the activity of a mixture of antioxidants (Peyrat-Maillard *et al.* 2003).

## Antimicrobial Activities of Lichen Extracts

The broth microdilution method was used to determine the antimicrobial susceptibilities of the lichen extracts assessed in this study. The MICs of the methanol extracts of the lichen species ranged from 64 to 512 μg/mL against the bacterial strains tested in this study (Table 4). The methanol extracts of *R. farinacea*, *R. fastigiata* and *R. fraxinea* had the greatest antimicrobial effects against *E. coli* E245 and *E. coli* O157:H7 with an MIC of 64 μg/mL. *R. fastigiata* was the most effective extract among the three lichen species, and *R. farinacea* showed poor activities against *E. coli* E101 and *S. aureus* 33591. This optimally effective extraction of *R. fastigiata* to pathogens may be due to its levels of evernic, fumarprotocetraric and lecanoric acids. The depside evernic acid has antifungal activities toward some plant pathogens (Halama and van Haluwin 2004), and lecanoric acid has antioxidant activities (Luo *et al.* 2009). In addition,

**TABLE 4.** MINIMUM INHIBITORY CONCENTRATION (ΜG/ML) OF THREE *RAMALINA* SPECIES METHANOL EXTRACTS

| | MIC | | |
|---|---|---|---|
| Isolates ID | *R. farinacea* | *R. fastigiata* | *R. fraxinea* |
| *Escherichia coli* E101 | 512 | 128 | 256 |
| *E. coli* E103 | 128 | 128 | 128 |
| *E. coli* E121 | 128 | 128 | 128 |
| *E. coli* E224 | 128 | 128 | 128 |
| *E. coli* E245 | 64 | 64 | 64 |
| *E. coli* E246 | 128 | 64 | 128 |
| *E. coli* E248 | 128 | 128 | 128 |
| *E. coli* E300 | 128 | 128 | 128 |
| *E. coli* 25922 | 128 | 128 | 128 |
| *E. coli* O157H7 | 64 | 64 | 64 |
| *Staphylococcus aureus* 25923 | 128 | 128 | 64 |
| *S. aureus* 33591 | 512 | 128 | 256 |

the strong antimicrobial influence of the depsidone fumarprotocetraric acid has been reported in the literature (Kosanić and Ranković 2011).

According to some studies (Burkholder *et al.* 1944; Silva *et al.* 1986; Rowe *et al.* 1989), lichens mainly inhibit Gram-positive bacteria; however, the methanol extract of *R. fraxinea* inhibited the growth of both *S. aureus* and *E. coli* in the present study. Furthermore, our results showed that there were no differences between the antimicrobial sensitivities of the *S. aureus* strains and those of the *E. coli* strains and the isolates. The micro-well dilution method was used to determine the MIC of acetone and methanol extracts of 34 lichen species against four bacterial strains in the literature (Shrestha *et al.* 2014). The lichen extracts demonstrated inhibitory effects against *S. aureus*, *Pseudomonas aeruginosa* and methicillin-resistant *S. aureus* with MIC values ranging from 3.9 to 500 μg/mL and also *E. coli* with MIC values ranging from 125 to 500 μg/mL. Although the acetone extracts were found to be more effective than methanol extracts in that literature, the methanol extracts of *Ramalina* species demonstrated significant inhibitory effects against *E. coli* and *S. aureus* with MIC values ranging from 64 to 512 μg/mL in our study.

## CONCLUSIONS

The extraction efficiencies of different solvent systems for extracting lichen acids from *Ramalina* species were examined. Lichen acids (fumarprotocetraric, lecanoric, evernic, stictic and usnic acids) were determined in the *Ramalina* samples by HPLC-DAD. These acids were quantified in different proportions depending on the extraction solvent. This is the first study to assess the levels of fumarprotocetraric and lecanoric acids in *R. fastigiata* extracts and the level of stictic acid in *R. farinacea* extract. Methanol was found to be the most suitable extraction solvent for lichen acids. According to the HPLC results, *R. fastigiata* and *R. farinacea* are rich in lichen substances and also possess high antioxidant capacities and total phenol contents compared with *R. fraxinea*. These results suggest that the tested lichen extracts and their lichen compounds could be utilized as antioxidant and antimicrobial agents. These findings could be of significance for the establishment of their future use in the pharmaceutical and food industries.

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