Scientific research article / https://doi.org/10.15446/rcciquifa.v52n3.110402

Chemical composition and *in vitro* antibacterial activity of three lichen species (Usnea sp., *Thamnolia vermicularis* subsp. *solida* and *Ramalina asperula*) collected from the Jauja and Huaral provinces-Peru

Fernando Carrasco[1,2]*, Wilfredo Hernández[1], Nino Castro[2], Geanina Angulo[2], Álvaro Quintero[2], Diego Zamudio[2], Carmen Tamariz-Angeles[3], Percy Olivera-Gonzales[3], Daniel Echevarría-Rodríguez[4]

[1] Facultad de Ingeniería, Universidad de Lima, Lima, Perú.

[2] Departamento Académico de Química Orgánica, Universidad Nacional Mayor de San Marcos, Calle German Amezaga 375, Lima, Perú.

[3] Centro de Investigación de la Biodiversidad y Recursos Genéticos de Ancash, Facultad de Ciencias,, Universidad Nacional Santiago Antúnez de Mayolo, Av. Centenario 200, 02002 Independencia, Huaraz, Ancash, Perú.

[4] Servicio Nacional de Sanidad Agraria del Perú– SENASA, Av. La Molina 1915, Lima, Perú

*Correspondence author: fccarras@ulima.edu.pe

Received: August 1, 2023
Corrected: September 19, 2023
Accepted: September 26, 2023

Summary

Introduction: Lichens are complex organisms conformed by a symbiotic association between algae and fungi; they are haven considerable interest owing to their pharmacological properties. **Objectives:** To determine chemical composition of the fatty acid (FA) and the percentage of usnic acid (UA), and to evaluate the *in vitro* antibacterial activity of the ethanolic extract obtained from the lichens *Usnea* sp.*, Thamnolia vermicularis* subsp. *solida* and *Ramalina asperula*. **Materials and Methods:** Organic extracts from the three lichens were obtained using a rotary evaporator, soxhlet extractor and ultrasound equipment. The chemical composition of FA was determined by gas chromatography-flame ionisation detector, and the percentage of UA was quantified by UV-Vis spectroscopy. The *in vitro* antibacterial activity of the lichen ethanolic extracts was evaluated against *E. coli* ATCC 25922,

Bacillus subtilis ATCC 11774*, Staphylococcus aureus* ATCC 43300 and *S. aureus* ATCC 29213 using the diffusion and microdilution methods. The minimum inhibitory concentration (MIC) was determined. Results: In the three tested lichens, the identified FAs were palmitic, stearic, oleic, linoleic, and linolenic acids; and the obtained percentage of UA was between 0.2 and 1.07 %. All the ethanolic extracts obtained from three lichens showed a high activity against *B. subtilis* (MIC = 0.5 mg/mL), while the ethanolic extracts obtained from *T. vermicularis* subsp. *solida* and *R. asperula* showed moderate activity against *S. aureus* ATCC 43300 (MIC = 3 and 1 mg/mL) and *S. aureus* ATCC29213 (MIC = 4 and 1 mg/mL). **Conclusions:** Our findings found for the three studied lichens confirmed the presence of some FAs and AU. These species provide an acceptable nutritional content and besides they can be considered as potential antibacterial agents.

Keywords: lichens, fatty acids, antimicrobial activity

Resumen

Composición química y actividad antibacteriana *in vitro* de tres especies de líquenes (*Usnea* sp., *Thamnolia vermicularis* subsp. solida and *Ramalina Asperula*) recolectadas en las provincias de Jauja y Huaral-Perú

Introducción: Los líquenes son organismos complejos conformados por una asociación simbiótica entre algas y hongos; tienen un interés considerable debido a sus propiedades farmacológicas. **Objetivos:** Determinar la composición química de los ácidos grasos (AG) y el porcentaje de ácido úsnico (AU), y evaluar la actividad antibacteriana *in vitro* del extracto etanólico obtenido a partir de los líquenes *Usnea sp*., *Thamnolia vermicularis* subsp. *solida* y *Ramalina asperula*. **Materiales y Métodos:** Los extractos orgánicos de los tres líquenes fueron obtenidos usando un rotavapor, extractor soxhlet y un equipo de ultrasonido. La composición química de los AG fue determinada mediante cromatografía de gases con detector de ionización de llama y el porcentaje de AU fue cuantificado mediante espectroscopia UV-Vis. La actividad antibacteriana *in vitro* de los extractos etanólicos de líquenes fue evaluada frente a las especies *E. coli* ATCC 25922, *Bacillus subtilis* ATCC 11774, *Staphylococcus aureus* ATCC 43300 y *S. aureus* ATCC 29213 mediante los métodos de difusión y microdilución. Se determinó la concentración inhibitoria mínima (MIC). **Resultados:** En los tres líquenes ensayados, los ácidos grasos identificados

fueron los ácidos palmítico, esteárico, oleico, linoleico y linolénico; y el porcentaje de AU obtenido estuvo entre 0,2 y 1,07 %. Todos los extractos etanólicos obtenidos mostraron una alta actividad frente a la especie *B. subtilis* (MIC = 0,5 mg/mL), mientras que los extractos etanólicos obtenidos a partir de *T. vermicularis* subsp. *solida* y *R. asperula* mostraron actividad moderada frente a la especie *S. aureus* ATCC 43300 (MIC = 3 y 1 mg/mL) y *S. aureus* ATCC29213 (MIC = 4 y 1 mg/mL). Conclusiones: Nuestros hallazgos encontrados para los tres líquenes estudiados confirmaron la presencia de algunos AG y AU. Estas especies aportan un aceptable contenido nutricional y además, pueden ser consideradas como potenciales agentes antibacterianos.

Palabras clave: líquenes, ácidos grasos, actividad antibacteriana.

Resumo

Composição química e atividade antibacteriana in vitro de três espécies de líquenes (*Usnea* sp., *Thamnolia vermicularis* subsp. *solida* e *Ramalina Asperula*) coletadas nas províncias de Jauja e Huaral-Peru

Introdução: Os líquenes são organismos complexos constituídos por uma associação simbiótica entre algas e fungos; eles são de considerável interesse devido às suas propriedades farmacológicas. **Objetivos:** Determinar a composição química dos ácidos graxos (AG) e a porcentagem de ácido úsnico (AU), e avaliar a atividade antibacteriana *in vitro* do extrato etanólico obtido dos líquenes Usnea sp., *Thamnolia vermicularis* subsp. *solida* e *Ramalina aspérula*. **Materiais e Métodos:** Os extratos orgânicos dos três líquenes foram obtidos utilizando evaporador rotativo, extrator soxhlet e equipamento de ultrassom. A composição química do FA foi determinada por cromatografia gasosa com detector de ionização de chama e a porcentagem de UA foi quantificada por espectroscopia UV-Vis. A atividade antibacteriana *in vitro* de extratos etanólicos de líquen foi avaliada contra as espécies *E. coli* ATCC 25922, *Bacillus subtilis* ATCC 11774, *Staphylococcus aureus* ATCC 43300 e *S. aureus* ATCC 29213 utilizando métodos de difusão e microdiluição. A concentração inibitória mínima (CIM) foi determinada. **Resultados:** Nos três líquenes testados, os ácidos graxos identificados foram os ácidos palmítico, esteárico, oleico, linoléico e linolênico; e o percentual de UA obtido ficou entre 0,2 e 1,07%. Todos os extratos etanólicos obtidos apresentaram alta atividade contra a espécie *B. subtilis*

(CIM = 0,5 mg/mL), enquanto os extratos etanólicos obtidos de *T. vermicularis* subsp. *solida* e R. asperula apresentaram atividade moderada contra as espécies *S. aureus* ATCC 43300 (CIM = 3 e 1 mg/mL) e *S. aureus* ATCC29213 (CIM = 4 e 1 mg/mL). **Conclusões:** Nossos achados para os três líquenes estudados confirmaram a presença de alguns AG e AU. Estas espécies fornecem um conteúdo nutricional aceitável e também podem ser consideradas como potenciais agentes antibacterianos.

Palavras-chave: líquenes, ácidos graxos, atividade antibacteriana.

Introduction

In the treatment of some diseases, the drug resistance of pathogenic microorganisms is attributed to the overuse of drugs by humans [1], generating a public health problem of major global impact [2]. The World Health Organization (WHO) has published a list of bacteria resistant to clinical use antibiotics with the aim of motivating researchers to discover novel drugs against certain types of bacterial strains, such as *E. coli* and *S. aureu*s, and thus avoid the resistance of mentioned strains to drugs [3]. In this sense, the research has been focused on the study of secondary metabolites present in plants in order to provide a natural structural model for the discovery of new drugs [4, 5]. In addition, many primary and secondary plant metabolites constitute a food source due to their glucose contents [5, 6] and other nutritional components such as fatty acids (FA) [7-9]. Lichens consist of a symbiotic association of an algae or cyanobacteria and a fungi [10, 11]. These complex organisms show high adaptability to severe climatic conditions probably due to the presence of lipids and FAs in their compositions, which enable cell membranes to continue to function at low temperatures [11]. FAs, such as linoleic acid, linolenic acid [7, 9] and arachidonic acid [8], are important in the diet of humans, thus making the study of lichens to be relevant in terms of nutrition [12]. Additionally, lichens produce other substances such as usnic acid (UA), nostistic acid and sekiakic acid, among others, which exert a broad spectrum of pharmacological properties such as antifungal, antibacterial [13] and antimycobacterial activities [14]. Recently, 34 lichen species from North American have been shown to be effective against methicillin-sensitive and methicillin-resistant *S. aureus* and *Pseudomonas aeruginosa* with MIC values ranging from 3.9 to 500 μg/mL. Besides, extracts from *Letharia vulpina*, *L. columbiana* and *Vulpicida canadensis* were effective against *E. coli* [15]. On the other hand, *Thamnolia vermicularis* acetonic extracts were used as a functional food in obese mice and the results of these tests demonstrated that these extracts exert anti-obesity activity and they can be considered as a natural resource for controlling obesity [16].

Our research group has previously studied the lichen *Thamnolia vermicularis* subsp. vermicularis and identified decarboxythamnolic acid and thamnolic acid by spectroscopic (FT-IR, ^{1}H NMR, and ^{13}C NMR) and mass techniques [17]. The aim of this work was to determine the chemical composition of FAs and the percentage of UA in *Usnea* sp., *Thamnolia vermicularis* subsp. *solida* and *Ramalina asperula*. The antimicrobial activity of ethanolic extracts of these lichen species was also evaluated against *E. coli* ATCC25922, *S. aureus* ATCC29213, *S. aureus* ATCC43300 (methicillin and oxacillin resistant) and *B. subtilis* ATCC11774.

Materials and methods

Microorganisms

Bacterial strains: Gram-negative *E. coli* ATCC 25922 and Gram-positive *S. aureus* ATCC 29213, *S. aureus* ATCC 43300 (methicillin and oxacillin resistant) and *B. subtilis* ATCC 11774 were obtained from the Centro de Investigación de la Biodiversidad y Recursos Genéticos de Ancash, Universidad Nacional Santiago Antúnez de Mayolo, Huaraz, Perú.

Lichens

Lichen species such as *Usnea* sp. and *Thamnolia vermicularis* subsp. *solida* were collected in the province of Jauja, department of Junín, Peru (4100 m above sea level). *Ramalina asperula* Kremp was collected in the province of Huaral, Lima, Peru (2000 m above sea level). The lichens were identified by biologist Daniel Ramos and the samples were deposited in the Herbarium of Southern Peru (Arequipa).

Preparation of the Lichen Extracts

Fresh and clean lichen samples were placed into an oven with circulating air at 40 °C for 3 days. Then, the dried material was ground as fine as 20 mesh and stored at 4 °C in sterilized amber glass bottles. A total of 100 g of each lichen was placed in a beaker containing 250 mL $CHCl_3$-MeOH (1:1 v/v) and left to stand for three days at room temperature. The extracts were separated by filtration and then the filtrate was concentrated under reduced pressure using a Buchi 110 rotary evaporator. Other lichen extracts in chloroform or ethanol were obtained by soxhlet extraction [1 g lichen in chloroform (150 mL); extraction time: 2 h)] and ultrasound extraction [20 g lichen in ethanol (50 mL); extraction time: 1 h]. The obtained extracts were concentrated.

Antimicrobial Activity Tests

The antimicrobial activity of the lichen ethanolic extracts was evaluated using diffusion (M02-A7 protocol, CLSI) and microdilution (M07-A8 protocol, CLSI) methods [18] against *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *S. aureus* ATCC 43300 and *B. subtilis* ATCC 11774. In a laminar flow cabinet, the samples were prepared by dissolving the dried ethanolic extracts at 200 mg/L in DMSO. The samples were then stored at −20 °C for 20 days.

Diffusion Method

Each ethanolic extract (5 µL) were added to sterile filter paper discs (5 mm diameter) and placed in Petri dishes containing Mueller and Hinton agar previously inoculated with bacteria prepared as previously described [19]. Disks of Ampicillin (25 µg) were used as reference antibiotic, and discs with 5 µL of DMSO were used as the negative control. Four replicates of all samples were prepared and incubated at 35 °C for 24 h. The inhibition halos were measured in those samples showing clear zones around the discs.

Microdilution Method

Samples showing an inhibition halo in the diffusion test were used in the microdilution assay. Serial dilutions of samples were made in Mueller and Hinton broth II to obtain final concentrations of 0.1, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/mL. Dilutions were transferred into sterile 96-well plates (100 µL per well) and then inoculated with 10 µL of bacterial suspension [19]. Contamination controls with or without extract and growth controls without extract were also prepared. Ampicillin (final concentration of 1 or 5 µg/mL in well) was used as reference antibiotic. Three replicates of all samples and controls were prepared and incubated at 35 °C for 24 h. To assess bacterial growth, 10 µL of 1% sterile tetrazolium violet was added to each plate and turbidity was observed [19]. The MIC value was defined as the minimum concentration of the reference drug or tested compound (in µg/mL) that inhibits bacterial growth. The assay was repeated for 7 days to evaluate the activity.

Fatty Acid Analysis via gas chromatography–flame ionisation detector

In a 100 mL flask, 10 mL of 0.5 N KOH solution were added to 200 mg of $CHCl_3$/MeOH organic extract and incubated for 20 min at 55 °C in a water bath. After adding 5 mL of diluted HCl (1:1) solution, the FAs were extracted with 10 mL of petroleum ether. The extract was washed with 10 mL water, dried with anhydrous sodium sulphate and then concentrated to dryness with gaseous N_2. The residue was dissolved in 10 mL of petroleum ether, and 1 mL of aliquot was then evaporated to dryness in

gaseous N_2. This extract was then dissolved in 10 mL of 5% $HClO_4$ methanolic solution and heated for 5 min at 55 °C. Finally, the esterified FAs of the three samples under study were extracted with 10 mL of petroleum ether.

Esterified FAs dissolved in petroleum ether were analysed in a GC-2010 Shimadzu gas chromatograph equipped with a fused silica column (75 m × 0.18 mm × 0.14 μm) at 140 °C and flame ionisation detector (FID). The injection was performed in split mode with a flow rate of 6.25 mL/min using an injector temperature of 260 °C. The carrier gas was helium with a run time of 45 min, and with an injection volume of 1 μL. The esterified FAs were identified by comparison with the retention times of the FAME standard mixture (Lot: LRAC3241), detected by FID, injected under the same conditions as the samples [18]. Calculations for the identification and quantification of esterified FAs by GC-FID were performed with the GCsolution Software (Shimazdu, ver. 2.44.00) and the results were expressed in mg FA per g of esterified extract according to the following formula:

$$\frac{mg\ FA}{g} = \frac{Asmp}{Astd} \cdot \frac{Wstd}{Wsmp} \cdot \frac{1}{200}$$

The percentage value of each FA in relation to the total concentration was determined using the following formula:

$$\%\ FA = \frac{mg\ FA/g}{mg\ FA\ \ g} \cdot 100$$

Where:

Asmp: Area of the sample recorded on the chromatogram

Astd: Area of the FAME standard mixture recorded on the chromatogram

Wstd: Weight of the FAME standard mixture

Wsmp: Weight of the sample

% FA: Percentage of the fatty acid

mg FA/g: Concentration of the fatty acid (mg/g)

Σ mg FA/g: Total concentration of fatty acids (mg/g)

Quantitative Analysis of Usnic Acid

UA standard (CAS No. 7562-61-0) was quantified using a Shimadzu UV-1601 UV-Vis spectrophotometer. Briefly, 5 mg of the standard UA were dissolved in 100 mL of chloroform; then, an aliquot of 100 µL was diluted with 50 mL of chloroform to obtain a final concentration of 0.1 mg/L. The maximum absorbance was recorded at 284 nm. Dilutions of 1/10, 1/50, 1/100, 1/500 and 1/1000 were made from the chloroformic extract to record absorbance values at 284 nm within the linear range of the calibration curve, which was previously defined from the analytical standard in the range of 0–10 ppm [17].

Results

Antibacterial Activity

From the diffusion method, all samples showed inhibitory activity against *B. subtilis* ATCC 11774. In addition, the *T. vermicularis* subsp. *solida* and *R. asperula* ethanolic extracts showed a moderate activity against *S. aureus* ATCC 29213 and *S. aureus* ATCC 43300. None of the tested extracts were active against *E. coli* ATCC 25922. These results were also confirmed using the microdilution method. All tested samples (MIC= 0.5 mg/mL) inhibited the growth of *B. subtilis* ATCC 11774 while the *T. vermicularis* subsp. *solida* and *R. asperula* ethanolic extracts stopped the growth of *S. aureus* ATCC 29213 and *S. aureus* ATCC 43300 when registering MIC values in the range of 1–4 mg/mL, as shown in Table 1 and Figure 1.

Table 1. Antibacterial activity of the lichen ethanolic extracts. The extract discs contained 1 mg of sample; the ampicillin disc contained 25 µg of ampicillin. [a] The values represent the means of 4 replicates ± the standard deviation.

Extracts	*B. subtillis* ATCC 11774		*S. aureus* ATCC 43300		*S. aureus* ATCC 29213	
	Halo (mm)[a]	MIC (mg/mL)	Halo (mm)[a]	MIC (mg/mL)	Halo (mm)[a]	MIC (mg/mL)
Thamnolia vermicularis subsp. *solida*	3.4 ± 0.5	0.5	2.9 ± 0.4	3	1.9 ± 0.2	4
Usnea sp.	6.9 ± 0.6	0.5	-	-	-	-
Ramalina asperula Kremp	8.5 ± 0.6	0.5	4.5 ± 0.6	1	3.8 ± 0.5	1
Ampicillin	33.0 ± 0.8	> 0.005	15.3 ± 0.5	<0.005	24.0 ± 0.0	0.005

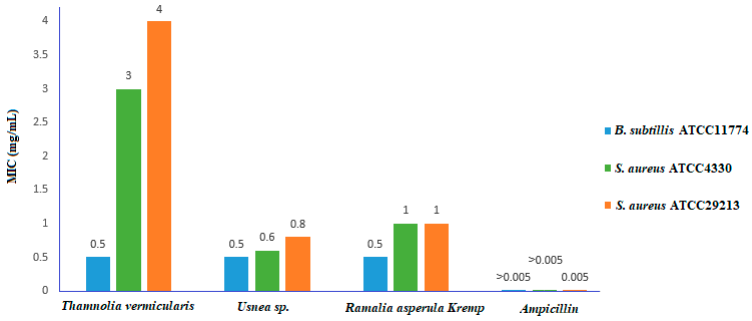

Figure 1. *In vitro* antibacterial activity expressed as MIC value (mg/mL) of lichen extracts and Ampicillin antibiotic tested

Identification and Quantification of FAs

The identification and quantification of the FAs from the three lichen species were carried out by comparing the retention times and areas of the chromatograms obtained for the samples (Figures 2-4) with the chromatograms for the FAME standard mixture using GC-FID. The results are shown in Table 2.

Table 2. Fatty acid (FA) concentration in *Usnea* sp., *Thamnolia vermicularis* subsp. *solida*, and *Ramalina asperula* Kremp. RT: retention time; SFA: saturated fatty acid; UFA: unsaturated fatty acid

Fatty acids	Formula	RT (min)	Concentration [mg FA/g sample], (% FA)		
			Thamnolia vermicularis subsp. *solida*	*Usnea* sp.	*Ramalina asperula* Kremp
Palmitic acid	C16:0	21.189	[18.4], (6.2)	[31.0], (7.2)	[22.7], (8.2)
Stearic acid	C18:0	24.724	[3.8], (1.2)	[12.3], (2.8)	[4.1], (1.5)
Oleic acid	C18:1 n-9	25.773	[21.3], (7.2)	[36.0], (8.4)	[18.8], (6.8)
Linoleic acid	C18:2 n-6	27.344	[162.4], (54.7)	[175.8], (40.8)	[107.6], (38.8)
Linolenic acid	C18:3 n-3	29.116	[48.4], (16.3)	[175.4], (40.9)	[124.1], (44.8)
Arachidonic acid	C20:4 n-6	32.428	[42.7], (14.4)	------	------
Total SFA			[22.3], (7.5)	[43.2], (10.0)	[26.8], (9.7)
Total UFA			[274.8], (92.5)	[388.2], (90.0)	[250.4], (90.3)
Total FA			[297.1], (100)	[431.4], (100)	[277.2], (100)

Quantitative Analysis of Usnic Acid

The calibration curve for the quantification of UA was linear in the range of 0–10 ppm where the obtained linear correlation equation was $Y = 0.0343\ X$. The percentages of UA determined for *Thamnolia vermicularis* subsp. *solida*, *Ramalina asperula Kremp* and *Usnea* sp. were 0.2, 1.01 and 1.07 %, respectively.

Discussion

The chemical characterization of the lichen extracts and the identification of FAs and UA were performed by gas chromatography (GC-FID) and UV-Vis spectroscopy, respectively. For extraction of FA, the volume ratio of the $CHCl_3$:MeOH mixture was 1: 1. This volume ratio was different with respect to that used in the literature (2:1 v/v) [10]. With the $CHCl_3$:MeOH (1:1 v/v) mixture used in this work, the solubility of low polarity compounds and the amount of unwanted metabolites dissolved in the organic extract was decreased. The esterification reaction was carried out using a 5% $HClO_4$ methanolic solution [20] in order to reduce the reaction time [21].

As shown in Table 2, saturated and unsaturated FAs were identified in the three assessed lichens (C16:0, C:18:0, C:18:1, C:18:2 and C:18:3). In all three studied lichens, stearic acid (C18:0)—a saturated FA—was found with the lowest concentration level, 3.8 and 4.1 mg/g sample in *Thamnolia vermicularis* subsp. *solida* and *Ramalina asperula Kremp*, respectively. In the present study, the FAs found are similar to those reported for the genus *Cladonia* [7] and *Stereocaulon scutelligerum* [22]. Furthermore, our results (2.8–4.1 mg/g sample) are comparable to the stearic acid concentrations found for others lichens containing the species *Cladonia cornuta* (L.) Hoff and *Cladonia pyxidata* (3.4 and 4.8 mg/g sample, respectively) [7]. In this work, arachidonic acid (C:20:4 n-6) was only detected in *Thamnolia vermicularis* subsp. *solida* and its concentration was 42.7 mg/g sample (Figure 1). This acid is one of the major unsaturated FAs that constitute brain membrane phospholipids and is found in lichens in varying proportions [8]. As shown in Table 2, the ratio of unsaturated FAs to saturated FAs for the studied lichens was approximately 10:1. This result could be related to the environmental conditions under which the lichens were collected [10].

The UA levels found in the chloroformic extracts of the tested lichens were in agreement with those previously reported for other lichens [5, 23].

All three lichen species (MIC = 0.5 mg/mL) showed significant inhibitory activity with respect to ampicillin (reference drug) against *B. subtilis* ATCC 11774. Furthermore, these lichen species were ten times more cytotoxic than the acetonic extracts of *Cladonia furcata* and *Cladonia subulata* against *B. subtilis* [24]. As shown in Table 1, *Thamnolia vermicularis* subsp. *solida* showed a moderate antibacterial activity against the two resistant *S. aureus* strains (MIC = 3–4 mg/mL). Our MIC values are close to those reported for the acetonic extracts of *D. miniatum* tested against *S. aureus* subsp. *aureus* ATCC 25923 (MIC = 3.75 mg/mL) [25]. The ethanol extract of *Ramalina asperula* Kremp showed a MIC value of 1.0 mg/mL which was similar to that found for the acetone extract of *Acarospora fuscata* (MIC = 1.25 mg/mL) tested against *S. aureus* ATCC 25923 [26]. The ethanol extract of *Thamnolia vermicularis* subsp. *solida* and *Ramalina asperula* were highly active against *S. aureus* compared to other *C. crispum, P. squamulosum and L. prophetae-eliae* species assayed against *S. aureus* ATCC 1885 (MIC = 1000, 500 and 125 mg/mL, respectively) [27]. The tested ethanolic extracts of the three studied species were inactive against the Gram-negative bacterium *E. coli*. This result is in agreement with previous reports related with the genus *Cladonia*, where none of the 42 studied species showed significant activity against *E. coli* [28]. The low activity of the lichen ethanolic extracts tested against Gram-negative bacteria may be attributed to the lipopolysaccharides and lipoproteins present in the bacterial cell wall, preventing easy permeability of external agents compared to the cell wall of Gram positive bacteria (composed of peptidoglycans (murein) and teichoic acid), which make them more resistant to certain antibiotics [29, 30].

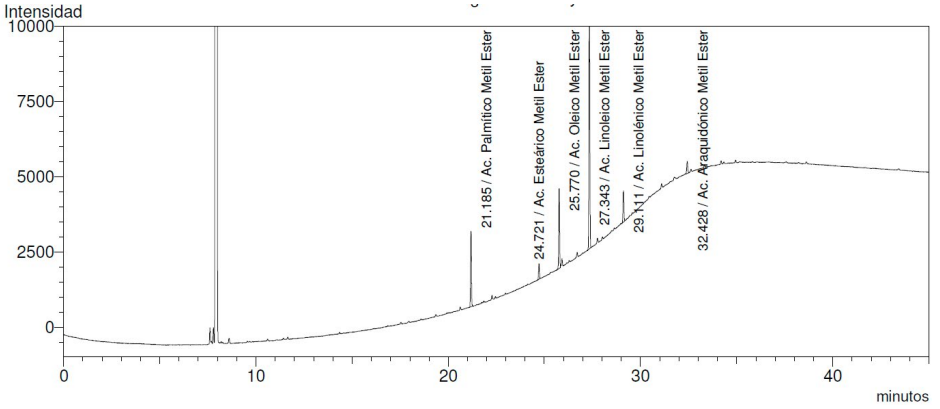

Figure 2. Chromatogram of the lichen *Thamnolia vermicularis subsp. solida*

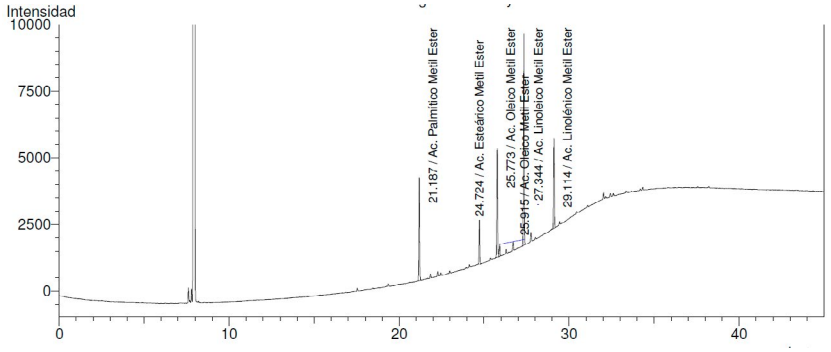

Figure 3. Chromatogram of the lichen *Usnea sp.*

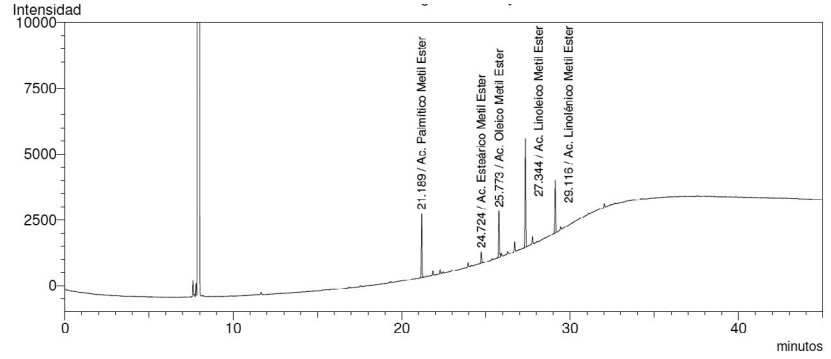

Figure 4. Chromatogram of the lichen *Ramalina asperula Kremp*

Conclusion

In this study, the palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid FAs were identified in the three studied lichens. The percentage of UA found was between 0.2 and 1.07 %. All three ethanolic extracts showed a significant activity against *B. subtilis* ATCC 11774 compared to the ampicillin (drug for clinical use) Furthermore, the ethanolic extracts of *Thamnolia vermicularis* subsp. *solida* and *Ramalina asperula* showed moderate inhibitory activity against *S. aureus* ATCC 43300 and *S. aureus* ATCC 29213. Our obtained results are an important contribution for food and pharmaceutical industries since the studied lichen species can be considered as potential nutritional and antibacterial agents.

Ethical approval

This study is part of a project that has been reviewed and approved by the Ethics Committee of the Universidad de Lima Scientific Research Institute.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Acknowledgments

W. H. and F. C. are grateful to the Universidad de Lima Scientific Research Institute for financial support to carry out this research work, as part of the execution of the research project entitled "Desarrollo de una crema fotoprotectora solar y antibacteriana elaborada de extractos de líquenes de Arequipa".

References

1. C.L. Ventola, The antibiotic resistance crisis: Part 2: Management strategies and new agents, *P. T.*, **40**(5), 344-352 (2015).

2. S.B. Levy, B. Marshall, Antibacterial resistance worldwide: Causes, challenges and responses, *Nat. Med.*, **10**(12), S122-S129 (2004). https://doi.org/10.1038/nm1145

3. World Health Organization, *Global priority list of antibiotic resistant bacteria to guide research, discovery, and development of new antibiotic*, WHO, Geneve, 2017. URL: https://remed.org/wp-content/uploads/2017/03/lobal-priority-list-of-antibiotic-resistant-bacteria-2017.pdf

4. B. Baral, B.L. Maharjan, Assessment of antimicrobial and phytochemical potentials of high altitudinal Nepalese lichens, *J. Microbiol. Biotechnol. Food Sci.*, **1**(2), 98-112 (2011).

5. A.P. Podterob, Chemical composition of lichens and their medical applications, *Pharm. Chem. J.*, **42**(10), 582-588 (2008). https://doi.org/10.1007/s11094-009-0183-5

6. C. Illana, Líquenes comestibles, *Bol. Soc. Micol. Madrid*, **33**, 273-282 (2009).

7. V.M. Dembitsky, Lipids of lichens, *Prog. Lipid Res.*, **31**(4), 373-397 (1992). https://doi.org/10.1016/0163-7827(92)90002-Z

8. S.M.M. Shanab, R.M. Hafez, A.S. Fouad, A review on algae and plants as potential source of arachidonic acid, *J. Adv. Res.*, **11**, 3-13 (2018). https://doi.org/10.1016/j.jare.2018.03.004

9. L.O. Hanus, M. Temina, V. Dembitsky, Biodiversity of the chemical constituents in the epiphytic lichenized ascomycete *Ramalina lacera* grown on difference substrates *Crataegus sinaicus*, *Pinus halepensis*, and *Quercus calliprinos*, *Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub.*, **152**(2), 203-208 (2008). https://doi.org/10.5507/bp.2008.031

10. R.A. Reis, M. Iacomini, P.A. Gorin, L.M. de Souza, M. Grube, L.M. Cordeiro, *et al.*, Fatty acid composition of the tropical lichen *Teloschistes flavicans* and its cultivated symbionts, *FEMS Microbiol. Lett.*, **247**(1), 1-6 (2005). https://doi.org/10.1016/j.femsle.2005.04.023

11. B.K. Dertien, L.J. De Kok, P.J.C. Kuiper, Lipid and fatty acid composition of tree-growing and terrestrial lichens, *Physiol. Plant.*, **40**(3), 175-180 (1997). https://doi.org/10.1111/j.1399-3054.1977.tb04053.x

12. D. Ivanova, D. Ivanov, Ethnobotanical use of lichens: lichens for food review, *Scrip. Sci. Med.*, **41**(1), 11-16 (2009). https://doi.org/10.14748/ssm.v41i1.456

13. A.S.N. Moreira, R. Braz-Filho, V. Mussi-Dias, I.J.C. Vieira, Chemistry and biological activity of *Ramalina* lichenized fungi, *Molecules*, **20**(5), 8952-8987 (2015). https://doi.org/10.3390/molecules20058952

14. S.N. Yilmaz, C.M. Yilmaz, Antimycobacterial activity of some lichen extracts, *Biol. Divers. Conserv.*, **10**(1), 65-68 (2017).

15. G. Shrestha, J. Raphael, S.D. Leavitt, L.L. St Clair, *In vitro* evaluation of the antibacterial activity of extracts from 34 species of North American lichens, *Pharm. Biol.*, **52**(10), 1262-1266. https://doi.org/10.3109/13880209.2014.889175

16. R.Y. Choi, J.R. Ham, J. Yeo, J.S. Hur, S.K. Park, M.J. Kim, *et al.*, Anti-obesity property of lichen *Thamnolia vermicularis* extract in 3T3-L1 cells and diet-induced obese mice, *Prev. Nutr. Food Sci.*, **22**(4), 285-292 (2017). https://doi.org/10.3746/pnf.2017.22.4.285

17. N. Castro, Contribución al estudio fitoquímico del liquen *Thamnolia vermicularis* Subsp. *vermicularis* S. Str., *Rev. Soc. Quím. Perú*, **76**(4), 322-329 (2010).

18. M. Danish, M. Nizami, Complete fatty acid analysis data of flaxseed oil using GC-FID method, *Data Brief*, **23**, 103845 (2019). https://doi.org/10.1016/j.dib.2019.103845

19. C. Tamariz-Angeles, P. Olivera-Gonzales, M. Santillán-Torres, Antimicrobial, antioxidant and phytochemical assessment of wild medicinal plants from Cordillera Blanca (Ancash, Peru), *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas – BLACPMA*, **17**(3), 270-285 (2018).

20. W.W. Christie, *Gas chromatography and lipids*, The Oily Press, Ayr, Scotland, 1989. p. 66-70.

21. M. Delange, D.M. Delange, Obtención y determinación de ácidos grasos de muy elevada masa molecular, *Rev. CENIC Cienc. Quím.*, **37**(1), 23-33 (2006).

22. T.H. Vu, D. Catheline, D. Delmail, J. Boustie , P. Legrand, F. Lohézic-le Dévéhat, Gas chromatographic analysis to compare the fatty acid composition of fifteen lichen species, with a focus on *Stereocaulon*, *Lichenologist*, **48**(4), 323-337 (2016). https://doi.org/10.1017/S0024282916000141

23. Y. Bazarnova, N. Politaeva, N. Lyskova, Research for the lichen *Usnea barbata* metabolites, *Z. Naturforsch C*, *J. Biosci.*, **73**(7-8), 291-296 (2018). https://doi.org/10.1515/znc-2017-0177

24. M. Kosanić, S. Ristić, T. Stanojković, N. Manojlović, B. Ranković, Extracts of five Cladonia lichens as sources of biologically active compounds, *Farmacia*, **66**(4), 644-651. https://doi.org/10.31925/farmacia.2018.4.13

25. Z. Karaahmet, K. Kinalioğlu, S. Aydin, Antioxidant and antibacterial potencies of *Xanthoparmelia conspersa* (Ehrh. ex Ach.) Hale and *Dermatocarpon miniatum* (L.) W. Mann. lichens from Black Sea Region in Turkey, *Gümüşhane Üniversitesi Fen Bilimleri Dergisi*, **9**(3), 415-424 (2019). https://doi.org/10.17714/gumusfenbil.462040

26. M. Kosanić, B. Ranković, T. Stanojković, P. Vasiljević, N. Manojlović, Biological activities and chemical composition of lichens from Serbia, *EXCLI J.*, **13**, 1226-1238 (2014).

27. T. Valadbeigi, M. Shaddel, Antibacterial and antifungal activities of gelatinose and non-gelatinose lichen species, *J. Arch. Mil. Med.*, **3**(4), e31610 (2015). https://doi.org/10.5812/jamm.31610

28. P.R. Burkholder, A.W. Evans, I. McVeigh, H.K. Thornton, Antibiotic activity of lichens, *Proc. Natl. Acad. Sci. U. S. A.*, **30**(9), 250-255 (1944). https://doi.org/10.1073/pnas.30.9.250

29. C.M. Plaza, C.P. Salazar, R.E. Plaza, M. Vizcaya, G. Rodriguez-Castillo, G. Medina-Ramirez, *In vitro* analysis of antibacterial and antifungal potential of lichen species of *Everniastrum* cf vexans, *Parmotrema blanquetianum*, *Parmotrema reticulatum* and *Peltigera laciniata*, *Drug. Design Dev. Ther.*, **2**(3), 125-134 (2018). https://doi.org/10.15406/mojddt.2018.02.00038

30. B. Ranković, M. Mišić, S. Sukdolak, Antimicrobial activity of extracts of the lichens *Cladonia furcata*, *Parmelia caperata*, *Parmelia pertusa*, *Hypogymnia physodes* and *Umbilicaria polyphylla*, *Biologia*, **64**(1), 53-58 (2009). https://doi.org/10.2478/s11756-009-0007-9

How to cite this article

F. Carrasco, W. Hernández, N. Castro, G. Angulo, Á. Quintero, D. Zamudio, C. Tamariz-Angeles, P. Olivera-Gonzales, D. Echevarría-Rodríguez, Chemical composition and *in vitro* antibacterial activity of three lichen species (*Usnea* sp.*, Thamnolia vermicularis* subsp. *solida* and *Ramalina asperula*) collected from the Jauja and Huaral provinces-Peru, *Rev. Colomb. Cienc. Quim. Farm.*, **52**(3), 1262-1277 (2023). https://doi.org/10.15446/rcciquifa.v52n3.110402