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Research article

Identification and determination of usnic acid and fatty acid from various lichen species in arequipa, Peru, as well as antibacterial and antioxidant capacity

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A B S T R A C T

Seven species of lichens such us *Umbilicaria* aff. *calvescens*, *Hypotrachyna enderythraea*, *Punctelia graminicola*, *Cladonia chlorophaea*, *Xanthoparmelia farinose*, *Psiloparmelia distincta* (rock and tree substrates; rs and ts), and *Usnea durietzii* were collected from the province of Arequipa - Peru. Their usnic acid (UA) and fatty acid (FA) amounts in methanol-acetone were determined by High-performance liquid chromatography with diode-array detection (HPLC-DAD) and Gas chromatography-flame ionization detector (GC-FID). The antimicrobial activities of these extracts were evaluated against *Staphylococcus aureus* ATCC strains (43300, 29213, 25923 and 700699), *Escherichia coli* strains (O157:H7 and ATCC 10536), *Salmonella enterica* sv *typhimurium* ATCC 14028, *Candida albicans* ATCC 90028 and *Candida. tropicalis* ATCC 750T. In addition, antioxidant capacity was also studied by Total phenolic content (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic Acid (ABTS) radical cation and Ferric Reducing Antioxidant Power (FRAP) assays. The UA contents in all the studied lichen species varied between 0.017 and 0.304 %. Among all the tested extracts, *Usnea durietzii* had the highest total FA concentration (5.81 mg/g). *P. distincta* (rs) was active against *S. aureus* strains (MIC = 0.02–0.2 mg/mL) as well as *C. albicans* (MIC = 4 mg/mL) and *C. tropicalis* (MIC = 3 mg/mL). *P. distincta* (ts) displayed moderate total phenol content (TPC = 29.1 ± 1.6 mg GAE/g) and antioxidant capacity evidenced by scavenging DPPH (IC_{50} = 1.45 ± 0.03 mg/mL) and ABTS (18.2 ± 0.2 mg TE/g) radicals, but it showed high values of FRAP (1953 ± 87 μM Fe^{2+}/g). Our findings indicate that *P. distincta* (rs) could be considered as a potential antimicrobial metabolites source whereas *P. distincta* (ts) and *Puntelia graminicole* for polyphenols with antioxidant compound.

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1. Introduction

Lichens form a symbiotic relationship involving a complex association between algae or cyanobacteria and fungi [1,2]. This association allows these organisms to adapt to challenging environmental conditions, including temperatures and habitat extremes with high humidity [3,4]. These conditions facilitate the presence of fatty acids (FAs) in different concentrations and diversity [1,5]. Furthermore, the impressive adaptive capabilities of these organisms also enable them to synthesize various secondary metabolites, such as depsides, depsidones, phenols, polysaccharides, lipids, ethers, and dibenzofurans [2,6,7]. Many of these metabolites are responsible for the broad pharmacological activity of lichen extracts, such as antimicrobial [8,9], antioxidant [10,11], anticancer [12, 13], and antifungal agents [14,15], among others. In this sense, usnic acid is a common metabolite produced only by lichens and it is considered as the best-studied lichen metabolite [16]. UA exhibits diverse pharmacological activities such as anti-inflammatory, analgesic, antiviral, antiprotozoal, antimicrobial, antituberculosis, and antiproliferative properties [16,17]. The concentration of this metabolite in lichen extracts will depend on the species and ecosystems where they grow [18]. Some properties exhibited by lichen extracts could be explained by their usnic acid concentration which has been reported in some species up to 6 % [16,18].

The World Health Organization (WHO) recently published the "Global priority list of antibiotic-resistant bacteria to guide research and development of new antibiotics" [19,20] to promote research and development of new antibiotics that act on certain pathogens of clinical importance and protect global public health. Moreover, it includes health-risk bacterial strains, such as *S. aureus*, *E. coli*, and *Salmonella*, which have been part of this study. In this sense, several lichen extracts have been demonstrated antibacterial activity [21–24]. It has been shown that methanol extracts of *U. filipendula* and *U. intermedia* exhibited activity against *E. coli* E245 and *E. coli* O157:H7 (MIC = 64 μg/mL) [25]. The acetonitrile extract of *Cladonia furcata* and ethanolic extract of *Umbilicaria polyphylla* demonstrate satisfactory antibacterial activity against *Bacillus mycoides*, *Bacillus subtilis*, *Enterobacter cloacae*, and *Klebsiella pneumoniae*, with minimum inhibitory concentration (MIC) of 0.78 μg/mL [10]. Similarly, EtOAc extracts of *Usnea baileyi*, *U. bismolliuscula*, and *U. pectinata* displayed high activity against *S. aureus* ATCC 25923 (MIC = 0.0625 mg/mL) [22]. Methanol extract of *Ramalina sinensis* exhibited broad antimicrobial activity against *B. subtilis* WT-A1, *S. aureus* MDC 5233, *Escherichia coli* VKPM-M17, *Pseudomonas aeruginosa* GRP3, and *Salmonella typhimurium* MDC 1754, with MIC from 0.9 to 1.8 mg/mL [26].

The antibacterial properties of lichens are attributed to usnic acid (UA) presence because it is a common metabolite in these organisms. However, the extract obtained in DCM from *Evernia prunastri* is approximately five times more active (MIC = 4 μg/mL) than UA (standard reference) (MIC = 21 μg/mL) when tested against *S. aureus* ATCC 29213. Furthermore, acetonitrile and n-hexane extracts from this species showed acceptable activity (MIC = 14 μg/mL and 21 μg/mL, respectively) compared with this standard compound [24]. These results indicate that UA is not the only metabolite responsible for the lichen antimicrobial activity. Moreover, lichens contain other metabolites that enhance their antimicrobial activity through a synergistic effect among the different metabolites found in lichen extracts [23]. Kosanić & Ranković (2011) [10] isolated fumarprotocetraric acid (from the acetonitrile extract of *Cladonia furcata*), gyrophoric acid (from the methanolic extract of *Umbilicaria polyphylla*), and physodic acid (from the acetonitrile extract of *Hypogymnia physodes*). These metabolites exhibited considerable antimicrobial activity against 15 bacterial strains, including *B. subtilis*, *Candida albicans*, and *S. aureus*, with MIC values ranging from 0.062 to 1.000 μg/mL.

On the other hand, antioxidant capacity has been reported in extracts of some lichens. For instance, the methanolic extract of *U. polyphylla* (1 mg/mL) displayed strong antioxidant capacity, with 90.08 % DPPH free radical scavenging (2,2-diphenyl-1-picrylhydrazyl) [10]. Similarly, the methanolic extract of *Parmelia sulcata* (1 mg/mL) achieved 71 % antiradical capacity in the DPPH assay [26]. Certainly, other biological activities have been reported, such as the ethanolic extract of *Himantormia lugubris* showed acceptable *in vitro* inhibitory activity against acetylcholinesterase (IC_{50}: 12.38 ± 0.09 μg/mL), butyrylcholinesterase (IC_{50}: 31.54 ± 0.20 μg/mL), and tyrosinase (IC_{50}: 22.32 ± 0.21 μg/mL) [27]. Moreover, the methanolic extracts of *Peltigera praetexata*, *Evernia prunastri*, *Ramalina sinensis*, and *R. farinacea* exhibited considerable cytotoxic activity against the HeLa tumor cell line (cervical carcinoma), with IC_{50} values ranging from 1.8 to 2.8 mg/mL [26].

In addition, different types of lichens are edible due to their high content of protein, carbohydrates, fats, fiber [28], and other metabolites in minor proportion such as fatty acids (FA) which are nutritionally important in the human diet [20,29,30]. Lichens have been shown to contain FAs, and some of them serve as chemotaxonomic markers [29,31]. Several studies focused on the composition of these compounds suggest that lichens exhibit considerable FA biochemical diversity [32]. The concentration of unsaturated FAs in lichens has also been reported to increase in low-temperature environments, which indicates that temperature is an important factor in FA metabolism [1]. The presence of FAs in lichens has been assessed through instrumental techniques such as gas chromatography coupled with flame ionization detection (GC–FID) and GC coupled with mass spectrometry (GC–MS) [31,33,34]. The FAs composition in 15 lichen species was determined through GC–FID, and the results were validated through GC–MS, then their profile of branched-chain FAs successfully differentiated between lichens of the *Stereocaulon* species and cyanolichens. Among the species studied, *Stereocaulon pileatum* exhibited the highest composition (28.8 %) of oleic acid, C18:1 (n-9), relative to the total FAs present in the species [34]. The total monounsaturated FAs in all the studied species ranged from 11.3 to 34.4 % [34].

Despite the lichens can be used as additives in functional foods, not only for their nutritional importance but also as a source of antioxidants and antibacterials that help prolong the shelf life of food [28,35], This is a contribution to the fight against climate change, due to a reduction in organic waste [36–39]. Moreover, lichens are a promising source of pharmacologically active compounds and they can combat bacterial resistance along with multiple diseases that afflict the population [40], contributing to the health and well-being of human beings [39,41]. Lichen studies in Peru predominantly center on taxonomic and ecological aspects, with 281 documented species [42,43]. The genus with the highest number of species is *Cladonia*, followed by *Hypotrachina* and *Heterodermia* [2]. However, the chemical and biological exploration of Peruvian lichens remains limited. In a recent study, Sepulveda et al. (2021) [43] conducted a metabolomic investigation, also, evaluated antioxidant capacity of ethyl lactate extract from *Hypotrachyna cirrhata* species

collected in Huaura (Lima) finding high antioxidant capacity through three different methods: DPPH (IC_{50} = 105.7 μg/mL), FRAP (IC_{50} = 196.4 μg/mL), and ABTS (IC_{50} = 69.28 μg/mL), these results were consistent with the high presence of total phenolic compounds (TPC = 153.6 mg GAE/g) observed in this species.

In this study, eight lichen samples collected from Arequipa province (Peru) belonging to seven species were evaluated. The studied samples were lichen extracts in MeOH:$(CH_3)_2CO$ (1:1) solvents. The quantities of UA and FA were determined using analytical techniques such as HPLC–DAD and GC–FID. Antimicrobial activity was assessed against the *S. aureus, E. coli, Salmonella,* and *Candida* strains using the agar diffusion and microdilution methods. Antioxidant capacity was studied using the DPPH, FRAP, and ABTS methods. Additionally, the concentration of total phenolic compounds (TPC) was investigated via ultraviolet–visible spectroscopy. Therefore, we think that our contribution can serve as a primary source for future research considering the pharmacological potential exposed in this work.

2. Materials and methods

2.1. Lichen material

The lichen samples were collected from the Arequipa province of Peru. And were identified by biologist Daniel Ramos Araníbar. A voucher specimen N° 009–2022 was deposited in the Herbarium of Scientific Institute "Michael Owen Dillon". known as "Herbario Sur Peruano" (HSP) (Table 1, Fig. 2A–H).

2.2. Preparation of Lichen extracts MeOH:$(CH_3)_2CO$ (1:1)

Solid impurities were removed from the collected samples using sterile metal tweezers. Next, the selected lichen material was washed with water on a sieve to remove dust. The clean biological material was placed in an oven with forced air circulation at 40 °C for 3 days. The dehydrated samples were ground using a mortar and pestle until a solid size corresponding to mesh 20. They were stored at 4–6 °C in amber and sterile glass containers.

For the maceration process, 50 g of each lichen was added to independent amber containers with a capacity of 500 mL containing 100 mL of a MeOH:$(CH_3)_2CO$ (1:1) solvent mixture. The solid-liquid mixture was left at room temperature for 3 days. Subsequently, the organic extract was separated through filtration and concentrated at room temperature. This procedure was repeated three times. The dry extract was stored at 4 °C for subsequent assays.

2.3. Quantitative determination of usnic acid via high-performance liquid chromatography coupled with diode array detection (HPLC–DAD)

2.3.1. Preparation of samples for HPLC–DAD

Two milligrams of lichen extract were separately placed into different volumetric flasks. Then, each sample was diluted and brought to a total volume of 25 mL with acetonitrile (MeCN). Subsequently, 1 mL of extract from each flask was extracted, filtered using 0.45 μm filters, and then transferred into a 2 mL amber autosampler vial.

2.3.2. Analytical conditions

All reagents and standard compounds employed in the experiments were HPLC grade and purchased from Sigma-Aldrich. UA standard was obtained from the Dr. Ehrenstorfer brand (purity >98 %, Lot. MKCK3869). The calibration curve was prepared to

Table 1
List of lichen species collected from the province of Arequipa-Peru, taxonomic and location data.

Especie	Order	Family	Subclass	Distrito (localidad), altitude	Coordinates
Umbilicaria aff. *calvescens* Nyl.	Umbilicariales	Umbilicariaceae	Umbilicariomycetidae	Pocsi (Tuctumpaya), 3356	S16°28′41.4"/ W71°19′08.5"
Hypotrachyna enderythraea (Zahlbr.) Hale	Lecanorales	Parmeliaceae	Lecanoromycetidae	Pocsi (Tuctumpaya), 3354	S16°28′40.8"/ W71°19′08.5"
Punctelia graminicola (B. de Lesd.) Egan	Lecanorales	Parmeliaceae	Lecanoromycetidae	Pocsi (Tuctumpaya), 3356	S16°28′41.4"/ W71°19′08.5"
Cladonia chlorophaea (Flörke ex Sommerf.) Spreng.	Lecanorales	Cladoniaceae	Lecanoromycetidae	Pocsi (Tuctumpaya), 3354	S16°28′40.8"/ W71°19′08.5"
Xanthoparmelia farinosa (Vain.) T.H. Nash, Elix & J. Johnst.	Lecanorales	Parmeliaceae	Lecanoromycetidae	Pocsi (Tuctumpaya), 3356	S16°28′41.4"/ W71°19′08.5"
Psiloparmelia distincta (Nyl.) Hale*	Lecanorales	Parmeliaceae	Lecanoromycetidae	Pocsi (Tuctumpaya), 3356	S16°28′41.4"/ W71°19′08.5"
Psiloparmelia distincta (Nyl.) Hale**	Lecanorales	Parmeliaceae	Lecanoromycetidae	Chiguata (Simbral), 4109	S16°23′16.9"/ W71°19′02.1"
Usnea durietzii Motyka	Lecanorales	Parmeliaceae	Lecanoromycetidae	Pocsi (Tuctumpaya), 3356	S16°28′40.8"/ W71°19′08.5"

quantify UA by injecting various concentrations of standard dissolved in MeCN, ranging from 20 to 200 mg UA/L. These standard solutions were directly introduced into the HPLC system using the autosampler.

Agilent Technologies 1200 HPLC equipment was equipped with a DAD and an autosampler. UA separation was accomplished using the reverse-phase chromatographic column from Knauer, C18, 5 μm, 150 × 4.6 mm. The flow rate was 2 mL/min. To identify UA at a wavelength of 285 nm, the mobile phase consisted of a mixture of MeCN, H_2O, and sodium citrate buffer (pH = 3) in a volume ratio of 60:36:4. Further, 20-μL aliquots from the extracts were injected into the HPLC system column. Each assay was conducted in triplicate. The UA peak identification in the samples relied on retention time comparison and UV spectrum with the pure UA used as the standard compound (Fig. 1a, b). The data were collected and analyzed using the ChemStation B.04.03 program (Table 2)

2.4. FA analysis

For this analysis, 200 mg of MeOH:$(CH_3)_2CO$ (1:1) organic extract was weighed and placed in a 50-mL flask, and 10 mL of 0.5-N KOH solution was added. The flask with the resulting mixture was placed in a water bath at 55 °C for 20 min. Subsequently, 5 mL of a 50 % HCl solution was added, and FAs were extracted using 10 mL of petroleum ether. Further, 1 mL of the extract was concentrated to dryness under a nitrogen atmosphere. This concentrate was redissolved with 10 mL of 5 % $HClO_4$ in methanol (v/v) and then heated at 55 °C for 5 min. The esterified FAs were extracted using 10 mL of petroleum ether and placed in 2 mL amber vials.

The esterified fatty acids were identified and quantified (mg FA/g of dry extract) using a Shimadzu GC–FID, model 2010. The dimensions of the column packed with fused silica were 75 m × 0.18 mm × 0.14 μm, and the oven temperature was set at 140 °C. The equipment's working characteristics are as follows: helium gas as the carrier, injection mode: split, at a flow rate of 6.25 mL/min, injector temperature: 260 °C, analysis time: 45 min, and injection volume: 1 μL. Identification and quantification of esterified fatty acids were performed by comparing the retention times with the FAME Mix standard (Lot: LRAD1445) and using GC Solution software (Shimadzu, version 2.44.00) [44,45].

2.5. In vitro antimicrobial activity

Samples were prepared for antimicrobial assays within a laminar flow hood (A2 biosafety level). Dried and refrigerated lichen extracts were dissolved with DMSO (10 % v/v) and sterile water (90 % v/v) at 200 mg/mL. These extracts were frozen and stored at −20 °C for up to 20 days. Antimicrobial assays were conducted using both diffusions (CLSI protocol M02-A7) [46] (refer to Fig. 4) and microdilution (CLSI protocol M07-A10) [47] methodologies in order to evaluate the antibacterial efficacy of lichen extracts against *S. aureus* ATCC strains (43300, 29213, 25923, and 700699), *E. coli* strains (O157:H7 and ATCC 10536) and *Salmonella enterica* sv *typhimurium* ATCC 14028. Moreover, the anticandidal activities against *Candida albicans* ATCC 90028 and *C. tropicalis* ATCC 750T were evaluated following M44-A and M27-2A (CLSI) methodologies with few modifications.

2.5.1. Antibacterial activity

For the diffusion method, sterile filter paper disks (diameter = 6 mm) with 1.0 mg of extract (for *S. aureus*) or 1.5 mg (for *E. coli* and *S. enterica*) were placed on Petri dishes containing Mueller–Hinton agar previously inoculated with bacteria. The experimental

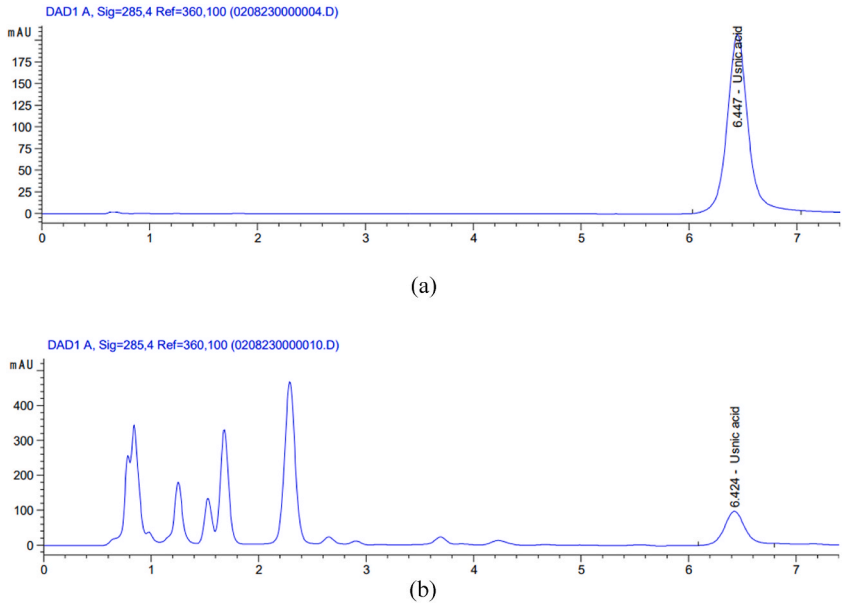

Fig. 1. Chromatograms of the (a) pure standard compound (usnic acid (UA)) and (b) extracted sample were recorded at 285.4 nm.

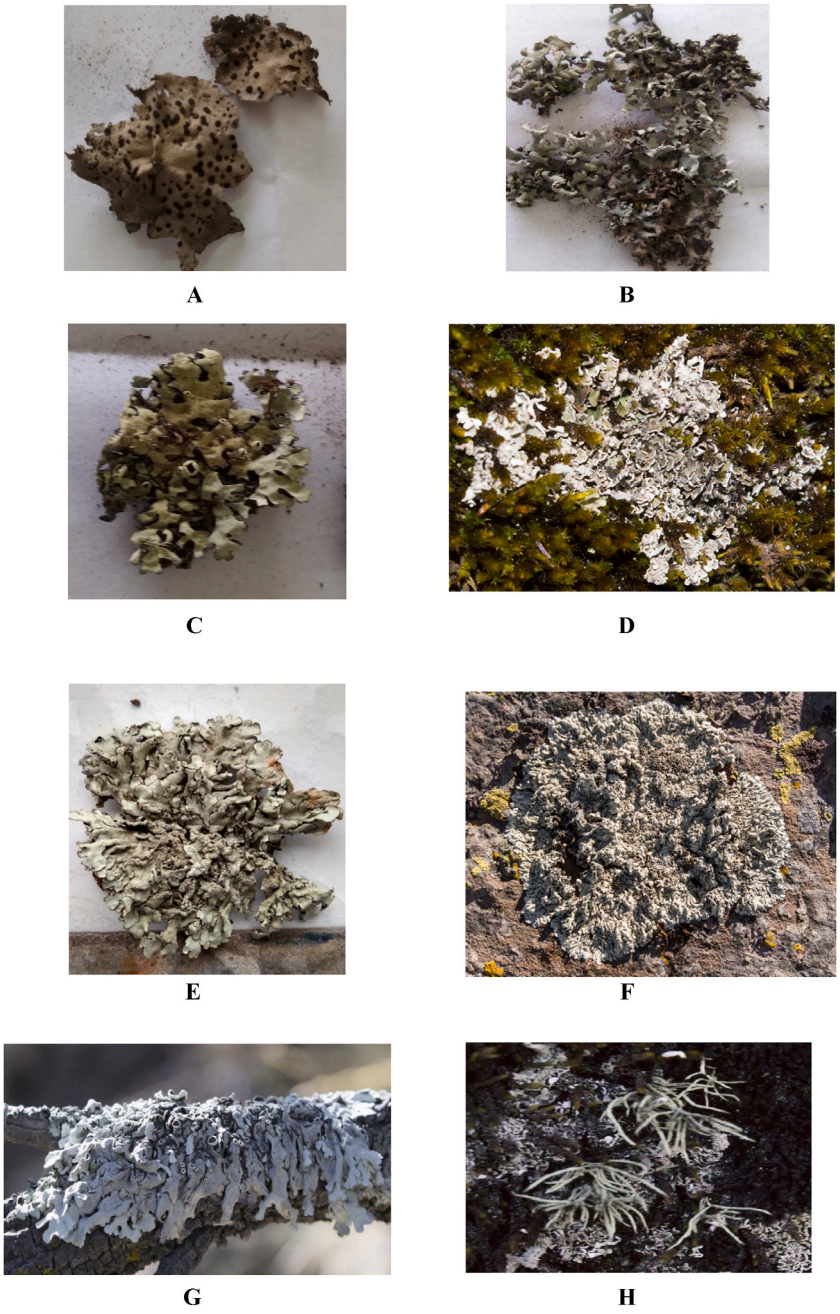

Fig. 2. Lichens collected from Arequipa province of Peru: **A**. *Umbilicaria* aff. *calvescens*, **B**. *Hypotrachyna enderythraea*, **C**. *Punctelia graminicola*, **D**. *Cladonia chlorophaea*, **E**. *Xanthoparmelia farinose*, **F**. *Psiloparmelia distincta* (rock substrate), **G**. *P. distincta* (tree substrate), and **H**. *Usnea durietzii*.

procedure adhered to established methodologies in literature [46–48]. Gentamicin (20 μg) [26] and UA (25 μg) [24] were used as reference antibiotics. Filter paper disk with 5 μL of DMSO (10 % v/v) served as a negative control. Independent assays were performed in triplicate, and reproducibility was confirmed through two assessments.

Extracts that showed inhibition zones in the diffusion test were considered to determine the minimum inhibitory concentration (MIC) following the microdilution method (Fig. 4, Table 4). Lichen extracts were diluted in Mueller–Hinton II broth at 0.1, 0.5, 1, 2, 3, 4, 6, 7, and 8 mg/mL, afterward, 100 μL was dispensed in sterile 96-well microtiter plates. They were inoculated with 10 μL of bacterial suspension [48]. Controls for contamination with and without the extract and growth controls were prepared. Gentamicin and UA were used as reference antibiotics. Plates containing the final mixture were incubated at 35 °C for 24 h, and bacterial growth was assessed by adding 10 μL of sterile 1 % tetrazolium violet [47,48]. The MIC value defined as the minimum inhibitory concentration for bacterial growth, was determined. All assays were conducted in triplicate and repeated 7 days after the initial assessment to verify

Table 2
Content of usnic acid in dry weight of lichen.

Species	% Usnic acid
Umbilicaria aff. calvenses	nd
Hypotrachyna enderytracea	0.035 ± 0.00
Punctelia graminicole	nd
Cladonia chlorophaea	nd
X. farinosa	0.18 ± 0.01
*P. distincta**	0.017 ± 0.00
*P. distincta***	0.042 ± 0.00
U. durietzii	0.304 ± 0.02

symbol (*): substrate rocks; symbol (**): substrate tree; The values represent the means of 3 replicates ± the standard deviation; nd: not detected.

antibacterial activity.

2.5.2. Anticandidal activity

For the diffusion method, disks containing 1.5 mg of dried extracts were placed on Petri dishes with Saboraud Agar previously inoculated with yeast [49]. Clotrimazole (20 μL) and UA (25 μg) disks were added as standard compounds. All plates were incubated at 35 °C for 48 h. Inhibition clear zones (halo) were considered as anticandidal activity.

Microdilution methods have been performed to determine the MIC values. Lichen extracts with anticandidal activity in diffusion test were diluted at 2, 4, 6, 8, and 10 mg/mL with Saboraud broth (SB) and dispensed (50 μL) in sterile 96-well microtiter plates. Clotrimazole was included as a reference antifungal and SB without extract as growth control. Candidal fresh culture was suspended at 0.08 of optical density at 620 nm and diluted at 1:50 followed by 1:20 in SB, and 50 μL was inoculated to extract dilution to achieve final extract concentration of 1, 2, 3, 4, and 5 mg/mL. Microplates were incubated at 35 °C for 48 h. Candidal growth was evaluated following the antibacterial activity methodology described previously [47,48].

2.6. Total phenol content

TPC in the lichen extracts were determined following microplate method [49]. Organic extracts were diluted with water to achieve 1.0 mg/mL. Subsequently, 50 μL of the aqueous extract and 50 μL of the Folin–Ciocalteu reagent (1:5) were dispensed into the wells, and incubated at room temperature for 10 min. Afterward, 100 μL NaOH (0.35 M) was added. Gallic acid standard dilutions at 0, 10, 20, 30, 40, and 50 mg/mL were prepared and used for the standard regression curve. The assay was performed in triplicate and microplates were kept in darkness for 3 min. Afterward, absorbances were measured at 760 nm in duplicate using a microplate spectrophotometer (Epoch, USA). The total soluble phenolic compounds of the lichen extracts were expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g) [50–52].

2.7. Antioxidant capacity

The antioxidant capacity of lichen extracts was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging [53,54], ABTS (2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging [55], and FRAP (ferric reducing antioxidant power) with 2,4,6-Tripyridyl-s-Triazine, TPTZ [56] methods. Lichen dry extracts were diluted at 200 mg/mL with DMSO for each test.

2.7.1. DPPH assay

Lichen extracts (200 mg/mL) were diluted with MeOH from 25 to 2000 μg/mL. Each diluted extract (100 μL) was dispensed into microplate wells in triplicate. Subsequently, 10 μL of DPPH, dissolved in MeOH at 10 mg/L, was added to each well. The plates with the final mixture were kept in the dark at 25 °C for 30 min. Ascorbic acid served as the analytical standard. The absorbance values were measured twice at 517 nm using a spectrophotometer for microplate (Epoch, USA). Antioxidant capacity was quantified as the IC_{50} value, indicating the extract concentration that inhibits 50 % of DPPH [54].

2.7.2. ABTS assay

ABTS radical cation assay was performed in microplate format [49]. Lichen extracts (100 mg/mL) were diluted with distilled water at 1000 μg/mL, and 10 mg were dispensed into microplate wells in triplicate. Afterward, 190 μL of ABTS reagent – previously adjusted to an optical density of 0.810 at 734 nm wavelength with acetate buffer pH 4.5 – was added to each well. Trolox at 0–20 μM and quercetin (20 μM) were used as analytical standard compounds, and samples were tested in duplicate. The microplates were placed in darkness at 24 °C for 30 min. The absorbance values were measured twice at 734 nm using a spectrophotometer (Epoch, USA). Antioxidant activities were expressed as Trolox (mg) equivalent per 1.0 g of lichen extract (Eq-Trolox mg/g), value obtained by interpolation of the lichen extract or quercetin absorbance in the linear regression from the Trolox standard curve.

Table 3
Concentration of fatty acids found in lichen extracts in the MeOH:$(CH_3)_2CO$ (1:1) solvent mixture.

Fatty acid	Formula	RT	Concentration [mg FA/g sample], (% AG)							
		(min)	*Umbilicaria aff. calvenses*	*H. enderythraea*	*Puntelia graminicole*	*Cladonia chlorophaea*	*X. farinosa*	*P. distincta**	*P. distincta***	*U. durietzii*
Butyric acid	C4:0	10.83	[0.32], (64.6)	[3.8], (100)	[3.6], (83.1)	[3.6], (100)	[3.4], (95.8)	[4.1], (72.7)	[3.4], (67.4)	[3.4], (58.5)
Hexanoic acid	C6:0	11.8	–	–	–	–	[0.15], (4.2)	–	–	–
Undecanoic acid	C11:0	17.885	–	–	–	–	–	–	[0.059], (1.2)	[0,055], (0.95)
Tridecanoic acid	C13:0	21.352	–	–	–	–	–	–	[0,067], (1.3)	[0,057], (0.98)
Myristic acid	C14:0	34.22		–	–	–	–	–	[0,072], (1.4)	[0,067], (1.15)
Palmitic acid	C16:0	28.72	[0,005], (1.0)	–	–	–	–	[0.11], (2.0)	–	[0.30], (5.16)
Henicosanoic acid	C21:0	38.73	[0.131], (26.5)	–	[0.14], (3.2)	–	–		[0.178], (3.5)	[0.33], (5.7)
Palmitoleic acid	C16:1	28.723	–	–	–	–	–	–	[0.060], (1.2)	[0,096], (1.65)
Trans acid-**9**-octadecenoic	C18:1	32.82	–	–	–	–	–	[0.09], (1.6)	–	–
Linoleic acid	C18:2 n-6	34.22	–	–	–	–	–	[0.14], (2.5)	–	–
Cis-8,11,14-eicosatrienoic acid	C20:3	41.237	–	–	–	–	–	–	[0.108], (2.1)	[0,166], (2.85)
Cis-11,14,17-eicosatrienoic acid	C20:3	42.564	–	–	–	–	–	–	[0,464], (9.2)	[0.36], (6.19)
Arachidonic acid	C20:4 n-6	36.32	[0.039], (7.9)	–	[0.59], (13.6)	–	–	[1.2], (21.3)	[0.349], (6.9)	[0.26], (4.47)
Cis-13,16-docosadienoic acid	C22:2 n-6	44.885	–	–	–	–	–	–	[0.287], (5.7)	[0.72], (12.4)
Total SFA			[0.46], (92.1)	[3.8], (100)	[3.7], (86.4)	[3.6], (100)	[3.55], (100)	[4.21], (74.6)	[3.78], (74.7)	[4.21], (72.4)
Total UFA			[0.039], (7.9)	–	[0.59], (13.6)	–	–	[1.43], (21.3)	[1.2], (25.3)	[1.6], (27.6)
Total FA			[0.5], (100)	[3.8], (100)	[4.3], (100)	[3.6], (100)	[3.55], (100)	[5.64], (100)	[5.04], (100)	[5.81], (100)

RT: Retention time; SFA: Saturated fatty acid; UFA: Unsaturated fatty acid; (*): Rock substrate; (**): Tree substrate.

Table 4
Antibacterial and anticandidal minimal inhibitory concentration (MIC) of the different lichen extracts in MeOH:$(CH_3)_2CO$ (1:1).

Species	MIC (mg/mL)							
	S. aureus				E. coli		C. albicans	C. tropicalis
	ATCC 43300	ATCC 2921	ATCC 7592	ATCC 700699	ATCC 10536	O157:H7	ATCC 90028	ATCC 750T
Umbilicaria aff. calvenses	–	–	–	–	(>8)	–	–	–
Hypotrachyna enderytracea	0.8	1	0.7	0.7	(>8)	(>8)	–	–
Punctelia graminicole	(NA)	–	(NA)	(NA)	(>8)	–	3	3
Cladonia chlorophaea	(NA)	(NA)	(NA)	(NA)	–	–	–	–
Xanthoparmelia farinosa	1	1	0.9	0.9	–	–	–	–
*Psiloparmelia distincta**	0.05	0.2	0.1	0.02	–	–	4	3
*Psiloparmelia distincta***	0.4	0.5	0.4	0.3	–	–	3	4
Usnea durietzii	0.3	0.3	0.4	0.3	(>8)	–	–	–
Usnic acid[c]	20	20	20	(>20)	–	–	–	–
Gentamicin[c]	100	10	10	(>100)	(<150)	(<150)	–	–
Clotrimazole[c]	–	–	–	–	–	–	1	2

MIC: Minimum inhibitory concentration by microdilution methodology.
[C] MIC values are expressed in (μg/mL); NA: No Activity; symbol (*): substrate rocks; symbol (**): substrate tree; symbol (–): not tested. Samples tested corresponding to antimicrobial activities exhibited in the previous Agar Diffusion Assay.

2.7.3. FRAP assay

Ferric reducing/antioxidant power (FRAP) assay was performed in microplate format [50] with a few modifications. Lichen extracts (200 mg/mL) were diluted with distilled water at 1000 μg/mL, and 10 mg were dispensed into microplate wells in triplicate. Afterward, 140 μL of FRAP reagent (TPZ + Fe^{3+} + acetate buffer at pH 3.5) was added to each well. The standard regression curve of Fe^{2+} was prepared with iron (II) sulfate at 0, 200, 400, 600, 800, and 1000 μM. Also, ascorbic acid (1 mM) was included as an analytical standard compound, and all samples were tested in duplicate. The microplates were placed in darkness at 24 °C for 30 min. The absorbance values were measured twice at 593 nm with a microplate spectrophotometer (Epoch, USA). The ferric reducing antioxidant power (FRAP) was expressed as Fe^{2+} (μM) per 1.0 g of lichen dry extract (μM Fe^{2+}/g), and this value was obtained through interpolating absorbance in Fe^{2+} standard regressions.

2.8. Statistical analyses

All assays considered three replicates, values and standard deviation were calculated. Also, for antimicrobial dilution methodology and antioxidant assays, ANOVA and Duncan's test ($P < 0.05$) were performed.

3. Results and discussion

3.1. Lichen identification

The eight lichen samples collected belong to seven different species, which are described in Table 1. *Psiloparmelia distincta* (Nyl.) Hale was found on two different substrates: rock substrate (rs) and tree substrate (ts) (Fig. 2F and G).

3.2. Quantification of usnic acid via HPLC

The quantitative assessment of UA was performed through HPLC–DAD. A composite mixture of MeCN: H_2O: sodium citrate buffer

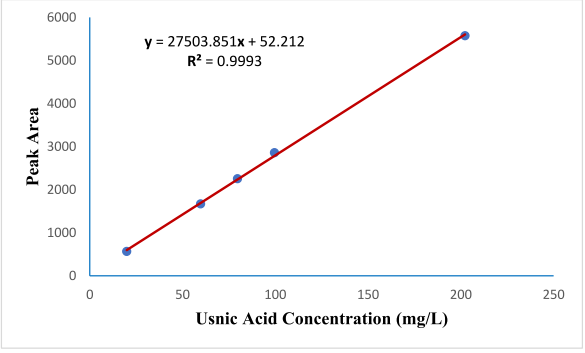

Fig. 3. Calibration curve of UA (standard) under HPLC conditions.

(60:36:4 v/v) was employed as the mobile phase. The retention time was ~6.4 min. The method's precision was confirmed with a standard deviation of less than 1.0 % (n = 10), and the recovery of UA was 99.78 %. The detection and quantification limits were 0.27 and 0.38 mg/L, respectively. The response was linear from 20 to 200 mg/L with a coefficient of determination (R^2) of 0.9993 [57–59]. The obtained linear equation used was y = 27503.851x + 52.212 (Fig. 3).

The results revealed that the UA content in lichen species varies within 0.017–0.304 % (Table 2), where *Usnea durietzii* showed the highest concentration of UA. Contrary, the lichen extracts of *Umbilicaria aff. calvenses*, *Punctelia graminicole*, and *Cladonia chlorophaea* did not exhibit detectable signals of UA in this study (LoD = 0.27 mg/L).

The UA concentration in *Xanthoparmelia farinosa* (0.18 %) and *Usnea durietzii* (0.304 %) of this research were in the same ranges that other lichens such as values reported for *Ramalina fraxinea* (0.13 %), *R. pollinaria* (0.22 %), *R. polimorpha* (0.27 %) [60], *Xanthoria pariatina* (0.13 %), *Rhizoplaca melanopthalma* (0.19 %), and *Umbilicaria leiocarpa* (0.27 %) [61]. Similarly, in this study, *Hypotrachyna enderytracea* and *P. distincta* reported a low UA concentration of 0.017%–0.042 %.

It is known that the content of secondary metabolites in the same species varies depending on environmental features like precipitation, temperature, and altitude conditions [62]. Furthermore, there is evidence of an inverse relation between altitude and UA content in lichens; for example, in *Parmelia flexilis*, the UA concentration varies between 5.13 % (sample collected at 841 m above sea level) and 1.66 % (sample collected at 2250 m above sea level) [62]. In this sense, the low UA concentration (less than 0.31 %) found in lichen extracts of this research could be in concordance with the high altitudes where they were collected (3356–4109 m above sea level).

3.3. FA quantification

The identification and quantification of FAs in all lichen samples were performed using a GC–FID. The only common FA present in all species under study parameters was butyric acid, and its concentration ranged from 3.4 to 4.1 mg/g (Table 3), although *Umbilicaria* aff. *calvenses* reported the lowest concentration of this compound (0.32 mg/g). Butyric acid emerged as the sole FA present in both *H. enderythraea* and *C. chlorophaea,* also *P. distincta*, sourced from both rock and tree substrates, only had butyric acid (C:4) and arachidonic acid (C20:4 n-6) as common FAs. In addition, *P. distincta* from rock substrate contained palmitic (C16:0), oleic (C18:1), and linoleic (C18:2 n-6) FAs at 0.11, 0.09, and 0.14 mg/g concentrations, respectively. However, *P. distincta* from tree substrate featured

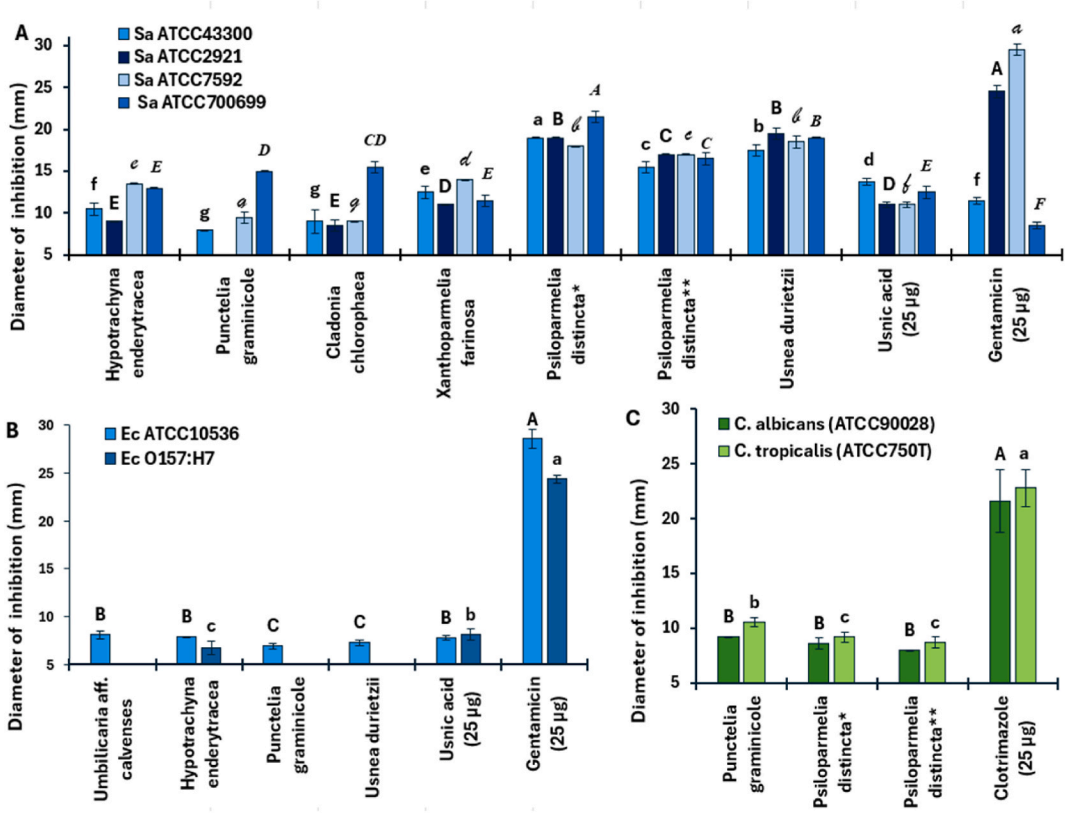

Fig. 4. Antimicrobial activities by diffusion methodology of different lichen extracts in MeOH:$(CH3)_2CO$ (1:1). **A**. Anti-*staphylococcus aureus* activity; **B**. Anti *E. coli* activity; **C**. Anti *Candidal* activity. Letters represent the different groups according to Duncan's test (P < 0.05). Symbol (*): substrate rocks; symbol (**): substrate tree.

saturated FAs such as undecylic (C11:0), tridecylic (C13:0), myristic (C14:0), palmitoleic (C16:1), and eicosenoic (C21:0) at concentrations ranging from 0.059 to 0.178 mg/g. Moreover, it contained unsaturated FAs such as dihomo-γ-linolenic (C20:3), arachidonic (C20:4 n-6), and docosadienoic (C22:2 n-6). Previous studies reported that MeOH: $CHCl_3$ (1:1) extract of *P. distincta* collected from Jauja province (Peru) at 4000–4200 masl, included FAs such as lauric (C12:0), stearic (C18:0), arachidic (C20:0), behenic (C22:0), and lignoceric (C24:0) [63], but these FAs were not identified in our study in this species. In the case of *U. durietzii*, this species exhibited higher concentration of total FAs (5.81 mg/g) than other species studied. Previous research demonstrated that *U. durietzii*, collected from Junin province, Peru (at 42000 m above sea level) and macerated with MeOH: $CHCl_3$ (1:1) contained FAs C16:0 and C16:1 [64], which were also identified in our study. However, FAs C17:0, C18:0, *cis*-oleic C18:1, and linoleic C18:2 [31] were identified in Junin sample, but they were not present in the same lichen species subjected in our research. Consequently, this study contributes new findings on the fatty acid composition of *P. distincta* and *U. durietzii*, using different solvents for the extracts of these species collected from the province of Arequipa.

3.4. Antimicrobial activity

The antibacterial activity of eight lichen extracts in MeOH:$(CH_3)_2CO$ (1:1) was assessed against bacterial strains of *S. aureus* ATCC (43300, 29213, 25923, and 700699), *E. coli* (O157:H7, ATCC 10536), and *Salmonella* (ATCC 14028) and anti-yeast activity against *C. albicans* (ATCC 90028) and *C. tropicalis* (ATCC 750T) (Figs. 4 and 5, and Table 4). In the diffusion methodology, seven extracts showed inhibition halo against all strains of *S. aureus* tested. They had significative differences with usnic acid and gentamicin (Fig. 4A), finding that lichens (1 mg/mL) and usnic acid (25 μg) inhibited better the strains ATCC43300 and ATCC 700699 than gentamicin (25 μg); contrary the strains ATCC 29213 and ATCC 25923 were better controlled by gentamicin. The extract from *P. distincta* (rs) exhibited considerable anti-staphylococcus activities with inhibition zone values ranging from 19 to 21.5 mm in all strains tested, so these activities were reflected in their MICs of 0.02–0.20 mg/mL (Fig. 4 and Table 4). The *S. aureus* ATCC 700699 strain presented high sensitivity (MIC = 0.02 mg/mL) toward *P. distincta* (rs) compared to the reference drug gentamicin (MIC = >0.100 mg/mL), further this extract was 2.5–15 times more active against all tested *S. aureus* strains than the extract from the same species collected from tree substrate. The extracts from *H. enderythraea*, *X. farinosa*, *P. distincta* (rock and tree substrates), and *U. durietzii* were active against *S. aureus* strains with inhibition halo values from 0.8 to 13.8 mm and MIC values of ≤1 mg/mL. Metabolites from the *Usnea* genus, such as UA, polyphenols, and depsides, have been considered the main components contributing to antibacterial and antifungal activities [65]. In this regard, the satisfactory antibacterial activity demonstrated by the organic extracts of *U. durietzii* against all *S. aureus* ATCC strains (43300, 29213, 25923, and 700699) was evidenced by diffusion assays (Figs. 4 and 5) and MIC values ranging from 0.3 to 0.4 mg/mL (Table 4). Concerning to MeOH:$(CH_3)_2CO$ (v/v, 1:1) organic extract of *X. farinosa* species, it was found that antibacterial against *S. aureus* ATCC 25923 (MIC = 0.9 mg/mL), was two and eight times less active than the ethanolic extract (MIC = 0.47 mg/mL) and acetonic extract (MIC = 0.12 mg/mL), respectively, of *Xanthoparmelia conspersa* evaluated against the same bacterial strain [66]. Similarly, the acetonic extract of *Xanthoparmelia stenophylla* demonstrated activity against *S. aureus* ATCC 6538 (MIC = 0.10 mg/mL) [67]. These findings suggest that *Xanthoparmelia* genus contains common metabolites that enhance its antibacterial activity against *S. aureus* strains.

According to the last results, it has been reported about the potential antibacterial activity of lichen extracts against Gram-positive bacteria [25,68–70]. However, their antibacterial activity against Gram-negative bacteria was limited [25]. In our study, extracts of *U. calvenses*, *H. enderytracea*, *P. graminicole*, and *Usnea durietzii* exerted weak inhibitory activity (<10 mm, MIC> 8 mg/mL) against *E. coli* (ATCC 10536); but only *H. enderytracea* extract showed low activity against *E. coli* (O157:H7) (6.7 mm, MIC >8 mg/mL) (Fig. 4B and Table 4). The other extracts did not show an inhibition zone against *E. coli*, also usnic acid (25 μg) showed weak activities against *E. coli* tested contrary to gentamicin (25 μg) that inhibited them highly (Fig. 4). Similar results were obtained against *Salmonella* (data

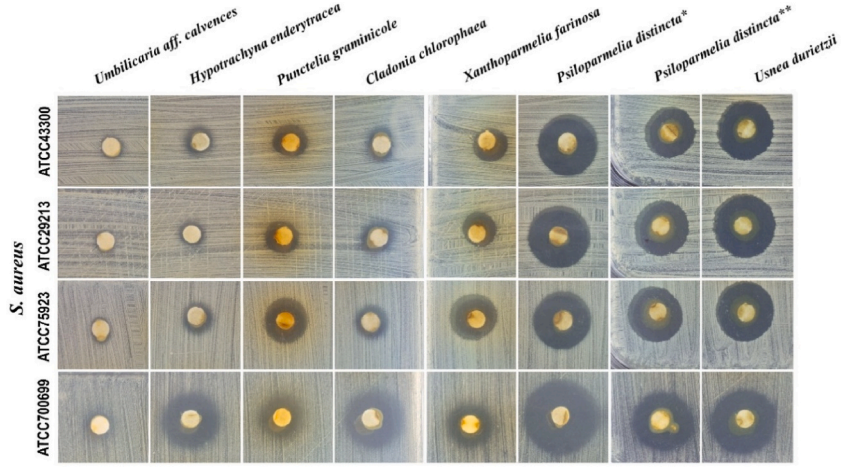

Fig. 5. Inhibition halos obtained for the eight lichen extracts in MeOH:$(CH_3)_2CO$ (1:1) assessed against the *Staphylococcus aureus* strain.

do not show). The inactivity of lichen extracts against Gram-negative bacteria may be related to cell wall permeability that gives them resistance to hostile environments [70,71].

The antifungal activity of the methanolic crude extracts is presented in Fig. 4C and Table 4. Only three lichen extracts (*P. graminicole* and *Psiloparmelia distincta* (rock and tree substrate) were active against *C. albicans* and *tropicalis* with inhibition zone values from 8 to 10.6 mm and MIC values ranging from 3 to 4 mg/mL. Concerning to standard controls, clotrimazole (25 μg) showed inhibition halos approx. one time more than positive lichens, but usnic acid (25 μg) did not exhibited activity (Fig. 4C), suggesting that other compounds different it could be responsible of antifungal activities of *Punctelia graminicole* and *Psiloparmelia distincta*. Extracts of these three lichens showed similar inhibitory activity against *C. albicans* compared with lichen extracts (in acetone and ethanol) obtained from *H. physodes* (MIC = 3.12 mg/mL) [71]. Furthermore, they were more active (around two times) than *C. furcata* extracts (in acetone and ethanol) (MIC = 6.25 mg/mL) [71]. However, *P. caperata* (0.78 mg/mL), *P. pertusa* (0.78 mg/mL), and *U. polyphylla* (1.56 mg/mL) [71] exhibited better antifungal activity compared to all our tested lichen extracts.

Methanol-acetone extracts of the *Punctelia graminicole* and *Psiloparmelia distincta* (rock and tree substrate) lichens inhibited growth of *C. tropicalis* at relatively low concentrations (MICs = 3, 3, and 4 mg/mL, respectively) (Table 4). These results are similar to MIC values found for ethanolic extracts of *Everniastrum vexans* (MIC = 4.7 mg/mL) and *Parmotrema blanquetianum* (MIC = 2.2 mg/mL). However, our results indicate higher antifungal activity against *C. tropicalis* compared to *Parmotrema reticulatum* (MIC = 11.9 mg/mL) and *Peltigera laciniata* (MIC = 14.8 mg/mL) (both lichens dissolved in ethanol) [72]. Despite that all the studied lichen extracts tested against *C. albicans* and *C. tropicalis* were less active than clotrimazole standard (1 and 2 μg/mL, respectively), antimicrobial activity found in our study places these lichens as a promising source for antifungal compounds. Moreover, due to the concentration of UA in the organic extracts of *P. distincta* (rock and tree substrates) that recorded lower values (0.017 % and 0.042 %, respectively), certain secondary metabolites different to UA present in *P. distincta* extract could enhance its antimicrobial activity, possibly through synergistic effect [23].

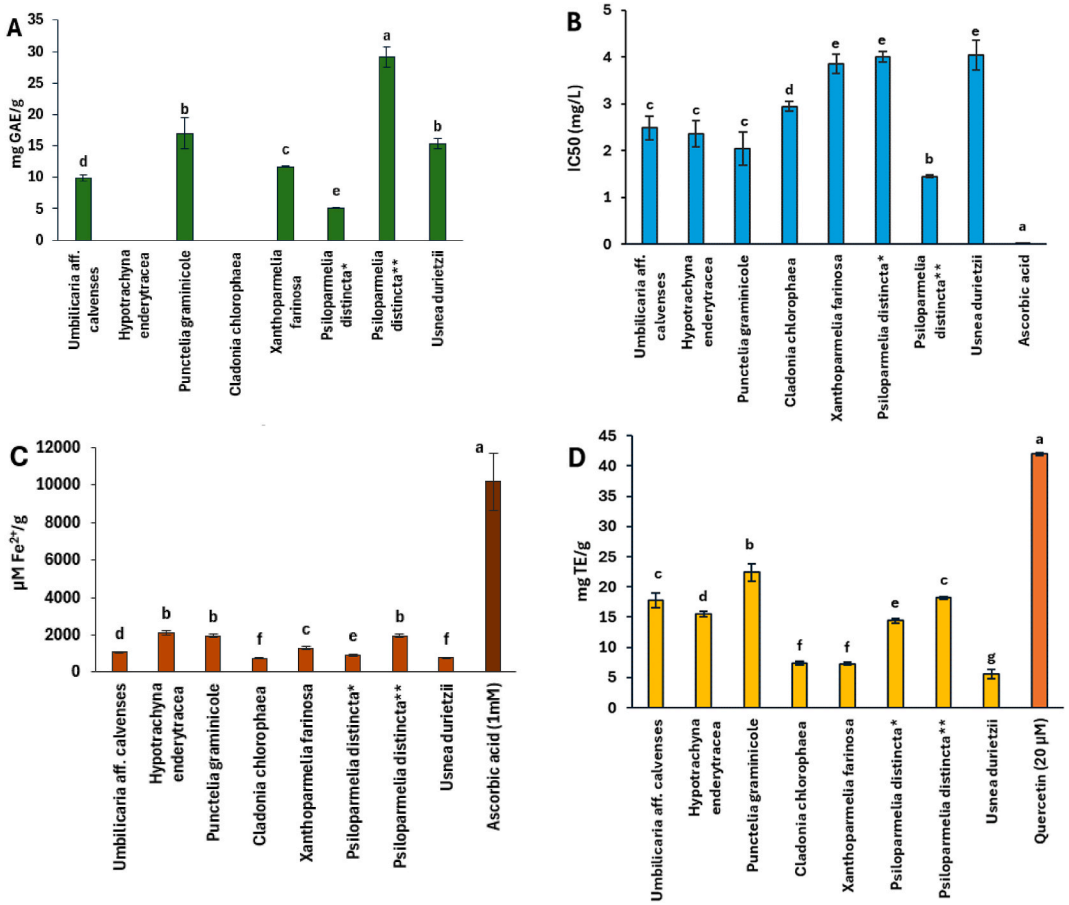

Fig. 6. Antioxidant capacities, and total phenolic content of the different lichen extracts in MeOH:(CH3)2CO (1:1). **A**. TPC, **B**. DPPH, **C**. ABTS, and **D**. FRAP. Symbol (*): substrate rocks; symbol (**): substrate tree. Values correspond to means ± SD of three replicates. Letters indicate the significance differences between each samples according to Duncan's test ($P < 0.05$).

3.5. Antioxidant capacities

The total phenolic content (TPC) and antioxidant capacities performed with DPPH, ABTS, and FRAP were presented in Fig. 6 and Table 5. Only extracts MeOH:$(CH_3)_2CO$ (v/v, 1:1) at 1 mg/mL from six studied lichens showed TPC ranged from 5.1 to 29.1 mg GAE/g, so extracts of *H. enderythraea* and *C. chlorophaea* did not report positive results (Fig. 6A–Table 5). The extract of *P. distincta* (ts) exhibited a higher TPC concentration (29.1 ± 1.6 mg GAE/g). This result is similar to methanolic extracts of *H. cirrhata* (31.11 ± 1.1 mg GAE/g) [52], and aqueous extract of *Bryoria fuscescens* (30.3 ± 1.960 mg GAE/g) [73]. On the contrary, *P. distincta* (rock substrate) extract showed the lowest TPC. This could be in relation with the influence of the substrate on the production of metabolites present in lichens.

Regarding antioxidant capacity, it was found less DPPH activity (high values of IC_{50}) in all samples compared to ascorbic acid, but *P. distincta* (ts) showed better scavenging activity among lichens (Fig. 6A–Table 5). The organic extracts of *P. distincta* (tree and rock substrates) inhibited the oxidative action of DPPH, with IC_{50} values of 1.45 and 4.0 mg/mL, respectively. These results are closely related to the obtained TPC values (29.1 and 5.1 mg GAE/g for tree and rock substrates, respectively). *X. farinosa* achieved considerable antioxidant activity (IC_{50} = 3.85 ± 0.21 mg/mL). However, hexane, methanol, and acetone extracts of *X. stenophylla*, exhibited a better response to DPPH radical elimination with IC_{50} values in the range of 81–95 μg/mL [67]. Regarding the extracts of *Umbilicaria* aff. *calvences* and *U. durietzii*, a better antioxidant response against DPPH radical would be expected due to their TPC contents (Fig. 6A and B).

FRAP assay involves the reduction of colorless Fe^{3+}- 2,4,6-tripyridyl-s-triazine complex (Fe^{+3}-TPTZ) into intense blue Fe^{2+}-TPTZ while interacting with an antioxidant compound. The calibration equation for Fe^{2+} was y = 0.0008X −0.0107, where x is the concentration of Fe^{2+} and y is the absorbance at 593 nm (R^2 = 0.9981). All the lichen extracts (2 mg/mL) exhibited moderate FRAP antioxidant capacity compared with ascorbic acid (1 mM) that showed approximately five times (10180 ± 152 μM Fe^{2+}/g) more than them (Fig. 6C–Table 5). *H. enderytracea* showed the greatest reducing power (2124 ± 127 μM Fe^{2+}/g), like *P. graminicole* and *P. distincta* (ts) both with the same FRAP value (1953 ± 79 or 87 μM Fe^{2+}/g, respectively). Similar results were obtained for methanolic extracts of *Lobothallia alphoplaca* (1673.32 μM Fe^{2+}/g) [74]. Extract of *P. distincta* (rs) exhibited less FRAP values (937 ± 56 μM Fe^{2+}/g) compared to *P. distincta* (ts). These findings followed the same trend in relation to TPC found. Furthermore, *C. chlorophaea* (759 ± 29 μM Fe^{2+}/g) and *U. durietzii* (778 ± 16 μM Fe^{2+}/g) exhibited close values of FRAP (Fig. 6C–Table 5). However, these results did not show a direct correlation between FRAP and TPC values, similarly to values obtained for methanolic extracts of *Umbilicaria vellea* (603.81 μM Fe^{2+}/g), *Rhizoplaca melanophthalma* (648.08 μM Fe^{2+}/g), *Pleopsidium flavum* (618.56 μM Fe^{2+}/g), *Physconia muscigena* (740.90 μM Fe^{2+}/g), and *Xanthoparmelia stenophylla* (646.72 μM Fe^{2+}/g) [74]. The extract of *Umbilicaria aff. calvenses* (1090 μM Fe^{2+}/g) and *Xanthoparmelia farinosa* (1290 ± 87 μM Fe^{2+}/g), showed similar FRAP values compared to the methanolic extracts of *Rhizoplaca chrysoleuca* (1112.16 μM Fe^{2+}/g) and *Dermatocarpon vellereum* (1222.30 μM Fe^{2+}/g), respectively [74].

ABTS assay of lichen extracts was performed at 1 mg/mL of concentration and quercetin (20 μM) as standard. The standard curve for Trolox was y = −0.0344x + 0.7528 with R^2 = 0.9906, where x is the trolox concentration and y is the absorbance at 734 nm measured at 30 min. ABTS scavenging activities of all extracts were less than its diluted standard, but the lichen extracts with better reducing power were *P. graminicole* (22.4 ± 1.4 mg TE/g) > *P. distincta* (ts) (18.2 ± 0.2 mg TE/g) and *U. calvenses* (17.8 ± 1.2 mg TE/g) > *H. enderytracea* (15.5 ± 0.4 mg TE/g) > *P. distincta* (sr) (14.4 ± 0.4 mg TE/g) (Fig. 6D–Table 5). There is a certain relationship between the TPC, FRAP, and ABTS results obtained for *P. graminicole* and *P. distincta* (ts) that showed better reducing power. *C. chlorophaea* and *H. enderytracea* did not show TPC values. Besides, *C. chlorophaea* (7.4 ± 0.3 mg TE/g) exhibited low FRAP and ABTS values, which are directly correlated with the TPC values. However, *H. enderytracea* exhibited the highest FRAP and ABTS values. The antioxidant capacity depends on the phenolic compounds and others such as hydrates and protein contained in the extract. However, some compounds can interact with the active centers of the phenolic compounds and generate an antagonistic effect, limiting their antioxidant capacity [67]. *C. chlorophaea, X. farinose* (7.4 ± 0.3 mg TE/g) and *Usnea durietzii* (5.6 ± 0.3) exhibited the lowest FRAP values. However, the reducing power of these extracts were superior to methanolic extracts of *Usnea filipendula* (1.75 ± 0.07 mg TE/g), *Usnea fulvoreagens* (1.81 ± 0.04 mg TE/g), and *Usnea intermedia* (1.41 ± 0.06 mg TE/g) [25], previously reported. The results showed also that the standard Quercetin (20 μM) (6 ± 0.0 mg TE/g) exhibited high $ABTS^+$ radical-scavenging activity in comparison with tested extracts (Fig. 6D–Table 5).

4. Conclusions

In this study, UA, FA, and TPC were identified and quantified in eight MeOH:$(CH_3)_2CO$ (1:1) extracts (v/v, 1:1) derived from seven lichen species, including *Umbilicaria aff. calvescens*, *H. enderythraea*, *Punctelia graminicola*, *C. chlorophaea*, *X. farinosa*, *P. distincta* (from rock and tree substrates), and *U. durietzii*, which were collected from the Arequipa province (Peru). Analytical characterization involved the HPLC–DAD and GC–FID methods. Moreover, the antimicrobial and antioxidant activities of these extracts were assessed. The results indicate that the UA content in all studied lichen species ranged from 0.017 % to 0.304 %, and the common FA identified in these species was butyric acid (C4:0). Regarding antibacterial activity, the lichen extract from *P. distincta* (rock substrate) exhibited higher activity (MIC = 0.02 mg/mL) against the *S. aureus* ATCC 700699 strain compared to other tested extracts as well as the reference drug gentamicin (MIC >0.100 mg/mL) and the reference UA compound (MIC >0.02 mg/mL). In addition, this extract exhibited the best antifungal activity against *C. albicans* and *C. tropicalis* (MIC = 4 and 3 mg/mL, respectively), like the extracts of *P. distincta* (tree substrate) and *Punctelia graminicole*, regarding the other studied extracts. None of the tested extracts showed antibacterial activity against Gram-negative strains (*E. coli* and *Salmonella*). The lichen extract from *P. distincta* (tree substrate) demonstrated superior inhibitory activity against DPPH (IC_{50} = 1.45 ± 0.03 mg/mL) compared to other tested extracts. In addition, the FRAP

Table 5
DPPH, ABTS, and FRAP antioxidant activities, and TPC of the different lichen extracts in the MeOH:$(CH_3)_2CO$ (1:1) solvent mixture.

Species	DPPH	FRAP	ABTS	TPC
	IC50 (mg/L)[a]	μM Fe^{+2}/g	mg TE/g	(mgGAE/g)[c]
Umbilicaria aff. Calvenses	2.48 ± 0.26	1090 ± 24	17.8 ± 1.2	9.9 ± 0.5
Hypotrachyna enderytracea	2.36 ± 0.28	2124 ± 127	15.5 ± 0.4	nd
Punctelia graminicole	2.04 ± 0.35	1953 ± 79	22.4 ± 1.4	17 ± 2.5
Cladonia chlorophaea	2.94 ± 0.10	759 ± 29	7.4 ± 0.3	nd
Xanthoparmelia farinosa	3.85 ± 0.21	1290 ± 87	7.3 ± 0.2	11.7 ± 0.1
*Psiloparmelia distincta**	4.0 ± 0.11	937 ± 56	14.4 ± 0.4	5.1 ± 0.1
*Psiloparmelia distincta***	1.45 ± 0.03	1953 ± 87	18.2 ± 0.2	29.1 ± 1.6
Usnea durietzii	4.04 ± 0.32	778 ± 16	5.6 ± 0.7	15.4 ± 0.8
Ascorbic acid[b]	28.03 ± 0.34		–	–
Usnic acid	–	10180 ± 1520	–	–
Quercetin (20 μM)	–	–	6 ± 0	–

FRAP: Ferric reducing antioxidant activities; TE: Trolox equivalent.
[a] IC50: Concentration of the compound (mg/mL) that causes a 50 % DPPH scavenging after 48 h of compound exposure.
[b] IC50 values expressed in (μg/mL).
[c] The total phenolic content (TPC) was expressed as milligrams of gallic acid equivalents (GAE) per gram of extract; NA: No Activity; symbol (*): substrate rocks; symbol (**): substrate tree; nd: not detected; symbol (–): not tested.

(1953 ± 87 μM Fe^{2+}/g dry extract) and ABTS values (18.2 ± 0,2 mg TE/g dry extract) have positive correlation with (TPC = 29.1 mg GAE/g) values. In general, it can be stated that the *P. distincta* (rs) and *P. distincta* (ts) species show promise as potential antimicrobial and antioxidant agents, respectively. *P. distincta* (ts) and *Punctelia graminicole* could be considered as potential antifungal agents. Further research into the identification of secondary metabolites in these lichen species and their potential biological applications is of interest within the field of pharmacology. This is the first time that a chemical study of lichen extracts of these species has been carried out, so our contribution can serve as a primary source for future research considering the pharmacological potential exposed in this work.

CRediT authorship contribution statement

Fernando Carrasco: Writing – original draft, Resources, Methodology, Investigation. **Wilfredo Hernández:** Writing – review & editing, Methodology, Conceptualization. **Nino Castro:** Writing – original draft, Resources, Methodology, Investigation. **Marco Guerrero:** Visualization, Investigation, Data curation. **Carmen Tamariz-Angeles:** Writing – review & editing, Methodology, Conceptualization. **Percy Olivera-Gonzales:** Writing – review & editing, Methodology, Conceptualization. **Daniel Echevarría-Rodríguez:** Visualization, Investigation, Data curation. **Cesar Raposo:** Investigation, Formal analysis, Data curation. **Lúcia A. Silva:** Investigation, Formal analysis, Data curation. **Jesus M. Rodilla:** Writing – original draft, Resources, Methodology, Investigation.

Data availability

The data used to support the findings of this study are included within the article. These data will be available when the researchers request it.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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