Antioxidant and UV photoprotective activity of lichenized fungi from Paramo de Sumapaz (Colombia)

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Research Article

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Posted Date: July 24th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-7005757/v1

Additional Declarations: No competing interests reported.

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Abstract

Lichenized fungi are particularly abundant in harsh environments. Paramos are unique neotropical mountain environments located in the northern Andes which face several treats due to human activities. The preset study evaluated the antioxidant and photoprotective activities of four paramo lichen extracts. Antioxidant activity was assessed using DPPH and FRAP assays. While photoprotective parameters (Sun protection factor - SPF, critical wavelength and spectral amplitude) were determined spectrophotometrically. Rock-inhabiting lichens such as *Thamnolia* performed better than phorophyte or soil-growing lichens, such as *Lobariella*, *Everniastrum* and *Peltigera*. All extracts showed DPPH inhibition higher than 70 % at 200 µg mL^{-1} and exhibited significant differences with BHT (92 % at 20 µg mL^{-1}). Furthermore, the extracts had moderate ferric ion reducing power and were statistically different from BHT. For protection against UV-B, the *in vitro* SPF were above 10 at 100 µg mL^{-1} for all extracts except extract from *Peltigera* older thalli, with *Lobariella* proving to be the most active. *Thamnolia* and *Lobariella* can be identified as potential broad-spectrum filters (λ_{crit} = 367.9 and 355.8 nm), while *Everniastrum* can be proposed as UV-B and UVA-2 filter and *Peltigera* presented the least photoprotective activity. Either younger or older *Peltigera* thalli were distinguished, obtaining differences in composition, antioxidant and photoprotective activity, which coincided with the optimal defense theory (ODT). Consequently, it is feasible to propose paramo lichen extracts, especially those growing on UV-exposed habitats, as sources of new photoprotective compounds. Indeed, they presented antioxidant capability as a remarkable improvement upon synthetic sunscreens.

Keywords: Lichenized fungi, antioxidant activity, photoprotective activity, paramo, ODT

Competing interests / Conflicts of Interest statement

The authors have no competing interests to declare that are relevant to the content of this article.

Authors contributions

David Gilberto Torres Vargas: Samples collection, extract obtention, laboratory experimentation, data analysis and article redaction.

Lissy Marcella Nuñez Arango: Samples collection, extract obtention, laboratory experimentation and data analysis.

Jaime Aguirre Ceballos: Advisement about article structure, guidelines, and references, article redaction, text revision especially to highlight the contributions and comparisons. Explanations about lichens ecology in Paramos, genres and species distribution.

Funding

Universidad Nacional de Colombia, which fund partially this work (Project HERMES: 35978).

Acknowledgments

We acknowledge to Colombian Environment and Sustainable Development Ministry for the Licence Nº 004 of 21st March 2018 added to the Contract Nº 121 of 2016, which covered the Project entitled "*Bioprospection of lichens from Paramo de Sumapaz as original natural source of dual substances with antioxidant and photoprotective activity for preventing sun-related skin diseases*".

We appreciate the accompaniment of Natural National Park Service during biological sampling. Special thanks to MSc Roberto Dávila Villamizar from Colombian Biodiversity Foundation for the taxonomic determination of lichen material.

Declarations

Ethics approval and consent to participate: Not applicable.

Introduction

The paramo is a *sui generis* neotropical high mountain environment, located in northern South America and southern Central America above 3 200 m up to 4 500 m. It is known for its biodiversity and harsh conditions such as low temperatures, elevated sun irradiation, abrupt changes in humidity and temperature during the day (Rangel Ch 2000; Castaño Uribe 2002). It extends from Colombia, Costa Rica, Ecuador and Venezuela to the drier Puna region in Peru (Rangel Ch 2000). Its flora consists mainly of small shrubs no taller than 10 m and *Calamogrostis* herbs, with lichenized fungi (LF) as ubiquitous component of the paramo flora (Rangel Ch 2000; Sipman et al. 2008). LF are composed by at least two different organisms, a fungus known as the mycobiont, an alga or photobiont, constituting a stable association known as holobiont, which can serve as habitat for bacteria and other organisms (Boustie et al. 2011; Zambare and Christopher 2012; Nguyen et al. 2013; Poulsen-Silva et al. 2025). LF are found in almost all terrestrial environments on Earth, including those too hostile for their separate bionts (McEvoy et al. 2006; Boustie et al. 2011; Zambare and Christopher 2012; Asplund and Wardle 2017; Beckett et al. 2021; Poulsen-Silva et al. 2025). LF are the dominant life-forms in c.a. 8% land surface, and high mountain environments are among the preferred sites of lichens (Boustie et al. 2011; Nguyen et al. 2013; Asplund and Wardle 2017; Beckett et al. 2021; Gasulla et al. 2021).

Although sunlight and ultraviolet radiation (UV-R) are necessary for processes like vitamin D biosynthesis, and have some health benefits like improvement of psoriasis and localised scleroderma, excessive exposure leads to oxidative stress and generate diseases, including cancer (Stiefel and Schwack 2015; Radice et al. 2016; Shanbhag et al. 2019). In lichens UV-R exposure causes irreversible damage to photobiont photosystems and DNA and generates Reactive Oxygen Species (ROS) (Cockell and Knowland 1999; Beckett et al. 2021; Veres et al. 2022; Mkhize et al. 2022). Exposome, a term that describes environmental factors including imbalanced diet, ionising radiation, solar UV radiation, heat exposure (photooxidative stress), can induce or accentuate oxidative stress and inflammatory processes (Wenk et al. 2000; Sies 2019; Shanbhag et al. 2019; Krutmann et al. 2021). ROS overproduction in cells and mitochondria due to UV-A and shortwave NIR leads to photooxidative stress, causing immunosuppression and accelerating the development of melanoma skin cancer in humans (Nash et al. 2006; Zastrow et al. 2009; Kolbe 2012; Sklar et al. 2013; Stiefel and Schwack 2015; Kammeyer and Luiten 2015; Sarkany 2017; Sies 2019). UV-B has enough energy to stimulate photoreactions in certain DNA bases, forming cyclobutane-pyrimidine dimers, which are associated with human squamous and basal cell carcinoma (Ravanat et al. 2001; Nash et al. 2006; Grether-Beck and Krutmann 2009; Stiefel and Schwack 2015). Skin cancer predominantly affects Caucasian populations, especially in high-latitude and mountainous regions, due to ozone depletion and the high population exposure to sunlight, and it is currently a global public health concern (Stiefel and Schwack 2015; Krutmann et al. 2021).

To provide physical and chemical protection against abiotic and biotic stressors, mycobionts biosynthesise and accumulate UV-screening secondary metabolites, photobionts dissipate energy through photochemical quenching and non-photochemical quenching, this latter corresponds to fluorescence and thermal dissipation. Whereas the whole LF employ strategies, such as thalli dehydration or curling (Huneck 1999; Luo et al. 2010;

Boustie et al. 2011; Solhaug and Gauslaa 2012; Nguyen et al. 2013; Beckett et al. 2021; Veres et al. 2022; Ureña-Vacas et al. 2022; Poulsen-Silva et al. 2025). Most lichen substances are phenolics, that are biosynthesised via two main pathways: the polyketide or acetic-malonic acid pathway, and the shikimic acid pathway (Huneck and Yoshimura 1996; Thadhani et al. 2011; Ureña-Vacas et al. 2022). These compounds have antioxidant activity and may influence lichen ecological interactions by deterring herbivory or protecting algal photosystems, the latter helps LF adapt to sudden variations in solar radiation (Appel 1993; Gauslaa and McEvoy 2005; Molnár and Farkas 2010; Nguyen et al. 2013; Ndhlovu et al. 2022).

Recently, there has been an increasing focus on LF secondary metabolites for the search of new and original drugs and cosmetic ingredients (Zambare and Christopher 2012; Nguyen et al. 2013; Varol 2018; Ureña-Vacas et al. 2022). Owing to its high biodiversity and environmental conditions, the paramo could be a source of new natural products with interesting activities. LF extracts and lichen substances exhibited various biological activities, including antibiotic, anti-inflammatory, antioxidant and photoprotective (Luo et al. 2010; Perico-Franco et al. 2015; Ureña-Vacas et al. 2022; Poulsen-Silva et al. 2025). The aim of this study is therefore to evaluate the antioxidant and photoprotective properties of different paramo lichen extracts in search of new alternative sunscreens.

Materials and Methods

Lichen samples

Samples of four species, *Lobariella sipmanii* (Moncada, Betancourt & Lücking), *Thamnolia vermicularis* (Sw.) Ach ex Schaerer, *Everniastrum* sp, and *Peltigera neopolydactyla* (Gyelnik), were collected between 2016 and 2018 in the Andabobos, Chisacá, and Alto Caicedo areas of Sumapaz National Natural Park (3700 - 3800 m), located in southern Bogotá DC, Colombia. The lichens were cleaned in wet state using an stereoscope to remove mosses, liverworts, and other plant material. A portion of the material was prepared, sent and deposited as a voucher specimen in Colombian National Herbarium (COL) under reference numbers from and 609185, 609186, 609190 and 609191 respectively.

The collected material was covered by licenses number 013 (August 24, 2009) and number 004 (March 21, 2018) from Colombian Environment and Land Development Ministry and collection draft licenses assigned to Universidad Nacional de Colombia by National Environmental Licensing Agency (ANLA).

Reagents and dissolvents

Reagents used as 2,2-diphenyl-1-picrylhydrazyl (DPPH), atranorin, and butylhydroxytoluene (BHT) were provided by Sigma-Aldrich. Disodium hydrogen phosphate, sodium dihydrogen phosphate, potassium ferricyanide, ferric chloride, trichloroacetic acid, and ethanol were provided by Merck. Avobenzone (AVO), Bemotrizinol (BMT), octyl methoxycinnamate (OMC) and benzophenone 3 (BP3) were supplied by their own commercial warehouses.

Extract preparation

Most phenolic antioxidant compounds from lichens are extracted more efficiently using acetone, rather than methanol or ethanol (Kosanić and Ranković 2011; Oran et al. 2016). Furthermore, acetone soaking is commonly used to evaluate the protective effects of lichen substances on thalli because acetone causes minimal damage to thalli and does not alter lipid composition compared to other dissolvents (McEvoy et al. 2006; Solhaug and Gauslaa 2012; Veres et al. 2022). Consequently, acetonic extracts were employed in this study, which were obtained from 2 g of grinded lichen material dissolved in 30 mL of pure acetone (Sigma-Aldrich). The extraction procedure involved two cycles of simple maceration, followed by two cycles of dynamic maceration until the samples were exhausted. The solvent was replaced daily after each cycle, and the extracts were filtered and concentrated via reduced-pressure evaporation.

Antioxidant activity

To evaluate antioxidant activity through proton transfer mechanism (Huang et al. 2005; Mishra et al. 2012), the scavenging activity with 2,2'-diphenyl-1-picrylhydrazyl (DPPH) of lichen extracts was evaluated quantitatively to obtain their EC_{50}, and inhibition percentages using the spectrophotometric Brand-Williams protocol at 517 nm in ethanol (Brand-Wiliams et al. 1995; Bondet et al. 1997). Ten concentration ratios were tested during 48 h to obtain EC_{50}, from 0.1 to 10 Extract:DPPH. For scavenging activity the steady-state of the curves at 200 µg mL^{-1} was taken to obtain the inhibition percentage.

$$\%\ Inhibition = \frac{(Abs\ DPPH_0\ -\ Abs\ DPPH_{ext})}{Abs\ DPPH_0} \times 100$$

Ferric ion reducing power (FRAP) was obtained using the ferricyanide assay at neutral pH (pH = 7) to better mimic the physiological conditions (Berker et al. 2007; Işıl Berker et al. 2010). In this assay, iron (II) ion are produced by reduction of ferric chloride and this ion is detected *in situ* by the formation of iron (II) ferricyanide complex or Prussia (Turnbull) blue. This assay is useful for determining antioxidant capacity by electron transfer mechanisms (Huang et al. 2005). Different dilutions of extracts between 10 and 200 µg mL^{-1} were prepared, 1 mL of this solution was mixed with 2.5 mL of phosphate buffer solution pH = 6.6 and then with 2.5 mL of potassium ferricyanide. Then 2.5 mL of 10% trichloroacetic acid was added, and the sample was centrifuged at 3000 rpm for 10 minutes. Finally an aliquot of 2.5 mL of the supernatant was made up to 5.0 mL with distilled water and 0.5 mL of ferric chloride 0.1% solution was added. The iron (II) ferricyanide complex can be detected spectrophotometrically at 700 nm (Berker et al. 2007; Işıl Berker et al. 2010). The higher the absorbance, the better the antioxidant activity of the lichen extracts.

Photoprotective activity

For the most antioxidant active extracts, *in vitro* photoprotective activity was also examined using Manzur methodology to determine SPF and Diffey parameters of spectral amplitude, critical wavelength and UVA/UVB

ratio (Manzur et al. 1986; Diffey 1994; Diffey et al. 2000; Dutra et al. 2004). Different dilutions of extracts and four common commercial sunscreens, AVO, BMT, BP3 and OMC between 10 and 200 µg mL^{-1} were prepared and spectroscopically measured between 290 and 400 nm.

$$SPF_{spectrophotometric} = CF \times \sum_{290\ nm}^{320\ nm} EE\ (\lambda) \times I\ (\lambda) \times Abs\ (\lambda)$$

$$Critical\ wavelength = 0.9 \times \int_{290\ nm}^{400\ nm} Abs\ (\lambda)\ d\lambda$$

Several UV ratios were determined for spectral amplitude, UV-A/UV-B ratio, and UVA1/ UV ratio (Diffey 1994; Diffey et al. 2000; Garoli et al. 2008; Hojerová et al. 2011). To investigate whether the lichen extracts could be proposed as broad-spectrum sunscreens according to FDA criteria.

$$UVA/UVB = \frac{\frac{\int_{320\ nm}^{400\ nm} Abs\ (\lambda)\ d(\lambda)}{\int_{320\ nm}^{400\ nm} d(\lambda)}}{\frac{\int_{290\ nm}^{400\ nm} Abs\ (\lambda)\ d(\lambda)}{\int_{290\ nm}^{400\ nm} d(\lambda)}} = \frac{\sigma\ UV-A}{\rho\ UV-B} \qquad \frac{\sigma\ UV-A}{\rho\ UV-B} = \frac{\int_{320\ nm}^{400\ nm} Abs\ (\lambda)\ d(\lambda)}{\int_{290\ nm}^{400\ nm} Abs\ (\lambda)\ d(\lambda)}$$

For UV-A/UV-B ratio values between 0 and 0.2 mean no protection, 0.2 to 0.4 moderate protection, 0.4 to 0.6 good UV-A protection, 0.6 to 0.8 optimal protection and > 0.8 maximum protection. The UVA-1/ UV ratio measures the degree of absorption in UVA-1 region, high UV-A protection is obtained for values greater than 0.70 (Hojerová et al. 2011; Federal Drug Administration 2019).

$$\frac{aUVA-1}{\lambda} = \frac{5}{3}\left[\frac{Abs_{290\ nm} + Abs_{400\ nm} + (4 \times \sum_{\lambda=295}^{\lambda=395} Abs_{\lambda}) + (2 \times \sum_{\lambda=300}^{\lambda=390} Abs_{\lambda})}{60}\right] \qquad \text{Ratio } \frac{UVA-1}{UV} = \frac{\frac{aUVA-1}{\lambda}}{\frac{aUV}{\lambda}}$$

$$\frac{aUV}{\lambda} = \frac{5}{3}\left[\frac{Abs_{290\ nm} + Abs_{400\ nm} + (4 \times \sum_{\lambda=295}^{\lambda=395} Abs_{\lambda}) + (2 \times \sum_{\lambda=300}^{\lambda=390} Abs_{\lambda})}{110}\right]$$

Statistical analysis

One-way ANOVA was performed on the obtained antioxidant and photoprotective data, prior verification of the statistical assumptions of variance normality and homoscedasticity using Shapiro-Wilk, Levene and Barlett tests performed in R version 4.5.0.

Results and Discussion

In the lichen symbioses, fungi provide protection against herbivory and UV-R. High irradiation, particularly UV, poses a major threat to photosynthetic organisms due to the potential damage to photosystems, and

excessive ROS production (Veres et al. 2022; Mkhize et al. 2022). Hence, antioxidant and photoprotective activities are essential for LF survival.

Antioxidant activity

Thamnolia vermicularis extracts exhibited the highest radical scavenging activity (**Fig. 1**), with an effective concentration (EC_{50}) approximately 13 times higher than BHT (0,136 mg / mg DPPH). Followed by extracts from *Lobariella sipmanii* and *Everniastrum* sp, which grow on shrub branches located at the edges of "*matorrales enanos*" typical of paramo vegetation (Castaño Uribe 2002), and *Peltigera neopolydactyla*, found on light-exposed ground. Paramo conditions comprise permanent exposure to strong direct sunlight, with fluctuating temperature and humidity (Rangel Ch 2000; Castaño Uribe 2002), LF that grow in exposed habitats are more tolerant to light stress than those growing in shaded habitats (Mkhize et al. 2022).

Phenolic compounds are primarily responsible for the antioxidant activity, acting as faster electron donors to substrates at neutral or acidic pH, counteracting radiation-induced damage (Appel 1993; Mishra et al. 2012). Although some phenolics exhibit reversibility in DPPH assay, which can lead to an underestimation of antioxidant activity (Mishra et al. 2012), this was not observed here. The time required to reach steady-state concentrations depends on the antioxidant; ascorbic and gallic acids react within minutes, whereas BHT needs several hours at low concentrations (Mishra et al. 2012). Lichen extracts required an even longer time to stabilise in order to accurately determine EC_{50} and scavenging activity.

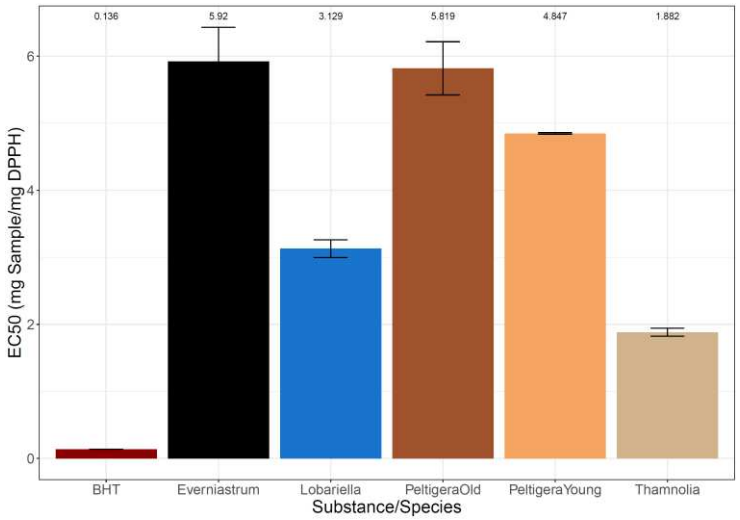

Figure 1 Determination of EC_{50} in mg Extract/mg DPPH using 1,1'-diphenylpicrylhydrazyl method of the lichenized fungi species of Paramo de Sumapaz.

At 20 µg mL^{-1}; BHT presented 92 % scavenging activity, with significant differences (p-value < 0.05) in its EC_{50} compared to the studied extracts. Whereas *Thamnolia vermicularis* was the most active extract with 94 % radical scavenging at 200 µg mL^{-1}, followed by *Lobariella sipmanii* (85 %). *Peltigera neopolydactyla* younger thalli (79 %), *Peltigera neopolydactyla* older thalli and *Everniastrum* sp obtained 75 and 70 % scavenging at

200 µg mL^{-1}, respectively. There were no significant differences between *Everniastrum* sp and *Peltigera neopolydactyla* older thalli extracts and between *Peltigera* younger and older thalli (p-value > 0.05). However, there were significant differences between *Peltigera* younger thalli and *Everniastrum* sp (p-value < 0.05). Interestingly, all Paramo species exhibited radical scavenging inhibition above 70%, which contrasts with more variable results observed for other lichens in different mountainous regions (Luo et al. 2006, 2010; Zambare and Christopher 2012).

Thamnolia vermicularis, a well-known ingredient in traditional Chinese medicine, has been reported to obtain 67 % DPPH radical scavenging activity at 2 µg mL^{-1} and moderate ferric reducing power (FRAP) compared to BHA (Luo et al. 2006). Luo *et al* (2010) studied several Himalayan species at altitudes between 2 000 to 4 000 m, thereon *Peltigera canina* and *P. pretextata* presented antioxidant activities approaching 85 % (Luo et al. 2010; Zambare and Christopher 2012). Himalayan *Everniastrum* species exhibited moderate scavenging activities (69.8 and 77.2 %) (Luo et al. 2010). Similar values were observed hereon for paramo *Everniastrum* species.

Atranorin and salazinic acid have been isolated from *Everniastrum cihrratum* in Bolivian high-mountain grasslands (puna), while decarboxythamnolic and thamnolic acid have been isolated from *Thamnolia vermicularis* (Vila et al. 2011).. Atranorin and salazinic acid are two of the most common lichen substances and have reported antioxidant activity (Studzinska-Sroka et al. 2017; Varol 2018). Among four LF species, *Peltigera laciniata* and *Thamnolia vermicularis* found in Merida paramo highlands of Venezuela exhibited the highest antiradical power in the DPPH assay with 2.72 and 0.95 mL/mg dw extract, respectively (Plaza et al. 2014). Besides, methyl orsellinate and lobariellin were isolated from *Lobariella pallida* in paramo de Sumapaz. Lobariellin was the most active compound for both the DPPH, FRAP, and lipid peroxidation assays, being also cytoprotective, whereas methyl orsellinate was inactive as radical scavenger and as ferric-reducing compound (Perico-Franco et al. 2015).

The FRAP assay allows assessing the ability of a substance to reduce free metal ions through electron transfer, as well as to prevent metal-catalysed *in vivo* formation of various ROS, including hydrogen peroxide or hydroxyl radicals (Wenk et al. 2000; Huang et al. 2005). The neutral pH ferricyanide method was selected due to its resemblance to the pH conditions prevalent within living organisms (Berker et al. 2007). All selected species exhibited low to moderate ferric reducing activity with a concentration-dependent behaviour. Significant differences with BHT (p-value < 0.05), and maximum reducing power at 500 µg mL^{-1} (**Fig. 2**). At lower concentrations, the lichen extracts had similar activity (p-value > 0.05). Nevertheless, at 200 µg mL^{-1} and 500 µg mL^{-1} *Thamnolia vermicularis* extract was the most active followed by *Peltigera*, *Lobariella* and showed no significant differences with the other species (p-value > 0.05) except with *Everniastrum* (p-value < 0.05).

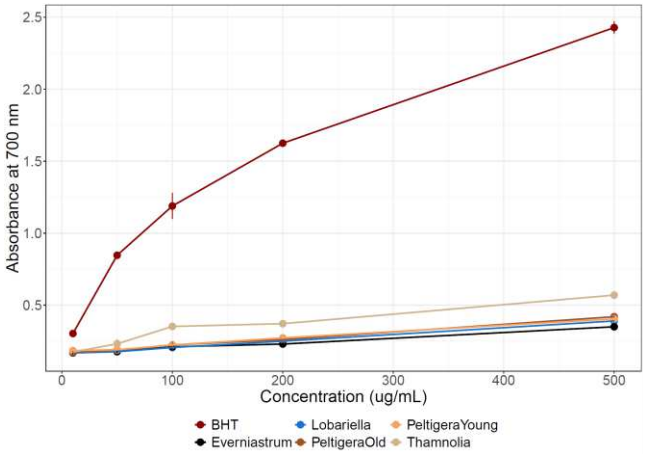

Figure 2 Ferric ion reducing power using ferricyanide method of BHT and lichenized fungi from Paramo de Sumapaz. Concentrations are between 10 and 500 µg mL^{-1}

The ferric-reducing activity of paramo lichens and Himalayan lichens is comparable. *Everniastrum*, *Peltigera* and *Lobariella* had FRAP absorbance values near 0.400, compared to the higher values seen in Himalayan *Everniastrum* species ($Abs_{700\ nm}$ between 0.70 and 0.85) (Luo et al. 2010). *Peltigera* species from Iceland and Hawaii have shown to have low antioxidant activity; however, those with cyanobacterial photobionts have been found to be remarkably effective antioxidants in FRAP and DPPH assays (Hagiwara et al. 2016). Lichens growing in tundra or polar conditions share similar environmental conditions with those growing in high-mountains; especially, temperature, irradiation, and humidity regimes (Boustie et al. 2011).

ROS production in lichen photosystems is unavoidable, even at moderate light intensities. Singlet oxygen and superoxide radicals (O_2^{*-}) are generated in photosystems, then disproportionated to hydrogen peroxide and reduced by Fe^{2+} or Cu^{+} to form reactive hydroxyl radicals ($^{*}OH$) (Beckett et al. 2021). Thus, ferric reducing activity is key for thalli survival. Furthermore, UV-R exposure accentuates ROS production and overwhelms light-dissipation mechanisms, causing oxidative damage to DNA and cell membranes, leading to oxidative stress (Sies 2019; Beckett et al. 2021). Consequently, the prevention of primary oxidation throughout scavenging activity provides additional protection against the harmful effects of UV radiation (Huang et al. 2005; Grether-Beck et al. 2014). Conversely several commercial sunscreens exhibited prooxidant and photosensitising activities; thus, lichen compounds with their constitutive antioxidant activity could be superior alternatives for photoprotection (Cockell and Knowland 1999; Gilbert et al. 2013).

Photoprotective activity

Photoprotection in lichenized fungi is a salient consideration, as excessive irradiation can result in a temporary or long-lasting reduction of photosynthesis, known as photoinhibition (Ndhlovu et al. 2022). Lichens mitigate UV exposure through a combination of mechanisms, such as pigment biosynthesis and accumulation, thalli dehydration, and light scattering (Varol 2018; Veres et al. 2022; Daminova et al. 2024). Lichen substances accumulate in thalli cortex and their concentrations varied seasonally and altitudinally, thereby significantly

reducing transmittance, effectively absorbing UV radiation, while absorbing less in the visible region. This provides protection from UV-R and intense photosynthetic active radiation (PAR) (Huneck and Yoshimura 1996; Gauslaa and McEvoy 2005; Boustie et al. 2011; Nguyen et al. 2013; Beckett et al. 2021; Ndhlovu et al. 2022). Several lichen compounds are produced in response to UV-B light, including dibenzofurans, depsides, depsidones and shikimate derivatives (Boustie et al. 2011). Various colourless compounds like pseudocypherellin A or methyl orsellinate may also offer UV-photoprotection, as have been found for atranorin or usnic acid (Varol et al. 2015; Studzinska-Sroka et al. 2017; Ndhlovu et al. 2022).

Broad-spectrum sunscreens must meet three criteria to be considered effective: Sun Protection Factor (SPF) *in vitro* or *in vivo* above 15, critical wavelength over 370 nm and spectral amplitude through UVA1/UV ratio higher than 0.7 (Hojerová et al. 2011; Stiefel and Schwack 2015; Federal Drug Administration 2019). *In vitro* approaches present some advantages such as low-training requirements, low-costs, high reproducibility and the absence of risk to human volunteers (Nash et al. 2006). SPF measurement accesses UV-B protection and indicates erythema prevention, the higher the SPF, the better the protection (Dutra et al. 2004; Nash et al. 2006; Stiefel and Schwack 2015). Nonetheless, most users apply less sunscreen than recommended (2 mg cm^{-2}); thus, more effective alternatives at low doses are required (Stiefel and Schwack 2015; Sarkany 2017).

In this study, *Lobariella sipmanii* was the sole species that showed UV-B protection at 100 µg mL^{-1}, achieving a SPF value of 17.37 ± 0.47 that exceeded the SPF ≥ 15 threshold (**Fig. 3**), which implies at least 90% erythema reduction after UV-B and UV-A2 (290 – 340 nm) exposure (Nash et al. 2006). While commercial sunscreens (OMC, BP3 and the broad-spectrum BMT) reached the maximum SPF for the *in vitro* procedure (SPF = 40) at 100 µg mL^{-1} (**Fig. 3**).

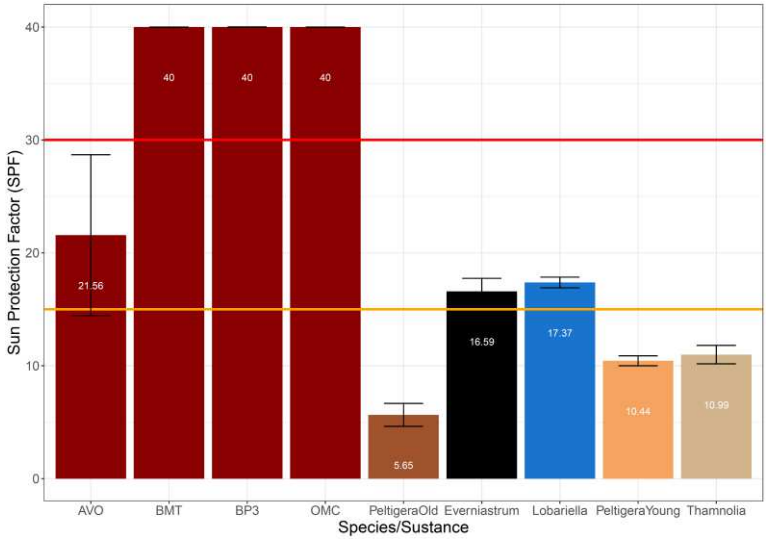

Figure 3 *In vitro* SPF of lichenized fungi extracts and several common commercial sunscreens (AVO, OMC, BP3 and BMT) at 100 µg mL^{-1}. Orange line represents the minimal threshold of 90% erythema reduction (SPF = 15) and red line 95% reduction (SPF = 30).

In second place, *Everniastrum* sp surpassed the threshold of SPF = 15 and offered good UV-B protection with a SPF value of 16.59 ± 1.16 at 100 µg mL^{-1}. *Thamnolia* and the younger thalli from *Peltigera neopolydactyla* exhibited moderate UV-B photoprotection with SPF values of 10.99 ± 0.81 and 10.44 ± 0.44 respectively. *Peltigera neopolydactyla* older thalli have SPF value of 5.65 ± 1.01 and do not offer UV-B photoprotection at 100 µg mL^{-1} (**Fig. 3**). No significant differences were observed (p-value > 0.05) between *Peltigera neopolydactila* younger thalli with *Thamnolia* and between *Everniastrum* with *Lobariella*. In contrast, *Lobariella* and *Peltigera* presented significant differences with the other lichen extracts (p-value < 0.05). Moreover, one-way ANOVA of SPF revealed significant differences for OMC and BMT and the lichen extracts (p-value < 0.05). While AVO presented no significant differences with *Lobariella* and *Everniastrum* extracts at 100 µg mL^{-1} (p-value > 0.05).

SPF values presented a concentration-dependent behaviour. At 200 µg mL^{-}1, *Lobariella* and *Everniastrum* extracts obtained SPF values around 30 and provided excellent UV-B protection at 200 µg mL^{-1}, indicating erythema reduction of about 95 %. Meanwhile *Thamnolia* and younger thalli *Peltigera* showed lower SPF values, 21,19 ± 0.35 and 17,39 ± 3.53 respectively (Supplementary material 1). UV-B photoprotection is relevant due to the generation of DNA damage throughout pyrimidine [2+2] dimerisation, dimerisation of adjacent adenines, as well as guanine oxidation, which are responsible for approximately 90 % of skin cancers worldwide (Ravanat et al. 2001; Grether-Beck and Krutmann 2009; Stiefel and Schwack 2015). Furthermore, excessive exposure to UV in lichens and in humans induces ROS production (Grether-Beck and Krutmann 2009; Mkhize et al. 2022; Ndhlovu et al. 2022). While UV-B is associated with mutations and sunburn, UV-A is mainly related to photo-ageing and skin cancer (Stiefel and Schwack 2015; Shanbhag et al. 2019). In this latter case, DNA mutations initiate most skin carcinogenic processes, with oxidative distress amplifying its harmful effects (Sarkany 2017; Klaunig and Wang 2018).

Consequently, *Lobariella sipmanii* has been identified as the most promising source for obtaining UV-B absorbing compounds, with the highest UV-B absorption at 100 µg mL^{-1}, followed by *Everniastrum* and *Thamnolia*, which highlights the potential of extremophile organisms for pharmaceutical or cosmetic applications. Nonetheless, further research is required to ascertain their ecological role before their potential utilisation as pharmaceutical or cosmetic actives (Varol 2018).

Critical wavelength and spectral amplitude

Skin photoprotection should encompass not only UV-B but also a broader range of wavelengths (Nash et al. 2006; Krutmann et al. 2021). Critical wavelength considers UV protection as a whole, rather than UV-A and UV-B as separate entities. This photoprotective parameter is related to spectral shape and is independent from sunscreen layer thickness and other undesirable variables (Nash et al. 2006). It is widely accepted by regulatory agencies as an indicator of UV-A photoprotection; the longer the critical wavelength, the broader the UV photoprotection (Stiefel and Schwack 2015). Lichen substances have been shown to function as sunscreens for thalli, because the removal of these compounds resulting in enhanced photoinhibition in various lichens; this

might be attributable to increased thalli transmittance and changes on thalli hydration state (Veres et al. 2022; Ndhlovu et al. 2022). Many lichen compounds absorb UV-R, violet, and blue light, thereby protecting the oxygen-evolving complex of photosystem II from the photoinhibitory effects of these wavelengths; the yellowish colouration of certain lichen compounds is a consequence of this necessity (Beckett et al. 2021). These compounds absorb photoinhibitory light and subsequently emit it at longer wavelengths through radiative mechanisms, such as fluorescence or thermal dissipation (Nguyen et al. 2013).

In this study, *Thamnolia* and *Lobariella* exhibited the highest UV-A protection, with critical wavelength values of 367.9 and 355.8 nm respectively (**Fig. 4**). Synthetic sunscreens AVO and BMT had critical wavelengths of 378.0 ± 1.4 and 367.9 ± 0.8 nm respectively and received four-star UV-A ratings (**Fig. 4**) (Diffey 1994). This behaviour was expected as AVO is the most commonly used UV-A sunscreen and BMT is a broad-spectrum sunscreen (Stiefel and Schwack 2015). *Thamnolia* presented no significant differences from BMT (p-value > 0.05). Whereas *Lobariella* ranked second among the lichen extracts and had no significant differences with *Everniastrum* (p-value > 0.05), it differed from *Peltigera, Thamnolia* and BMT, AVO, and OMC (p-value < 0.05). *Everniastrum*, *Lobariella* and *Thamnolia* were rated as three-star sunscreens (Diffey 1994), indicating the possible presence of compounds that offer broad-spectrum UV protection.

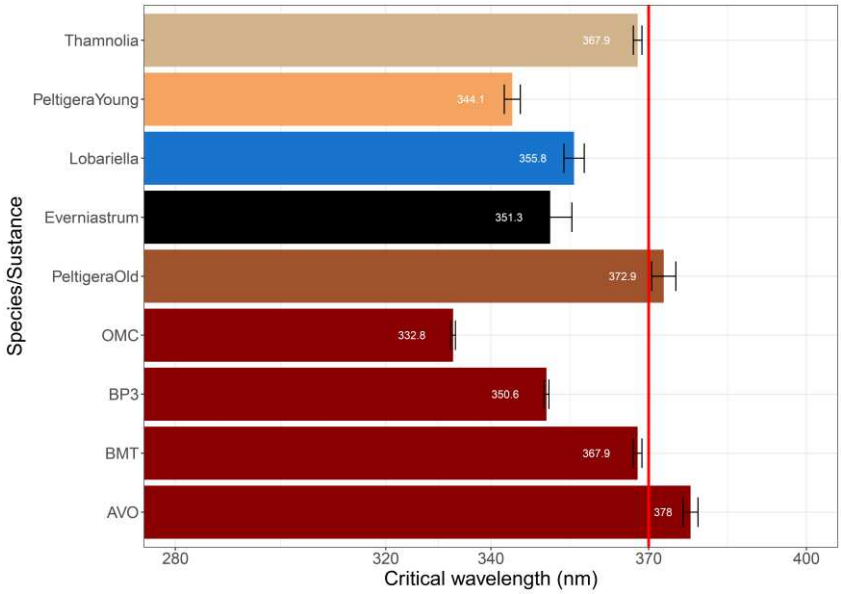

Figure 4 Critical wavelength of the lichen extracts from Paramo de Sumapaz. The red line is the minimal FDA criterium for broad-spectrum sunscreens λ = 370 nm (Federal Drug Administration 2019).

Due to the dependence between spectral form and critical wavelength (Nash et al. 2006), the case of *Peltigera neopolydactyla* older thalli requires further analysis. As will be shown afterwards this spectrum has a practically flattened form (**Fig. 6**), which consequently alters the critical wavelength calculation, yielding a result of 372.9 ± 2.3 nm. This result is potentially misleading because the other photoprotective results for this sample are the

less active from the analysed samples. Moreover, TLC-UV results of this extract (not shown) exhibited a reduced secondary metabolites content in comparison to the younger thalli.

UV-A is 20 times more intense than UV-B and penetrates deeper into human skin reaching the dermis and subcutaneous tissue (Stiefel and Schwack 2015). Hence, UVA photoprotection is also necessary, particularly UVA-1 (340 – 400 nm), due to its skin proinflammatory effects, its role in extracellular matrix degradation, and its contribution to ROS production in skin after sun exposure, which contribute to skin carcinogenesis, especially squamous cell carcinoma and melanoma (Zastrow et al. 2009; Stiefel and Schwack 2015; Krutmann et al. 2021). In contrast to UV-B, for which a clear standard, namely SPF, exists; there is no unique photoprotection measurement for UV-A that clearly communicates protection levels to consumers through labelling (Nash et al. 2006; Garoli et al. 2008; Sarkany 2017). The measurement of UV-A photoprotection is based on several spectrometric parameters, including critical wavelength, UVA/UVB ratio, UVA-1 / UV ratio, amongst others (Diffey 1994; Diffey et al. 2000).

The content of secondary metabolites in mountain-inhabiting LF varies with altitude and season, depending mainly in moisture rather than in irradiation, showing higher amounts in spring, where the conditions are more humid, compared to autumn. Furthermore, after acetone rinsing, *Xanthoria parietina* resynthesized parietin only in humid conditions (Veres et al. 2022). In our case, the absence of annual seasonality and higher daily environmental moisture variability in paramos may result in more constant secondary metabolites content, but this hypothesis required further validation.

Nonetheless, under stable environmental conditions, highly exposed LF exhibited higher photoinhibition resistance but lower plasticity. Besides, LF exhibited species-specific differences in tolerance to photoinhibition when subjected to abrupt variations in light intensity, with these differences also being influenced by habitat variability (Mkhize et al. 2022). LF surviving in more extreme microhabitats exhibited better adaptation to dehydration and photoinhibition, owing to the prevalence of their constitutive protection mechanisms over induced mechanisms. Organisms reliant on induced mechanisms are normally unable to adequately respond to sudden condition changes characteristic of harsh environments (Gasulla et al. 2021; Mkhize et al. 2022). Chemical defenses can be categorised as either constitutive or facultative (Asplund et al. 2009). Moreover, these preventive mechanisms obviate the necessity for using repair mechanisms; hence, the higher damage prevention, the greater advantage for the organism (Cockell and Knowland 1999).

Furthermore, the UV-A radiation around 355 nm causes a peak in free-radical production due to photosensitizers such as riboflavin and porphyrins (Zastrow et al. 2009). Accordingly, an UVA/UVB ratio value of 0.8 is considered to represent adequate UVA protection (**Fig. 5**) (Diffey 1994). BMT, BP3, and *Thamnolia vermicularis* extract achieved a two-star rating, meaning moderate UVA photoprotection (Diffey 1994). AVO was rated four-stars, giving maximum UV-A photoprotection, and was statistically significant different from all lichen extracts and sunscreens (p-value < 0.05). None of the extracts evaluated fulfil the criteria for UVA-1 ratio and UVA/UVB ratio (**Fig. 5**).

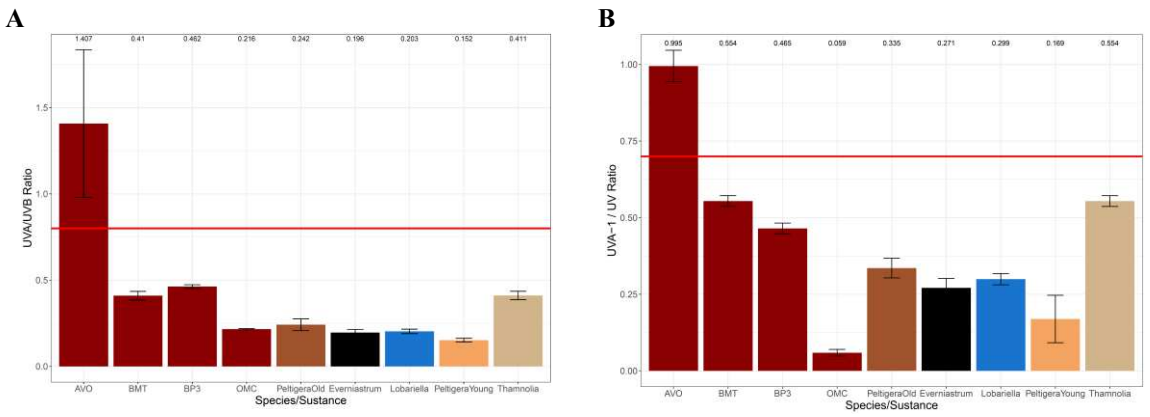

Figure 5 *In vitro* UVA/UVB ratio (**A**) and UVA1 / UV ratio (**B**) of lichen extracts from Paramo de Sumapaz. The red line corresponds to FDA criteria for broad-spectrum sunscreens (Hojerová et al. 2011; Federal Drug Administration 2019).

Thamnolia vermicularis extract obtained UVA/UVB and UVA-1 ratios comparable to BMT (p-value > 0.05); therefore, this lichen extract had the best performance with moderate UV-A and UVA-1 photoprotection. Whereas lichen extracts did not differ significantly from OMC, BP3 and BMT for UVA/UVB ratio (p-value > 0.05), they varied more for UVA-1 ratio (p-value < 0.05). *Lobariella* and *Everniastrum* obtained a one-star rating for UVA protection at 100 µg mL^{-1}, indicating low UV-A photoprotection and no observed differences within them (p-value > 0.05); thus they were grouped as low UV-A and UVA-1 protectors (p-value > 0.05). Conversely, *Peltigera* should not be suggested as a photoprotector, as it obtained the lowest FPS, critical wavelength, UVA/UVB ratio and UVA-1/UV ratio. Indeed, misleading outcomes due to the flattened spectral form were found for the UVA-1/UV ratio for *Peltigera neopolydactyla* older thalli, which were similar to critical wavelength observations.

Effective UV-screening molecules must include aromatic groups, substituents with non-bonding or π electrons, and a mixture of electron-donating (long hydrocarbon chains, hydroxyl, amine) and electron-withdrawing (carbonyl, amide, ester) groups (Nguyen et al. 2013; Varol 2018). For both commercials organic sunscreens and lichen substances, aromatic rings linked to carbonyl groups and at least one ortho-hydroxyl group are structural features typical from photoprotective compounds (Radice et al. 2016; Varol 2018). Lichen depsides depsidones, dibenzofurans, chromones and diphenyl ethers are UV-B absorbers, while pulvinic acid derivatives are UV-A absorbers (Nguyen et al. 2013). Atranorin and usnic acid evidenced UV-photoprotection against UV-B induced damage (Varol 2018). Depsides and depsidones such as gyrophoric acid and salazinic acid have UV-B photoprotective properties and could serve as UV-A boosters (Radice et al. 2016; Ureña-Vacas et al. 2022). Despite their potential as sunscreens, under natural conditions, lichen substances not only defend thalli from excessive UV-R irradiation, avoiding photoinhibition, but also from lichenicolous fungus and potential grazers (McEvoy et al. 2006; Asplund et al. 2009; Nguyen et al. 2013; Ndhlovu et al. 2022).

Commercial sunscreens like AVO, BP3 and OMC present drawbacks such as allergenicity, photoinstability, photosensitisation and endocrine disruption (Gilbert et al. 2013; Stiefel and Schwack 2015). Conversely, lichen compounds are photostable compared to commercial sunscreens, blocking UV-R but not PAR, and preventing DNA damage associated with singlet oxygen (Nguyen et al. 2013). *Thamnolia* and *Lobariella* extracts provide low UV-A and UVA-1 protection, but moderate to high UV-B photoprotection and antioxidant activity. This latter feature is lacking in currently used sunscreens, which make lichen substances better alternatives for photoprotection. Exogenous antioxidants offer several benefits including the mitigation of the UV molecular photooxidative processes, and the amelioration of wrinkled, photoaged, discretely pigmented skin (Shanbhag et al. 2019; Krutmann et al. 2021). Moreover, future studies on the lichen extracts UV-R absorption could deepen our comprehension of the LF photoprotective mechanisms. Spectrophotometric parameters like SPF, critical wavelength, and UVA/UVB ratio may further enhance the comprehension of light-physiological processes in photosynthetic organisms. Finally, this awareness of potential applications could also support conservation efforts in threatened areas like paramos.

Peltigera neopolydactyla and optimal defense theory (ODT)

Peltigera neopolydactyla is a green-algae photobiont lichen. In this study, thalli showed subtle morphological differences and were therefore treated separately for extraction and assays. Some thalli contained higher concentrations of UV-absorbing compounds and exhibited greater antioxidant activity than others (**Fig. 1 & 2**). A possible explanation of these variations is thalli age because mature thalli generally produce less variety and lower amounts of lichen substances. UV spectroscopy of both *Peltigera* extracts at 50 µg mL^{-1} confirmed the aforementioned observation (**Fig. 6**).

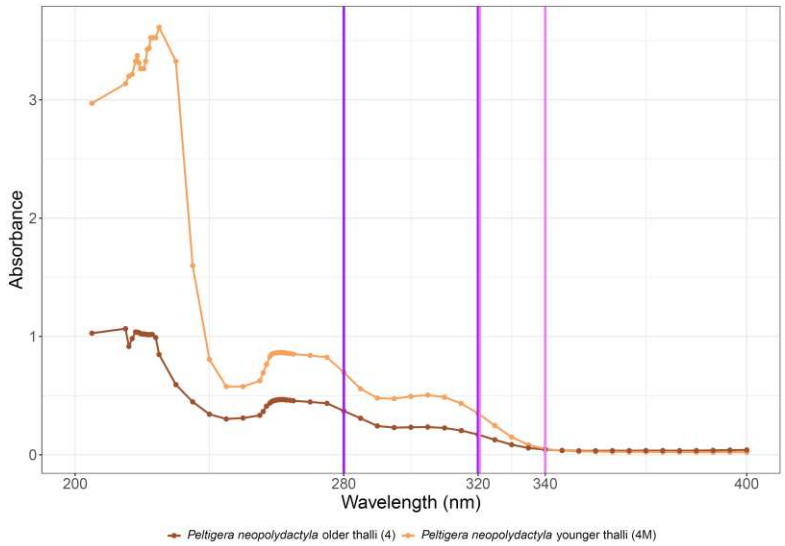

Figure 6. UV spectra of *Peltigera neopolydactyla* (4 & 4M) at 50 µg mL^{-1}. The purple lines highlight UV-B region (280 – 320 nm) and the pink lines focus on UV-A 2 region (320 – 340 nm).

According to optimal defense theory (ODT), organisms efficiently allocate energy efficiently, prioritising defensive mechanisms like secondary metabolites synthesis, particularly in tissues critical for survival (Hunziker et al. 2021). The allocation of secondary metabolites can be explained by protection against both photoinhibition and herbivory, depending on the role of plant structure in its fitness and its vulnerability to attack from other organisms (Asplund et al. 2010; Hunziker et al. 2021). Young tissues are nutrient-rich, more photosynthetically productive, making them more valuable for plant fitness. Consequently, they are more susceptible to herbivory and must produce higher levels of secondary metabolites, as has been reported for lichens and for vascular plants (Stephenson and Rundel 1979; Seriña Ramírez et al. 1991; Asplund et al. 2010; Hunziker et al. 2021).

For other lichenized fungi with green-algae photobionts from mountainous regions like Mongolia have pulvinic acid derivatives, which are yellowish or orange coloured. These lichen substances absorb UV and photosynthetic active radiation, attenuate incident radiation and bind to metal ions in a pH-dependent manner (Hauck et al. 2009; Boustie et al. 2011). Similarly, for radial-growth foliose lichen *Lasallia pustulata*, secondary metabolites were more concentrated in the younger, outer part of thalli (Seriña Ramírez et al. 1991). Nevertheless, herbivory protection may also be a factor, in the fruticose lichen *Letharia vulpina*, younger thalli contain higher concentrations of vulpinic acid (Stephenson and Rundel 1979). Pulvinic acid derivatives have mainly an anti-herbivory role, the most widely distributed derivative is vulpinic acid, which is toxic to vertebrates and may act as feeding deterrent or antiherbivore compound (Rundel 1978; Stephenson and Rundel 1979).

Metabolite partitioning does neither affect fast-growing plants, nor does it appear to increase their fitness. Indeed, higher metabolite concentrations are associated with longer growing seasons and higher temperatures, both of which favour herbivory (Kooyers et al. 2017). Among these factors, thallus growth rate may be relevant for paramo lichens, *Peltigera* thalli can grow 2 - 4 cm per year in moist environments (Smith 1961). Slow-growing and resource-limited organisms like LF often have constitutive defenses, such as secondary metabolites. Nonetheless, when the concentration of secondary metabolites is high due to other environmental factors, such as UV-irradiation, organisms rarely show induced responses to herbivory (Asplund et al. 2009). Finally, an alternative hypothesis that could explain the observed differences between *Peltigera* thalli is that the examined samples belong to different chemotypes (Asplund and Wardle 2017), despite both thalli were collected simultaneously at the same area.

Conclusions

The most active extract for radical scavenging and ferric ion reducing activity was *Thamnolia vermicularis*, followed by *Lobariella sipmanii* and *Everniastrum* sp for DPPH scavenging, and for *Peltigera neopolydactyla* and *Lobariella* in FRAP assay. This indicates that *Thamnolia*, and *Lobariella* were the most effective antioxidant extracts, acting through both proton-mediated and electron transfer mechanisms.

Lobariella sipmanii, a paramo lichen, can be proposed as UV-B photoprotectant because it obtained the highest *in vitro* SPF value, while *Thamnolia vermicularis* and *Lobariella sipmanii* had the largest critical wavelength values. Hence, these two species can be proposed as sources of broad-spectrum sunscreens and are rated as three-star sunscreens according to Diffey classification system. Alternatively, the *Everniastrum* sp extract can be proposed as a UV-B screening agent. Despite having moderate antioxidant activity, *Peltigera neopolydactyla* was the least photoprotective extract. Although critical wavelength and spectral amplitude indexes obtained spectrophotometrically are easy to obtain, some caution must be implemented due to their dependence on the shape of absorbance curve, particularly with extracts whose metabolite concentration is low, such as *Peltigera neopolydactyla* older thalli, to avoid misleading interpretations.

Species inhabiting highly exposed habitats not only exhibit antioxidant and photoprotective activities but also demonstrate differences between habitats. *Thamnolia*, which grow on rocks, exhibited better antioxidant activity, critical wavelength and spectral amplitude than phorophyte-inhabiting (*Lobariella*, *Everniastrum*) and soil-growing lichens (*Peltigera*). This may indicate that environmental conditions induce protective mechanisms in organisms. Additionally, the eventual commercial uses of lichens and their compounds could help in conservation efforts, especially on endangered areas like Paramos.

Although both samples of *Peltigera neopolydactyla* were collected simultaneously from the same sites, morphological differences were observed, as well as variations in the concentrations of secondary metabolites, antioxidant activity and photoprotective activity. These variations can be explained by optimal defense theory (ODT), which suggests that younger thalli contain higher concentrations of phenolic compounds due to their role in lichen reproduction and survival. This is consistent with previous reports for vascular plants and LF.

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