Phytochemistry Letters 70 (2025) 104063

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Phytochemistry of crustose lichens from Mount Rossman, Ellsworth Land, Antarctica

Rodrigo A. Contreras [*], Marisol Pizarro, Pablo Zamora [1], Gustavo E. Zúñiga [**]

Laboratorio de Fisiología y Biotecnología Vegetal, Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile, Chile

ARTICLE INFO

Keywords:
Antarctica
Lichens
Phytochemistry
Sugars
Union Glacier

ABSTRACT

Lichens are symbiotic organisms that inhabit all climate zones of this planet, including even those in Antarctica, thanks to their sophisticated biochemical machinery. This study investigated the phytochemical composition of three lichen species (*Pleopsidium* sp., *Sarcogyne* sp., and *Rusavskia* sp.) collected from Mount Rossman, Antarctica. We used liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) to putatively assign key secondary metabolites, including anthraquinones and various depsides and depsidones, important for UV protection and antimicrobial defense. We also quantified the lichens' sugar profiles by high-performance liquid chromatography with refractive index detection (HPLC-RID) using external standards. Here, we provide new perspectives on how these lichens utilize metabolic strategies to survive under extreme desiccation conditions in their terrestrial environment and highlight their biotechnological and pharmaceutical applications.

1. Introduction

Lichens are fascinating organisms formed through a symbiotic partnership between a fungus (mycobiont) and a photosynthetic partner (photobiont), which may be an alga or cyanobacterium (Arun et al., 2023; Morillas et al., 2022). In this relationship, the photobiont carries out photosynthesis, converting inorganic carbon into organic molecules like sugar alcohols. These sugar alcohols are then transferred to the mycobiont, which processes them into essential sugars and other organic compounds that support the symbiosis and help both partners maintain balance (Arun et al., 2023). This unique cooperation allows lichens to thrive in diverse environments, including some of the most extreme places on Earth, like Antarctica (Spribille et al., 2022).

In Antarctica, lichens are the only visible organisms that have managed to survive in regions like Union Glacier, displaying remarkable resilience to harsh conditions such as freezing temperatures, intense UV radiation, and long periods of dehydration (Beckett et al., 2021; Bednaříková et al., 2020; Singh et al., 2022). On Mount Rossman (79°47'S, 82°48'W), lichens play a vital ecological role by colonizing barren surfaces and helping with soil formation, thus contributing to the stability of the local ecosystem. However, despite their importance, there is still limited knowledge about the chemical composition of these Antarctic lichens. Understanding their chemical profile is crucial to elucidating the biochemical strategies that enable them to survive in extreme environments (Goga et al., 2020).

Lichens from the *Rusavskia*, *Sarcogyne* and *Pleopsidium* genera have attracted attention for their impressive adaptations to extreme climates and the wide range of secondary metabolites they produce. These compounds, including phenolic acids, depsidones, depsides, and other phenolic substances, play key ecological roles such as protecting against UV light, inhibiting microbial growth, and defending against herbivores and pathogens (Canini et al., 2023; García et al., 2020; Scur et al., 2022). Additionally, these metabolites function as antioxidants, helping the lichens neutralize free radicals generated by environmental stress, and thus promoting their longevity and stability (Ndhlovu et al., 2024).

This study aimed to putatively identify *Rusavskia* sp., *Sarcogyne* sp. and *Pleopsidium* sp. collected from Mount Rossman, and to characterize their phytochemical profiles and sugar profiles using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) and high-performance liquid chromatography with refractive index detection (HPLC-RID), respectively. These methods provide a detailed interpretation of the compounds present, offering valuable insights into the chemical diversity and metabolic adaptations that allow these lichens to endure such hostile conditions (Devashree et al., 2021).

* Correspondence to: The Not Company, 3550 Quilín Ave., Macul, Santiago, Chile, 454 Shotwell St., San Francisco, CA 94110, USA.
** Correspondence to: Facultad de Química y Biología, Universidad de Santiago de Chile, L. B. O'Higgins Ave. #3363, Estación Central, Santiago, Chile.
E-mail addresses: rodrigo.contrerasar@usach.cl (R.A. Contreras), gustavo.zuniga@usach.cl (G.E. Zúñiga).
[1] Fundación Chile, Santiago, Chile.

https://doi.org/10.1016/j.phytol.2025.104063

Available online 22 October 2025

By tentatively assigning the secondary metabolites in these lichens, this research sheds light on their ecological functions and opens doors to potential biotechnological or pharmaceutical applications. In the context of climate change and the search for new bioactive compounds, this study enhances our understanding of Antarctic lichens and their role in the resilience of polar ecosystems.

2. Material and methods

2.1. Biological samples

Three different lichen samples were collected from the moraine of Mount Rossman (79°47'S, 82°48'W) during the first Union Glacier expedition, which was part of the 51st Antarctic Scientific Expedition organized by the Chilean Antarctic Institute. The lichens were initially placed in paper bags for preservation and transported to Union Glacier Station. After a week of preservation at the station, the samples were transferred to a laboratory in Santiago, Chile, for further analysis. The total dry masses of the collected samples were approximately 230 mg (sample 1), 350 mg (sample 2) and 280 mg (sample 3), which limited the feasibility of large-scale purification or structural elucidation techniques.

2.2. Identification using 18S rDNA sequencing

For genomic DNA extraction from the lichen samples, we used the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, USA), following the manufacturer's protocol, with adaptations for 10 mg samples. The purified DNA was then quantified and its quality was assessed using spectrophotometric techniques. Once extracted, the DNA samples were sent to Macrogen Inc. (Seoul, South Korea) for sequencing of the 18S rDNA region, which was used to identify the species through comparison with GenBank sequences.

2.3. Metabolite analysis

2.3.1. HPLC analysis of sugars

To analyze the sugar content, 10 mg lichen samples were extracted with 80 % ethanol over one week using the diffusion method. After extraction, the samples were filtered through 0.45 μm PVDF membranes, collected in glass vials, and freeze-dried at −60 °C. The sugar profiles were analyzed using high-performance liquid chromatography (HPLC) with a refractive index detector. The dried extract was resuspended in 100 μl of a solution of 50 mg/l EDTA-Ca^{2+}. The sugar profile was analyzed using a Sugar-Pak I column (300 × 6.5 mm) (Waters Corp., MS, USA) at 75 °C, with an isocratic mobile phase of 50 mg/l EDTA-Ca^{2+} at a flow rate of 0.35 ml/min. Standards were used to detect and quantify the different sugars present (Pizarro et al., 2019).

2.3.2. LC-MS/MS analysis of phytochemicals

For the phytochemical analysis, 10 mg lichen samples were extracted by diffusion over three days in a 1:1 methanol:chloroform mixture. The extract was filtered through 0.45 μm PVDF membranes and injected into a liquid chromatography-mass spectrometry (LC-MS/MS) system (Agilent 1200s-6410). The elution was conducted using a binary solvent system at 25 °C. The mobile phase consisted of (A) 10 mM ammonium acetate (pH 4.0) and (B) 10 mM ammonium acetate in 95 % acetonitrile (pH 4.0). A Zorbax Eclipse C18 column (15 × 4.6 mm, 5.0 μm) was used as the stationary phase. The elution system progressed as follows: 0–5 min, 0–50 % B; 5–10 min, 50–52 % B; 10–20 min, 52–100 % B; 20–35 min, 100–65 % B; and 35–40 min, 65–0 % B, with a flow rate of 0.6 ml/min. Ionization was performed using electrospray ionization with a capillary voltage of 4000 V, a nebulization pressure of 3.0 bar, nitrogen flow of 10 l/min, and a gas temperature of 330 °C in negative mode. The metabolites were putatively assigned using the METLIN MS/MS database (Contreras et al., 2019), following Metabolomics Standards Initiative (MSI) Level 2 confidence criteria (Sumner et al., 2007), as no co-injection with authentic standards.

2.4. Statistical analysis

Results were analyzed using one-way ANOVA ($p < 0.05$) and Tukey's post hoc test, with three biological replicates and two technical repetitions.

3. Results

3.1. Morphological description

The collected samples were brought to the laboratory for a detailed analysis of their physical characteristics. Each of the three samples displayed notable differences in color, texture, and overall appearance (Fig. 1). The key observations for each sample were as follows:

- **Sample 1** featured a crustose thallus with bright yellow-green spots, similar to those seen in the other samples, and was also firmly attached to the substrate. These bright areas indicated the possible presence of specific pigments. The surface was rough and embedded in the substrate with an irregular distribution, forming compact clusters. As with the other samples, no vegetative or reproductive structures were visible (Fig. 1a).
- **Sample 2** had a deep black thallus attached to the rock. Its structure was irregular and fragmented, with growth occurring in scattered patches across the rock's surface. The texture was rough to the touch. As with Sample 1, no signs of vegetative propagation or reproductive structures were detected (Fig. 1b).
- **Sample 3** had a crustose thallus tightly bound to the rocky surface, and a compact and continuous structure without any lobes or leafy extensions. Its color was a striking bright orange. The surface of the thallus appeared slightly granular, with fine and consistent wrinkles and well-defined edges. No vegetative structures, such as isidia, soredia, or visible reproductive structures, were observed, which could suggest that this sample was not in a reproductive phase (Fig. 1c).

3.2. 18S identification

We extracted genomic DNA from three lichen samples collected at Mount Rossman, amplified the 18S rDNA region by PCR, and then sequenced the PCR products for species identification. The sequences obtained through this process are summarized as follows:

Sample 1

CAGATACCGTCGTAGTCTTAACCA-
TAAACNNNGCCGACTAGGGATCGGNCGGTGTTATTACNTT-
GANCCGTTCGGNACCTTACGA-
GAAATCAAAGTCTTTGGGTTCTGNGGGGAGTATGNTCGCNAGNCT-
GAAACTTAAAGACATTGNCGGNAGGGCACCAA-
CAGGCGTGGANCCTGCGGCNTAACTGACTCAA-
CACGGGAAACTCACNNCAGGTCCAGACATAATAAGGATTGACA-
GATTGA-
GAGCTCTTTCTTGATCTTATGGGNGGTGGTGCATGGCCGTTTT-
TAGTTGGTGGAGTGATTTGTCTGCTNAATTGCGATAACGAACGA-
GACCTTAACCTGCTAAA-
TAGCCCGGTCAGCTTTGGCTGGCCGCCGGCTTCTTA-
GAGGGACTATCGGCTCAAGCCGATGGAAGNTTGAGGCAAAAA-
CAGGTCTGTGATGCCCTTAGATGTCCTGGGCCGCACGNGCGTTA-
CACTGACAGAGA-
CAACGAGNTCATCACCTTGGCCGAAAGGTCCGGGTAATCTTGT-
TAAACTCTGTCGTGCTGGGGATA-
GAGCATTGCAANTATTGCTCTTCAACGAGGAATGCCTAGTAAGCG-
CAAGTCATCAGCTTNCGTTGATTACGTCCCTGCCCTTTGTACA-

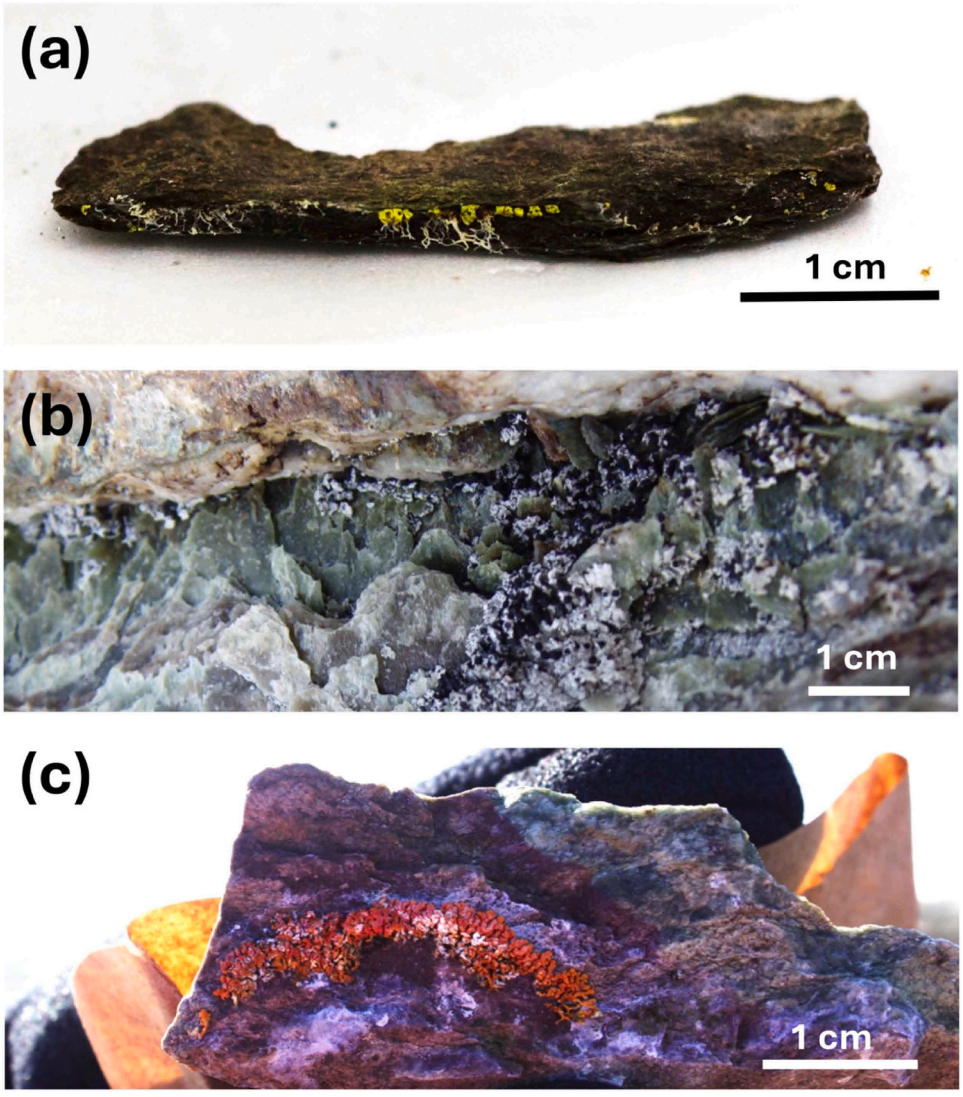

Fig. 1. Lichens collected at Mount Rossman, near Union Glacier, Antarctica, growing on rocks, displaying distinct thallus pigmentation and surface microstructures. (a) Lichen with yellow-green crustose thallus emerging from the rock surface. (b) Lichen with dark crustose thallus tightly adhered to the rock, with scattered darkened surface zones. (c) Lichen with bright orange crustose thallus forming a continuous band-like growth, partially embedded in the rock. The scale bar in each image is 1 cm.

CACCGCCCGTNGCTACTACCGATTGAATGGCTCAGTGAGGCCTTCG-
GACTGGCTCAGGGAGGTCGGCAACGACCAACCCAGAGC

Sample 2

CAGTTATCGTTTATTTGATAGTACCTNGCTACTTGGA-
TAACNGTGGTAATTCTAGAGCTNANACNTGCTAA-
NAAAACCTCGACTTCGGAAGGGGTGTATNTATTAANA-
TAAAAAACCAATGCCCTTCGGGNCTCCNTGGTNATTNA-
TAATNNCTCAACGAATCGCATGGCNTTGCGCCGGCGATGGTNCATT-
CAAATTTCTGCCCANTCAACTTTCGATGGTAGGA-
TAGTGGNCTACCNTGGTGTNAACGGGTAACGGGGAANTAGGGTNC-
TATTCCGNAGAGGGAGCCTGAGAAACGGNTACCA-
CATCCAANGANGCAGCANGGCGCGCAAATTACCCAATCCC-
GACNCGNGGAGGTAGTGACAATAAATNCTGATA-
CAGGGCCCTTTCGGGTCTTGTNATTGGAATGAGTA-
CAATTTAANTCCCTTAACGAGGANCAATTGGAGGGCAAGTCTGGTG-
GACGATTNAGTCTCGGCGGAGGGTGGGCTGCGGCCCCN-
CACGCTGGCGCCAGGAACNAGTTGCCCNNAGCTCTCGTGAGCNT-
GAGGGTTGATTNAGTGGGGCTTTATGCTNTGCAAGGTGTTCGG-
CACGGGNANGGCCATTNCTGGCTNCATCCCGTGGGTGAGGGTCACTT-
GACCGCCACCCTTAACGCGCGCANGGACGCCGGTGAGTTGAA-
GATGCATCTNACTTGAGGGACGTGCNTATCCCGCCCGTAAAGGGTCA-
GAGTAGCCTGCATG

Sample 3

GGAA-
CACCCTAATGNTCCAGGTACCTCNCNCTAAGCTGAAAGGCCGGGG-
CACGGTTACAATCCTCGNGATATCA-
CAATGAGTNACCCGCAGCCNCGGGCCTACAGTGAA-
CACTNTCCCANNTTCACTACGGCAGTGGTNCANAGATTA-
GATGGGTGTGGGTNGCGTGGACTCGATNTGAGGGGAGCCCATGAG-
CAAANGCAGCTCTNCCGACTNGACCATTGGTTGA-
TATGGTAANNGTCCGGCGGAGCTTTCACGGGCAGCAGA-
CAGTGCAGNNTCCCCCCAAGTTGTTTGCGAGTTCTACTTGAGCTT-
NAATNCAGTCTGCAATCGTGTGATTGCGAATTCAA-
GACTNACGGGAGCCTGCGGCTTAATTTGACTCAA-
CACGGGNAAACTCACCAGGTCCAGNNCNAATAAGGATTGACA-
GATTGAGAGCTCTTTCTTGATCTTGTGGATGGTGGTAGAA-
CAGTNCAGGTGCCACCTNCAGCTACGGCA-
TAGNTNGCCCGTCTCCGCGCGGGCGA-
CACTCTCAAGGTGCTGAAAGCCTCCGGTGTGAAGGCACGGAGGTAT-
CAGCANCCGGGCGTGCTCTCTGGCGNGCNTTGGCCCACA-

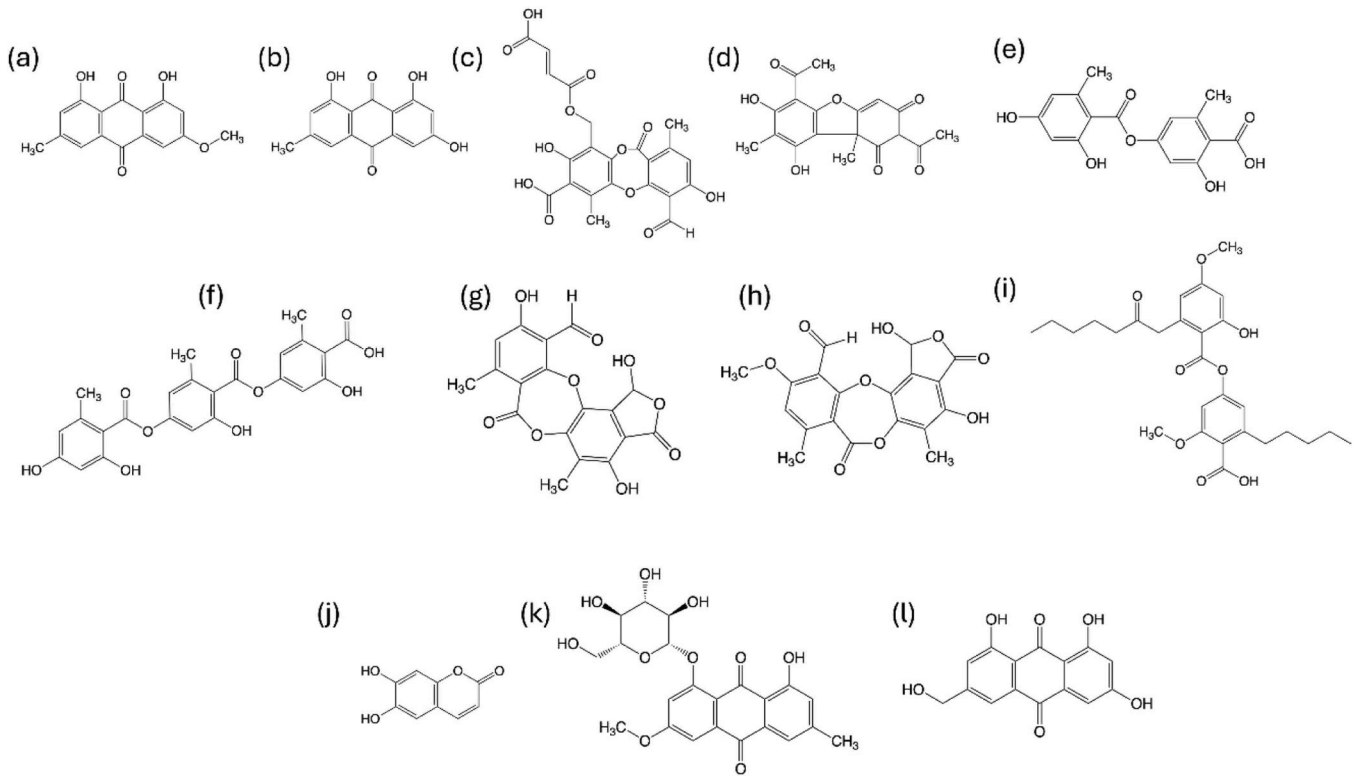

Fig. 2. Chemical structures of the more abundant secondary metabolites identified in the collected lichens. (a) parietin (physcion); (b) emodin; (c) fumarprotocetraric acid; (d) usnic acid; (e) lecanoric acid; (f) gyrophoric acid; (g) norstictic acid; (h) stictic acid; (i) confluentic acid; (j) esculetin; (k) rheochrysin; (l) citreorosein (purpurin).

GACTAAATGGGAGTGGGCTGGTGCTCTNCAGTGGAGCTCCAGTTG-
TAAGATA-
TAGCCGNACCCGGGGCTGCAAGGCTCCCGGNATCANNTG-
CATGGCCGTTCTTAGTTGGTGGAGTGATTG

3.3. BLASTn analysis

We compared the sequences obtained from the lichen samples against those in GenBank using BLASTn to find similarities with known species. The results for each sample are outlined below:

- **Sample 1** had a 94.18 % sequence match with *Pleopsidium chlorophanum* (accession **DQ525570.1**), with 100 % query coverage and an E-value of 0.0. Another close match was *Pleopsidium gobiense* (accession **DQ525567.1**), which showed 94.13 % identity with 100 % query coverage.
- **Sample 2** showed 92.81 % identity with *Sarcogyne privigna* (accession **KJ766792.1**), with 100 % query coverage and an E-value of 0.0. Additional matches included *Keissleriella taminensis* (accession: **AB797306.1**), which had 87.55 % identity and 63 % query coverage.
- **Sample 3** had 95.84 % identity with *Rusavskia elegans* (accession **KJ766790.1**), with 100 % query coverage and an E-value of 0.0. Another notable match was *Xanthoria elegans* (accession **AJ535296.1**), which showed 96.81 % identity and 100 % query coverage. The species was identified as *R. elegans* based on the currently accepted taxonomic denomination within Teloschistaceae.

These findings suggest that the sampled species are closely related to those in the genera *Pleopsidium*, *Sarcogyne*, and *Rusavskia*, respectively, based on their high sequence similarity to previously identified lichen species.

3.4. Metabolite identification

The metabolite profiles of all three lichen samples were analyzed using LC-MS/MS, and the putatively assigned metabolites are summarized in Table 1.

- **Sample 1 (*Pleopsidium* sp.):** A total of 16 metabolites were putatively assigned in this sample. The most abundant compounds were parietin, emodin, and fumarprotocetraric acid. Additionally, smaller quantities of usnic acid and lecanoric acid were putatively assigned.
- **Sample 2 (*Sarcogyne* sp.):** This sample also contained 16 metabolites. Gyrophoric acid was the most dominant, followed by stictic acid and norstictic acid. Other notable compounds, such as confluentic acid and psoromic acid, were putatively assigned.
- **Sample 3 (*Rusavskia* sp.):** 15 metabolites were putatively assigned in this sample. Parietin was among the prominent compounds, as in Sample 1, along with emodin and rheochrysin. Fumarprotocetraric acid and lecanoric acid were also putatively assigned in smaller quantities.

3.5. Sugar analysis

We quantitatively analyzed the sugar and sugar alcohol profiles for the three lichen samples, with the findings summarized in Table 2.

- **Hexoses:** Glucose was not detected in any of the samples. However, fructose was quantified in Sample 3 at a concentration of 13.5 $\pm$ 1.41 mg/g.
- **Disaccharides:** Sucrose was quantified in all three samples, with concentrations of 1.27 $\pm$ 0.09 mg/g in Sample 1, 1.33 $\pm$ 0.08 mg/g in Sample 2, and 1.74 $\pm$ 0.12 mg/g in Sample 3. Trehalose was not detected in any of the samples.

Table 1
Metabolite profile putatively assigned using LC–MS/MS in lichen samples collected from the moraine of Mount Rossman. *Pleopsidium* sp. (Sample 1), *Sarcogyne* sp. (Sample 2), and *Rusavskia* sp. (Sample 3). Values are presented as a percentage of the total metabolite content (% ± standard deviation). Metabolites are grouped by chemical family, including anthraquinones, depsides, depsidones, coumarins, alkaloids, pulvinic derivatives, biflavonoids, and dibenzofurans (n = 3). Significant differences were determined using one-way ANOVA with a Tukey post hoc test ($p < 0.05$). Putative metabolite assignments were based on LC–MS/MS fragmentation patterns and METLIN spectral matching following MSI Level 2 criteria (Sumner et al., 2007). Unassigned molecular features correspond to MS-detected ions without a confirmed spectral library match.

Family	[M-H]⁻	Molecule	*Sample 1*	*Sample 2*	*Sample 3*
Alkaloid	251.2	Orellanine	1.20 ± 0.08 %[a]	1.65 % ± 0.15 %[b]	–
Anthraquinone	251.2	Anthraquinone−2-carboxylic acid	2.44 ± 0.39 %	–	–
Anthraquinone	253.2	Chrysophanol	2.13 ± 0.29 %[b]	1.86 % ± 0.12 %[a]	1.83 % ± 0.07 %[a]
Anthraquinone	285.2	Citreorosein	–	–	1.14 % ± 0.09 %
Anthraquinone	269.2	Emodin	9.15 ± 3.11 %[c]	1.69 % ± 0.25 %[a]	5.45 % ± 0.01 %[b]
Anthraquinone	299.3	Fallacinol	1.19 ± 0.05 %[a]	2.26 % ± 0.15 %[b]	1.30 % ± 0.01 %[a]
Anthraquinone	283.3	Parietin	13.96 ± 1.72 %[b]	–	9.39 % ± 0.01 %[a]
Anthraquinone	445.4	Rheochrysin	–	–	3.80 % ± 0.02 %
Anthraquinone	299.3	Xanthorin	–	–	1.49 % ± 0.34 %
Biflavonoid	537.5	Skyrin	2.71 ± 0.55 %[a]	2.07 % ± 0.21 %[a]	–
Coumarin	177.1	Esculetin	–	3.71 % ± 0.27 %	–
Depside	373.3	Atranorin	2.99 ± 0.50 %[b]	1.86 % ± 0.28 %[a]	1.50 % ± 0.02 %[a]
Depside	499.6	Confluentic acid	–	4.69 % ± 0.28 %	–
Depside	375.4	Dihydroatranorin	1.59 ± 0.08 %	–	–
Depside	467.4	Gyrophoric acid	1.40 ± 0.17 %[a]	14.33 % ± 1.38 %[b]	–
Depside	317.3	Lecanoric acid	3.60 ± 0.81 %[b]	2.25 % ± 0.07 %[a]	2.14 % ± 0.11 %[a]
Depside	371.3	Norstictic acid	2.18 ± 0.58 %[a]	6.79 % ± 0.61 %[b]	–
Depside	443.5	Perlatolic acid	–	1.40 % ± 0.07 %	–
Depside	374.3	Protocetraric acid	2.82 ± 0.11 %	–	–
Depside	385.3	Stictic acid	–	9.17 % ± 0.97 %	–
Depside	419.3	Thamnolic acid	–	–	0.92 % ± 0.02 %
Depsidone	305.3	Calycin	1.94 ± 0.16 %[b]	–	0.94 % ± 0.10 %[a]
Depsidone	471.4	Fumarprotocetraric acid	5.29 ± 0.97 %[b]	–	2.34 % ± 0.03 %[a]
Depsidone	357.3	Psoromic acid	–	3.15 % ± 0.10 %	–
Depsidone	321.3	Vulpinic acid	–	1.82 % ± 0.16 %	–
Dibenzofuran	343.3	Usnic acid	4.24 ± 0.85 %[c]	2.71 % ± 0.12 %[b]	1.25 % ± 0.08 %[a]
Pulvinic derivative	307.3	Pulvinic acid	–	–	1.42 % ± 0.10 %
		Unassigned molecular features	41.17 ± 0.65 %	38.63 ± 0.65 %	57.69 ± 0.65 %

Table 2
Profile of sugars identified using HPLC-RID in lichen samples collected from the moraine of Mount Rossman. Samples include *Pleopsidium* sp. (Sample 1), *Sarcogyne* sp. (Sample 2), and *Rusavskia* sp. (Sample 3). Sugar content was measured in milligrams per gram (mg/g) of fresh weight. Values are expressed as the mean ± standard deviation. Different classes of sugars are reported, including hexoses, disaccharides, oligosaccharides, and sugar alcohols (n = 3). Significant differences were determined using one-way ANOVA with a Tukey post hoc test ($p < 0.05$).

Sugars (mg/g)		Sample 1	Sample 2	Sample 3
Hexoses	Fructose	–	–	13.5 ± 1.41
Disaccharides	Sucrose	1.27 ± 0.09[a]	1.33 ± 0.08[a]	1.74 ± 0.12[b]
Oligosaccharides	Stachyose	–	0.02 ± 1.5E−3[a]	0.03 ± 0.00[a]
	Verbascose	1.27 ± 0.11[c]	0.49 ± 0.03[b]	0.05 ± 0.00[a]
Sugar alcohols	Glycerol	1.02 ± 0.10	–	–
	Ribitol	32.31 ± 2.50[c]	4.95 ± 0.43[b]	0.06 ± 0.01[a]
	Mannitol	4.42 ± 0.51[a]	4.45 ± 0.51[a]	4.50 ± 0.38[a]
	Arabitol	1.38 ± 0.18[a]	3.48 ± 0.39[b]	7.92 ± 0.59[c]
	Pinitol	–	1.12 ± 0.12[b]	1.5E−5 ± 0.00[a]
	Iso-erythritol	–	–	1.30 ± 0.11

- **Oligosaccharides:** Stachyose was quantified in Sample 2 (0.02 ± 0.0015 mg/g) and Sample 3 (0.03 ± 0.00 mg/g). Verbascose was quantified in all three samples, with the highest concentration in Sample 1 (1.27 ± 0.11 mg/g), followed by Sample 2 (0.49 ± 0.03 mg/g) and Sample 3 (0.05 ± 0.00 mg/g).
- **Sugar alcohols:** Glycerol was quantified only in Sample 1 (1.02 ± 0.10 mg/g). Ribitol was quantified in all samples, but in varying amounts: Sample 1 contained 32.31 ± 2.50 mg/g, Sample 2 had 4.95 ± 0.43 mg/g, and Sample 3 had 0.06 ± 0.01 mg/g. Mannitol was quantified in similar concentrations across all three samples at approximately 4.4–4.5 mg/g. Arabitol was quantified in all samples, increasing from 1.38 ± 0.18 mg/g in Sample 1–7.92 ± 0.59 mg/g in Sample 3. Pinitol was quantified in Sample 2 (1.12 ± 0.12 mg/g) and detected at trace levels in Sample 3 (1.5E-5 ± 0.00 mg/g). Lastly, iso-erythritol was quantified only in Sample 3, at a concentration of 1.30 ± 0.11 mg/g.

4. Discussion

Understanding how organisms persist in environments that appear inhospitable to most life forms provides valuable insight into evolutionary selection under extreme stress. The metabolic traits observed in crustose lichens from Mount Rossman suggest that biochemical adaptation is central to their ecological success in Antarctica. Instead of relying on rapid growth or dormancy strategies, these lichens deploy specialized secondary metabolites and compatible solutes to actively buffer against UV damage, microbial colonization and desiccation stress, a pattern consistent with other extremophile lineages (Armstrong, 2017; Gutt et al., 2021).

Antarctica's harsh conditions, low temperatures, intense UV radiation and prolonged water scarcity exert strong selective pressure on exposed epilithic communities (Kappen, 2000). In this context, the putatively assigned anthraquinones and depsidic compounds detected in *Pleopsidium*, *Sarcogyne* and *Rusavskia* reflect not only stress mitigation

but also chemotaxonomic signatures characteristic of their respective lineages. Members of Teloschistaceae, such as *Rusavskia* and *Pleopsidium*, are known to accumulate parietin and related anthraquinones, whereas *Sarcogyne*, within Verrucariaceae, is associated with depsides such as gyrophoric acid, reinforcing the taxonomic placement inferred from 18S rDNA sequencing (Scur et al., 2022). Although BLASTn returned both *Xanthoria elegans* and *Rusavskia elegans* as closest matches for Sample 3, we adopted the latter name in agreement with current Teloschistaceae taxonomy (Arup et al., 2013), where *Xanthoria elegans* is considered a synonym of *Rusavskia elegans*.

The ecological roles of these metabolites align with their chemical nature. Parietin, emodin and related anthraquinones act as UV-screening pigments, while depsides such as gyrophoric, stictic and fumarprotocetraric acids exhibit antimicrobial or antioxidant functions, providing chemical defense on exposed rock surfaces (Petrová et al., 2022; Sepúlveda et al., 2022). These compounds, although only putatively assigned by LC–MS/MS, are consistent with previously reported metabolite patterns within these genera and likely contribute to competitive exclusion of microbial colonizers and oxidative stability during freeze–thaw transitions (Faiz ul Rasul et al., 2024; Ndhlovu et al., 2024).

Sugars and sugar alcohols, in turn, were quantified using external standards and not tentatively inferred, supporting their role as osmoprotectants and metabolic reserves. Ribitol and arabitol are key photobiont-derived polyols that sustain the mycobiont under carbon-limited states while simultaneously regulating osmotic balance during cyclic dehydration and rehydration events (Gasulla et al., 2021). The marked difference in ribitol content between samples suggests species-specific modulation of carbohydrate allocation strategies, potentially reflecting microhabitat variability in irradiance and moisture regimes.

Taken together, the coexistence of lineage-specific secondary metabolites and differential sugar alcohol accumulation indicates a dual adaptive strategy: chemotaxonomic specialization rooted in evolutionary history, combined with dynamic metabolic allocation shaped by local environmental constraints. This supports the view that crustose lichens in Antarctica are not just passive stress-tolerant organisms but chemically active systems capable of fine-tuned metabolic regulation in response to one of the most extreme terrestrial environments on Earth.

5. Conclusions

This study underscores the remarkable biochemical diversity of Antarctic crustose lichens and the pivotal role their secondary metabolites play in helping them survive under extreme conditions. Compounds such as parietin, fumarprotocetraric acid, and usnic acid were putatively assigned and are consistent with known UV-protective and antimicrobial chemotypes in extremophilic lichens. Additionally, the sugars and sugar alcohols quantified in the samples, such as ribitol and sucrose, are likely vital for maintaining osmotic balance and serving as metabolic reserves under repeated dehydration cycles. The metabolic profiles observed in *Pleopsidium* sp., *Sarcogyne* sp., and *Rusavskia* sp. highlight the essential contribution of these species to chemically mediated resilience in Antarctic ecosystems, reinforcing their value as models for stress-adapted chemical strategies.

Although further structural confirmation would be required for full metabolite validation, the putatively assigned chemical signatures reported here contribute to the chemotaxonomic characterization of these genera and expand current knowledge on their adaptive metabolic repertoire.

CRediT authorship contribution statement

Rodrigo A. Contreras: Writing – review & editing, Writing – original draft, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Gustavo E. Zúñiga:** Writing – original draft, Project administration, Methodology, Funding acquisition. **Pablo Zamora:** Funding acquisition, Conceptualization. **Marisol Pizarro:** Formal analysis.

Declaration of Competing Interest

The authors declare no conflict of interest. This research was conducted independently, and the funding sources were not involved in the design, execution, interpretation, or writing of the study. The authors affirm that they have no financial, personal, or other relationships that could inappropriately influence or bias the manuscript's content.

Acknowledgements

We thank the Chilean Antarctic Institute (INACH) for its logistical support. We are also grateful to the Ministry of Science, Technology, Knowledge, and Innovation of Chile (MinCiencia), and the Armed Forces of Chile (Scientific Conjunct Station). This work was funded by FONDECYT grants 1140189 (GEZ) and 3160274 (RAC).

Data availability

Data will be made available on request.

References

Armstrong, R.A., 2017. Adaptation of lichens to extreme conditions. In: Shukla, V., Kumar, S., Kumar, N. (Eds.), Plant Adaptation Strategies in Changing Environment. Springer Singapore, pp. 1–27. https://doi.org/10.1007/978-981-10-6744-0_1.

Arun, A.B., Girish, S., Ravi, L., 2023. Chapter 9 - photobiont symbiotic association in lichens. In: Dharumadurai, D. (Ed.), Microbial Symbionts. Academic Press, pp. 161–175. https://doi.org/10.1016/B978-0-323-99334-0.00009-8.

Arup, U., Søchting, U., Frödén, P., 2013. A new taxonomy of the family Teloschistaceae. Nord. J. Bot. 31 (1), 016–083. https://doi.org/10.1111/j.1756-1051.2013.00062.x.

Beckett, R.P., Minibayeva, F., Solhaug, K.A., Roach, T., 2021. Photoprotection in lichens: adaptations of photobionts to high light. Lichenologist 53 (1), 21–33. https://doi.org/10.1017/S0024282920000535.

Bednaříková, M., Váczi, P., Lazár, D., Barták, M., 2020. Photosynthetic performance of Antarctic lichen *Dermatocarpon polyphyllizum* when affected by desiccation and low temperatures. Photosynth. Res. 145 (2), 159–177. https://doi.org/10.1007/s11120-020-00773-4.

Canini, F., Borruso, L., Newsham, K.K., D'Alò, F., D'Acqui, L.P., Zucconi, L., 2023. Wide divergence of fungal communities inhabiting rocks and soils in a hyper-arid Antarctic desert. Environ. Microbiol. 25 (12), 3671–3682. https://doi.org/10.1111/1462-2920.16534.

Contreras, R.A., Pizarro, M., Köhler, H., Zamora, P., Zúñiga, G.E., 2019. UV-B shock induces photoprotective flavonoids but not antioxidant activity in Antarctic *Colobanthus quitensis* (Kunth) bartl. Environ. Exp. Bot. 159, 179–190. https://doi.org/10.1016/j.envexpbot.2018.12.022.

Devashree, Pandey, A., Dikshit, A., 2021. Lichens: fungal symbionts and their secondary metabolites. In: Singh, J., Gehlot, P. (Eds.), New and Future Developments in Microbial Biotechnology and Bioengineering. Elsevier, pp. 107–115. https://doi.org/10.1016/B978-0-12-821005-5.00007-7.

Faiz ul Rasul, H., Afzal, F., Khalid, W., Ahmad, M., Gull, S., Faiz ul Rasool, I., Ilyas, M., Javed, M., 2024. Antioxidant properties of lichen. In: Das, A.K., Sharma, A., Kathuria, D., Ansari, M.J., Bhardwaj, G. (Eds.), Chemistry, Biology and Pharmacology of Lichen, pp. 153–167. https://doi.org/10.1002/9781394190706.ch12.

García, R., Márquez, G., Acosta Hospitaleche, C., 2020. Richness of lichens growing on eocene fossil penguin remains from Antarctica. Polar Biol. 43 (12), 2011–2019. https://doi.org/10.1007/s00300-020-02761-9.

Gasulla, F., del Campo, E.M., Casano, L.M., Guéra, A., 2021. Advances in understanding of desiccation tolerance of lichens and Lichen-Forming algae. Plants 10 (4). https://doi.org/10.3390/plants10040807.

Goga, M., Elečko, J., Marcinčinová, M., Ručová, D., Bačkorová, M., Bačkor, M., 2020. Lichen metabolites: an overview of some secondary metabolites and their biological potential. In: Mérillon, In.J.-M., Ramawat, K.G. (Eds.), Co-Evolution of Secondary Metabolites. Springer International Publishing, pp. 175–209. https://doi.org/10.1007/978-3-319-96397-6_57.

Gutt, J., Isla, E., Xavier, J.C., Adams, B.J., Ahn, I.-Y., Cheng, C.-H.C., Colesie, C., Cummings, V.J., di Prisco, G., Griffiths, H., Hawes, I., Hogg, I., McIntyre, T., Meiners, K.M., Pearce, D.A., Peck, L., Piepenburg, D., Reisinger, R.R., Saba, G.K., Wall, D.H., 2021. Antarctic ecosystems in transition – life between stresses and opportunities. Biol. Rev. 96 (3), 798–821. https://doi.org/10.1111/brv.12679.

Kappen, L., 2000. Some aspects of the great success of lichens in Antarctica. Antarct. Sci. 12 (3), 314–324. https://doi.org/10.1017/S0954102000000377.

Morillas, L., Roales, J., Cruz, C., Munzi, S., 2022. Lichen as multipartner symbiotic relationships. Encyclopedia 2 (3), 1421–1431. https://doi.org/10.3390/encyclopedia2030096.

Ndhlovu, N.T., Minibayeva, F., Beckett, R.P., 2024. A role for secondary metabolites in desiccation tolerance in lichens. Microbiol. Res. 15 (1), 225–235. https://doi.org/10.3390/microbiolres15010016.

Petrová, K., Bačkorová, M., Demčišáková, Z., Petrovová, E., Goga, M., Vilková, M., Frenák, R., Bačkor, M., Mojžiš, J., Kello, M., 2022. Usnic acid isolated from *Usnea antarctica* (Du Rietz) reduced in vitro angiogenesis in VEGF- and bFGF-Stimulated HUVECs and ex ovo in quail chorioallantoic membrane (CAM) assay. Life 12 (9). https://doi.org/10.3390/life12091444.

Pizarro, M., Contreras, R.A., Köhler, H., Zúñiga, G.E., 2019. Desiccation tolerance in the Antarctic moss *Sanionia uncinata*. Biol. Res. 52 (1). https://doi.org/10.1186/s40659-019-0251-6.

Scur, M.C., Kitaura, M.J., de Paula, J.B., Spielmann, A.A., Lorenz, A.P., 2022. Contrasting variation patterns in *Austroplaca hookeri* and *Rusavskia elegans* (Teloschistaceae, lichenized Ascomycota) in maritime Antarctica. Polar Biol. 45 (1), 101–111. https://doi.org/10.1007/s00300-021-02976-4.

Sepúlveda, B., Cornejo, A., Bárcenas-Pérez, D., Cheel, J., Areche, C., 2022. Two new fumarprotocetraric acid lactones identified and characterized by UHPLC-PDA/ESI/ORBITRAP/MS/MS from the Antarctic lichen *Cladonia metacorallifera*. Separations 9 (2). https://doi.org/10.3390/separations9020041.

Singh, J., Singh, R.P., Khare, R., 2022. Higher rate of pigment synthesis in antarctic plants: a strategy of survival under UV radiations. In: Khare, In.N. (Ed.), Assessing the Antarctic Environment from a Climate Change Perspective: An Integrated Approach. Springer International Publishing, pp. 255–275. https://doi.org/10.1007/978-3-030-87078-2_15.

Spribille, T., Resl, P., Stanton, D.E., Tagirdzhanova, G., 2022. Evolutionary biology of lichen symbioses. N. Phytol. 234 (5), 1566–1582. https://doi.org/10.1111/nph.18048.

Sumner, L.W., Amberg, A., Barrett, D., Beale, M.H., Beger, R., Daykin, C.A., Fan, T.W.-M., Fiehn, O., Goodacre, R., Griffin, J.L., Hankemeier, T., Hardy, N., Harnly, J., Higashi, R., Kopka, J., Lane, A.N., Lindon, J.C., Marriott, P., Nicholls, A.W., Viant, M. R., 2007. Proposed minimum reporting standards for chemical analysis. Metabolomics 3 (3), 211–221. https://doi.org/10.1007/s11306-007-0082-2.