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Chemical profiling of lithophytic holobiont depside of hammered shield lichen

Vikrant Arya[1], Amandeep Kaur Gill[2*], Arbind Kumar Choudhary[3]

[1]Department of Pharmaceutical Sciences, Government Pharmacy College Rakkar, Himachal Pradesh, India
[2]School of Pharmaceutical Sciences, CT University, Ludhiana, Punjab, India
[3]Department of Pharmacology, Government Erode Medical College and Hospital, Tamil Nadu, India

ABSTRACT: *Background*: Lithophytic holobionts survive harsh environments by synthesizing specialized secondary metabolites. This study outlines the distinct chemotype of the rock-associated, extremophilic hammered shield lichen, i.e., *Parmelia sulcata*. *Methods*: Crude ethanolic extract was obtained by cold maceration of the hammered shield lichen and fractionated using silica-gel column chromatography. Nonpolar fractions were profiled by GC-MS, with compound annotations based on NIST library matching and relative peak-area normalization. *Results*: GC-MS profiling of *P. sulcata* fraction L10-L13 revealed a dominant orcinol-pathway chemotype, comprising primarily orcinol (43.39%) and atraric acid (20.92%). *Conclusion*: Chromatography-assisted approach successfully separated bioactive depsides. The isolated lichen depsides (*P. sulcata*) represent favorable structural scaffolds for treating several chronic diseases.

1. INTRODUCTION

Lithophytic holobionts, composite organisms comprising a macroscopic host and its associated microbiota living in rock-crevice habitats, represent a largely untapped reservoir of novel secondary metabolites (Gordon et al., 2013; Rolshausen et al., 2023).

The extreme abiotic stressors inherent to lithophytic environments, including desiccation, severe UV radiation, and nutrient scarcity, drive the evolution of unique biochemical adaptations (Wierzchos et al., 2015).

Parmeliaceae is one of the most diverse groups within the lichenized fungi (Tsurykau et al., 2019). Members of this genus in the family Parmeliaceae serve as important environmental biomonitoring tools. The genus is widely distributed from the Arctic to the Antarctic, stretching from cold to temperate regions of the world (Berry, 1941). Apart from their applicability as biosensor agents, they also exhibited diverse pharmacological activities due to the existence of varied chemical constituents. Recent studies on several species confirm therapeutic properties, viz., antioxidant, antimicrobial, anti-tumor, anti-microbial, etc. The phenolics present in several species, viz., green shield lichen, shield lichen, and salted shield lichen, exhibit antioxidant, antimicrobial, and anticancer activities when evaluated in pharmacological models (Rankovic et al., 2011) . Usnic acid, reported in shadow green shield lichen (*P. soredians*), was reported to be an antioxidant (Caviglia et al., 2001). Depsides reported in *P. dilatata* and *P. aurulenta* have potential as anti-cancer agents when evaluated in several cell lines, viz., Hep-G2, A-549, and HL-60 (Nugraha et al., 2020). *P. cirrhatum* has antifungal properties (Shahi et al., 2003). Polysaccharides of palm ruffle lichen (*P. tinctorum*) and stone flower (*P. perlata*) have radioprotective and antimicrobial effects (Xu et al., 2014). Depsides and phenolics of *P. cetrata* exhibited anthelmintic properties (Nugraha et al., 2021).

* Corresponding author.
E-mail address: amansatnamgill@gmail.com (Amandeep Kaur Gill)

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Some species of this genus (*P. caperata, P. pertusa, Parmelia sulcata (P. sulcata), P. saxatilis*) possess antimicrobial activities (Candan et al., 2007; Ingolfsdottir et al., 1998; Rankovic et al., 2007). Collectively, the existing scientific research on Parmelia highlighted its significance not only in environmental monitoring but also in therapeutics. The Phytopharmacological status of the Genus Parmelia has been documented in Table 1.

Lichen-derived constituents from the Parmelia genus were promising candidates for drug discovery. Compounds like usnic acid, lecanoric acid, atranorin, etc., confer wide pharmacological activities when evaluated in biological models. Overall, the therapeutic profile of Parmelia marked its relevance in the development of natural remedies with a multifaceted approach.

1.1. Hammered shield lichen

It was first described by the Irish Botanist and Mycologist Thomas Taylor in 1836, who classified it in the genus Parmelia within the family Parmeliaceae. It consists of the thallus of the lichen *P. sulcata* Taylor, belonging to the family Parmeliaceae. It is commonly found as saxicolous and corticolous. Cracked/hammered shield lichen is a saxicolous/corticolous species found in zones experiencing temperate to cold climates stretching across the Northern and Southern hemispheres (Molina et al., 2011).

1.2. Ethnomedicinal background of hammered shield lichen

Shield lichen has been used in dyeing applications (wool) since the dawn of time. The Metis (Indigenous community of Canada) employed this lichen as a paste on the gums of teething newborns to ease their suffering (Rogers, 2012). The morphological resemblance of the thallus of *P. sulcata* to the human brain is attributed to its ethnomedicinal usage in cranial-/brain-related disorders (Rai, 2012; Rizzini, 1952). The literature review endorsed its ethnomedicinal relevance in treating several diseases and disorders.

1.3. Chemical profiling of hammered shield lichen

Hammered shield lichen encompasses a plethora of distinct chemical constituents, as represented in Figure 1. Some of the major constituents include Atraric acid (1), Salazinic acid (depsidone) (2), Atranorin (3), Decalactone (4), 9-Octadecynoic acid (5), Farnesane (6), 1-Acetoxynonadecane (7), and Succinic acid, hexyl 2-phenylethyl ester (8) (Ari et al., 2015; Kyslychenko et al., 2019; Mitrovic et al., 2011).

2. MATERIALS AND METHODS

2.1.Collection and preparation of lithophytic lichen

The biological material for this study comprised a lithophytic holobiont: *P. sulcata* (Parmeliaceae). Lichen was manually harvested from phyllite rock-associated crevices during the dry season to ensure minimal moisture content. The intact thalli were collected, manually cleaned to remove substrate debris, air-dried in the dark at 25–30°C for 10 days, and coarsely powdered. The collected sample was subjected to authentication from the Raw Materials Herbarium and Museum, Delhi (RMHD), CSIR National Institute of Science Communication and Policy Research,

Table 1
Phytopharmacological potential of Genus Parmelia.

Species	Constituents	Pharmacology	References
P. saxatilis	Depsidone (Salazinic acid)	Antimycobacterial	Ingolfsdottir et al. (1998)
P. soredians	Dibenzofuran derivative (Usnic acid)	Antioxidant	Caviglia et al. (2001)
P. cirrhatum	Phenolics	Antifungal	Shahi et al. (2003)
P. caperata, P. pertusa	Phenolics	Antimicrobial	Rankovic et al. (2007)
P. sulcata	Depsidone (Salazinic acid)	Antimicrobial	Candan et al. (2007)
P. perlata	Polysaccharide	Antimicrobial	Esimone et al. (2007)
P. saxatilis, P. caperata, P. sulcata	Phenolics	Antioxidant, antimicrobial, and anticancer	Kosanic et al. (2012)
P. tinctorum	Polysaccharide	Radioprotective	Xu et al. (2014)
P. dilatata, P. aurulenta	Depsides	Anticancer (Hep-G2, A-549, and HL-60)	Nugraha et al. (2020)
P. cetrata	Depsides	Anthelmintic	Nugraha et al. (2021)

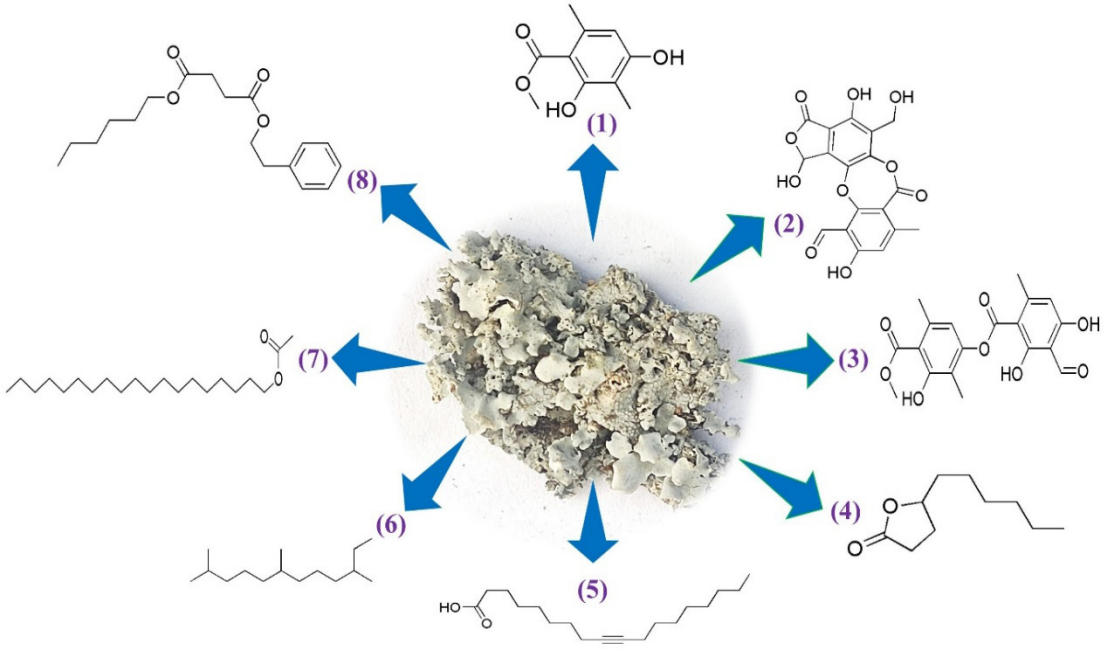

Figure 1. Reported constituents of *P. sulcata.*

having the authentication number: NIScPR/RHMD/Consult/2023/4429-30-2.

2.2. Extraction procedures

To isolate distinct chemotypes, cold maceration was employed based on the thermal stability of the target secondary metabolites. To preserve thermolabile lichen depsides and depsidones, 50 g of powdered *P. sulcata* thallus was subjected to cold maceration. The total extractive yield of lichen extract was 4.4 %w/w.

2.3. Isolation

Lichen crude extract was subjected to dry column chromatography to isolate specific compound classes. Briefly, the dried extract was packed onto silica gel (100–200 mesh) and loaded into a glass chromatography column. Elution was performed using a stepwise gradient mobile phase of chloroform, methanol, and water ($CHCl_3$:MeOH:H_2O), initiating at 8:2:0.01 and concluding at 8:8:0.8. Fractions were collected in 250 mL aliquots and monitored using thin-layer chromatography (TLC) under UV light (254 and 366 nm) and post-derivatization with 5% ethanolic sulfuric acid. Based on matching retardation factors (R_f), the lichen extract yielded 21 subfractions, which were subsequently pooled into active working groups, notably L10–L13. The isolation and characterization protocol has been summarized in Figure 2.

2.4. Characterization via GC-MS

The GC-MS analysis of L10–L13 was conducted using an Agilent 7890B gas chromatograph paired with an Agilent 5977A mass selective detector, utilizing an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm) with helium as the carrier gas at a flow rate of 1 mL/min. Sample (L10–L13) was injected in split mode with a 10:1 split ratio. The oven temperature program started at 50°C with a hold for 2 minutes, followed by an increase at 10°C per minute to 150°C, where it was held for 2 minutes. The temperature then increased at 5°C per minute to 250°C, with another 5-minute hold, and finally, it was ramped at 10°C per minute to 280°C, holding for 5 minutes (Kumari et al., 2024). Peak identification was conducted by comparing the acquired mass spectra against the National Institute of Standards and Technology (NIST) Mass Spectral Library. Peaks were annotated when the library similarity index was ≥80% and the fragmentation pattern was consistent with the proposed compound class. Quantification was based on relative total-ion-current (TIC) peak-area normalization without an external or internal standard; therefore, the reported percentages indicate semiquantitative relative abundance rather than absolute concentration. Instrumental artifacts, including cyclosiloxanes and borinic/silyloxy arsenic

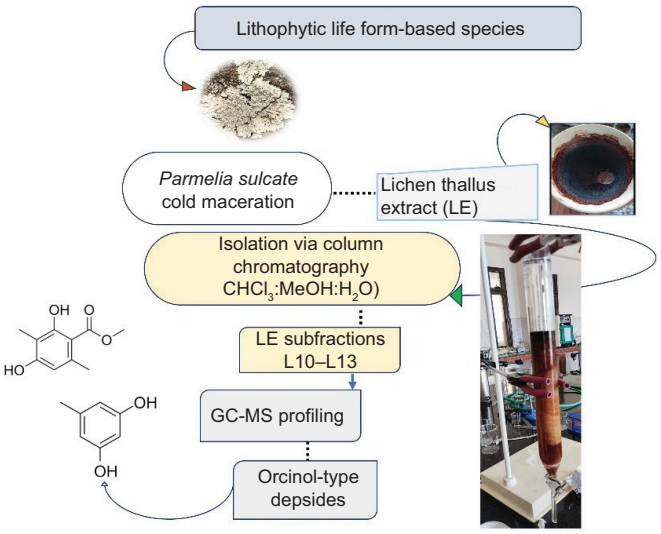

Figure 2. Isolation and characterization protocol for LE.

derivatives, were manually identified and excluded from pharmacological interpretation. No additional spectroscopic confirmation by FTIR, NMR, or LC-MS was performed; accordingly, the compound assignments should be interpreted as GC-MS library–based tentative annotations.

3. RESULTS

3.1. Sample profile

Lithophytic holobiont was collected from rock-associated crevice habitats and authenticated before chemical processing. *P. sulcata* Taylor (lichen; Parmeliaceae) was harvested as an air-dried thallus. Lichen was thoroughly air-dried, coarsely powdered as required for further processes. Authentication details, extraction yield, solvent system, and GC-MS reporting approach are summarized in Table 2.

3.2. GC-MS chemical profiling

Several compounds were identified via GC-MS (Table 3) in isolated fraction of hammered shield lichen, viz., Cyclopentasiloxane decamethyl (0.82%), Cyclohexasiloxane dodecamethyl (0.58%), Orcinol (43.39%), Cyclopentene, 1,2,3,3,4 pentamethyl (1.25%), Benzaldehyde, 2,4-dihydroxy-6-methyl (5.28%), Methyl orsellinate (8.79%), Methyl hematommate (2.40%), Atraric acid (20.92%), Ethyl 2,4-dihydroxy-6-methylbenzoate (2.61%), n-Hexadecanoic acid (1.57%), Bacteriochlorophyll-c-stearyl (0.52%), cis-11-Hexadecenal (1.54%), and Tris(tertbutyldimethylsilyloxy)arsane (4.33%).

Table 2
Sample authentication, collection metadata, and other conditions.

Parameters	Summary
Voucher/authentication	NIScPR/RHMD/Consult/2023/4429-30-2; RMHD, CSIR-NIScPR, New Delhi, India
GPS coordinates	Latitude: 32.214262°, Longitude: 76.383294°
Area of collection	Kharota region of District Kangra, Himachal Pradesh, India
Organism class	Lichen
Family	Parmeliaceae
Life form	Lithophytic
Substrate	Rock/crevice (Phyllite)
Plant part under investigation	Thallus
Drying method	Shade drying
Preprocessing	Thoroughly dried, coarsely powdered
Lichen extract ID	LE
Extraction method	Cold maceration
GC-MS characterized fraction	L10–L13
Starting material	Thoroughly dried, coarsely powdered thallus
Solvent system	Ethanol 96% (v/v)
Extraction method	Cold maceration (static immersion)
Duration	7 days at ambient temperature
Temperature (extraction)	Ambient (20–25°C)
Concentration temperature	≤45°C
Concentration method	Low-temperature drying to prevent depside hydrolysis
Isolation technique	Column chromatography (dry packing)
Stationary phase	Silica gel (100–200 mesh)
Mobile phase system	$CHCl_3$:MeOH:H_2O
Gradient range	8:2:0.01 → 8:8:0.8
Gradient type	Gradient
Detection in TLC	TLC (UV 254/366 nm; 5% H_2SO_4 charring)
Pooling rule	Similar R_f across adjacent subfractions
Downstream chemical profiling via GC-MS	18 peaks
Key compounds identified	Orcinol (43.39%), Atraric acid (20.92%), Methyl orsellinate (8.79%)

The fraction is dominated by orcinol-type aromatic polyketide metabolites characteristic of the lichen secondary metabolome (Table 1). Orcinol ($C_7H_8O_2$, MW 124.13, RT 14.975 min; TIC area 5260508; 43.39% relative TIC area; peak height 650349 counts; base ion m/z 124, 95, 67) was the single most abundant compound, confirming its role as the major lichen polyketide building block (Peak 3). Atraric acid (benzoic acid, 2,4-dihydroxy-3,6-dimethyl methyl ester; $C_{10}H_{12}O_4$, MW 196.20, RT 22.039

min; TIC area 2536574; 20.92% relative TIC area; peak height 647258 counts; base ion m/z 164, 136, 196[M+,58]) was the second major compound (Peak 9). Together, orcinol and atraric acid accounted for 64.31% of the total TIC area, establishing orcinol-type depsides as the dominant chemical signature. Secondary lichen-specific metabolites included methyl orsellinate ($C_9H_{10}O_4$; RT 21.027 min; 8.79%), 2,4-dihydroxy-6-methylbenzaldehyde (RT 18.798 min; 5.28%), 3,5-di-*tert*-butylphenol (RT 17.698 min; 4.80%), methyl haematommate (RT 21.186 min; 2.40%), and ethyl orsellinate (RT 22.610 min; 2.61%). Collectively, lichen-specific depsides and depsidones (Peaks 3, 6–10) accounted for ~81.38% of the total TIC area. Minor fatty acid constituents included n-hexadecanoic acid (palmitic acid; 1.57%) and *cis*-11-hexadecenal (1.54%). Three TBS-As artifact peaks (RT 39.097, 40.538, 43.714 min; combined 4.33%) and two siloxane contaminant peaks (RT 5.416, 12.763 min; combined 1.40%) were excluded from pharmacological interpretation, as illustrated in Figure 3.

It is evident from the literature survey that Ari et al. (2015) had also reported several components in *P. sulcata* extract, viz., Atraric acid, n-Hexadecanoic acid, Dodecanamide, 9-Octadecenal, Methyl isoheptadecanoate, 2-Methylnonadecane, 2-Nonadecanone, Ethyl icosanoate, Squalene, etc.

Orcinol remains the major constituent of several lichens (Krishna et al., 2022) and has been reported to exhibit free radical scavenging and anti-apoptotic properties (Leal et al., 2018; Sanchez-Lira et al., 2017). Our findings also complement the reported data, demonstrating that lichens contain significant phenolic and fatty acid compounds, methylated benzoate derivatives, and terpenoid components. These data will prove substantial in affirming the metabolite composition of *P. sulcata*.

The predominance of orcinol-pathway phenolics indicates that the L10–L13 fraction of *P. sulcata* is enriched in low-molecular-weight aromatic polyketides. Such molecules are pharmacologically relevant because oxidative stress, apoptosis, microbial persistence, and dysregulated cell-survival pathways contribute to chronic inflammatory, metabolic, neurodegenerative, and cancer-related disorders. Previous lichen studies reported antioxidant and anti-apoptotic effects for orcinol/depside-related molecules (Leal et al., 2018; Sanchez-Lira et al., 2017), whereas Parmelia metabolites have shown antimicrobial and anticancer activities in biological models (Kosanic et al., 2012; Nugraha et al., 2020; Ari et al., 2015).

The principal limitation of this study is the reliance on GC-MS library matching for compound annotation. FTIR, NMR, and LC-MS confirmation were not performed, and no purified reference standards were used for absolute quantification. Hence, the % values should be interpreted as relative peak-area abundances. Future studies should combine orthogonal spectroscopy, preparative isolation, purified standards, and in vitro/in

Table 3
GC-MS annotated peak list: L10–L13 fraction (*P. sulcata*).

Peak no.	Compound	RT	Mol. formula	Mol. weight (g/mol)	Height	Area	Peak area (%)
1	Cyclopentasiloxane, decamethyl	5.416	$C_{10}H_{30}O_5Si_5$	370.77	12434	99664	0.82
2	Cyclohexasiloxane, dodecamethyl	12.763	$C_{12}H_{36}O_6Si_6$	444.9236	31689	70783	0.58
3	Orcinol	14.975	$C_7H_8O_2$	124.13	650349	5260508	43.39
4	Cyclopentene, 1,2,3,3,4-pentamethyl	15.928	$C_{10}H_{18}$	138.25	31840	151952	1.25
5	Phenol, 3,5-bis (1,1-dimethylethyl)	17.698	$C_{14}H_{22}O$	206.3239	233953	582083	4.80
6	Benzaldehyde, 2,4-dihydroxy-6-methyl	18.798	$C_8H_8O_3$	152.15	70355	640433	5.28
7	Methyl orsellinate	21.027	$C_9H_{10}O_4$	182.1733	217690	1066171	8.79
8	Methyl haematommate	21.186	$C_{10}H_{10}O_5$	210.1834	98831	291039	2.40
9	Atraric acid	22.039	$C_{10}H_{12}O_4$	196.1999	647258	2536574	20.92
10	Ethyl orsellinate	22.610	$C_{10}H_{12}O_4$	196.20	45604	316484	2.61
11	1,2-Oxaborole, 2,3,4-triethyl-2,5-dihydro-5,5-dimethyl	22.833	$C_{11}H_{21}BO$	180.10	15742	79856	0.66
12	n-Hexadecanoic acid	26.839	$C_{16}H_{32}O_2$	256.4241	47495	190097	1.57
13	Bacteriochlorophyll-c-stearyl	27.415	$C_{52}H_{72}MgN_4O_4$	841.5	25291	63004	0.52
14	cis-11-Hexadecenal	30.145	$C_{16}H_{30}O$	238.41	19493	187108	1.54
15	1,2-Oxathiane, 6-dodecyl-, 2,2-dioxide	31.039	$C_{16}H_{32}O_3S$	304.5	22404	64668	0.53
16	Tris(tertbutyldimethylsilyloxy)arsane	39.097	$C_{18}H_{45}AsO_3Si_3$	468.7262	40649	130971	1.08
17	Tris(tertbutyldimethylsilyloxy)arsane	40.538	$C_{18}H_{45}AsO_3Si_3$	468.7262	19394	215578	1.78
18	Tris(tertbutyldimethylsilyloxy)arsane	43.714	$C_{18}H_{45}AsO_3Si_3$	468.7262	18406	177630	1.47

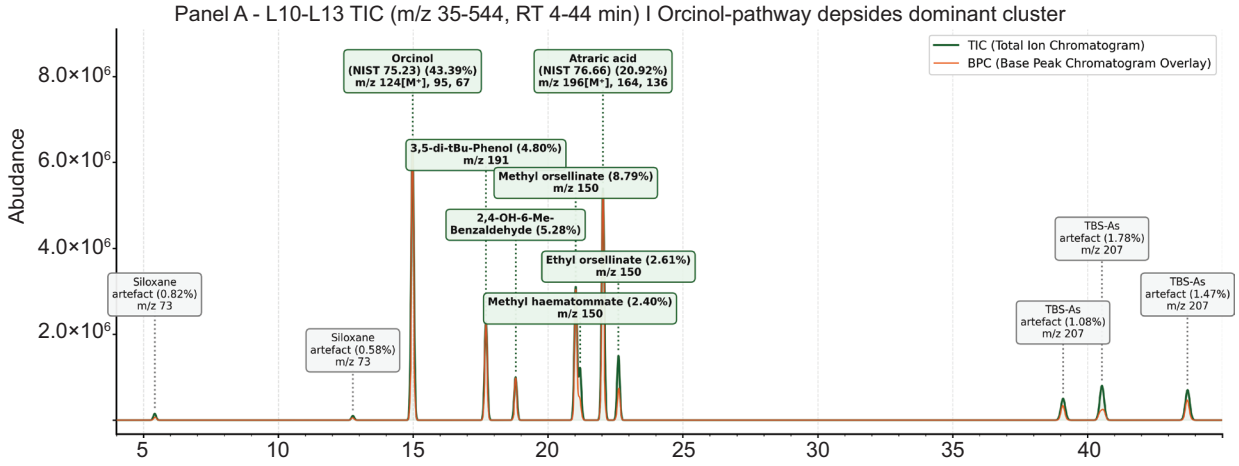

Figure 3. GC-MS profiles of purified fraction (L10–L13).

vivo pharmacological assays to confirm the identified molecules and evaluate their relevance to chronic disease management.

4. CONCLUSION

This research provides significant insight into the chemical composition of lithophytic lichen, i.e., *P. sulcata*. The data revealed the predominance of orcinol-type depsides and atraric acid in hammered shield lichen, which affirms the lichen's ability to synthesize distinct phenolic compounds. Column chromatography–guided isolation seems to be a reliable method of separating active metabolites from *P. sulcata*. By utilizing GC-MS profiling, results demonstrated that the nonpolar fractions of this species are characterized by a distinct orcinol-pathway chemotype. These results advance our understanding of the chemical diversity within selected lithophytic life forms and also highlight their role in their survival.

AUTHOR CONTRIBUTIONS

This research was done by collaborating with the authors. All the authors revised and approved the final article.

Experimental design, Conceptualization, Manuscript writing: VA; Isolation protocol design, Proofreading: AKG; GC-MS data interpretation, manuscript editing: AKC.

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CONFLICT OF INTEREST

None.

DATA AVAILBAILITY

Materials and data are provided upon request.

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ORCID

Vikrant Arya 0000-0002-6808-1754
Amandeep Kaur Gill 0000-0001-7412-3247
Arbind Kumar Choudhary 0000-0001-8910-1745

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