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Characterisation, Phytochemical Analysis, and Ethnobotanical Significance of Temperate Lichen Species of Uttarakhand, India

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Abstract

Background: Lichens are known to be symbiotic organisms consisting of fungi and algae. They have been found to produce unique secondary metabolites and display bioactive properties. **Aim:** This characterisation and phytochemical study were undertaken on lichens belonging to the *Parmotrema austrosinense, Dermatocarpon vellereum*, and *Heterodermia diademata*, which were obtained from the temperate regions of *Tehri, Pauri*, and Chamoli districts of Uttarakhand. **Methods:** These lichens were preserved in the Lucknow Garden (LWG) herbarium of the National Botanical Research Institute (NBRI), Lucknow. Phytochemical screening was done on the lichens using petroleum ether, acetone, and methanol. The ethnobotanical importance and uses of lichens were studied through literature surveys and interactions with people in the Garhwal region. **Results:** Methanol was found to be the most effective solvent in extracting the highest amount of extract from smaller samples and giving the highest number of positive phytochemical screening results. **Conclusion:** This study successfully identifies key secondary metabolites and confirms the ethnobotanical significance, supports the medicinal, ecological, and conservation value of temperate lichens in Uttarakhand.

Major Findings: Major findings include the superior efficiency of methanol as an extraction solvent, the consistent presence of bioactive secondary metabolites across all studied species, and the validation of their traditional therapeutic applications, thereby supporting their potential for future pharmacological exploration.

Keywords: Ethnobotany, Phytochemical Analysis, Secondary Metabolites, Thin-Layer Chromatography, Uttarakhand Himalaya

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1. Introduction

Lichens, which are symbiotic combinations of fungi and algae or cyanobacteria, have been found to have interesting uses. This association of fungi and algae/cyanobacteria, the algal component provides the necessary energy, and the fungal component provides the necessary support and protection[1]. One of the major reasons for the flexibility of lichens is the formation of secondary metabolites like usnic acid, lobaric acid,

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gyrophoric acid, and atranorin, which protect against environmental stress[2]. Lichens have been found to have unique chemical structures because of their symbiotic association with fungi and algae/cyanobacteria, and this chemical structure is absent in plants[3,4].

Lichens have been used in Ayurveda and Unani medicine as antibiotics, anti-inflammatory agents, herbal contraceptives, and remedies for digestive disorders, skin diseases, and tuberculosis[5,6]. They are also employed in perfumes, spices, agrochemicals, and environmental monitoring due to pollutant absorption[7]. Despite their importance, many species remain underexplored for phytochemical composition and ethnobotanical significance. Different bioactive substances have been reported in earlier research on the three species under investigation. Atranorin, lecanoric acid, usnic acid, and methyl orsellinate, which have antibacterial, antioxidant, and anti-inflammatory qualities, are found in *P. austrosinense*[8-10]. Gyrophoric acid, stictic acid, and fumarprotocetraric acid are found in *D. vellereum*, which supports the use of traditional dyes and has a phenolic and terpenoid-rich profile[11-13]. Atranorin, physodic acid, lobaric acid, and protolichesterinic acid are found in *H. diademata* and are linked to wound healing, antibacterial activity, and cytotoxic effects[10,13-14]. *Parmotrema austrosinense* (Zahlbr.) Hale, *D. vellereum* Zschacke, and *H. diademata* (Taylor) *D.D. Awasthi,* which were obtained from the Uttarakhand districts of Tehri, Pauri, and Chamoli, are the subjects of this study.

These species were chosen due to their potential for new bioactive chemicals, past ethnobotanical use, and ecological value. The goals of the study are to describe the chosen lichen species taxonomically, to use methanol, acetone, and petroleum ether for phytochemical screening to find the best solvent for bioactive substances, to use literature and experiences with the local people to record ethnobotanical applications, to evaluate the effectiveness of extraction, and to compare phytochemical yields in various solvents. There are over 20,000 lichen species in the world, of which about 2,900 are found in India, 540 are endemic, mostly in the Western Himalayas[15,16].

Chemical characterisation, which frequently uses methods like TLC (Thin-Layer Chromatography), GC-MS (Gas Chromatography-Mass Spectrometry), and HPLC (High-Performance Liquid Chromatography), is essential for medicinal and ecological applications[17]. The diverse temperatures of Uttarakhand, which range from alpine regions to tropical lowlands, are perfect for researching lichen biodiversity. Secondary metabolite production is influenced by temperate elevations (800–2400m), which provide minimal disruptions and good growth[18]. This study supports the medicinal, ecological, and conservation value of temperate lichens in Uttarakhand and offers insights into their biochemical variety through the integration of morphological, phytochemical, and ethnobotanical investigations.

2. Materials and Methods

2.1 Study Area and Sampling Sites

Samples of lichen were carefully gathered from three different districts in the Garhwal Himalayas of Uttarakhand: Tehri, Pauri, and Chamoli. These areas, which are distinguished by low levels of industrial pollution and abundant biodiversity, mark the transition from subtropical to temperate climate zones.

2.1.1 Sample 1 (Tehri Garhwal)

Gathered from *Vernicia fordii* (Tung) bark near Jawar on Jakher-Dang Road. Temperatures in the region range from 5°C to 40°C, creating a niche for a variety of lichen adaptations.

2.1.2 Sample-2 (Pauri Garhwal)

Acquired from a cliff stone close to Prem Nagar on the Pauri-Srinagar Road. Altitude-specific lichen characteristics are supported by the site's notable altitudinal variance and monsoon-influenced climate shifts.

2.1.3 Sample 3 (Chamoli)

Collected from big stones in the Mandal Village Forest (Khalla) Orchid Conservation Area. Because of the region's terrain, which results in below-freezing winter temperatures, certain species are able to adapt to particular ecological niches.

In addition to their pristine biological circumstances, these locations were chosen because they are home to indigenous populations whose traditional knowledge of lichens is still mostly unknown. Table 1 lists these locations' precise characteristics, and Figure 1 shows how they are distributed geographically.

Table 1. Parameters of the sampling site

Sample No.	Date of Sampling	Sampling Site	Location	Substratum	Humidity	Temperature	Altitude
Sample 1	10/07/2022	Site-1: Jakher-Dang Road, Near Jawar village, Tehri Garhwal, Uttarakhand	30°14′29.9″N 78°40′11.5″E	Bark and twigs of Tung tree (*Vernicia fordii*)	52%	32°C	1100-1200m
Sample 2	27/06/2021	Site-2: Pauri-Srinagar Road, Near Prem Nagar, Pauri Garhwal, Uttarakhand	30°07′57.4″N 78°47′29.5″E	Stone of a cliff	73%	34°C	1600-1700m
Sample 3	23/12/2021	Site-3: Orchid Conservation Area, Khalla, Near Mandal Village Forest area, Chamoli, Uttarakhand	30°26′49.2″N 79°16′25.5″E	Big stones	60%	12°C	1700-1800m

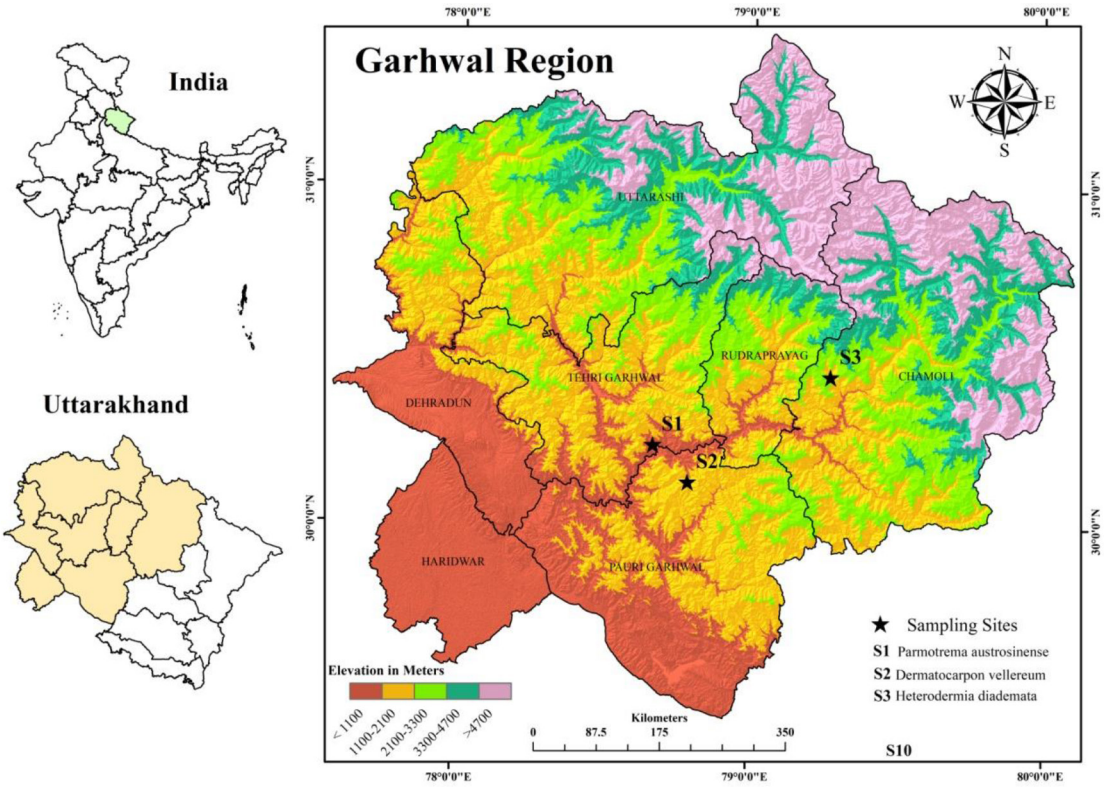


Figure 1. Illustrates the geographical locations of the sampling sites.

2.2 Sample Collection and Management

Using chisels and knives, lichen specimens were taken from stones and the bark of *Vernicia fordii* (Tung). Sampling locations were photographed and recorded. Samples were transported in zip-lock bags and kept in newspaper compartments[19]. After that, the specimens were cleaned of unnecessary material, weighed (20g), and divided for identification, phytochemical investigation, and submitted to the LWG herbarium at NBRI for accession. Samples for later use were kept cold[19-21].

2.3 Lichen Identification

Standard taxonomic procedures were used for identification[22]. Through dissection and compound microscopy, morphological characteristics (thallus colour, texture, cilia, and rhizines) were investigated. In order to examine the distribution of the cortex, algal layer, and medulla, anatomical analysis used vertical thallus slices (with potato-embedding). The K, C, KC, and PD chemical spot tests were carried out in accordance with Nylander[23] and Asahina[24].

Additionally, Solvent System C (toluene: acetic acid, 170:30) was used for TLC[25]. Merck 60F silica plates were treated with acetone extracts, and *Parmelinella wallichiana* (salazinic acid and atranorin) was used as a control. Spots were visualised with 10% sulfuric acid at 110°C and matched with standard R_f classes[25].

2.4 Phytochemical Analysis

Lichen extracts were prepared via sequential Soxhlet extraction using petroleum ether, acetone, and methanol[26-28]. Qualitative screening for secondary metabolites was performed as follows[29-32]:

i. Tannins: 5% $FeCl_3$ addition to extract.
ii. Alkaloids: 1% HCl extraction followed by Dragendorff's reagent.
iii. Saponins: Frothing test via vigorous shaking with distilled water.
iv. Glycosides: Keller-Kilani test using glacial acetic acid, 2% $FeCl_3$, and conc. H_2SO_4
v. Flavonoids: 10% NaOH addition.
vi. Triterpenoids/ Steroids: Salkowski test using chloroform and conc. H_2SO_4; Steroid presence was confirmed by acetic anhydride and H_2SO_4 (blue-green ring).

3. Results and Discussion

3.1 Microscopic Identification

3.1.1 Sample 1 Characteristics

The thallus of the lichen found on tree bark (*corticolous* habitat) attains a diameter of up to 10cm. Its lobes are rounded and measure about 15 to 20mm wide, lacking cilia. The upper surface of the lobes exhibits a glaucous to pale grey hue, with faint whitish spots, while marginal soralia appear linear. The soredia present vary in texture from farinose to granular. On the lower surface, the central region is black, encircled by a broad margin of ivory-colored tissue that is mottled with yellow-brown patches, lacking any structures. The medulla, situated within the thallus, is white. Apothecia, reproductive structures, are infrequent and measure up to 5mm in diameter, featuring perforations as shown in Figure 2.

3.1.2 Sample 2 Characteristic

The thallus of the lichen is found on a rock (*saxicolous*), often monophyllous, and reaches a diameter of up to 12cm. It exhibits an umbilicate structure and possesses substantial thickness, displaying a leathery texture. On the upper surface, the colouration varies from light brownish to brownish red, and it is characterised by a dense, white, and thick pruinose covering. In contrast, the lower surface is black in colour and is distinguished by the presence of densely distributed, thick, and stubby rhizomorphs resembling coral structures, as shown in Figure 3.

3.1.3 Sample 3 Characteristics

The thallus of the lichen is found growing on rocks (*saxicolous*), and it reaches a diameter of up to 15cm. It exhibits a branched structure, with linear lobes that are approximately 2.5mm wide. In the central region, secondary lobules are infrequently observed. The lobes

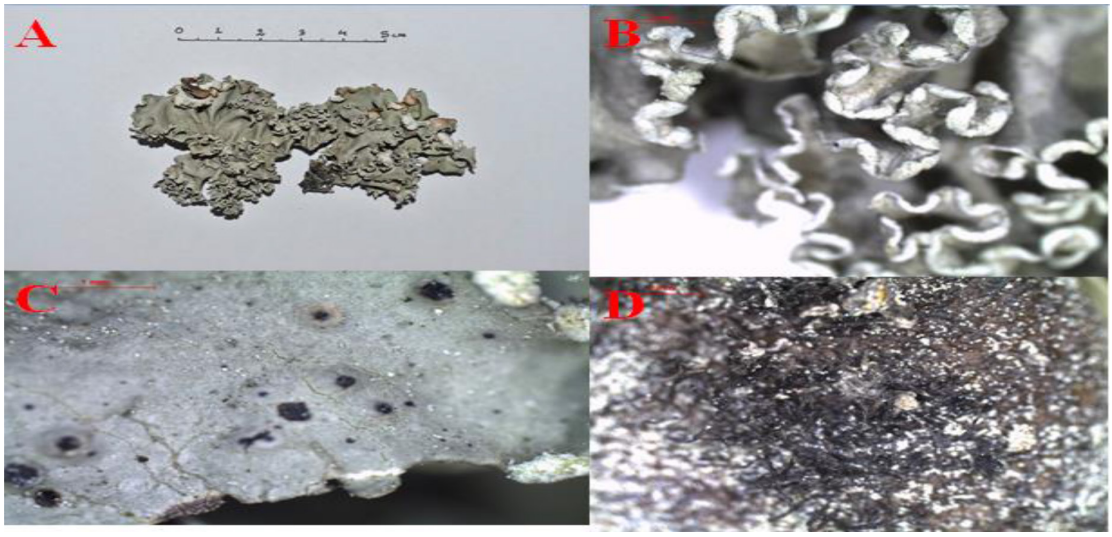


Figure 2. Showing morphological and anatomical characters of Sample 1. **(A).** Thallus of Sample 1, **(B).** Lobes of Sample 1, **(C).** Showing perforations, **(D).** Lower surface of Sample 1.

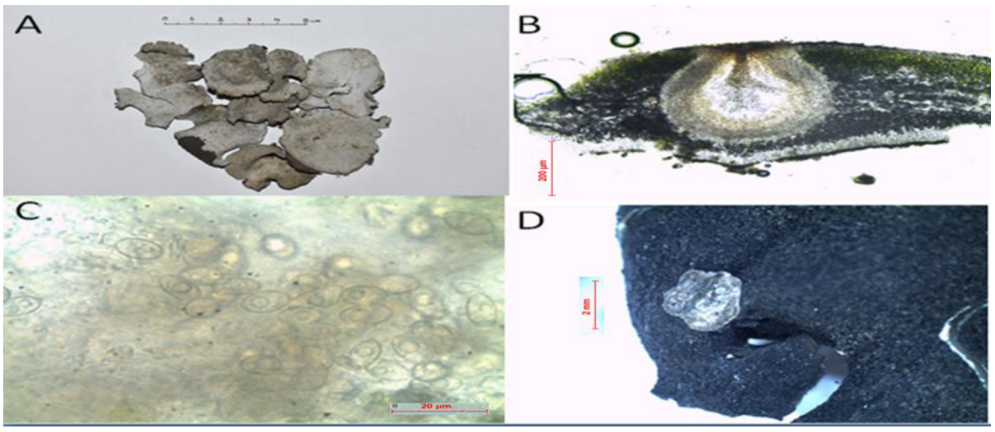


Figure 3. Showing morphological and anatomical characters of Sample 2. **(A).** Thallus of Sample 2. **(B).** Ostiole of Sample 2. **(C).** Spores of Sample 2. **(D).** Showing rhizomorphs on the posterior side of Sample 2.

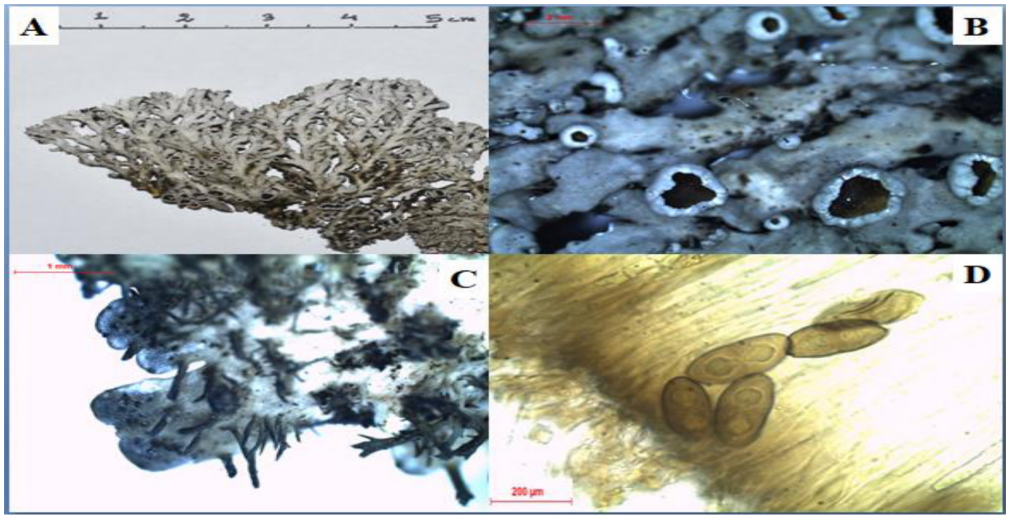


Figure 4. Showing morphological and anatomical characters of Sample 3. **(A).** Thallus of Sample 3. **(B).** Rhizines of Sample 3. **(C).** Apothecia of Sample 3. **(D).** Ascospores of Sample 3.

are coated on both upper and lower surfaces. The upper surface displays a grey to grey-white colouration and lacks the presence of isidia and soredia. The lower surface is pale brown and features sparse rhizines that match its colour. Apothecia, the reproductive structures, are typically numerous and have a diameter of up to 7mm. The cortex of the apothecial receptacle is relatively thin. The ascospores, reproductive cells produced within the apothecia, measure (16-) 22-32 (-40) x 10-18um and lack sporoblastidia as shown in Figure 4.

3.2 Colour Spot

3.2.1 Sample 1

During the application of colour spot tests to Sample 1, the cortex region displayed no colour change in response to any of the tests (K, C, KC, and PD), signifying the absence of detectable chemicals in the cortex of Sample 1. In the medulla region, the K and PD tests yielded negative results, indicating no discernible colour changes. Conversely, the C and KC tests yielded positive outcomes, resulting in a red colouration when applied to the medulla. This red colouration is indicative of the presence of Lecanoric acid within the medulla of Sample 1. The gathered observations and associated colour change results have been systematically documented in Table 2.

3.2.2 Sample 2

Sample 2 was subjected to colour spot tests (K, C, KC, and PD) on both the cortex and medulla regions.

Table 2. Observation of the colour spot test result of Sample 1

Sample 1	K Test		C Test		KC Test		PD Test		Result
Cortex	-ve	No color change	-ve	No color change	-ve	No color change	-ve	No color change	Lecanoric acid present
Medulla	-ve	No color change	+ve	Red color	+ve	Red color	-ve	No color change	

Table 3. Shows the observation of the colour spot test result of Sample 2

Sample no. 2	K test		C test		KC test		PD Test		Result
Cortex	-ve	No color change	-ve	No color change	-ve	No color change	-ve	No color change	Not Detected
Medulla	-ve	No color change	-ve	No color change	-ve	No color change	-ve	No color change	

Table 4. Shows the observation of the colour spot test result of Sample 3

Sample 3	K Test		C Test		KC Test		PD Test		Result
Cortex	+ve	Yellow color	-ve	No color change	-ve	No color change	-ve	No color change	Salazinic acid present
Medulla	+ve	Yellow color	-ve	No color change	-ve	No color change	-ve	No color change	

The outcome of these tests revealed no observable colour changes, suggesting the absence of detectable chemical compounds within the thallus. The recorded observations and corresponding colour change results have been tabulated in Table 3.

3.2.3 Sample 3

Following the execution of colour spot tests (K, C, KC, and PD tests) on Sample 3, the K test demonstrated affirmative outcomes in both the cortex and medulla. The application of K reagent resulted in a yellow colouration, indicative of the presence of Salazinic acid. Conversely, the C, KC, and PD tests rendered negative results in both the cortex and medulla, signifying the absence of observable colour changes subsequent to their application. A comprehensive summary of the recorded observations and corresponding colour change results has been meticulously organised within Table 4.

3.3 Thin Layer Chromatography (TLC)

Thin-Layer Chromatography (TLC) analysis provided valuable chemotaxonomic insights for the identification of the lichen samples based on their secondary metabolite profiles. *Parmelinella wallichiana* (C1PW), used as a reference species, exhibited two well-characterised compounds: Salazinic acid at R_f class 2 and atranorin at R_f class 7. These compounds are known markers of the genus *Parmelinella* and support its identification. Salazinic acid, a depsidone with known antioxidant and antimicrobial properties, and atranorin, a widespread depside involved in Ultraviolet (UV) protection, served as reliable chemical standards. *Stereocaulon pomiferum* (C2SP) showed three distinct spots corresponding to stictic acid, lecanoric acid, and atranorin at R_f classes 3, 3, and 7, respectively. Stictic acid and lecanoric acid are characteristic compounds found in the genus *Stereocaulon*, further validating the morphological identification. Among the unidentified test samples:

vii. Sample 1 (S1) displayed chemical similarities with the control *S. pomiferum*, showing the presence of lecanoric acid (R_f class 3) and atranorin (R_f class 7). This chemical profile, in combination with morphological and anatomical features, suggests that Sample 1 may belong to the *Stereocaulon* genus or a related group.

viii. Sample 2 (S2) did not reveal any detectable secondary metabolites under the TLC conditions used. This could indicate either the absence of extractable lichen substances in the tested sample or limitations in solvent extraction and detection sensitivity for this particular lichen.

ix. Sample 3 (S3) exhibited spots for atranorin (R_f class 7) and zeorin (R_f class 5). Zeorin is a triterpenoid commonly associated with certain *Parmeliaceae* members, supporting its tentative placement within this family.

The presence and identity of these secondary metabolites not only reinforce morphological

Table 5. Shows the observations of TLC plate results

Controls and Samples	R_f Classes	Chemicals Present
C1PW- Control 1 *Parmelinella wallichiana*	R_f class 2 R_f class 7	Salazinic acid Atranorin
C2SP-Control 2 *Stereocaulon pomiferum*	R_f class 3 R_f class 3 R_f class 7	Stictic acid Lecanoric acid Atranorin
Sample 1	R_f class 3 R_f class 7	Lecanoric acid Atranorin
Sample 2	_	_
Sample 3	R_f class 7 R_f class5	Atranorin Zeorin

identification but also highlight their chemotaxonomic importance in lichen classification. The chromatographic data, when matched with literature and standard references[25], validate the utility of TLC as a reliable and specific technique for lichen characterisation. Minor additional spots were observed across all samples, but were excluded from taxonomic interpretation due to a lack of a standard match or diagnostic value. Visual and tabular representations of these results are provided in Figure 5 and Table 5.

3.4 Final Identification

Upon completion of the comprehensive identification procedures, the specimens were successfully characterised as follows: Sample 1 was confirmed to be *P. austrosinense* (Zahlbr.) Hale, Sample 2 was identified as *D. vellereum* Zschacke, and Sample 3 was determined to be *H. diademata* (Taylor) D.D. Awasthi. Subsequently, these identified specimens were appropriately curated and preserved within the LWG Herbarium of the National Botanical Research Institute (NBRI), each assigned a distinct accession number for future reference. Specifically, the accession numbers assigned were as follows: Sample 1: 63589, Sample 2: 58770, and Sample 3: 61627.

3.6 Extraction and Yield Determination of Lichen Extracts

The process of extracting lichen constituents involved the preparation of three distinct extracts from each Sample, utilising solvents of ascending polarity: petroleum ether, acetone, and methanol. These extracts

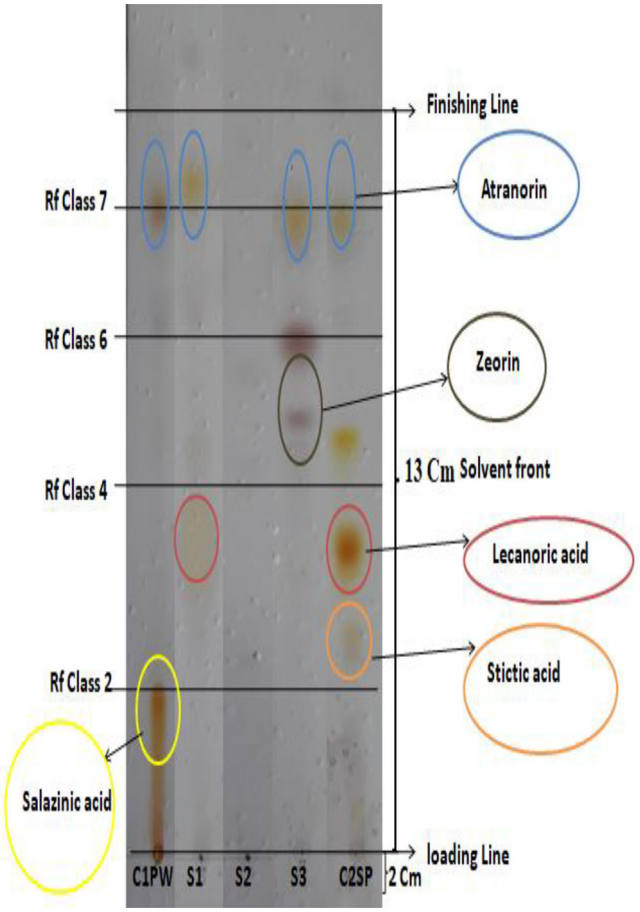


Figure 5. TLC plate showing different chemical spots at different R_f classes.

were procured through the utilisation of the Soxhlet apparatus (1g sample per 10ml of each solvent is used for extraction). The quantification of extract yield was subsequently undertaken, resulting in the determination of the extract yield for each solvent and Sample. The obtained results have been systematically presented in Table 6. Evidently, the data highlights that methanol consistently exhibited the highest yield across all samples in comparison to petroleum ether and acetone extracts.

3.6 Phytochemical Analysis

Following the preliminary phytochemical assessment, methanol was identified as the most effective solvent for extracting phytochemical compounds from all three Samples. The conclusions were validated by aligning the observed outcomes with the anticipated reactions in the test tubes. The conclusive results were then systematically documented and represented in Table 7.

Table 6. Quantitative yield of successive solvent extraction

Sample and Solvent Name	Quantity of Solvent Before Extraction	Quantity of Extract Obtained	Quantity of Solvent Utilised	Powder Weight Before Extraction	Powder Weight After Extraction	Powder Utilised in Extraction	Extraction Yield (%)
Sample 1: *Parmotrema austrosinense*							
Petroleum Ether	100 ml	85 ml	15 ml	10g	9.85g	0.15g	1.50%
Acetone	90.85 ml	82 ml	8.85 ml	9.85g	9.10g	0.75g	7.61%
Methanol	90.10 ml	83 ml	7.1 ml	9.10g	8.32g	0.78g	8.57%
Sample 2: *Dermatocarpon vellereum*							
Petroleum Ether	100 ml	81 ml	19 ml	10g	9.43g	0.57g	5.70%
Acetone	90.43 ml	84 ml	6.43 ml	9.43g	9.31g	0.12g	1.27%
Methanol	90.31 ml	85 ml	5.31 ml	9.31g	8.58g	0.73g	7.84%
Sample 3: *Heterodermia diademata*							
Petroleum Ether	100 ml	82 ml	18 ml	10g	9.47g	0.53g	5.30%
Acetone	90.47 ml	80 ml	10.47 ml	9.47g	9.24g	0.23g	2.43%
Methanol	90.24 ml	85 ml	5.24 ml	9.24g	8.50g	0.74g	8.01%

Note: Values represent the quantitative recovery from a single successive extraction process per species.

Table 7. Observation for qualitative phytochemical analysis of all samples

Test Name	Solvent	Expected Outcome	Sample 1 Result	Sample 2 Result	Sample 3 Result
Alkaloids	Petroleum ether	Reddish brown precipitate	Negative	Negative	Negative
	Acetone		Negative	Negative	Negative
	Methanol		Positive	Positive	Negative
Flavonoids	Petroleum ether	Yellow to orange color	Positive	Positive	Negative
	Acetone		Positive	Positive	Positive
	Methanol		Positive	Positive	Positive
Glycosides	Petroleum ether	Brown ring formation	Negative	Positive	Positive
	Acetone		Positive	Positive	Positive
	Methanol		Positive	Positive	Positive
Saponins	Petroleum ether	Stable foam formation	Negative	Negative	Negative
	Acetone		Positive	Positive	Positive
	Methanol		Positive	Positive	Negative
Steroids	Petroleum ether	Blue-green color ring	Positive	Positive	Positive
	Acetone		Negative	Positive	Negative
	Methanol		Negative	Negative	Negative
Tannins	Petroleum ether	Blue, Green, Black colors	Negative	Negative	Negative
	Acetone		Positive	Positive	Positive
	Methanol		Positive	Positive	Positive
Triterpenoids	Petroleum ether	Red-brown color at interphase	Positive	Positive	Negative
	Acetone		Positive	Negative	Negative
	Methanol		Positive	Negative	Negative

4. Importance and Significance of the Study

Lichens have garnered scientific attention due to their multifaceted significance and diverse benefits (Tables 8 and 9). These entities, often residing in challenging habitats, contribute to ecological dynamics, biogeochemical cycling, and environmental monitoring. In terrestrial ecosystems, lichens act as vital indicators of air quality and environmental health due to their sensitivity to pollutants. Their capacity to accumulate heavy metals and toxins from the atmosphere underscores their role as bioindicators, aiding in the assessment of air pollution levels and ecological disturbances. Beyond their environmental role, lichens hold pharmacological potential. Their bioactive compounds, including secondary metabolites, exhibit antimicrobial, anti-inflammatory, and antioxidant properties. Such attributes have spurred interest in their utilisation for traditional medicine and pharmaceutical research, particularly in addressing various ailments. Lichens' ecological significance is pronounced as they create microhabitats, fostering biodiversity by providing shelter and substrates for various organisms. Their capacity to fix nitrogen enhances soil fertility and nutrient cycling in ecosystems with limited nutrient availability. This, in turn, influences plant growth and ecosystem productivity. In cultural contexts, lichens carry historical and traditional importance. They have been utilised for dyeing textiles, providing insights into ancient artisanal practices. Additionally, lichens' role in folklore, rituals, and spiritual beliefs underscores their cultural value, connecting societies with nature and fostering a sense of identity. However, prudent utilisation is essential, as certain lichen species contain toxic compounds that can be harmful to humans and animals. Expert guidance and sustainable practices are imperative for safe utilisation while preserving their ecological integrity. In sum, lichens encompass a spectrum of importance ranging from environmental monitoring and ecological interactions to pharmaceutical applications and cultural significance. Investigating their biological, chemical, and ecological intricacies unlocks a treasure trove of benefits with the potential to influence scientific, medicinal, and cultural landscapes. Enumerated below are several ethnobotanical applications and the pivotal significance associated with the identified lichen specimens, namely *P. austrosinense, D. vellereum,* and *H. diademata.*

The present study involves the systematic collection and chemical analysis of lichen samples from the Garhwal region of Uttarakhand, India. Although previous reports have documented lichen samples from locations such as Chopta forest in Baniyakund, Rudraprayag, Govind Wildlife Sanctuary in Uttarkashi, and Khirsu-Pauri[33-37], the current research focuses on distinct sites that have not been previously explored for their lichen diversity. The selected sites for this study include Site 1: Jakher-Dang Road near Jawar village, Tehri Garhwal; Site 2: Pauri-Srinagar Road near Prem Nagar, Pauri Garhwal; and Site 3: Orchid Conservation Area near Mandal Village Forest area, Chamoli. Despite exhibiting similar habitats, these sites encompass different altitudes. The rationale for the collection of lichen species from various altitudes and habitats lies in the hypothesis that organisms alter their chemical composition in response to habitat variations to endure challenging environmental conditions[20]. Lichen specimens were collected from the chosen sites and subsequently identified based on established taxonomic keys, specifically utilising the "Compendium of Macrolichens of India, Nepal, and Sri Lanka" authored by Awasthi[22]. Although conventional identification methods were employed, contemporary research suggests the incorporation of molecular techniques to ensure precise and accurate identification[7]. This is attributed to the existence of morphologically and anatomically similar species with different chemical compositions and genetic variations arising from distinct habitats[20]. Universal primers, specifically the Internal Transcribed Spacer 1 (ITS1) and Internal Transcribed Spacer 4 (ITS4) sequences, have been employed as molecular tools for the precise identification of lichen species through a process known as DNA barcoding. DNA barcoding is a molecular technique that utilises these universal primers to target and amplify the ITS1 and ITS4 regions within the lichen DNA. These regions, located within the ribosomal DNA, have proven to be highly conserved within the lichen fungal symbionts and have variable regions that can discriminate between different lichen species. By amplifying and sequencing the ITS1 and ITS4 regions of lichen DNA, researchers can generate unique genetic profiles or "barcodes"

Table 8. Importance and uses of *P. austrosinense*, *D. vellereum,* and *H. diademata*

Lichen Species	Uses and Importance	References
Parmotrema austrosinense	Used as a spice in local traditional cuisine - B-glucosidase inhibitor (anti-diabetic potential) - Antibacterial, antifungal, and antimicrobial properties - Rich source of usnic acid and other bioactive secondary metabolites	8,9,13,20
Dermatocarpon vellereum	- Source of natural dyes for fabrics and textiles - Contains phenolic compounds with potential antioxidant properties - Traditionally used in colouring and preservation practices	10,11,13
Heterodermia diademata	- Applied on wounds and cuts for healing and infection prevention (especially with *Banmara* leaves in Sikkim) - Used as a spice and a natural dye - Paste of thalli used as plaster - Antiseptic, anti-lipoxygenase, and free radical scavenging properties - Exhibits significant antimicrobial and antifungal activity	10,11,13,14,20

Table 9. Ethnobotanical relevance of selected lichen species from Garhwal, Uttarakhand (Based on field data)

Lichen Species	Local Name	Date of Collection	Nearest Village	Traditional Uses	Mode of Application	Informant Names and Demographics
Parmotrema austrosinense	*Chharila (छरिला)*	10 July 2022	*Jawar (Tehri* Garhwal*)*	Wound healing, fever, and respiratory problems	Paste for wounds; decoction for cough and fever	1. Kundan Singh (M, 63) 2. Gulab Devi (F, 68) 3. Ramesh Rawat (M, 55) 4. Kamla Negi (F, 49) 5. Mahesh Rana (M, 60)
Dermatocarpon vellereum	No specific name	27 June 2021	Prem Nagar *(Pauri)*	Colouring of wool/cotton, relief in joint pain, skin rash	Boiled for dyeing; decoction/ topical for joint pain	1. Hemwati Devi (F, 71) 2. Chandraman Nautiyal (M, 67) 3. Pushpa Rawat (F, 58) 4. Surendra Bisht (M, 64) 5. Laxmi Devi (F, 62)
Heterodermia diademata	*Pahadi Chatta*	23 December 2021	*Mandal (Chamoli)*	Burns, sore throat, skin infections	Paste for burns; infusion as a gargle	1. Tejram Bhatt (M, 48) 2. Rajkumari Devi (F, 60) 3. Deena Ram (M, 53) 4. Nandu Singh (M, 50) 5. Shanta Devi (F, 57)

for each lichen species. These barcodes serve as a molecular signature for species identification, allowing for rapid and accurate discrimination between different lichen taxa. This approach is particularly valuable in cases where traditional morphological or chemical characteristics may not provide sufficient resolution for distinguishing between closely related lichen species[7]. The accurate identification of lichen species is crucial to harness their potential properties effectively. In this study, the collected lichen samples were identified as follows: Sample 1 – *P. austrosinense*, Sample 2 – *D. vellereum*, and Sample 3 –*H. diademata*. These samples were preserved in the LWG herbarium of the National Botanical Research Institute (NBRI) in Lucknow for future reference. The analysis of the chemical composition of the lichen samples involved the application of both primitive and advanced methods, including TLC, GCMS, High-Performance Thin Layer Chromatography (HPTLC), and Nuclear Magnetic Resonance (NMR) spectroscopy[7]. To initiate chemical analysis, the selection of an appropriate solvent for the extraction of compounds from the crushed lichen material was paramount. This study employed two methods to determine the optimal solvent for extraction: successive extraction using solvents of increasing polarity (petroleum ether, acetone, and methanol) and the assessment of residue weight on filter paper post-extraction[19,38]. Additionally, preliminary phytochemical tests were conducted to identify the solvent yielding the most favourable

outcomes for phytochemical extraction. Ultimately, the results indicated that methanol was the most effective solvent for extracting and testing the phytochemical constituents in the collected lichen samples. This research investigates lichen species from newly surveyed altitudinal sites in Garhwal, Uttarakhand, which have not been previously explored in published literature. Furthermore, it compares the efficiency of different solvents for secondary metabolite extraction, resulting in methanol being the most effective solvent, thus enhancing methodological clarity in lichen phytochemical research.

The present study explores the collection, taxonomic identification, and preliminary phytochemical profiling of three temperate lichen species—*Parmotrema austrosinense*, *D. vellereum*, and *H. diademata*—from ecologically distinct altitudinal zones in the Garhwal region of Uttarakhand. By selecting varied environmental habitats, this investigation sought to capture not only species diversity but also examine how altitude and substratum influence the secondary metabolite profiles of lichen species[20]. Taxonomic identification was carried out using standard morphological characters, chemical spot tests, and TLC, with expert validation conducted at the LWG Herbarium, CSIR-NBRI, Lucknow. All three species were confirmed and catalogued based on available taxonomic keys[12,22]. Their substrata—ranging from bark and twigs (Parmotrema), to rock surfaces (Dermatocarpon), to large stones in forest ecosystems (Heterodermia)—reflect habitat specificity and suggest ecological adaptations that may influence their biosynthetic pathways.

Preliminary phytochemical screening of methanolic extracts demonstrated the presence of major classes of bioactive compounds, including flavonoids, alkaloids, saponins, terpenoids, tannins, and glycosides. Among the solvents tested (data not shown), methanol consistently yielded the highest diversity of secondary metabolites, supporting its utility as an effective polar solvent for lichen extraction. In addition to chemical profiling, the study incorporates a detailed ethnobotanical field survey, which reveals that local villagers across Tehri Garhwal, Pauri Garhwal, and Chamoli districts retain traditional knowledge regarding the medicinal and utilitarian applications of lichens. The structured interviews conducted with local informants provided significant insights into how these species are used for healing wounds, treating respiratory disorders, soothing burns, managing skin ailments, and even dyeing textiles. These applications reflect a deep-rooted connection between indigenous communities and their surrounding biodiversity, underscoring the socio-ecological relevance of lichen species[39-42].

The field data documented in the study revealed multiple independent reports of traditional use for each species, with consistent patterns across different informants and villages. For example, *P. austrosinense* was widely applied as a wound dressing and fever remedy in paste or decoction form, while *D. vellereum* was used both medicinally and as a natural dye. *Heterodermia diademata* was especially noted for its role in treating burns and sore throats. The cross-verification of uses by multiple villagers from the same regions adds ethnographic robustness to these findings. Furthermore, environmental parameters such as temperature, humidity, altitude, and substratum—recorded at the time of collection—may also influence the metabolic output of these lichens. It is well-established that lichens adapt to abiotic stress by altering the production of secondary metabolites, many of which have ecological roles like UV protection, allelopathy, or antimicrobial defense[10,43]. Taken together, the study presents a valuable integration of chemical and ethnobotanical evidence, providing a foundation for deeper pharmacological, ecological, and conservation-oriented research. Given the growing interest in bioactive compounds from natural sources, these findings underscore the need for further chromatographic and bioassay-guided studies on Himalayan lichens to validate and explore their pharmacological potential.

5. Limitations of the Study

i. Geographical Scope: The study is restricted to three specific lichen species (*P. austrosinense*, *D. vellereum*, and *H. diademata*) collected from the temperate regions of *Tehri, Pauri,* and *Chamoli* districts. Consequently, the chemical profiles may vary in different climatic zones or altitudes.

ii. Analytical Depth: While preliminary phytochemical screening and TLC were used for metabolite identification,

advanced quantitative techniques like GC-MS and NMR were not employed in this preliminary phase.

iii. Pharmacological Validation: The study documents traditional uses (e.g., wound healing, fever) through ethnobotanical interactions but does not provide *in vivo* clinical data to validate these medicinal claims.

iv. Statistical Analysis: Extraction data represents the absolute yield from specific biomass samples processed via a single-run successive extraction method; further replicates would be required for a detailed statistical distribution.

6. Conclusion

The present study establishes a comprehensive baseline for the phytochemical and ethnobotanical profile of *P. austrosinense*, *D. vellereum*, and *H. diademata* from the Garhwal Himalayas. By integrating morphological validation with standardised TLC, this research successfully identifies key secondary metabolites and confirms methanol as the most efficient solvent for capturing maximum chemical diversity. Beyond the laboratory findings, the documentation of traditional knowledge reveals a deep-rooted cultural reliance on these species for therapeutic purposes, such as wound healing and respiratory relief. These insights emphasise that lichens are not merely biological specimens but are integral to the local traditional pharmacopoeia. Consequently, this work advocates for the urgent conservation of these underexplored altitudinal regions to prevent the loss of both biodiversity and associated indigenous knowledge. Future research utilising advanced quantitative tools like GC-MS and NMR is recommended to further explore the specific bioactive molecules responsible for the observed medicinal properties.

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8. Ethical Approval and Consent to Participate

The study was conducted in accordance with the ethical standards of the institutional research committee. Prior informed oral consent was obtained from all individual participants (local informants) involved in the ethnobotanical survey. All participants were briefed on the purpose of the study and voluntarily agreed to the public disclosure of the information provided.

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Author Contribution

Sachin Singh: investigation, original draft writing. Anjali Patil: Original draft writing, review, and editing. Vishal Khandelwal, Nitin K Prabhakar, Manali Kaintura, and Shivam Kumar: review, editing, data analysis, and formatting. Mamta Arya: conceptualise and supervision. All authors have read and agreed to the published version of the manuscript.