https://doi.org/10.48047/AFJBS.6.5.2024. 10023-10054

AfricanJournalofBiological Sciences

Comprehensive Study of Alpine Lichen Species in Uttarakhand: Characterization, Phytochemical Analysis, and Ethnobotanical Relevance

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Article History
Volume 6, Issue 5, 2024
Received: 22 May 2024
Accepted: 03 Jun 2024
doi:***10.48047/AFJBS.6.5.2024. 10023-10054***

Abstract

Lichens, complex symbiotic entities composed of fungi and algae, produce unique secondary metabolites with notable bioactive properties. This study aimed to collect, classify, and conduct preliminary phytochemical analysis on five lichen species: *Stereocaulon foliolosum*, *Everniastrum cirrhatum*, *Usnea longissima*, *Lobaria retigera*, and *Peltigera polydactylon*. Specimens were sourced from the alpine zones of Rudraprayag and Uttarkashi districts in Uttarakhand. To determine the optimal solvent for secondary metabolite profiling, we used petroleum ether, acetone, and methanol. Methanol was identified as the most effective solvent for phytochemical assessment. Specimens were preserved in the LWG herbarium at the National Botanical Research Institute (NBRI), Lucknow, for future reference. The uses and significance of these lichen species were documented through literature review and interactions with indigenous residents of the Garhwal region in Uttarakhand.

Keywords: Lichens, Taxonomy, Phytochemicals, *Everniastrum cirrhatum*, *Lobaria retigera*, *Peltigera polydactylon*, *Stereocaulon foliolosum* and Usnea longissima

Introduction

Lichens are symbiotic associations between fungi and algae (or cyanobacteria) that exhibit unique attributes and diverse applications. This symbiosis, characterized by mutual coexistence (Ahmadjian, 1993), involves a photosynthetic algal component (photobiont) providing energy and a fungal component (mycobiont) offering structural support. Lichens can adapt to extreme environments globally, from tundras to deserts, thanks to their distinct chemical compounds (Arya *et* al., 2021) which protect them against harsh conditions (Lawrey, 1986). These unique compounds arise

from the combined contributions of both the fungal and algal partners (Holm Hansen, 1968).

Historically, lichens have been utilized in traditional Indian medicine systems such as Ayurveda and Unani (Nayaka *et* al., 2010). They serve various roles, including natural dye production, perfume extraction, fodder, medicinal sources, agrochemicals, spices, and in lichenometry for dating substrates (Shah, 2014; Singh, 2019b). Their sensitivity to pollution makes them excellent bioindicators (Garty, 2001; Arya *et* al., 2020). Identifying lichen species and understanding their chemical makeup is essential for harnessing their potential, employing microscopic, chemical, and molecular methods (Nayaka, 2014; Singh *et* al., 2019). Over a thousand chemicals identified in lichens exhibit valuable properties (Molnar and Farkas, 2010; Stocker-Wörgötter, 2013), with the pharmaceutical industry exploring their medicinal applications. Techniques such as TLC, GCMS, and HPLC are used to analyze their chemical composition (Singh and Arya, 2019), with solvent choice impacting chemical yield due to varying polarities. Globally, around 20,000 lichen species exist, with approximately 2,900 in India, including 540 endemic species under the phylum Ascomycota, distributed across eight lichen-geographic regions (Nayaka *et* al., 2003; Feuerer and Hawksworth, 2007). Uttarakhand, with its diverse topography and altitudinal range, exhibits substantial lichen diversity, particularly in the Western Himalayan region. Pristine areas are recommended for sampling high-quality and abundant lichen specimens.

Uttarakhand's climate ranges from sub-tropical conditions in valleys to temperate and alpine climates at higher elevations, influenced by monsoons. Temperature variations range from 16°C to 40°C during summer, dropping below freezing in the elevated mountain regions during winter. The terai and bhabar areas adjacent to the plains exhibit a typical tropical climate, while alpine conditions prevail above 2400 meters (Chauhan, 2010), leading to a wide daily temperature range from tropical to frigid.

This study involved collecting, taxonomically identifying, and phytochemically evaluating five distinct lichen species to determine the optimal solvent for maximizing phytochemical yield. Solvents of ascending polarity—petroleum ether, acetone, and methanol—were used. Preliminary phytochemical analyses identified bioactive compounds. The ecological uses and significance of the selected lichen samples from the alpine region were documented through literature review and interactions with local inhabitants of the Garhwal region in Uttarakhand.

This work builds upon and advances the existing knowledge in several significant ways:

1. **Enhanced Taxonomic Understanding**: By collecting and taxonomically identifying lichen species from underexplored regions of Uttarakhand, this study adds to the growing database of lichen biodiversity in India, particularly in the Western Himalayan region.
2. **Phytochemical Insights**: The comprehensive phytochemical evaluation using solvents of varying polarities provides new insights into the optimal conditions for extracting bioactive compounds from lichens. This methodological advancement can improve the efficiency of phytochemical extraction processes.
3. **Ethnobotanical Relevance**: Documenting the ecological uses and significance of these lichen species through interactions with local inhabitants contributes to the preservation and appreciation of traditional knowledge. It also highlights potential new applications of lichens in various fields, including medicine and agriculture.
4. **Biotechnological Applications**: By identifying specific bioactive compounds, this research opens avenues for their potential use in pharmaceutical and biotechnological industries. This could lead to the development of new drugs and bioactive agents derived from lichens.
5. **Environmental Indicators**: The study reinforces the importance of lichens as bioindicators, offering a deeper understanding of their sensitivity to environmental changes. This can aid in monitoring and managing ecosystem health in Uttarakhand and similar regions.

This study not only enriches the existing scientific knowledge of lichen species in Uttarakhand but also lays the groundwork for future research and applications in various scientific and industrial domains.

Methodology

Sample 1: Collected from large rocks along the trek to Tungnath Temple, Kedarnath Wildlife Sanctuary, Rudraprayag, at approximately 3,680 meters above sea level.

Sample 2: Obtained from a Quercus leucotrichophora (oak) tree behind Madhyamaheshwar Temple in Rudraprayag, within the Kedarnath Wildlife Sanctuary.

Sample 3: Collected from Betula utilis (bhoj patra) trees near Budha Madhyamaheshwar, 1.5 kilometers northeast of Madhyamaheshwar Temple, at approximately 3,500 meters above sea level.

Sample 4: Gathered from a rock near Gangad village, Govind Wildlife Sanctuary, Uttarkashi, at around 2,700 meters above sea level.

Sample 5: Collected from a rock between Osla and Gangad villages on the Har Ki Dun trek, within Govind Wildlife Sanctuary, at approximately 2,580 meters above sea level. Table 1 presents the parameters of the sampling sites, while Figure 1 illustrates their geographical locations

Table 1: Parameters of sampling sites

Sample no.	**Date of sampling**	**Sampling Site**	**Location**	**Substratum**	**Humidity**	**Temperature**	**Altitude**
Sample 1	22/09/2021	Site-1: Way to Tungnath temple, chopta , Rudraprayag	30°29'20.80" N 79°12'26.50" E	on rock	78%	10^0C	3100-3200m
Sample 2	02/10/2021	Site-2: Forest area near Madhyamaheshwar temple, Rudraprayag	30°38'22.43" N 79°13'21.25" E	Bark of oak tree (*Quercus leucotrichophora*)	82%	12^0C	3400-3500m
Sample 3	02/10/2021	Site-3: Budha Madhyamaheshwar, Rudraprayag	30°37'59.80" N 79°12'40.93" E	Bark of bhoj patra tree (Betula utilis)	85%	14^0C	3400-3500m
Sample 4	15/08/2022	Site-4: Near Gangad village, Govind wild life	31°6'25.07"N 78°19'33.52" E	on rock	60%	20^0C	2700-2800m

		Sanctuary, Uttarkashi					
Sample 5	15/08/2022	Site-5: Between osla and Gangad, Govind wild life Sanctuary, Uttarkashi	31°6'41.81"N 78°20'19.59" E	on rock	62%	18^0C	2700-2800m

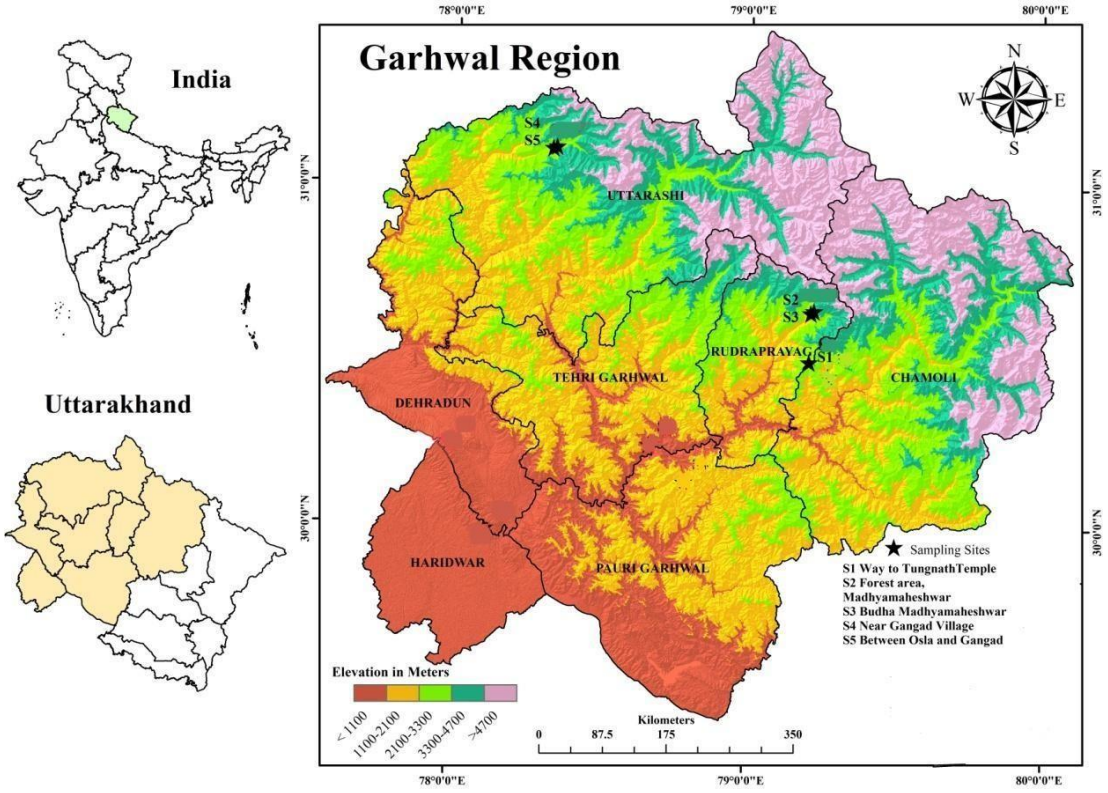

Figure 1: Illustrates the geographical locations of the sampling sites.

Collection Process

Lichen specimens were meticulously collected from the bark of Quercus leucotrichophora (oak) trees, twigs of Betula utilis (bhoj patra) trees, and rocks using chisels and knives. The sampling sites were thoroughly documented and photographed. Each sample was placed in newspaper compartments and securely stored in zip-lock polythene bags for safe transportation to the laboratory (Singh and Arya, 2023).

Sample Segregation and Allocation

The lichen samples were carefully detached from their substrates and cleaned using scalpels and blades to remove extraneous material. Cleaned specimens were weighed and designated for various purposes:Identification and authentication at the LWG herbarium at NBRI for validated accession numbers, phytochemical analysis, for future analysis samples were stored in a refrigerated environment for optimal preservation (Nayaka, 2014; Singh and Arya, 2023).

Figure 2: (A) Scraping of lichen sample 1 from a big rock situated on the trek to Tungnath Temple, (B) Scratching of lichen sample 2 frombark of a oak tree (*Quercus leucotrichophora*) in a forest near Madhyamaheshwar Temple, (C) Collecting of lichen sample 3 from bhojpatra (*Betula utilis*) tree in a forest near Budha Madhyamaheshwar, (d) lichen sample 4 attached on a big rock near Gangad village in between trek to Harkidun, (E)) View of Har-kidun trek, (F)Scratching of lichen sample 5 from a big rock situated in between Gangad and Osla village, trek to Har-kidun.

Lichen Identification

The collected lichen specimens were identified following protocols from the "Compendium of Macrolichens of India, Nepal, and Sri Lanka" (Awasthi, 2007).

Microscopic Examination

Morphological Examination: Both dissecting and compound microscopes were used. Key traits such as thallus color, structure, and granular formations were documented, including measurements of marginal lobes, presence of cilia, lower surface color, and rhizine characteristics. Photographic documentation was performed.

Anatomical Examination: Thallus anatomy was studied using temporary mount slides observed under compound microscopes at magnifications of 4X, 10X, 40X, and 100X. Measurements included the thickness of different thallus layers, algal type and distribution, and fungal hyphae arrangement. Vertical sections of the thallus were prepared by embedding in potato slices and slicing thinly with a sharp blade.

Chemical Identification Approaches

Color Spot Tests (Nylander and Flora, 1856; Asahina, 1934)

K Test: A 10% KOH solution was applied to the thallus surface, observing color changes. For medulla testing, the upper surface was scratched to expose the medulla before applying the reagent.

C Test: A solution of 2g calcium hypochlorite in 30 ml distilled water was applied to both cortex and medulla, observing color changes.

KC Test: Sequential application of K and C reagents to the cortex and medulla, followed by observation of color changes.

PD Test: A mixture of 3g sodium sulphite, 0.3g para-phenylenediamine, and 0.15ml detergent liquid in 30ml distilled water was applied to both cortex and medulla, and color changes were recorded.

Thin Layer Chromatography (TLC) (Culberson, 1972)

Extraction of Lichen Substances: Lichen samples were dissolved in 1 ml acetone.

Sample Loading: Pre-prepared Merck 60F 20X20 TLC plates were used. Acetone extracts and standard lichen were spotted on the TLC plates.

Solvent Preparation and TLC Tank Setup: Solvent C (Toluene acid, 170:30 ratio) was freshly prepared.

Running the Sample: TLC plates were placed in the TLC tank with solvent. Once the solvent reached the finish line, plates were removed.

Visualization: A 10% H_2SO_4 spraying reagent was used to visualize lichen substances, with plates briefly heated in a hot air oven at 110°C.

Identification of Spots: Spots were classified into seven Rf classes for comparison with standard lichen spots.

Qualitative Phytochemical Analysis (Sofowora, 1993; Trease and Evans, 1989; Harborne, 1973; Patil et al., 2019; Sethi et al., 2022)

Preparation of Lichen Extract

Lichen samples were washed, dried, ground, and sequentially extracted using petroleum ether, acetone, and methanol in a Soxhlet apparatus.

Phytochemical Tests

Tannins Test (Ferric Chloride Test): A 5% $FeCl_3$ solution was added to 2 ml of the crude lichen extract.

Alkaloids Test (Dragondroff‘s Test): A 1% HCl solution was mixed with 2 ml of the crude lichen extract, steamed for 10 minutes, and 6 drops of Dragondroff‘s reagent were introduced.

Saponins Test (Frothing Test): A test tube containing 2 ml of crude lichen extract and 5 ml of distilled water was vigorously shaken to induce frothing.

Glycosides Test (Keller-Kilani Test): A 2% $FeCl_3$ solution was mixed with 2 ml of glacial acetic acid and 2 ml of crude lichen extract, followed by addition to 2 ml concentrated H_2SO_4.

Flavonoids Test: A 10% NaOH solution was mixed with 2 ml of crude lichen extract.

Triterpenoid Test (Salkowski Test): A 2 ml portion of crude lichen extract was mixed with 1 ml chloroform, followed by the addition of concentrated H_2SO_4.

Steroid Test: A 2 ml portion of crude lichen extract was mixed with acetic anhydride and a few drops of concentrated H_2SO_4, forming a blue-green ring indicating the presence of steroids.

Results

Microscopic identification

Sample 1 Characteristics: The thallus of Sample 1, which exhibits a saxicolous habitat, typically reaches heights of 3.5 cm. It displays limited branching, a decorticated surface, and subglabrous characteristics. The pseudopodetia are brownish, with flattened, leafy phyllocladia measuring 2 mm in length, evenly distributed up to the apex. Brown, protosacculatecephalodia, measuring 1-2 mm in diameter, possess a cortex with elongated or isodiametric cell lumina enclosing Nostoc. Terminal apothecia reach up to 2 mm in diameter. Vermiform ascospores feature 6-14 septa and measure approximately 250 µm. Several of these traits are visually represented in Figure 3.

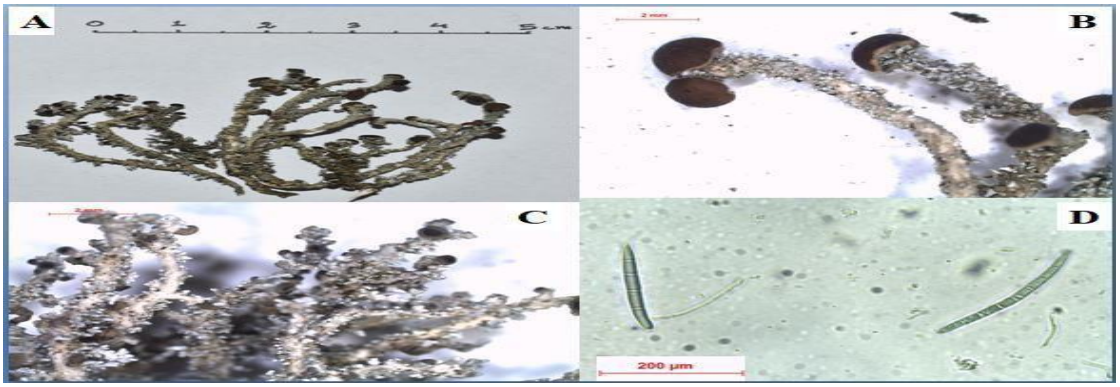

Figure 3: (A) Thallus of Sample 9, (B)Pseudopodetia, (C) Thallus having apothecia, (D) Ascospores

Sample 2 Characteristic: The thallus of this corticolous species, which grows on tree bark, exhibits a sub-erect to pendulous form with a diameter of up to 12 cm. The thallus lobes measure 2-4 mm in width. The upper side of the thallus is grey to dark grey and lacks soredia and isidia. The lower side is black-brown and may feature elongated rhizinal structures. Apothecia, measuring up to 6 mm in diameter, possess hollow stalks. Ascospores measure 15-18 (-30) x 7-12 µm. Some of these characteristics are visually depicted in Figure 4.

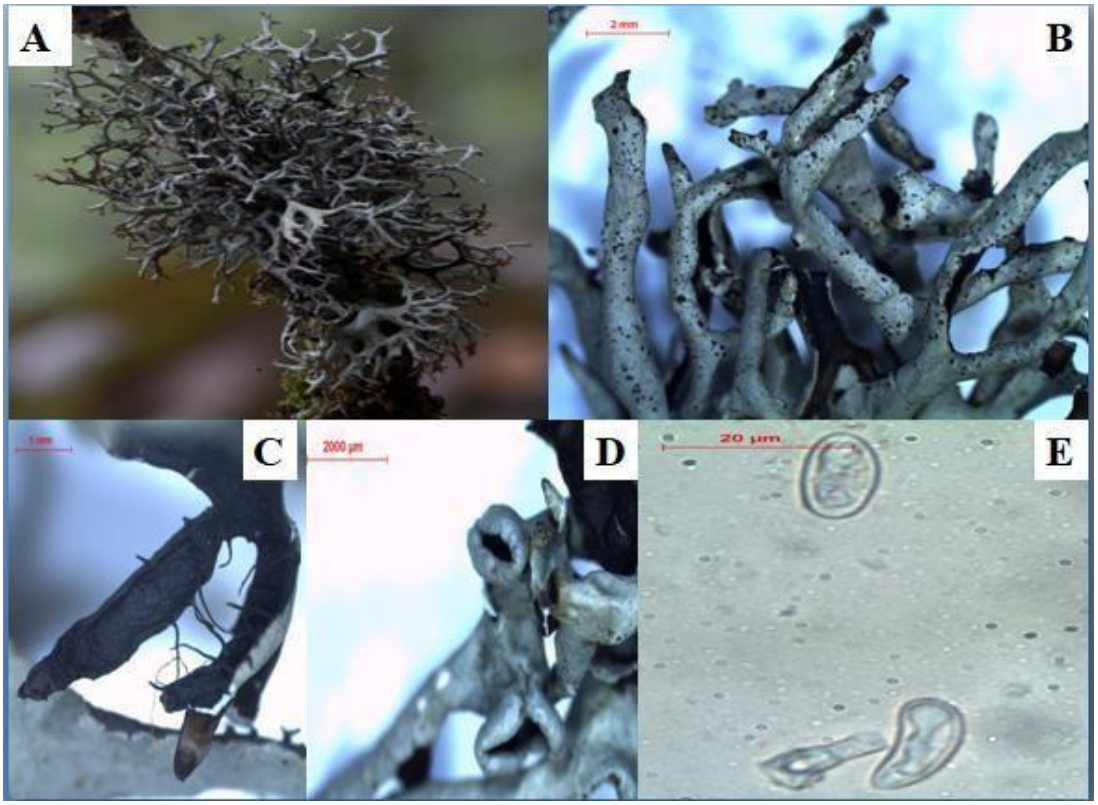

Figure 4: (A) Thallus of Sample 2, (B) Divaricated branches, (C) lower side rhizines, (D) Apothecia, (E) Ascospores

Sample 3 Characteristics: This corticolous species, which grows on trees, exhibits a pendulous growth form with filamentous branches extending up to 60 cm, and occasionally several meters in nature. The thallus ranges in color from pale yellow to greyish green, and sometimes light brown, with a diameter of approximately 1 mm. Densely arranged lateral branchlets, perpendicular to the main axis, measure 3 cm in length. The filamentous branches typically have a decorticated surface, though they may appear pulverulent or powdery in rare instances. The cortex of the lateral branchlets is persistent, often cracked near the base, and occasionally bears soredia or isidia. The thallus's central axis is solid, colorless, and bordered by a ciliate margin. These characteristics are depicted in Figure 5.

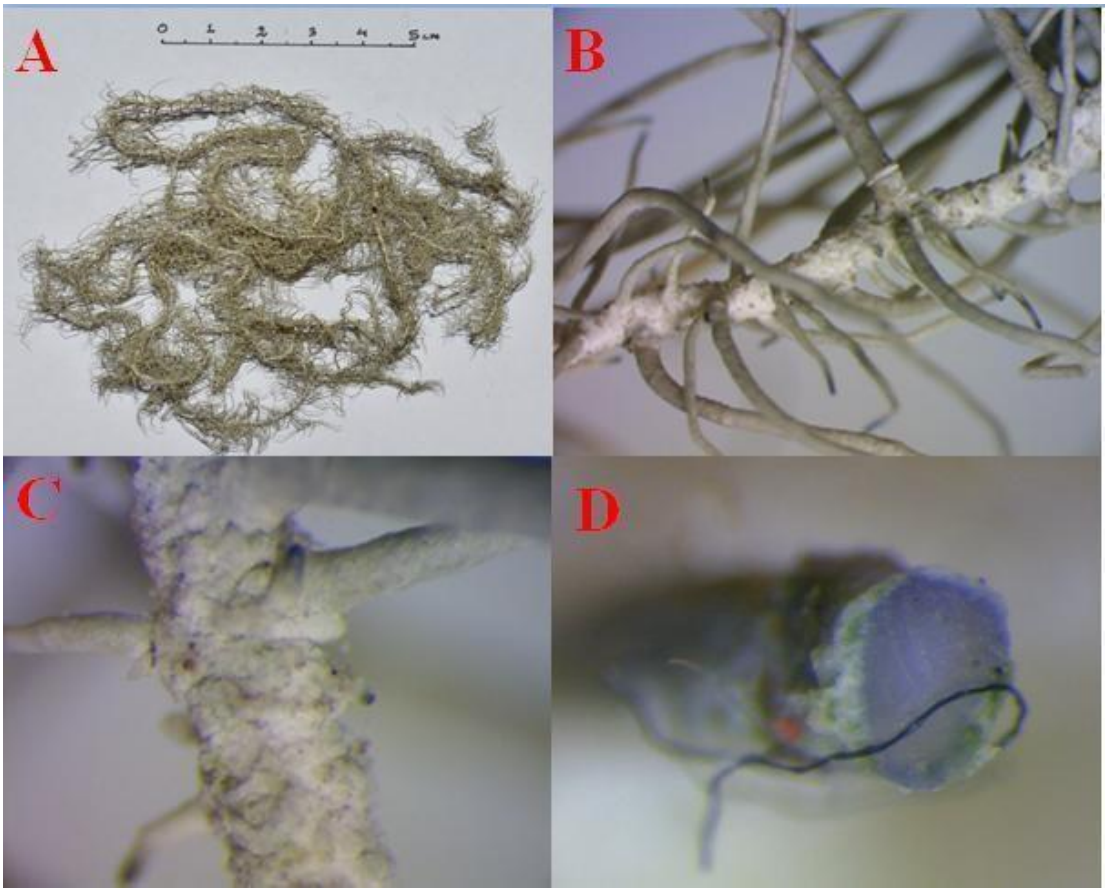

Figure 5: (A) Thallus of Sample 10, (B)pendulous growth form of thallus, (C) Filamentous branches of thallus, (D) Vertical section of central axis changes white to blue on applying Iodine on it (I+ Blue)

Sample 4 Characteristics: The thallus of this saxicolous species is firmly attached to a rock surface, exhibiting a loose adnate growth pattern and extending up to 30 cm in diameter or larger. The thallus lobes are substantial, measuring 10-30 mm in width. The upper side of the thallus is pale brown, darkening towards the margins, with scrobiculate features and reticulately ridged patterns. Isidia formations, which can be granular, cylindrical, and range from simple to coralloid, are commonly found on these ridges, along with occasional minute lobules. The lower side of the thallus is dark brown to black, with tomentose characteristics and sparse rhizines occurring in grooves and convex areas. The convex areas are devoid of rhizines and exhibit a pale brown coloration. The associated phycobiont is identified aNostoc. These characteristics are depicted in Figure 6.

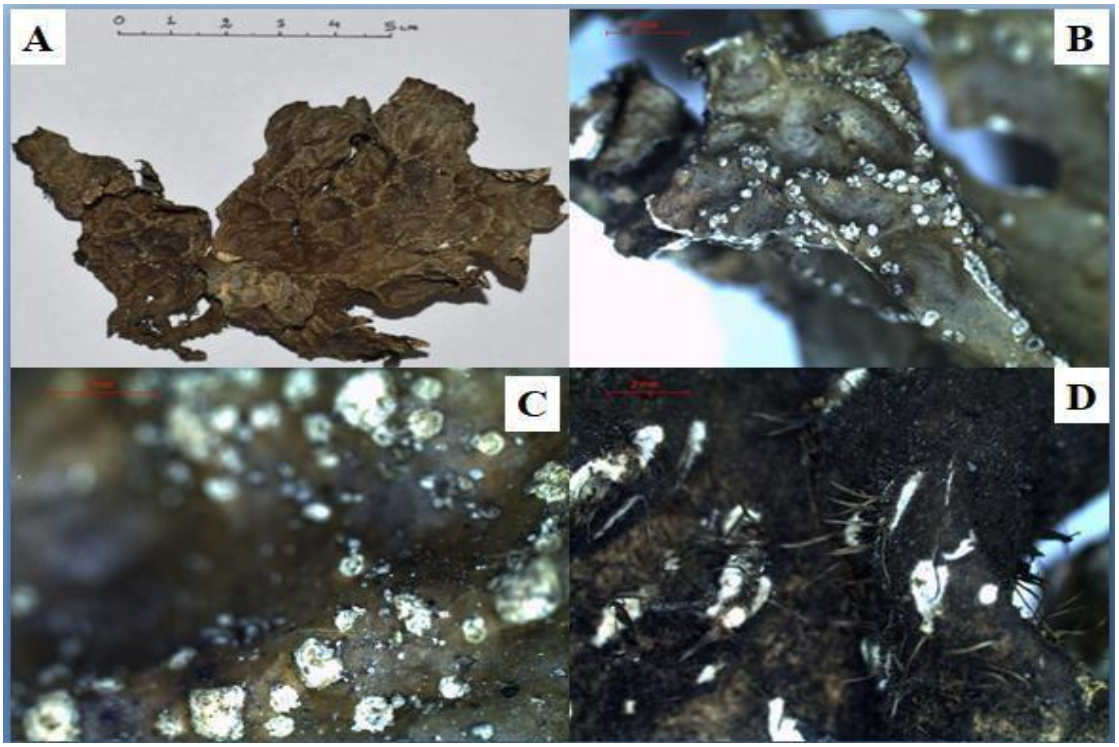

Figure 6: (A) Thallus of Sample 4, (B) Isidia on ridges, (C) Isidia, D) lower side rhizines

Sample 5 Characteristics: This species exhibits a saxicolous thallus, typically exceeding 10 cm in size. Thallus lobes measure 4-5 cm in length and 10-15 mm in width, often featuring phyllidia. The upper surface is greyish-brown and etomentose, lacking isidia and soredia. The lower surface displays a distinct reticulate pattern with brown-black veined structures and round or elongated interspaces. Rhizines are confluent and fasciculate. The photobiont is Nostoc. Apothecia are vertically oriented, clustered in groups of 2-7, saddle-shaped, and measure 4-7 mm in diameter, maturing to a reflexed form. Ascospores are acicular, possessing 5-9 septa and measuring approximately 150 µm. These features are depicted in Figure 7.

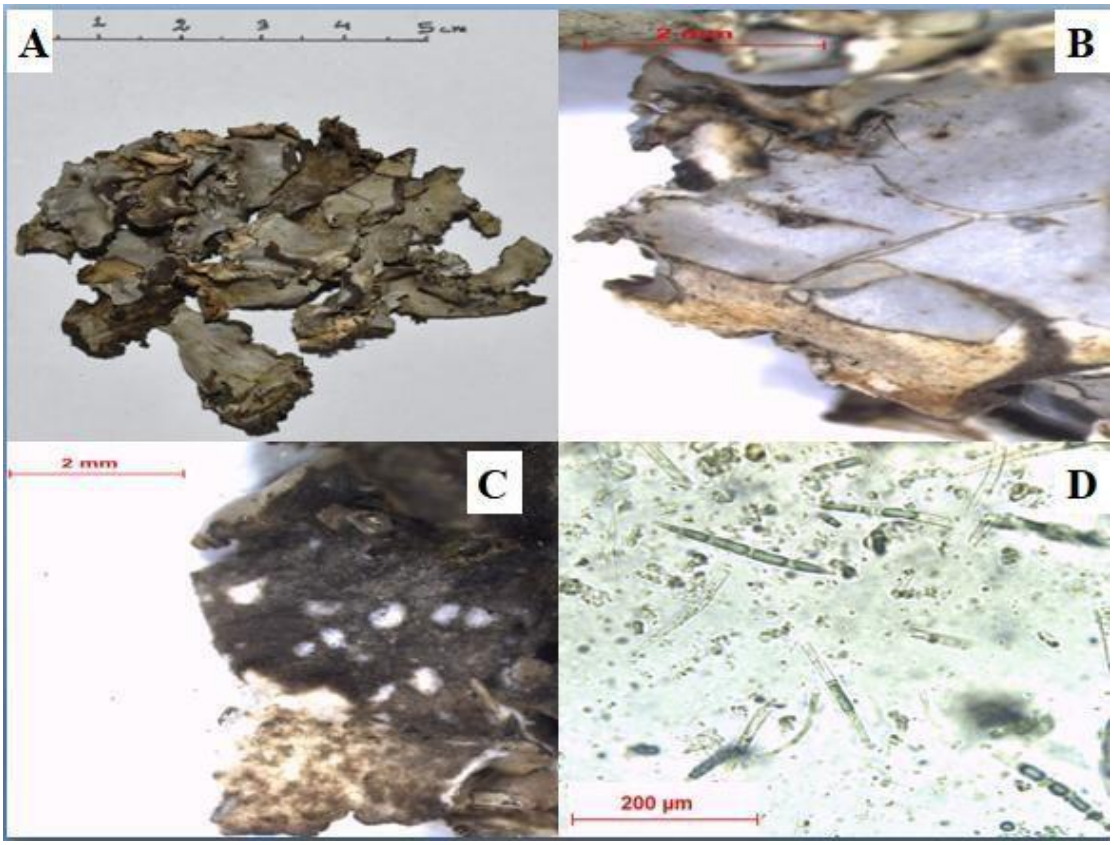

Figure 7: (A) Thallus of Sample 8, (B) Lobes of the thallus, (C) lower side of the thallus, (D) Ascospores

Color spot results

Sample 1-Upon subjecting the sample to comprehensive color spot tests, distinct color changes were observed in both the cortex and medulla. In the cortex, the application of K+ and P+ reagents resulted in a yellow coloration, indicating positive results. In the medulla, the K+ reagent alone produced a yellow color change, confirming the presence of atranorin. No discernible color changes were observed for the remaining tests, which were thus categorized as negative. Detailed observations and results for Sample 1 are presented in Table 2.

Table 2: Observation of color spot test of sample 1.

Sample 1	**K test**		**C test**		**KC test**		**Pd test**		**Result**
Cortex	+ve	Yellow	-ve	No color change	-ve	No color change	+ve	Yellow	Atranorin present
Medulla	+ve	Yellow	-ve	No color change	+ve	Yellow	-ve	No color change	

Sample 2-Sample 2 underwent color spot tests, revealing distinct color changes in both the medulla and cortex. Upon application of the K+ reagent, a yellow coloration was observed, transitioning to orange and eventually brown-red in both regions. Similarly, the P+ reagent induced an orange coloration in the cortex and medulla, subsequently turning red. Notably, no observable color changes were noted in the remaining tests. These results collectively indicate the presence of salazinic acid in Sample 2. Detailed test observations are provided in Table 3.

Table 3: Observation of color spot test of sample 2

Sample 2	**K test**		**C test**		**KC test**		**Pd test**		**Result**
Cortex	+Ve	Yellow to orange	-Ve	No color change	-Ve	No color change	+Ve	light orange	Salazinic acid present
Medulla	+Ve	Yellow to orange	-Ve	No color change	-Ve	No color change	+Ve	Orange to red	

Sample 3- Following a comprehensive series of tests on Sample 3, no discernible color changes were observed, indicating negative results for all conducted tests and suggesting the absence of the targeted chemicals. However, it is noteworthy that the I+ reagent induced a blue coloration, facilitating the identification of this sample. Detailed observations and results for Sample 3 are provided in Table 4.

Table 4: Observation of color spot test of sample 3

Sample 3	**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	No chemical found
Medulla	-ve	No color change	-ve	No color change	-ve	No color change	-ve	No color change	

Sample 4- After subjecting Sample 4 to a comprehensive testing protocol, no discernible color changes were detected. Consequently, all tests performed on this sample were classified as negative, indicating the absence of the targeted chemicals. Further analysis, such as Thin Layer Chromatography (TLC), is warranted to confirm the presence of chemicals. Detailed observations and results for Sample 4 are provided in Table 5.

Table 5: Observation of color spot test of sample 4

Sample 4	**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	No chemical found
Medulla	- ve	No color change	- ve	No color change	- ve	No color change	- ve	No color change	

Sample 5- Following an extensive array of tests on Sample 5, no discernible color changes were observed. Consequently, all tests conducted on this sample yielded negative results, suggesting the absence of the targeted chemicals. Detailed observations and results for Sample 5 are provided in Table 6.

Table 6: Observation of color spot test of sample 5

Sample 5	**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	No chemical found
Medulla	−ve	No color change	−ve	No color change	−ve	No color change	-ve	No color change	

Thin Layer Chromatography (TLC) Results

Parmelinella wallichiana (C1PW) and *Stereocaulon pomiferum* (C22SP) were utilized as reference controls for comparative analysis in Thin Layer Chromatography (TLC).

Parmelinella wallichiana (C1PW) exhibited three distinct chemical spots at Rf class 1, 2, and 7, corresponding to Consalazinic acid, Salazinic acid, and Atranorin, respectively. Similarly, *Stereocaulon pomiferum* (C2SP) showed three spots at Rf class 3, 3, and 7, representing Stictic acid, Lecanoric acid, and Atranorin. In Sample 1, two spots at Rf class 4 and Rf class 7, displaying pale green-grey and yellow-orange colors, respectively, indicated the presence of lobaric acid and atranorin. Sample 2 exhibited three spots at Rf class 2, Rf class 5-6, and Rf class 7, with bright orange, faded grey, and yellow-orange colors, respectively, indicating the presence of salazinic acid, protolichesterinic, and atranorin. Sample 3 displayed three spots at Rf class 2, Rf class 5, and Rf class 4-5, showing alate grey, straw yellow, and yellow colors, respectively, suggesting the presence of fumarprotocetraric, barbaric acid, and evernic acid. Sample 4 displayed three unknown spots at Rf class 1, Rf class 3, and Rf class 8, all faded grey, implying the possible presence of triterpenoids. Sample 5 exhibited a single spot at Rf class 7, light yellow in color, indicating the presence of Tenuiorin. Detailed observations and results for these samples are provided in Table 7, while visual representations of the color spots on the TLC plate are available in Figure 8.

Table 6: Shows the observations of TLC plate results

Controls and Samples	**Spot color**	**Rf Classes**	**Chemicals present**
C1PW- Control 1 *Parmelinella wallichiana*	Yellowish Dark Orange Yellow	Rf class 1 Rf class 2 Rf class 7	Consalazinic acid Salazinic acid Atranorin
C2SP- Control 2 *Stereocaulon pomiferum*	Light Yellow Dark Orange Yellowish	Rf class 3 Rf class 3 Rf class 7	Stictic acid Lecanoric acid Atranorin
Sample 1	Yellow-orange	Rf Class 7	Atranorin
	Pale green grey	Rf Class 4	Lobaric acid

Sample 2	Bright orange	Rf class 2	Salazinic acid
	Faded grey	Rf class 5-6	Protolichesterinic acid
	Yellow-orange	Rf class 7	Atranorin
Sample 3	Alate- grey	Rf Class 2	Fumarprotocetraric
	Straw yellow	Rf Class 5	Barbatic acid
	Yellow	Rf class 4-5	Evernic acid
Sample 4	Faded grey	Rf class1	Unknown
	Faded grey	Rf class3	Unknown
	Faded grey	Rf class8	Unknown
			May be Triterpenoids
Sample 5	Light Yellow	Rf class 7	Tenuiorin

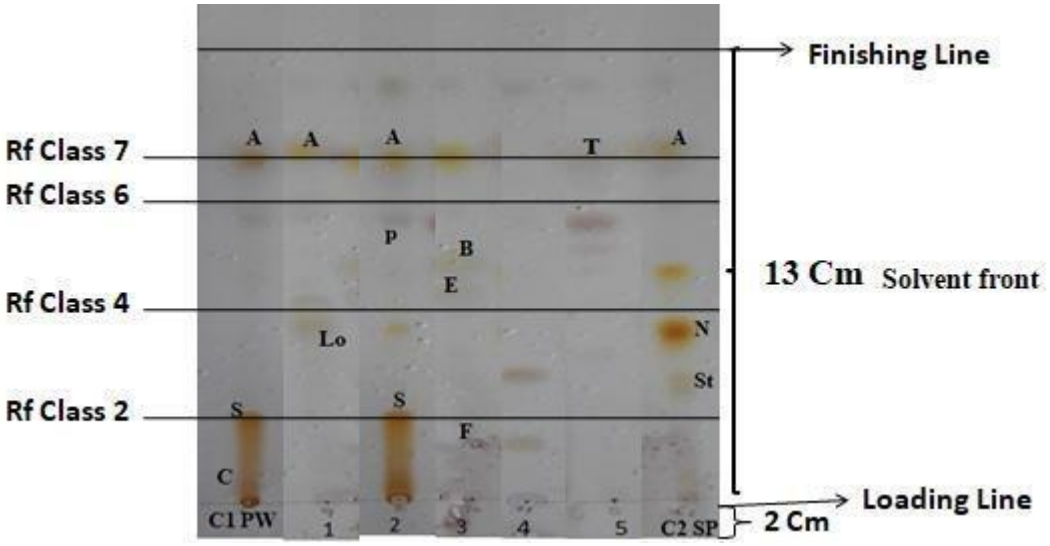

Figure 8: TLC plate showing different color spots at different Rf classes having C1PW- *Parmelinella wallichiana* and C2 SP- *Stereocaulon pomiferum* as a control, 1 to 5 samples to be tested, Chemicals indicated by color spots: C- consalazinic acid, S- Salazinic acid, A- Atranorin, P- Protolichesterinic acid, L- Lecanoric acid, T- Tenuiorin, Lo- lobaric acid, F- Fumarprotocetraric, E- Evernic acid, B- Barbatic acid.

Final Identification Results

Following thorough identification procedures, the specimens were successfully categorized as follows: Sample no. 1 was identified as *Stereocaulon foliolosum*, Sample no. 2 as *Everniastrum cirrhatum*, Sample no. 3 as *Dolichousnea longissima*, Sample no. 4 as *Lobaria retigera*, and Sample no. 5 as *Peltigera polydactylon*. Subsequently, these identified specimens were curated and preserved within the LWG Herbarium of the National Botanical Research Institute (NBRI), with each specimen assigned a unique accession number for future reference. Specifically, the accession numbers assigned were as follows: Sample no. 1: LWG-61629, Sample no. 2: LWG-63590, Sample no. 3: LWG-58771, Sample no. 4: LWG-63587, Sample no. 5: LWG-63586.

Extraction and Yield Determination of Lichen Extracts

The lichen constituents were extracted from each sample using three solvents of increasing polarity: petroleum ether, acetone, and methanol, employing the Soxhlet apparatus. Subsequently, the yield of each extract was quantified, allowing for the determination of the extract yield for every solvent and sample. The resulting data is systematically presented in Table 7 Methanol consistently demonstrated the highest yield across all samples compared to petroleum ether and acetone extracts.

Table 7: Sample Utilization for Successive Solvent Extraction

Sample no. 1- *Stereocaulon foliolosum*			
Solvent used for extraction	Powder weight before extraction	Powder weight after extraction	Powder utilized in extraction
Petroleum ether	10 grm	9.75grm	0.25grm
Acetone	9.75 grm	9.20grm	0.55grm
Methanol	9.20grm	8.40grm	0.80grm
Sample no. 2- *Everniastrum cirrhatum*			
Solvent used for extraction	Powder weight before extraction	Powder weight after extraction	Powder utilized in extraction
Petroleum ether	10 grm	9.60grm	0.40grm
Acetone	9.60grm	9.18grm	0.42grm
Methanol	9.18grm	8.42grm	0.76grm

Sample no. 3- *Usnea longissima*			
Solvent used for extraction	Powder weight before extraction	Powder weight after extraction	Powder utilized in extraction
Petroleum ether	10 grm	9.65grm	0.35grm
Acetone	9.65grm	9.11grm	0.54grm
Methanol	9.11grm	8.33grm	0.78grm
Sample no. 4-*Lobaria retigera*			
Solvent used for extraction	Powder weight before extraction	Powder weight after extraction	Powder utilized in extraction
Petroleum ether	10 grm	9.55grm	0.45grm
Acetone	9.55grm	9.24grm	0.31grm
Methanol	9.24grm	8.50grm	0.74grm
Sample no. 5- *Peltigera polydactylon*			
Solvent used for extraction	Powder weight before extraction	Powder weight after extraction	Powder utilized in extraction
Petroleum ether	10 grm	9.62grm	0.38grm
Acetone	9.62grm	9.32grm	0.30grm
Methanol	9.32grm	8.55grm	0.77grm

Phytochemical Analysis

After conducting the initial phytochemical evaluation, it was determined that methanol served as the most efficient solvent for extracting phytochemical compounds from all five samples. These conclusions were substantiated by comparing the observed outcomes with the expected reactions in the test tubes. The definitive results were systematically recorded and depicted in Table 8.

Table 8: Presents the qualitative phytochemical analysis observations for all lichen samples. The summary of preliminary phytochemical results for the lichen samples includes *Stereocaulon foliolosum* (SF), *Everniastrum cirrhatum* (EC), *Usnea longissima* (UL), *Lobaria retigera* (LR), and *Peltigera polydactylon* (PG). The tests conducted include Alkaloids(Alko), Flavonoids (Flavo), Glycosides (Glyco), Saponins (Sapo), Steroids (Stero), Tannins (Tan), and Triterpenoids (Trit).

Table 8: Preliminary analysis results

Preliminary phytochemical results of all lichen samples

Sample initials	**Solvent used**	**Test initials**	**Alko**	**Flavo**	**Glyco**	**Sapo**	**Stero**	**Tan**	**Trit**	**Total test positive**
SF	Petroleum ether		-	-	+	-	+	-	+	3
	Acetone		-	+	+	+	-	+	+	5
	Methanol		-	+	+	+	-	+	+	5
EC	Petroleum ether		-	+	+	-	+	-	-	3
	Acetone		-	+	+	+	-	+	-	4
	Methanol		-	+	+	+	-	+	-	4
UL	Petroleum ether		-	+	-	-	+	-	+	3
	Acetone		-	+	+	+	-	+	+	5
	Methanol		-	+	+	+	-	+	+	5
LR	Petroleum ether		-	-	-	-	+	-	+	2
	Acetone		-	+	+	+	-	+	+	5
	Methanol		-	+	+	+	-	+	+	5
PG	Petroleum ether		-	-	+	-	+	-	+	3
	Acetone		-	+	+	-	-	-	+	3
	Methanol		+	+	+	-	-	+	+	5

Importance and Significance of lichens

Lichens have gained considerable scientific attention owing to their multifaceted significance and diverse ecological contributions. Inhabiting challenging environments, they play crucial roles in ecological dynamics, biogeochemical cycling, and environmental monitoring. Particularly in terrestrial ecosystems, lichens serve as vital indicators of air quality and environmental health due to their sensitivity to pollutants. Their ability to accumulate heavy metals and toxins from the atmosphere makes them valuable bioindicators for assessing air pollution levels and ecological disturbances.

Beyond their environmental role, lichens exhibit pharmacological potential, with bioactive compounds such as secondary metabolites displaying antimicrobial, anti-inflammatory, and antioxidant properties. This has led to exploration in traditional medicine and pharmaceutical research for addressing various ailments. Ecologically, lichens create microhabitats that foster biodiversity by providing shelter and substrates for diverse organisms. Their nitrogen-fixing capacity enhances soil fertility and nutrient cycling, influencing plant growth and ecosystem productivity. Culturally, lichens hold historical and traditional importance, having been utilized for dyeing textiles and carrying symbolic significance in folklore, rituals, and spiritual beliefs. However, cautious utilization is crucial as certain lichen species contain toxic compounds harmful to humans and animals. Expert guidance and sustainable practices are necessary for safe utilization while preserving ecological integrity.

In summary, lichens encompass a wide spectrum of importance spanning environmental monitoring, ecological interactions, pharmaceutical applications, and cultural significance. Understanding their biological, chemical, and ecological intricacies unveils a wealth of benefits with potential impacts on scientific, medicinal, and cultural realms. Below in Table 9 there are several ethnobotanical applications and pivotal significance associated with identified lichen specimens: *Stereocaulon foliolosum, Everniastrum cirrhatum, Usnea longissima, Lobaria retigera, and Peltigera polydactylon*

Table 9: Importance and uses of *Stereocaulon foliolosum, Everniastrum cirrhatum, Usnea longissima, Lobaria retigera, Peltigera polydactylon*

S.No.	Lichen species	Uses and Importance	References
1.	*Stereocaulon foliolosum*	Used for Urinary trouble, blister of the tongue, Anti-mycobacterial	(Saklani and Upreti 1992), (Gupta, 2007)
2.	*Everniastrum cirrhatum*	Widely used as spices, food, and medicines. Used to make a crude drug named ‗Chharila' applied to	(Jain, 2016), (Pol *et* al. 2017), (Prashith Kekuda *et* al.

		wounds, diseases of the blood and heart, stomach disorders, enlarged spleen, bronchitis, bleeding piles, dyspepsia, scabies, leprosy, sore throat, toothache and pain in general, kidney stones, painful urination, haemorrhoids, lack of menstruation, menstrual pain, broken bones, rheumatism, reducing swelling. It is also used as a carminative, aphrodisiac, diuretic, sedative and astringent. Antioxidative, cardioprotective, anticancer, antifungal, cytotoxic, antiobesity (pancreatic lipase inhibitory), antimicrobial, antihelmintic, insecticidal, anti-mycobacterial, antibacterial, amylase inhibitory	2012), (Anil,2011), (Swathi *et* al. 2010), (Gupta 2007), (Joshi 2011), (Nayaka, 2010), (Thadhani, 2017)
3.	*Usnea longissima*	The Baiga tribes of Madhya Pradesh used the species along with other ingredients for treating bone fracture. The Chinese used it as an expectorant in the name ―Sun-Lo‖. In China it is used for stopping sweating dizziness, cold and cough. The species is also used in the treatment of gastric, used to treat cancer, tuberculosis and ulcers Antimicrobial, cytotoxic,	(Jain,2016), (Thippeswamy, 2011), (Yu,2016), (Atalay, 2011), (Lee, 2005)

		antifungal, antioxidant, antiplatelet, antithrombotic	
4.	*Lobaria retigera*	Constituents of Chinese medicine, Used as spice in North India, Antitumor, Anti-dermatophytic, antibacterial, antifungal	(Upreti *et* al. 2005), (Pathak 2016), (Takahashi, 1974)
5.	*Peltigera polydactylon*	Paste of thalli applied on cut injury to stop bleeding	Sinha, 2005

Discussion

Ecological Significance

Lichens, remarkable symbiotic organisms composed of fungi and algae, have garnered significant attention in scientific research due to their multifaceted significance across ecological, pharmaceutical, and cultural domains. This discussion delves into the diverse attributes and implications associated with five identified lichen species: *Stereocaulon foliolosum*, *Everniastrum cirrhatum*, *Usnea longissima*, *Lobaria retigera*, and *Peltigera polydactylon*.

In ecological terms, lichens play pivotal roles in various ecosystems. They serve as bioindicators of environmental health and air quality due to their sensitivity to pollutants (Smith, 2009). The ability of lichens to accumulate heavy metals and toxins from the atmosphere makes them valuable tools for monitoring air pollution levels (Nash, 2008). Moreover, lichens contribute to biogeochemical cycling and nutrient cycling, particularly through their nitrogen-fixing capacity, enhancing soil fertility and influencing ecosystem productivity (Lange & Kilian, 1993). The presence of these lichen species in alpine zones of Uttarakhand highlights their role in maintaining ecological balance in harsh environments.

Pharmaceutical and Bioactive Potential

Lichens are known for their diverse array of secondary metabolites, which possess significant pharmaceutical potential. The phytochemical analysis of the five lichen samples in this study revealed the presence of various bioactive compounds, including alkaloids, flavonoids, glycosides, saponins, steroids, tannins, and triterpenoids. These compounds exhibit antimicrobial, anti-inflammatory, antioxidant, and anticancer properties, making lichens valuable in traditional medicine and pharmaceutical research (Boustie & Grube, 2005).

For instance, *Stereocaulon foliolosum* has demonstrated anti-mycobacterial properties, potentially useful in treating tuberculosis (Gupta, 2007). *Everniastrum cirrhatum* has a broad spectrum of medicinal applications, including antioxidative, cardioprotective, and anticancer activities (Jain, 2016). *Usnea longissima* has been used traditionally for treating bone fractures and has antimicrobial and cytotoxic properties (Jain, 2016). *Lobaria retigera* and *Peltigera polydactylon* also exhibit antibacterial and antifungal activities, further underscoring the therapeutic potential of lichens (Pathak, 2016; Sinha, 2005).

Cultural and Ethnobotanical Significance

Lichens have historically been used in various cultural and ethnobotanical contexts. They have been utilized for dyeing textiles, in traditional medicine, and in cultural rituals. For example, *Everniastrum cirrhatum* is widely used in traditional Indian medicine for treating a variety of ailments, from respiratory issues to gastrointestinal disorders (Swathi et al., 2010). The use of lichens in traditional practices highlights their significance beyond ecological and pharmaceutical domains, bridging cultural heritage and biodiversity conservation.

Building Upon and Advancing Existing Knowledge

This study builds upon and advances existing knowledge in several ways:

1. **Comprehensive Characterization**: By employing microscopic identification, color spot tests, and thin-layer chromatography (TLC), this study provides a detailed characterization of the five lichen species. The combination of these

methods allows for accurate identification and a deeper understanding of the chemical composition of lichens.

2. **Phytochemical Profiling**: The phytochemical analysis conducted in this study adds to the existing knowledge of lichen metabolites. By identifying specific compounds present in each lichen species, this research contributes to the growing database of bioactive compounds derived from lichens.
3. **Ecological Insights**: The study highlights the ecological roles of lichens in the alpine zones of Uttarakhand, contributing to the understanding of their environmental significance in these regions. This knowledge is crucial for conservation efforts and for monitoring the health of these ecosystems.
4. **Ethnobotanical Relevance**: Documenting the traditional uses and cultural significance of these lichens enriches the ethnobotanical literature. This aspect of the study underscores the importance of preserving traditional knowledge and integrating it with modern scientific research.
5. **Pharmaceutical Potential**: By identifying bioactive compounds with potential therapeutic applications, this study paves the way for future pharmacological research and the development of new medicines derived from lichens.

Therefore, this study provides a comprehensive analysis of five lichen species, highlighting their ecological, pharmaceutical, and cultural significance. The findings enhance our understanding of lichens' multifaceted roles and pave the way for future research and applications in various fields. The integration of traditional knowledge with modern scientific techniques exemplifies a holistic approach to studying these remarkable organisms.

Conclusion

Our comprehensive investigation into the diverse lichen species found within the alpine zones of Uttarakhand has yielded valuable insights into their ecological significance, phytochemical composition, and potential ethnobotanical applications. Through meticulous field sampling, specimen identification, and phytochemical analysis, we have illuminated the intricate relationships between lichens and their environment, as well as their pharmacological and cultural importance. Our findings underscore the role of lichens as bioindicators of environmental health, particularly their sensitivity to air pollution and their ability to accumulate heavy metals and

toxins. This sensitivity makes lichens valuable for monitoring environmental changes and pollution levels. Additionally, the presence of bioactive compounds in lichens, including secondary metabolites with antimicrobial, anti-inflammatory, and antioxidant properties, highlights their pharmaceutical potential. These compounds hold promise for the development of novel drugs to address various ailments. Furthermore, our study sheds light on the ecological role of lichens in enhancing soil fertility, nutrient cycling, and biodiversity in alpine ecosystems. By creating microhabitats and fixing nitrogen, lichens contribute significantly to ecosystem productivity and resilience. Our investigation into the ethnobotanical applications of lichens reveals their historical and cultural significance, from dyeing textiles to their roles in folklore and spiritual beliefs. However, while our study has provided a broad overview of the potential uses and importance of lichens, it is crucial to interpret these findings with a nuanced and balanced perspective. The pharmacological potential of lichen-derived compounds, while promising, requires further in-depth research and clinical validation. Additionally, the sensitivity of lichens to environmental changes necessitates careful monitoring to accurately assess the impact of pollution and climate change. Future research should aim to explore the full pharmacological potential of lichen-derived compounds and their applications in traditional medicine. Continued monitoring of lichen populations is essential for assessing environmental health and the efficacy of conservation efforts. By integrating scientific inquiry with cultural knowledge, we can harness the multifaceted benefits of lichens while ensuring their sustainable utilization and conservation. Overall, our study highlights the importance of interdisciplinary research in unlocking the diverse benefits of lichens and their integral role in ecosystem health, human well-being, and cultural heritage. By bridging scientific knowledge with traditional wisdom, we can pave the way for a more harmonious relationship between humans and the natural world. This study contributes to the growing body of literature on lichens and serves as a foundation for future investigations aimed at further elucidating their ecological, pharmacological, and cultural significance.

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