Phytochemical analysis of Corticolous macrolichens and their substrates: assessing habitat specificity in Madhyamaheshwar Valley, Garhwal Himalaya, Uttarakhand

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Abstract
This study investigates the phytochemical composition of five corticolous macrolichen species (Hypotrachyna nepalensis, Parmotrema tinctorum, Ramalina conduplicans, Dolichousnea longissima and Usnea orientalis) and their associated bark substrates (Berberis jaeschkeana, Betula utilis, Rhododendron arboreum, Rhododendron campanulatum and Sorbus foliolosa) in Madhyamaheshwar valley. Phytochemical analysis using various solvent extracts (chloroform, ethyl acetate, ethanol and methanol) revealed distinct chemical profiles for each species. Parmotrema tinctorum exhibited a wide range of compounds including flavonoids, glycosides, saponins, tannins, phenols and terpenoids while saponins were commonly present in ethanolic extracts of most lichens.

No alkaloids or steroids were detected in any of the lichen samples and terpenoids were absent in Hypotrachyna nepalensis and Usnea orientalis. Bark extracts from Berberis jaeschkeana showed the highest chemical diversity including flavonoids, glycosides, saponins, tannins, phenols and terpenoids while other bark substrates contained glycosides and terpenoids but lacked saponins in some cases. The study confirms that Corticolous macrolichens do not derive nutrients from their bark substrates, relying instead on environmental photosynthesis for sustenance. These findings underscore the role of environmental and physiographic conditions in supporting lichen diversity, highlighting their habitat preference for attachment and stability rather than chemical nourishment.

Keywords: Corticolous macrolichens, Phytochemical composition, Substrate specificity, Ecological adaptation, Madhyamaheshwar valley.

Introduction

Lichen is a mutualistic association between fungi and algae. The term "mycobiont" specifically denotes the fungal component of lichen whereas "phycobiont" specifically refers to the algal component. Their resemblance makes them look like a single plant. Lichens thrive on rocks, trees, tree barks, soil and leaves in various climatic conditions. There are over 17,000 species of lichen, with more than 800 lichen products used for activities such as dyeing, perfume, floral decoration, monitoring pollutants and medical applications. This mutualistic interaction produces lichen chemicals which are extracellular secondary metabolites. The chemicals are frequently found in the fungal hyphae's thalli and they usually crystallize. More than 800 lichens secondary compounds have been discovered, the majority of which are unique to lichens.

Large numbers of plants and lichens have been employed in diverse research projects concerning phytochemical and medicinal applications. Lichens and their secondary compounds possess a variety of medicinal properties[25,37]. Throughout the course of human history, lichens have been used for a variety of purposes including the coloring of fabrics, the control of diseases and the consumption of some species as food.

Additionally, certain lichens are the subject of interest for their potential use as food preparation ingredients, particularly in the preparation of delicious dishes and spices[39]. European uses of lichens, particularly *Usnea* sp., have been widely adopted globally, influencing their use in other cultures' traditional medicine[8]. Lichen-derived bioactive compounds show great promise for biopharmaceutical applications including antimicrobial, antioxidant and cytotoxic agents as well as for developing new health technologies[24]. Lichen extracts represent important natural sources of antibacterial agents, showing considerable potential for the development of future antimicrobial treatments. The antibacterial activity of these extracts is largely determined by their unique phytochemical composition[29,30].

The substrate specificity of lichens that inhabit trees is regulated by elements including pH levels, texture, light and moisture[7,14,36]. The structure of the corticolous lichen community, for example, may be influenced by the amount of bark cracking. Certain species have a preference for old cracked bark, while others prefer the smooth bark of young trees. The duration until colonization is also a significant factor[33]. Larger trees usually have more species and

distinctive lichen communities because they are older and contain a larger surface area favorable to colonization[20,23,33]. It has also been shown that substrate pH affects lichen development[9,38]. As an example, because of atmospheric acidification, many lichen species cannot photosynthesize well on bark with a low pH[10,11,13,17]. This sensitivity is likely due to loss of photosystem II in the photosynthetic pathway responsible for the production of chlorophyll A and B under acidic conditions[10].

Since the locations of lichens are highly specific, even slight changes in the surrounding environment may lead to variations in the species' structure[28,31].

Since ancient times, people have traditionally used tree bark for various purposes. As an example, the outer bark of the Himalayan birch tree (*Betula utilis*) was used as paper to write texts in Sanskrit for centuries[35]. Extensive research on bark for systematic industrial utilization started in 1930[2]. Tree bark contains significant amounts of cellulose, hemicellulose, lignin and various extracts that are underutilized. Some of these compounds consist of sugars, starch, terpenoids, suberin and tannin[6,12,27]. Tree bark contains valuable chemicals, making it an excellent resource for biorefineries and pharmaceuticals[15,16]. Understanding of these chemical compounds will significantly contribute to accelerating the development of many different green products through the use of effective technologies for conversion[26].

Present study stands out by integrating both ecological and phytochemical analyses, focusing specifically on the Madhyamaheshwar valley, an underexplored region in Indian lichenology. Unlike previous research that primarily examined either medicinal or ecological aspects, this study uniquely investigates the distribution patterns of *Corticolous macrolichens* in relation to microclimatic conditions and their substrate specificity, offering a comprehensive view of how environmental factors shape the phytochemical profiles of lichens. The aim of this study was to explore the secondary metabolites in lichens and their substrates, to assess the ecological factors influencing their distribution and to contribute to the pharmacological potential of lichens in Madhyamaheshwar valley.

Material and Methods

Collection of lichen material: A sample of Corticolous macrolichen, *Hypotrachyna nepalensis*, *Parmotrema tinctorum*, *Ramalina conduplicans*, *Dolichousnea longissima* and *Usnea orientalis* (Fig. 1), along with their associated substrates (bark) *Rhododendron campanulatum*, *Rhododendron arboreum*, *Berberis jaeschkeana*, *Betula utilis* and *Sorbus foliolosa* were collected from the Madhyamaheshwar valley, situated at 30˚36`22″ N to 30˚37`59″ N latitude and 79˚11`12″ E to 79˚21`07″ E longitude, with elevations ranging from 1600 to 3600 m. asl located in Rudraprayag district, a part of Garhwal Himalayan region of Uttarakhand (Fig. 2 and table 1).

The lichens samples identified morphological, anatomical and chemical characteristics with the help of the current literature[3]. The shrub and tree species were identified based on specific morphological features including leaf structure (shape, arrangement and size), bark characteristics and the features of flowers and fruits, as detailed in Hooker's earlier works (1872–1897)[18] in the Laboratory of Forest Ecology at Department of Botany and Microbiology, H.N.B. Garhwal University and lichens were confirmed and authenticated in the laboratory of National Botanical Research Institute (CSIR-NBRI) in Lucknow and the accession numbers for the lichen samples provided are as follows: *Hypotrachyna nepalensis* (LWG - 61633), *Parmotrema tinctorum* (LWG - 61623), *Ramalina conduplicans* (LWG - 61630), *Dolichousnea longissima* (LWG - 61632) and *Usnea orientalis* (LWG - 61635).

Preparation of extracts of lichens and their substrates: The collected corticolous macrolichen samples, along with their associated substrates (bark), were air-dried at room temperature to remove any moisture. Once completely dried, the lichen thalli and substrates were ground separately into a fine powder using a mixer grinder.

Table 1
Corticolous macrolichens and their substrates collected from the different elevations from Madhyamaheshwar valley

Lichen species	Accession number	Sampling site	Lichens substrate	Latitude	Longitude	Elevation
Hypotrachyna nepalensis	LWG - 61633	Budha Madhyamaheshwar (S8)	*Rhododendron campanulatum*	30˚63`38″ N	79˚21`07″ E	3487 m. a.s.l.
Parmotrema tinctorum	LWG - 61623	Nanu-chatti (S4)	*Rhododendron arboreum*	30˚61`07″ N	79˚19`62″ E	2191 m. a.s.l.
Ramalina conduplicans	LWG - 61630	Budha Madhyamaheshwar (S8)	*Berberis jaeschkeana*	30˚63`23″ N	79˚21`06″ E	3510 m. a.s.l.
Dolichousnea longissima	LWG - 61632	Budha Madhyamaheshwar (S8)	*Betula utilis*	30˚63`37″ N	79˚21`07″ E	3487 m. a.s.l.
Usnea orientalis	LWG - 61635	Budha Madhyamaheshwar (S8)	*Sorbus foliolosa*	30˚63`37″ N	79˚21`07″ E	3486 m. a.s.l.

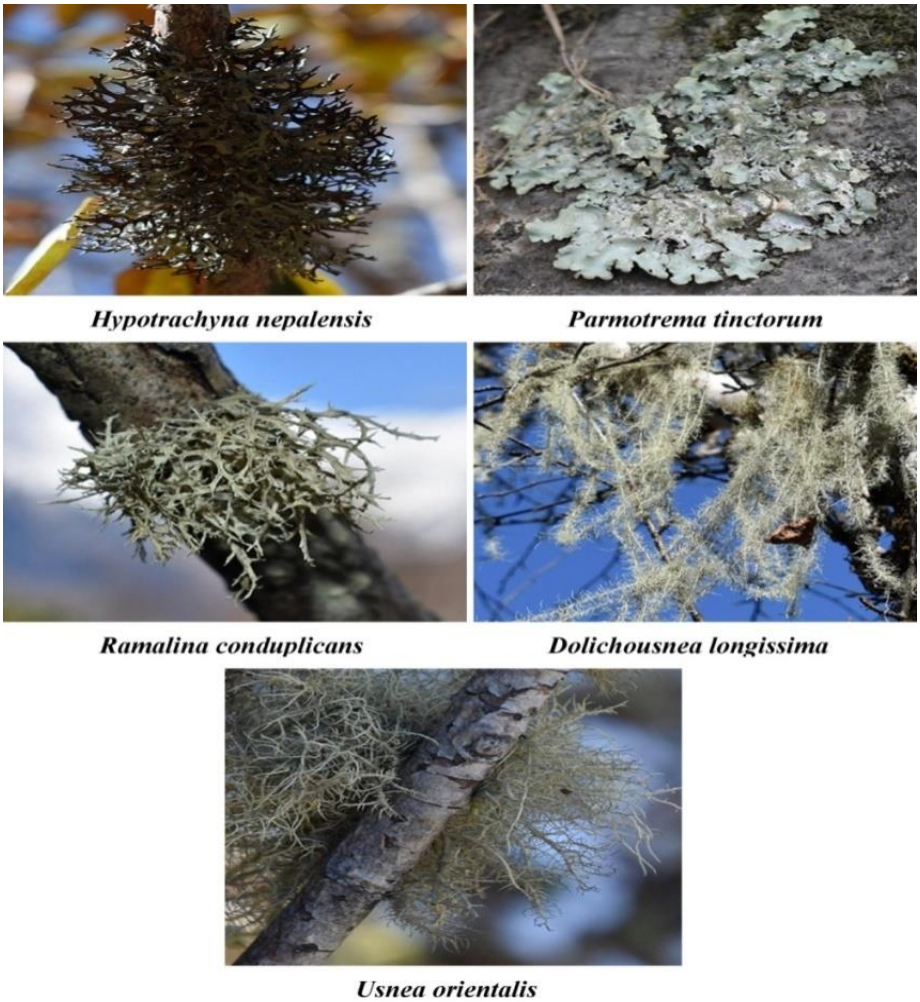

Figure 1: Selected species of Corticolous macrolichens for phytochemical screening

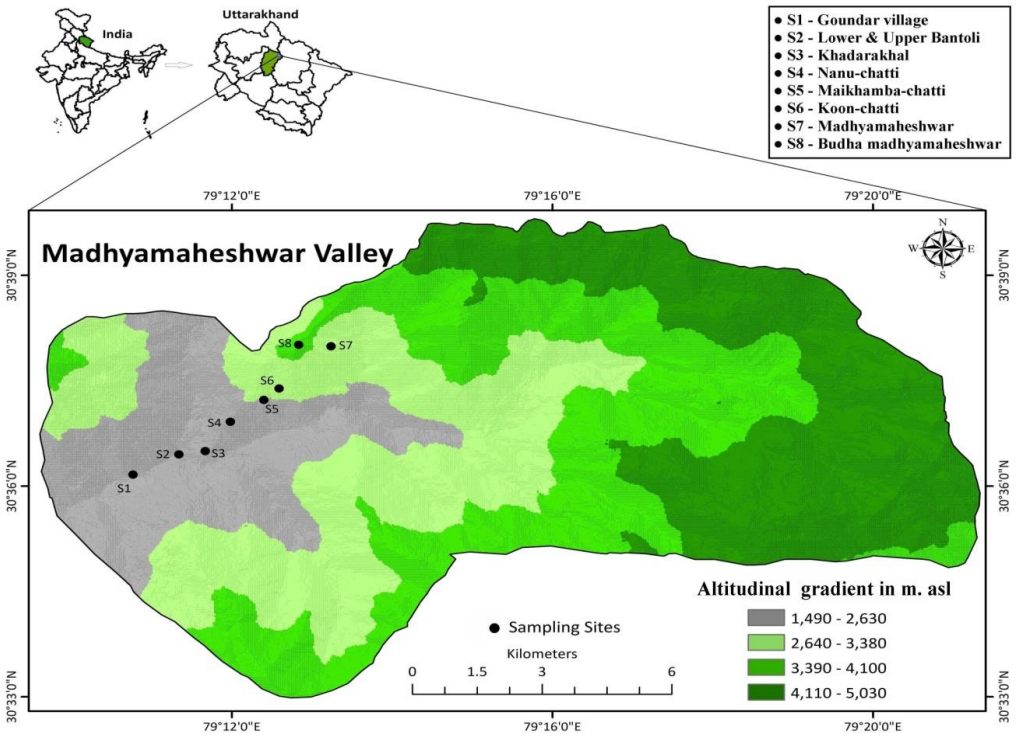

Figure 2: Geographical map of the study area

The powdered samples were then stored in airtight containers at 4°C for preservation. The extraction process involved taking 10 grams each of powder of lichen and powder of bark samples separately, with 100 ml of solvent used for each 10-gram sample. The solvents used in the extraction included non-polar chloroform, ethyl acetate and polar solvents like ethanol and methanol. The extraction was performed using a Soxhlet apparatus under hot continuous extraction conditions, with the extraction cycle run for 4 hours at the respective boiling points of each solvent (chloroform: 61.2°C, ethyl acetate: 77.1°C, ethanol: 78.4°C, methanol: 64.7°C). After extraction, the mixture was filtered through Whatmann no. 1 filter paper. The filtrates were then used for subsequent phytochemical screening.

Qualitative Testing of Phytochemicals: Phytochemical screening of the crude extract was conducted using specific reagents to detect the presence of various bioactive compounds including flavonoids, glycosides, saponins, steroids, tannins, phenols, terpenoids and alkaloids. The investigation was carried out following standard procedures as outlined by Rashmi and Rajkumar[32].

Results

Conducting a phytochemical investigation is extremely important for revealing new sources of therapeutically and scientifically valuable compounds. The results of the present investigation are shown in table 2 and table 3.

Table 2
Occurrence of preliminary phytochemical constituents of corticolous macrolichens used as spices extracted with different solvents

S.N.	Lichen samples	Flavonoids				Glycosides				Saponins				Steroids				Tannins				Phenols				Terpenoids				Alkaloids			
		Chloroform	Ethyl acetate	Ethanol	Methanol	Chloroform	Ethyl acetate	Ethanol	Methanol	Chloroform	Ethyl acetate	Ethanol	Methanol	Chloroform	Ethyl acetate	Ethanol	Methanol	Chloroform	Ethyl acetate	Ethanol	Methanol	Chloroform	Ethyl acetate	Ethanol	Methanol	Chloroform	Ethyl acetate	Ethanol	Methanol	Chloroform	Ethyl acetate	Ethanol	Methanol
1	*Hypotrachyna nepalensis*	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-
2	*Parmotrema tinctorum*	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	+	-	-	+	+	+	+	+	-	-	-	-	-
3	*Ramalina conduplicans*	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-
4	*Dolichousnea longissima*	+	+	-	+	+	+	+	+	-	-	+	+	-	-	-	-	-	-	+	+	-	-	+	+	-	+	+	-	-	-	-	-
5	*Usnea orientalis*	-	+	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[+] Presence of compound
[-] Absence of compound

Table 3
Occurrence of preliminary phytochemical components of corticolous macrolichens substrata using various solvent extracts

S. N.	Lichen substrata (Bark samples)	Flavonoids				Glycosides				Saponins				Steroids				Tannins				Phenols				Terpenoids				Alkalo			
		Chloroform	Ethyl acetate	Ethanol	Methanol	Chloroform	Ethyl acetate	Ethanol	Methanol	Chloroform	Ethyl acetate	Ethanol	Methanol	Chloroform	Ethyl acetate	Ethanol	Methanol	Chloroform	Ethyl acetate	Ethanol	Methanol	Chloroform	Ethyl acetate	Ethanol	Methanol	Chloroform	Ethyl acetate	Ethanol	Methanol	Chloroform	Ethyl acetate	Ethanol	Methanol
1	*Berberis jaeschkeana*	-	+	+	+	-	+	-	+	-	-	+	-	-	-	-	-	-	-	+	+	-	-	+	+	+	-	-	+	-	-	-	-
2	*Betula utilis*	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	+	-	-	-	-	-	-	-
3	*Rhododendron arboreum*	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-
4	*Rhododendron campanulatum*	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	+	+	-	-	-	-	-	-
5	*Sorbus foliolosa*	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[+] Presence of compound
[-] Absence of compound

A total of 5 different species of Corticolous macrolichens and their substrates were carefully collected from Madhyamaheshwar valley in Rudraprayag district of Uttarakhand for the purpose of investigating their secondary metabolites. From these lichens and their substrate (bark) samples, a total of 40 extracts were completely prepared using solvents including chloroform, ethyl acetate, ethanol and methanol.

An initial analysis was performed to identify the presence of various compounds in the mentioned extracts including flavonoids, glycosides, saponins, steroids, tannins, phenols, terpenoids and alkaloids. The extracts obtained from *Parmotrema tinctorum* exhibited a wide range of constituents including flavonoids, glycosides, saponins, tannins, phenols and terpenoids. These constituents were found in different extracts obtained using chloroform, ethyl acetate, ethanol and methanol as solvents. Only the ethanolic extracts of *Hypotrachyna nepalensis, Parmotrema tinctorum, Ramalina conduplicans, Dolichousnea longissima* and *Usnea orientalis* contained saponins. No terpenoids were found in the extracts of *Hypotrachyna nepalensis* and *Usnea orientalis*. Only *Usnea orientalis* lacked tannins and phenols and *Ramalina conduplicans* lacked flavonoids.

The ethanol and methanolic extracts exhibited higher activity and contained nearly all of the tested phytochemicals in the lichen samples.

The bark extracts of *Berberis jaeschkeana* contained the largest number of constituents. These constituents included flavonoids obtained from extracts of ethyl acetate, ethanol and methanol, glycosides obtained from ethyl acetate and

methanolic extract, saponins obtained from extracts of ethanol, tannins and phenols obtained from extracts of ethanol and methanol and terpenoids from chloroform and methanolic extracts.

The ethanol and methanolic extracts exhibited higher activity and contained nearly all of the tested phytochemicals in the bark samples. The bark extracts of *Betula utilis*, *Rhododendron arboreum* and *Rhododendron campanulatum* contained glycosides. Saponins were absent in the bark extracts of *Betula utilis*, *Rhododendron arboreum* and *Rhododendron campanulatum*. Tannins and phenols were only absent in *Rhododendron arboreum* bark extracts. Flavonoids were only absent in the bark extracts of *Rhododendron campanulatum*. Presence of terpenoids was found in *Berberis jaeschkeana, Betula utilis*, *Rhododendron arboreum* and *Rhododendron campanulatum* bark extracts. *Sorbus foliolosa* contained glycosides exclusively in ethyl acetate, ethanol and methanol extracts while all other secondary metabolites such as flavonoids, saponins, steroids, tannins, phenols, terpenoids and alkaloids were absent in all tested extracts.

There was no presence of steroids and alkaloids in any of the bark extracts that were examined.

Notably, alkaloids and steroids were absent in all solvent extractions from both the lichens and their bark substrates which explained the absence of a red-brown precipitate for alkaloids and the absence of a green ring at the interphase for steroids during the experiment. The phytochemical screening of lichens and their substrates revealed that the components present in lichens and their substrates were distinct from each other. It indicates the variation in both constituents. This proves that lichens and the substrates contain their own distinct components. This leads to the conclusion that lichens do not extract any nutrients from bark. Lichens choose habitat simply for attachment, so that they can remain stable in one place and take nourishment from the environment through photosynthesis.

The distinct phytochemical profiles of lichens and their substrates suggest that lichens are highly habitat-specific, choosing substrates primarily for physical attachment and stability rather than for chemical nourishment. The variation in secondary metabolites between lichens and their substrates indicates that lichens synthesize their own compounds through their symbiotic relationship, utilizing environmental factors like light and moisture, rather than extracting nutrients from the bark or other surfaces they inhabit. This reinforces the idea that lichens are adapted to specific ecological niches where their chemical compositions help them to cope with environmental stressors, but their habitat choice is driven by the need for a stable attachment site rather than nutrient acquisition.

Ecological factors influencing lichen abundance and diversity in Madhyamaheshwar valley: The extensive field survey in Madhyamaheshwar valley revealed a greater abundance of the selected corticolous macrolichens for phytochemical analysis i.e. *Hypotrachyna nepalensis*, *Parmotrema tinctorum*, *Ramalina conduplicans*, *Dolichousnea longissima* and *Usnea orientalis*. The lichens were collected from the different substrate (bark of trees) which were acclimatized in various microclimatic habitats. We noticed a significant variation in their abundance; the same lichen was observed in lesser numbers in one habitat and higher in the other. Consequently, we found the specific substrates for *Ramalina conduplicans* are *Rhododendron*, *Berberis jaeschkeana*, *Sorbus foliolosa*, *Oak* and *Betula utilis* (Fig. 3).

Substrates for *Parmotrema tinctorum* are *Rhododendron*, *Oak* and *Sorbus foliolosa* (Fig. 4) and substrates for *Hypotrachyna nepalensis* and *Usnea orientalis* are *Rhododendron, Oak, Sorbus foliolosa* and *Betula utilis* (Fig. 5 and fig. 6). *Dolichousnea longissima* has only two substrates *Quercus semecarpifolia* and *Betula utilis* at an altitude of 2900 to 3600 m. a.s.l. (Fig. 7). These observations suggest that the microclimatic conditions of lower attitudes are not favorable for *Dolichousnea longissima*. Due to this it prefers to grow in higher elevations. Higher altitude is also the specific region where lichens thrive in large numbers.

During the study we also noticed that the abundance of *Corticolous macrolichens* is rich in the moist habitats where the substrates and lichens both are able to absorb the atmospheric moisture and humidity from the environment. This is a main reason that Corticolous macrolichens retained their thallus structure for longer time. The abundance of lichens is still in good condition in the studied sites of Madhyamaheshwar valley. The appropriate climatic condition and less anthropogenic disturbances provide an ideal condition to flourish the diversity of lichens.

Discussion

Baral and Mahajan[4] identified volatile oils, polyoses, saponins, quinones, flavonoid glycosides and coumarins in *Hypotrachyna nepalensis*. In contrast, the present study observed only flavonoids, glycosides, saponins, tannins and phenols, indicating a difference in the phytochemical profiles, possibly due to regional variations or environmental factors. Reddy et al[33] reported the presence of tannins, alkaloids, saponins, glycosides, flavonoids, proteins, triterpenoids, carbohydrates and steroids in *Parmotrema tinctorum*. Similarly, Rashmi and Rajkumar[32] also identified tannins, alkaloids, proteins and carbohydrates in this species. The current study identified only flavonoids, glycosides, saponins, tannins, phenols and terpenoids. This difference suggests that the chemical composition of lichens might vary with physiographic factors, season and methodological variations.

Similarly, Reddy et al[33] documented tannins, alkaloids, saponins, glycosides, flavonoids, proteins and triterpenoids in *Ramalina conduplicans*. In contrast, the present study

detected only glycosides, saponins, tannins, phenols and terpenoids. These discrepancies could be attributed to differences in ecological conditions such as variations in temperature, moisture and air quality which may affect the lichens chemical composition.

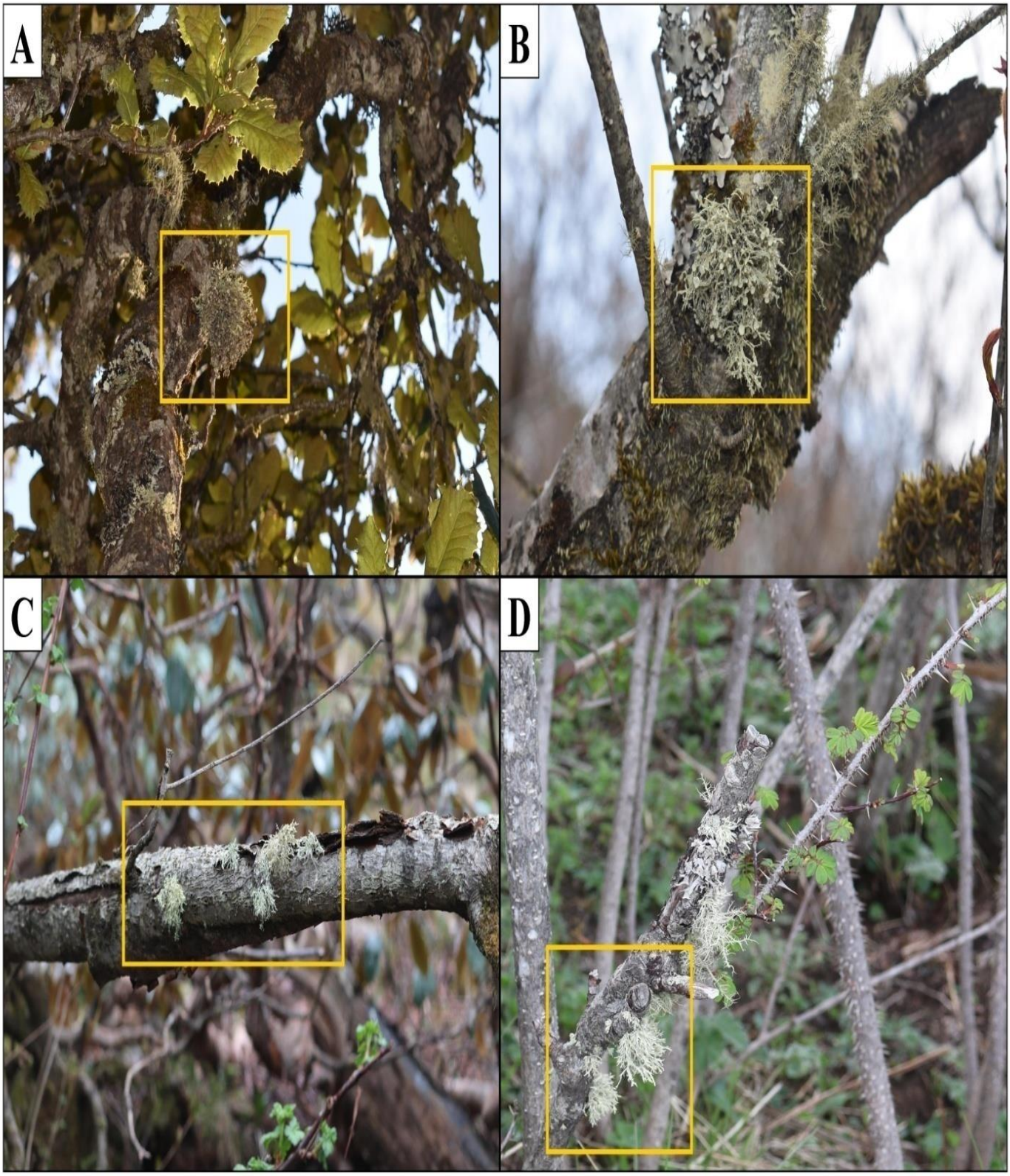

Figure 3: ***Ramalina conduplicans*** **growing on their specific substrates: A -** ***Quercus semecarpifolia,*** **B -** ***Sorbus foliolosa,*** **C -** ***Rhododendron campanulatum*** **D -** ***Berberis jaeschkeana***

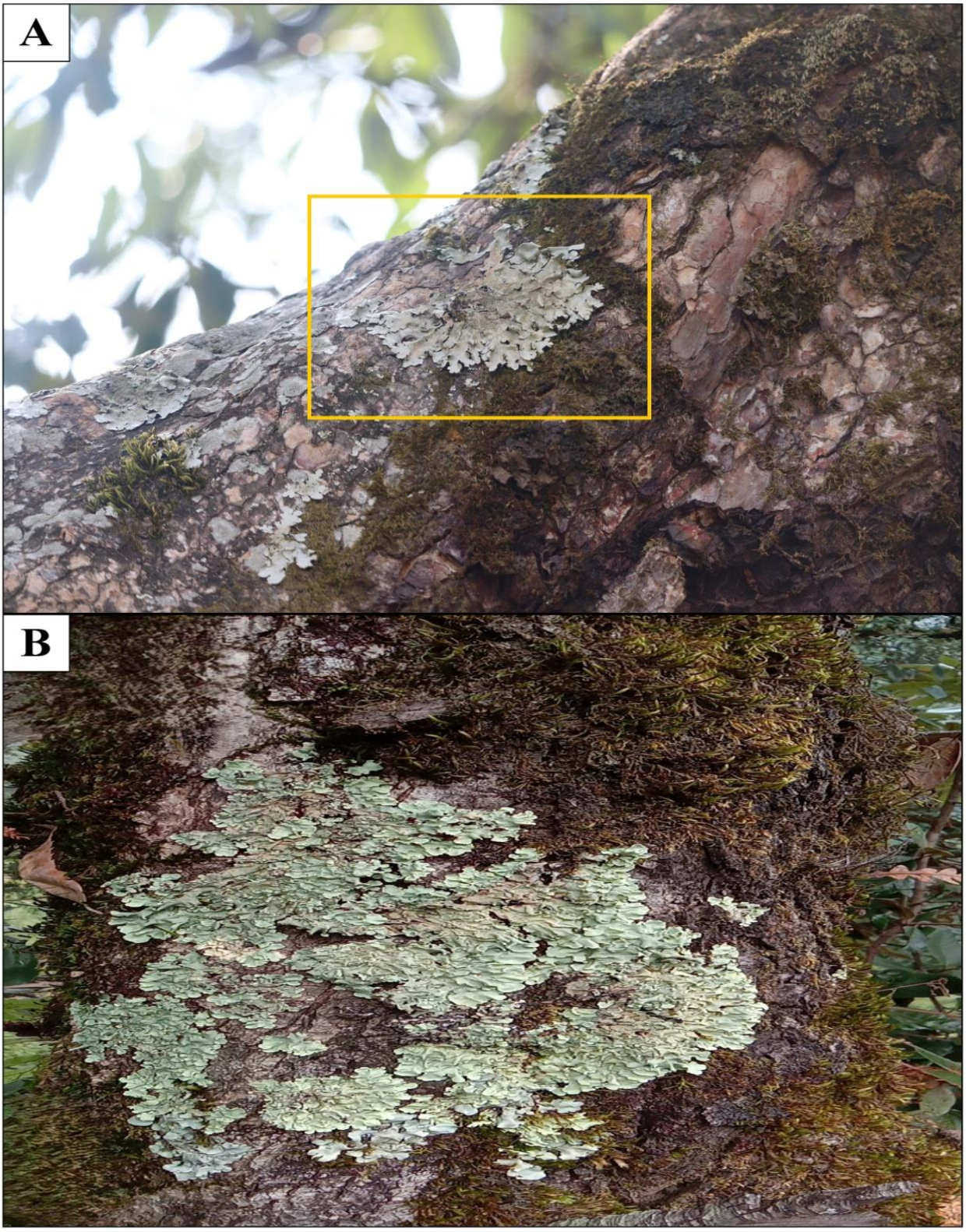

Figure 4: *Parmotrema tinctorum* growing on their specific substrates: A - *Rhododendron arboreum*, B - *Quercus leucotrichophora*

Singh et al[34] reported the presence of flavonoids, glycosides, saponins, steroids, tannins and triterpenoids in *Dolichousnea longissima*. The present study, however, only detected flavonoids, glycosides, saponins, tannins, phenols and terpenoids, indicating a variation in the lichens phytochemical content. This could reflect regional adaptation or differing environmental influences on the biosynthesis of secondary metabolites.

Bhandarkar et al[5] found tannins, alkaloids, saponins, glycosides, flavonoids, proteins, triterpenoids and steroids in *Usnea orientalis*, while the present study identified only flavonoids, glycosides and saponins. This highlights the potential for variation in phytochemical composition between studies, perhaps due to differing extraction methods, sample preparation and environmental factors. Regarding the tree barks, Alamzeb et al[1] found alkaloids, glycosides, flavonoids, steroids, saponins, reducing sugars and terpenoids in *Berberis jaeschkeana*. However, the present study identified flavonoids, glycosides, saponins, tannins, phenols and terpenoids, indicating some differences in the detected phytochemicals.

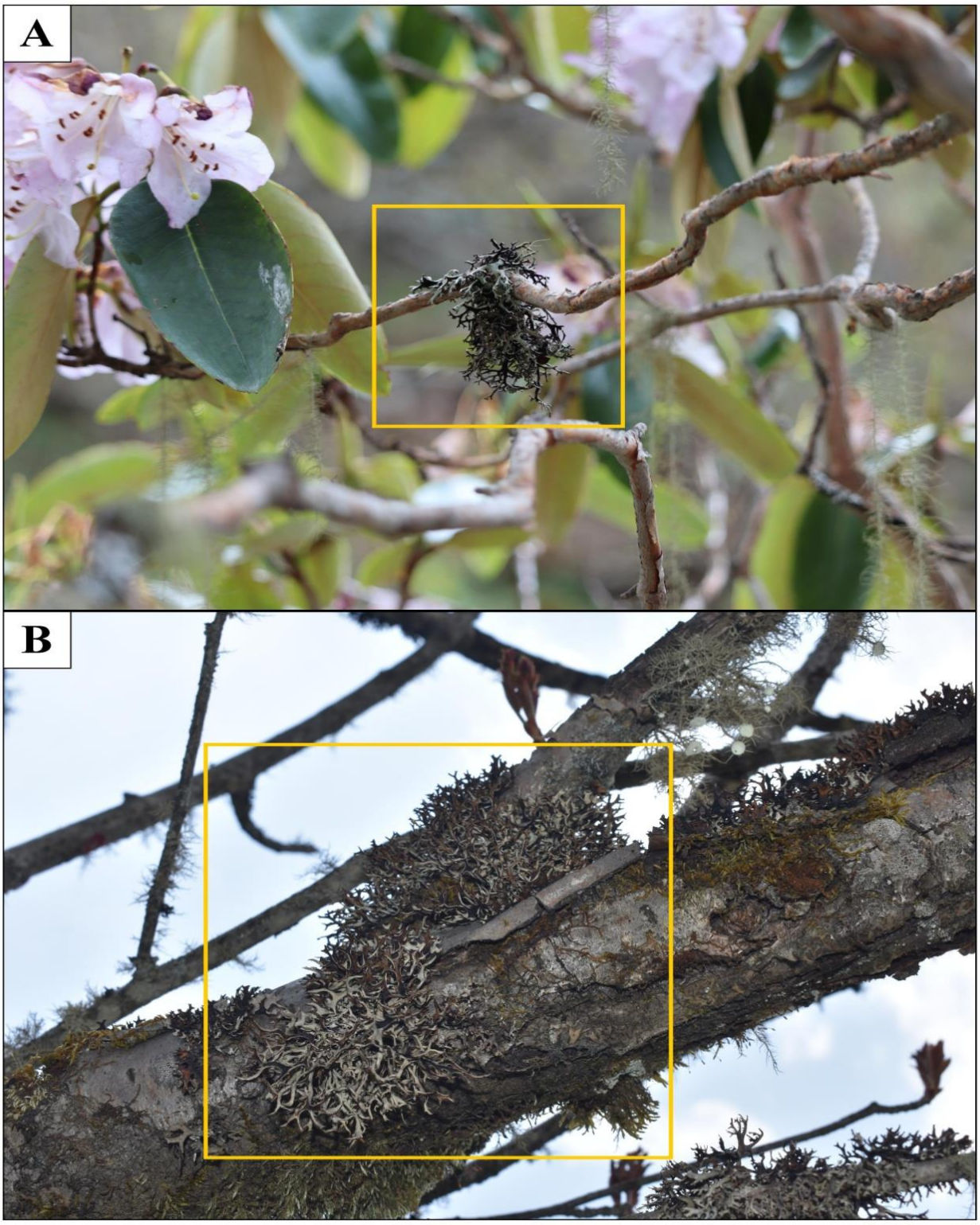

Figure 5: *Hypotrachyna nepalensis* growing on their specific substrates: A - *Rhododendron campanulatum*, B - *Sorbus foliolosa*

Figure 6: *Usnea orientalis* growing on their specific substrates: A - *Rhododendron campanulatum* B - *Sorbus foliolosa*

Similarly, Joshi et al[19] documented alkaloids, carbohydrates, flavonoids, saponins and triterpenes in *Betula utilis*, while the current analysis showed the presence of flavonoids, glycosides, tannins, phenols and terpenoids. This study also provides the first phytochemical profiles for *Rhododendron arboreum*, *Rhododendron campanulatum* and *Sorbus foliolosa* barks. In *Rhododendron arboreum*, flavonoids, glycosides and terpenoids were detected. *Rhododendron campanulatum* bark contained glycosides, tannins, phenols and terpenoids whereas *Sorbus foliolosa* bark contained only glycosides, with no other phytochemicals identified. The preference of *Dolichousnea longissima* for *Quercus semecarpifolia* and *Betula utilis* at altitudes of 2900 - 3600 m. asl supports the concept of regionally varying habitat relationships in lichens.

Lõhmus et al[22] highlighted that lichens exhibit habitat specificity, which is strongly influenced by microclimatic factors such as altitude and substrate availability, shaping distribution patterns in temperate ecosystems. The findings of this study align with the results of Lakatos et al[21] who observed that Corticolous macrolichens thrive in moist habitats, where both substrates and lichens can absorb atmospheric moisture. Such conditions enable lichens to maintain their thallus structure, contributing to a diverse lichen population in less disturbed environments.

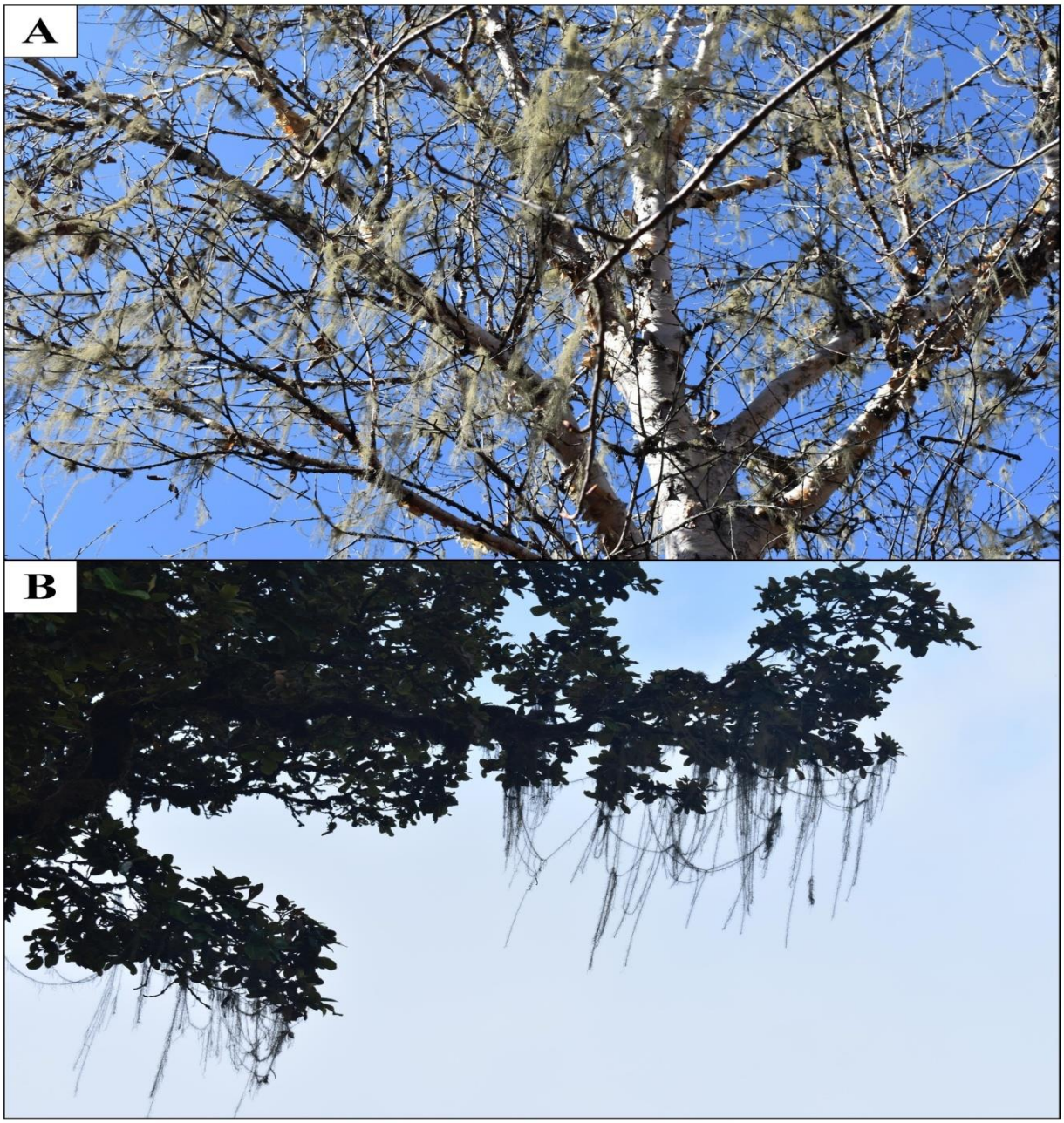

Figure 7: *Dolichousnea longissima* growing on their specific substrates: A - *Betula utilis*, B - *Quercus semecarpifolia*

In our present study, we also noted that the abundance of Corticolous macrolichens is higher in moist habitats where both substrates and lichens are able to absorb atmospheric moisture and humidity from the environment. This is a key factor that allows Corticolous macrolichens to retain their thallus structure for a longer period. The abundance of lichens remains in good condition at the studied sites in Madhyamaheshwar valley. The appropriate climatic conditions and low anthropogenic disturbances create an ideal environment for the flourishing of lichen diversity.

Conclusion

Thus, it can be concluded that this study underscores the importance of Madhyamaheshwar valley as a biodiversity hotspot, offering rich insights into the ecological and pharmacological roles of Corticolous macrolichens. The present findings also emphasize the distinct chemical composition between lichens and their substrates, affirming that lichens do not extracts nutrients from bark but rather utilize it for attachment while deriving nourishment through photosynthesis. This ecological insight underscores the intricate relationship between lichens, their own substrates and environmental factors crucial for their survival.

The results also provide a base for further research into the medicinal potential of lichen-derived compounds, especially given their diverse bioactive profiles. Future studies should aim to explore the full therapeutic potential of these compounds as well as the environmental factors that influence lichen growth and distribution.

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References
1. Alamzeb M., Khan M.R., Ali S. and Shah S.Q., Antimicrobial properties of extracts and compounds isolated from *Berberis jaeschkeana*, *Bangladesh Journal of Pharmacology*, **8(2)**, 107-109 **(2013)**

2. Austin D.F., Bark, The formation, characteristics and uses of bark around the world, *Economic Botany*, **48**, 89–89 **(1994)**

3. Awasthi D.D., A key to the macrolichens of India and Nepal, *The Journal of the Hattori Botanical Laboratory*, **65**, 207-302 **(1988)**

4. Baral B. and Maharjan B.L., Assessment of antimicrobial and phytochemical potentials of high altitudinal Nepalese lichens, *Journal of Microbiology, Biotechnology and Food Sciences*, **1(2)**, 98-112 **(2011)**

5. Bhandarkar S.V., Mohale D.S., Chandewar A.V. and Kochar N.I., Phytochemical investigation and antimicrobial activity of lichen species *Usnea orientalis* (Motyka), *African Journal of Biological Sciences*, **6**, 317-331 **(2024)**

6. Chen H., Chauhan P. and Yan N., Barking" up the right tree: biorefinery from waste stream to cyclic carbonate with immobilization of CO 2 for non-isocyanate polyurethanes, *Green Chemistry*, **22(20)**, 6874-6888 **(2020)**

7. Cleavitt N.L. et al, Influence of tree composition upon epiphytic macrolichens and bryophytes in old forests of Acadia National Park, Maine, *The Bryologist*, **112(3)**, 467-487 **(2009)**

8. Crawford S.D., Lichens used in traditional medicine, Lichen secondary metabolites: bioactive properties and pharmaceutical potential, 31-97 **(2019)**

9. Fritz Ö., Brunet J. and Caldiz M., Interacting effects of tree characteristics on the occurrence of rare epiphytes in a Swedish beech forest area, *The Bryologist*, **112(3)**, 488-505 **(2009)**

10. Garty J., Karary Y. and Harel J., Effect of low pH, heavy metals and anions on chlorophyll degradation in the lichen Ramalina duriaei (De Not.) Bagl, *Environmental and Experimental Botany*, **32(3)**, 229-241 **(1992)**

11. Gauslaa Y., The Lobarion, an epiphytic community of ancient forests threatened by acid rain, *The Lichenologist*, **27(1)**, 59-76 **(1995)**

12. Giannotas G., Kamperidou V. and Barboutis I., Tree bark utilization in insulating bio-aggregates: a review, *Biofuels, Bioproducts and Biorefining*, **15(6)**, 1989-1999 **(2021)**

13. Gilbert O.L., Field evidence for an acid rain effect on lichens, *Environmental Pollution Series A, Ecological and Biological*, **40(3)**, 227-231 **(1986)**

14. Gustafsson L. and Eriksson I., Factors of importance for the epiphytic vegetation of aspen Populus tremula with special emphasis on bark chemistry and soil chemistry, *Journal of Applied Ecology*, **32(2)**, 412-424 **(1995)**

15. Hamad A.M., Ates S., Olgun Ç. and Gür M., Chemical composition and antioxidant properties of some industrial tree bark extracts, *Bio Resources*, **14(3)**, 5657-5671 **(2019)**

16. Harkin J.M., Bark and its possible uses, Forest Products Laboratory, US Forest Service, 91 **(1971)**

17. Hawksworth D.L. and McManus P.M., Lichen recolonization in London under conditions of rapidly falling sulphur dioxide levels and the concept of zone skipping, *Botanical Journal of the Linnean Society*, **100(2)**, 99-109 **(1989)**

18. Hooker J.D., Flora of British India, **7**, 1872-1897 **(1982)**

19. Joshi H., Saxena G.K., Singh V., Arya E. and Singh R.P., Phytochemical investigation, isolation and characterization of betulin from bark of *Betula utilis*, *Journal of Pharmacognosy and Phytochemistry*, **2(1)**, 145-151 **(2013)**

20. Kantvilas G. and Minchin P.R., An analysis of epiphytic lichen communities in Tasmanian cool temperate rainforest, *Vegetatio*, **84**, 99-112 **(1989)**

21. Lakatos M., Rascher U. and Büdel B., Functional characteristics of corticolous lichens in the understory of a tropical lowland rain forest, *New Phytologist*, **172(4)**, 679-695 **(2006)**

22. Lõhmus A., Motiejūnaitė J. and Lõhmus P., Regionally varying habitat relationships in lichens: the concept and evidence with an emphasis on North-temperate ecosystems, *Journal of Fungi*, **9(3)**, 341 **(2023)**

23. McMullin R.T., Duinker P.N., Cameron R.P., Richardson D.H.S. and Brodo I.M., Lichens of coniferous old-growth forests of southwestern Nova Scotia, Canada: diversity and present status, *Bryologist*, **111**, 620–637 **(2008)**

24. Murugesan P., Phytochemical analysis and antimicrobial activity of edible lichen, *Journal of Drug Delivery and Therapeutics*, **10(2-s)**, 102-104 **(2020)**

25. Nayaka S. and Asthana S., Diversity and distribution of lichens in India vis a vis its Lichenogeographic Regions, In Conference: Biodiversity Conservation, Status, Future and Way Forward, National Academy of Biological Science, Chennai, 79-96 **(2014)**

26. Pandey S. and Pant P., Possibilities and challenges for harnessing tree bark extracts for wood adhesives and green chemicals and its prospects in Nepal, *Forest Science and Technology*, **19(1)**, 68-77 **(2023)**

27. Pásztory Z., Mohácsiné I. R., Gorbacheva G. and Börcsök Z., The utilization of tree bark, *Bio Resources*, **11(3)**, 7859-7888 **(2016)**

28. Pharo E.J. and Beattie A.J., The association between substrate variability and bryophyte and lichen diversity in eastern Australian forests, *The Bryologist*, **105(1)**, 11-26 **(2002)**

29. Prabhakar N.K., Gangwar R., Adhikari R.S., Nayaka S., Arya M. and Mehta J.P., Phytochemical Profiling and Antibacterial Efficiency of Hypotrachyna cirrhata and Parmotrema tinctorum Lichen Extracts from Madhyamaheshwar Valley, Garhwal Himalaya, Uttarakhand, *Environment and Ecology*, **43(1)**, 175-184 **(2025)**

30. Prabhakar N.K., Gangwar R., Arya M. and Mehta J.P., Exploring Antibacterial Potential: Dolichousnea longissima and Hypotrachyna nepalensis Lichens from Madhyamaheshwar Valley, Garhwal Himalaya, Uttarakhand, *Journal of Mountain Research*, **19(2)**, 119-127 **(2024)**

31. Ranius T., Johansson P., Berg N. and Niklasson M., The influence of tree age and microhabitat quality on the occurrence of crustose lichens associated with old oaks, *Journal of Vegetation Science*, **19(5)**, 653-662 **(2008)**

32. Rashmi S. and Rajkumar H.G., Preliminary phytochemical screening of different solvent extracts of lichens from Kodagu district, Karnataka, *Journal of Pharmacognosy and Phytochemistry*, **3(4)**, 209-212 **(2014)**

33. Reddy A.M., Devi A., Mohabe S., Akkulanna S. and Nayaka S., Qualitative phytochemical analysis of three solvents extracts of some selected macrolichens from Seshachalam Biosphere Reserve, Andhra Pradesh, *Cryptogam Biodiversity and Assessment*, **2(1)**, 19-25 **(2017)**

34. Singh S., Bhandari N., Debbarma B., Patil A., Kumar S. and Arya M., Comprehensive study of alpine lichen species in Uttarakhand: characterization, phytochemical analysis and ethnobotanical relevance, *African Journal of Biological Sciences*, **6**, 10023-10054 **(2024)**

35. Singh S., Yadav S., Sharma P. and Thapliyal A., Betula utilis: A potential herbal medicine, *Int J Pharm Biol Arch*, **3**, 493-498 **(2012)**

36. Spribille T., Thor G., Bunnell F.L., Goward T. and Björk C.R., Lichens on dead wood: species-substrate relationships in the epiphytic lichen floras of the Pacific Northwest and Fennoscandia, *Ecography*, **31(6)**, 741-750 **(2008)**

37. Upreti D.K., Divakar P.K. and Nayaka S., Commercial and ethnic use of lichens in India, *Economic Botany*, **59(3)**, 269-273 **(2005)**

38. Wolseley P. and James P., Factors affecting changes in species of Lobaria in sites across Britain 1986–1998, *Forest Snow and Landscape Research*, **75**, 319-338 **(2000)**

39. Yusuf M., A review on trends and opportunity in edible lichens, Lichen-Derived Products: Extraction and Applications, 189-201 **(2020)**.

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