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# Characterization, Comprehensive Phytochemical Analysis, and Ecological Significance of Two Lichen Species Indigenous to the Shivalik Ranges of Uttarakhand

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**Abstract:** Lichens are composite organisms consisting of fungi and algae, possessing unique secondary metabolites not found in higher plants. These compounds exhibit valuable bioactive properties. This study aimed to collect, identify, and conduct preliminary phytochemical analysis of two lichen species (*Parmotrema tinctorum* and *Parmotrema praesorediosum*) from the Shivalik Ranges in Rajaji National Park (Motichur and Phanduwala blocks). Three solvents of increasing polarity (petroleum ether, acetone, and methanol) were used to determine the optimal solvent for revealing the secondary metabolite composition. Voucher specimens were deposited in the LWG herbarium of National Botanical Research Institute (NBRI), Lucknow, for authentication and future reference. Methanol was found to be the most suitable solvent for phytochemical analysis of the identified lichen species. The uses and significance of *Parmotrema tinctorum* and *Parmotrema praesorediosum* were documented from literature and through interactions with local residents of the Garhwal region in Uttarakhand.

**Keywords:** Lichen • *Parmotrema tinctorum* • *Parmotrema praesorediosum* • Phytochemical Analysis • Shivalik Ranges • Uttarakhand

## Introduction

Lichens are fascinating organisms that result from a unique symbiotic relationship between a fungus and an alga (Schwendener 1867) or cyanobacterium. This partnership allows them to survive and thrive in a wide range of environments, from the extreme conditions of tundra regions to the arid deserts. The fungus, known as the mycobiont, provides structural support and shelter to the lichen, whiles the alga or cyanobacterium, known as the photobiont, conducts photosynthesis and provides nutrients through its ability to harness sunlight. One of the remarkable features of lichens is their production of a diverse array of chemical compounds (Julia Levy and Jack1973). These compounds play a crucial role in their adaptation to specific habitats and their ability to withstand harsh conditions (Arya *et al* 2021). The chemical composition of lichens is distinct and different from that of higher plants (Lawery 1986), making them a rich source of unique bioactive compounds. The chemical compounds produced by lichens have significant ecological and practical importance (Arya *et al* 2019). They have been used for centuries in ancient and traditional systems of Indian medicine, such as Ayurveda and Unani, for their therapeutic properties (Upreti 2015). Lichens also find applications in various industries, including natural dyes (Singh *et al* 2019), perfumes, fodder, agrochemicals, and spices (Dayan and Romagni 2001). Additionally, lichens are utilized in "Lichenometry," a method used to estimate the age of geological substrates based on lichen growth rates. Accurate identification and characterization of lichens are essential for harnessing their potential benefits. Various methods, including microscopic examination, chemical tests, and molecular characterization (Singh *et al* 2018), are employed to identify and study lichens. These methods enable scientists to determine the specific species of lichens and analyze their chemical composition (Nayka 2014; Singh and Arya 2019). In India, lichens are abundant, with around 2,900 species

recorded; representing a significant proportion of the world's known lichen diversity. The Western Himalayan region, particularly Uttarakhand, is a hotspot for lichen diversity (Upreti and Negi 1998, Sheikh *et al* 2006, Nayka *et al* 2011, Singh and Sinha 2010, Goni *et al* 2015, Goni and Sharma 2015, Mishra 2015). Rajaji National Park, located in the Shivalik ranges in Uttarakhand remains relatively unexplored in terms of lichen studies. This research focuses on two specific sites within Rajaji National Park: Phanduwala and Motichur. These sites offer unique ecological conditions and undisturbed environments for studying lichens. The aim of this study is to identify and characterize the abundant lichen species present in these locations and analyze their phytochemical composition. By understanding the diversity and chemical profile of the lichens found in Shivalik ranges in Uttarakhand, this study will contribute to a broader knowledge of lichens and their ecological significance in the region.

## Material and Methods

**Sampling Site:** The Phanduwala-Kansrao-Motichur trail is a 40 km route that traverses undisturbed stretches of dense forest within Rajaji National Park. It is characterized by a dense forest cover and runs parallel to the Suswa River, which forms the park's northern boundary. The trail connects the three forest ranges and can be accessed through the Asarori gate or the Ramgarh gate. To reach Phanduwala, one can take the Asarori gate, located opposite the Karvapani gate on the Saharanpur-Dehradun highway. The Motichur Range, situated in the Haridwar district, is an essential part of the park's ecosystem and can be easily reached via road, private vehicle, taxi, or bus. Rajaji National Park is relatively unexplored in terms of lichens, making it an ideal location for sampling lichens from this pristine and restricted area. This untouched region offers the opportunity to collect lichen samples of high quality and quantity for further study and analysis.

**Collection, Segregation and distribution of lichen samples:** Lichen samples were collected by scraping the samples from bark of Shorea robusta trees. The samples were documented, stored in compartments made of newsprint paper, and placed in zip lock polythene bags for transportation to the laboratory. Unwanted portions were removed, and the clean samples were weighed and distributed for identification, authentication, and phytochemical analysis. Herbarium packets were prepared for preservation, and samples were dispatched to NBRI for accession numbers. Samples for future analysis were stored in a refrigerator (Nayka 2014).

**Identification of lichen sample:** Lichens are identified based on their morphology, anatomy, and chemistry (Singh *et al* 2019). The micro and macrolichen keys by Awasthi (1991, 2007) are important references for identifying lichens in India. It is essential for beginners to have a glossary of technical terms, and the illustrated glossary provided in "Lichen flora of Great Britain and Ireland" by Purvis *et al* (1992) can be highly useful. Morphology and anatomy were done by dissecting and compound microscopes. For chemistry color spot test (Nylander and Flora 1856 and Asahina 1934) and Thin Layer Chromatography (TLC) **(**Culberson 1972) was performed (Nayka 2014 and Singh *et al* 2019).

**Preparation of lichen extract and determination of yield of the extract:** Lichen samples were washed and dried. They were then finely ground and subjected to successive extraction using solvents of increasing polarity (petroleum ether, acetone, and methanol) in a Soxhlet apparatus. The colored solvents were collected, filtered, and further analyzed. The

yield of the extract was determined through the difference between the initial weight of the powdered lichen sample and the final weight of the lichen sample obtained before and after each solvent change, respectively.

**Procedure for phytochemical tests:**

Seven phytochemical tests have been performed such as test for Tannins, Flavonoids, Alkaloids, Saponins, Glycosides, Triterpenoid, Steroid and results were recorded on the basis of observations by some modification in following protocols **(**Harborne *et al* 1973, Yadav *et al* 2011, Rooplatha *et al* 2013, Geetha and Geetha 2014, Rashmi and Rajkumar 2014).

## Results

**Sampling Sites:** Two samples were collected from two different sites of Raja ji national park, Sample 1 from Phanduwala block, Ramgarh range and Sample 2 from Motichur block, Motichur range and the physical parameters were noted, details of sampling sites are given in Table 1.

**Table 1: Physical parameters of sampling sites**

| Sample no. | Date of sampling | Sampling site | Location | Substratum | Humidity | Temp. | Altitude |
|---|---|---|---|---|---|---|---|
| Sample 1 | 26/06/2022 | Phanduwala block, Ramgarh range, (Rajaji National Park), Dehradun, Uttarakhand | 30°11'40.6" N 78°02'47.3"E | Bark of sal tree (*Shorea robusta*) | 52% | 29°C | 600-700m |
| Sample 2 | 25/08/2022 | Motichur block, Motichur range (Rajaji National Park), Haridwar, Uttarakhand | 30°02'45.7" N 78°12'02.9"E | Bark of sal tree (*Shorea robusta*) | 73% | 30°C | 500-600m |

**Identification of Lichen**

**Microscopic identification:**

Characteristics of Sample 1- eciliate; upper side grey to darker, emaculate; isidia granular to filiform, becoming coralloid or rarely flattened; lower side centrally black, wide marginal zone tan to brown, nude.

Characteristics of Sample 2: eciliate; upper side grey to darker, emaculate, soralia usually marginal, linear or crescent-shaped; soredia granular; lower side centrally black, narrow marginal zone lighter tan, nude.

**Color Spot Test :** After performing color spot test on both the samples observations were recorded, in sample 1 all tests on cortex K, C, KC, PD and on medulla K and PD remains colorless and gives negative results while C and KC gives + Red color on medulla which indicates that sample 1 contain Lecanoric acid and for sample 2 all the tests on cortex and medulla are negative which means chemicals of sample 2 are not revealed by this test, another test such as TLC should be performed for further identification up to species level according to the key .

**TLC (Thin Layer Chromatography)**:

Sample 1 gives one spot at Rf class 7 and sample 2 gives spot at Rf class 4 which matched with the control C1-*Parmelinella wallichiana* and C2- *Stereocaulon pomiferum* and finally recognized as Atranorin and Lecanoric acid.

After performing all identification tests the samples are identified as Sample 1- ***Parmotrema tinctorum* (Despr. Ex Nyl.) Hale**, Sample 2- ***Parmotrema praesorediosum* (Nyl.) Hale**.

These specimens have been stored in the LWG Herbarium of the National Botanical Research Institute (NBRI) with accession numbers **Sample no. 1: 63581 and Sample no. 2: 63584** for future references and further studies**.**

**Preparation of lichen extract and determination of yield of the extract**

Three extracts of each sample with different solvents in increasing polarity (petroleum ether, acetone and methanol) has been prepared with the help of Soxhlet apparatus and the final yield of the extract has been calculated as shown in Table 2 which shows methanol gives the maximum yield in both the samples than other two solvents which were petroleum ether and acetone.

**Table 2: Sample powder utilized during successive extraction by three different solvents with increasing polarity (petroleum ether, acetone, and methanol)**

| **Sample no. 1- *Parmotrema tinctorum*** | | | |
|---|---|---|---|
| Solvent used for extraction | Powder weight before extraction | Powder weight after extraction | Powder utilized in extraction |
| Petroleum ether | 10 gm | 9.90gm | 0.10gm |
| Acetone | 9.90 gm | 9.75gm | 0.15gm |
| Methanol | 9.75gm | 9.18gm | 0.57gm |
| **Sample no. 2- *Parmotrema praesorediosum*** | | | |
| Solvent used for extraction | Powder weight before extraction | Powder weight after extraction | Powder utilized in extraction |
| Petroleum ether | 10 gm | 9.47gm | 0.53gm |
| Acetone | 9.47 gm | 9.12gm | 0.35gm |
| Methanol | 9.12gm | 8.30gm | 0.82gm |

**Phytochemical Analysis**

After performing phytochemical test, it is observed (shown in Table 3) that methanol is the best solvent to check the phytochemicals as it gives positive result in sample 1 for five tests (Tannins, Flavonoids, Alkaloids, Glycosides, Triterpenoid and positive results in sample 2 for six tests (Tannins, Flavonoids, Saponins, Alkaloids, Glycosides, Triterpenoid in different solvents with increasing polarity (petroleum ether, acetone, methanol).

**Table 3: Observation for Qualitative Phytochemical Analysis of Sample 1 and sample 2**

| **Test name** | **Solvent** | **Expected outcome** | **Sample 1 Observation** | **Sample 1 Result** | **Sample 2 Observation** | **Sample 2 Result** |
|---|---|---|---|---|---|---|
| Alkaloids | petroleum ether | Reddish brown precipitate | No precipitation | Negative | Reddish brown ppt. | Positive |
| | Acetone | | No precipitation | Negative | Reddish brown ppt. | Positive |
| | Methanol | | No precipitation | Negative | Reddish brown ppt. | Positive |
| Flavonoids | petroleum ether | Yellow to orange color | Yellow to orange color | Positive | Yellow to orange color | Positive |
| | Acetone | | Yellow to orange color | Positive | Yellow to orange color | Positive |
| | Methanol | | Yellow to orange color | Positive | Yellow to orange color | Positive |

| | | | | | | |
|---|---|---|---|---|---|---|
| Glycosides | petroleum ether | Brown ring formation | No Brown ring | Negative | Brown ring formation | Positive |
| | Acetone | | Brown ring formation | Positive | Brown ring formation | Positive |
| | Methanol | | Brown ring formation | Positive | Brown ring formation | Positive |
| Saponins | petroleum ether | Stable foam formation | No Stable foam | Negative | No Stable foam | Negative |
| | Acetone | | No Stable foam | Negative | No Stable foam | Negative |
| | Methanol | | No Stable foam | Negative | Stable foam formation | Positive |
| Steroids | petroleum ether | Blue green color ring | Blue green color ring | Positive | Blue green color ring | Positive |
| | Acetone | | No ring formation | Negative | No ring formation | Negative |
| | Methanol | | No ring formation | Negative | No ring formation | Negative |
| Tannins | petroleum ether | Blue, Green, Black color | Remain same | Negative | Remain same | Negative |
| | Acetone | | Dark blue black | Positive | Dark blue black | Positive |
| | Methanol | | Dark blue black | Positive | Dark blue black | Positive |
| Triterpenoids | petroleum ether | Red brown color at interphase | Red brown color at interphase | Positive | Red brown color at interphase | Positive |
| | Acetone | | Red brown color at interphase | Positive | Red brown color at interphase | Positive |
| | Methanol | | Red brown color at interphase | Positive | Red brown color at interphase | Positive |

**Ethnobotanical uses**

Lichens have diverse ethnobotanical uses. They have been used in traditional medicine for treating coughs, respiratory issues, skin problems, and wounds. Certain lichen species provide natural dyes for coloring textiles and leather. Some lichens, like Iceland moss, have been consumed as food during scarcity. Lichens have practical applications as animal fodder and tinder for starting fires. They hold cultural and spiritual significance in rituals and symbolize purity, harmony, or longevity. Caution is necessary as some lichens may contain toxic compounds. Expert guidance and sustainable harvesting practices are important for safe utilization. Some of the ethnobotanical use and importance of two identified lichen samples *Parmotrema tinctorum* and *Parmotrema praesorediosum* are enlisted below in Table 4.

**Table 4: Importance and uses of *Parmotrema tinctorum* and *Parmotrema praesorediosum*.**

| S.No. | Lichen species | Traditional uses and Biological activities | References |
|---|---|---|---|
| 1. | *Parmotrema tinctorum* | As flavouring agent, spices, Natural dyes, Used for bleeding from uterus and from external injuries, sores and swelling, chronic dermatitis and localized swelling, antibacterial, antioxidant, antiglycation, inhibitory, antifungal | Upreti, 2015; Shukla, 2014; Raj 2014; Prashith Kekuda 2016 |

| | | | |
|---|---|---|---|
| 2. | *Parmotrema praesorediosum* | As spices, Beta-glucosidase inhibitor, antimicrobial, antibacterial, antifungal | Upreti, 2005; Lee & Kim 2000; Balaji & Hariharan 2007 |

**Conclusion:**

This study contributes to the characterization, preliminary phytochemical analysis, and ethnobotanical documentation of two lichen species from the Shivalik Range in Uttarakhand. The findings enhance our understanding of the bioactive compounds present in these lichens and their potential for various applications in traditional medicine, cultural practices, and other domains. Out of three solvents of increasing polarity (petroleum ether, acetone, and methanol) methanol considered as the optimal solvent for revealing the maximum secondary metabolite composition. *Parmotrema tinctorum* and *Parmotrema praesorediosum* demonstrate significant ethnobotanical value due to their medicinal properties, natural dye sources, cultural importance, contribution to biodiversity, and potential as environmental indicators. Further investigations are warranted to explore the specific bioactive constituents, pharmacological activities, and sustainable management practices for these lichen species.

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