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Phytochemical Analysis of Lichens from Garhwal Himalaya: A Qualitative Analysis of Secondary Metabolites Across Different Solvents

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ABSTRACT

Lichens are symbiotic organisms formed by a partnership between fungi (mycobiont) and algae (phycobiont), thriving on surfaces like rocks, bark of trees and soil across diverse climates. Humans use around 17,000 lichen species for applications in decoration, pollution monitoring, fragrance, dyeing and medicine. Lichens produce unique secondary metabolites with a wide range of biological activities, including anticancer, antimicrobial, anti-inflammatory, analgesic and antioxidant properties. Their secondary compounds have gained growing interest in the pharmaceutical industry for potential therapeutic uses. This study provides a comprehensive analysis of the phytochemical diversity in five lichen species, i.e., *Flavoparmelia caperata*, *Heterodermia diademata*, *Hypotrachyna cirrhata*, *Nephromopsis laii* and *Parmotrema nilgherrensis*, collected from the Madhyamaheshwar Valley, Garhwal Himalaya. The extraction was done using a range of solvents including chloroform, ethyl acetate, ethanol and methanol. The study uncovered significant variability in secondary metabolites among the lichens. *Flavoparmelia caperata* was found to contain flavonoids, glycosides, saponins, tannins and phenols ; *Heterodermia diademata* exhibited flavonoids, glycosides, tannins, phenols and terpenoids, *Hypotrachyna cirrhata* showed flavonoids, saponins, steroids, tannins and phenols ; *Nephromopsis laii* was identified with glycosides, tannins and phenols ; and *Parmotrema nilgherrensis* had glycosides, saponins, tannins and phenols. Notably, no alkaloids were detected in any of the species. This study is the first to present a detailed phytochemical analysis of lichens from the Madhyamaheshwar Valley highlighting their rich chemical diversity and potential pharmacological applications. These findings lay a crucial foundation for future research into their therapeutic uses and the development of novel natural products.

Keywords Lichens, Phytochemistry, Secondary metabolites, Pharmacological potential.

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INTRODUCTION

Lichen is a symbiotic partnership between fungus and algae, forming a single thallus (Alexopoulos and Mims 1979). The mycobiont is fungal part while the phycobiont is algal part in lichen. Lichens appear to thrive on surfaces such as rocks, tree bark, trunks and soil. They are found thriving in a variety of climates. Humans employ around 17,000 lichen species and 800 lichen-derived items for decorative purposes, pollution observation, fragrance, dyeing process and medicine field (Nayaka and Asthana 2014, Upreti *et al.* 2005). They can produce numerous types of secondary compounds each one unique to their abilities (Lawrey 1986). The pharmaceutical industry has shown an increasing interest in utilizing lichen secondary metabolites for the treatment of various diseases (Huneck 1999). Lichen compounds exhibit a broad spectrum of biological activities such as anti-cancer, antimicrobial, anti-inflammatory, antipyretic, analgesic, antiproliferative, cytotoxic, antioxidant and antihelminthic properties (Ingólfsdóttir *et al.* 2000, Kumar and Müller 1999, Vijayakumar *et al.* 2000, Vinayaka *et al.* 2009).

MATERIALS AND METHODS

Collection of lichen samples

Lichen specimens were systematically collected from various locations in the Madhyamaheshwar Valley, Garhwal Himalaya, Uttarakhand (Figs. 1–2). The sampling covered an altitudinal range from 1685 m to 3335 m above sea level, within latitudes 30°60'20" N to 30°63'89" N and longitudes 79°17'76" E to 79°22'86" E. Specimens were collected from tree bark and rocks. The collection sites and their respective details are summarized in Table 1.

Identification

The lichen samples were identified based on their morphological, anatomical and chemical characteristics, following the guidelines provided in the literature (Awasthi 1988). The analysis was conducted at the Forest Ecology Laboratory, Department of Botany and Microbiology, HNB Garhwal University. Subsequently, the samples were confirmed and authenticated at the Lichenology Laboratory CSIR-National Botanical Research Institute in Lucknow. The accession numbers assigned to the identified lichen samples are as follows: *Flavoparmelia caperata* (LWG – 65153), *Heterodermia diademata* (LWG - 61631), *Hypotrachyna cirrhata* (LWG - 62400), *Nephromopsis laii* (LWG - 61634) and *Parmotrema nilgherrensis* (LWG - 61626).

Sample extraction procedures

The lichens thalli were washed with tap water and then air-dried at room temperature for 24 hrs to ensure the complete removal of moisture. After being air-dried the lichen samples were ground into a fine powder with the help of mixer grinder. Each 10 g sample was placed into separate thimbles and for each sample 100 ml of solvent was used. Extraction was carried out using a *Soxhlet apparatus* with four different solvents of varying polarities: Chloroform, Ethyl acetate, Ethanol and Methanol. After the extraction process was completed the extracts were filtered using Whatman No. 1 filter paper. The filtrate was subsequently utilized for phytochemical analysis.

Qualitative assessment of phytochemicals

An initial screening of secondary metabolites was carried out on all four extracts of the selected lichen

Table 1. Sampling locations and geographical coordinates of selected lichen species.

Sl. No.	Lichen species	Accession number	Sampling location	Elevation	Latitude	Longitude
1	*Flavoparmelia caperata*	LWG - 65153	Nanu-chatti	2261 m. asl	30°61'30" N	79°19'92" E
2	*Heterodermia diademata*	LWG - 61631	Goundar village	1685 m. asl	30°60'20" N	79°17'76" E
3	*Hypotrachyna cirrhata*	LWG - 62400	Koon-chatti	2957 m. asl	30°62'34" N	79°21'13" E
4	*Nephromopsis laii*	LWG - 61634	Madhya-maheshwar	3297 m. asl	30°63'87" N	79°22'21" E
5	*Parmotrema nilgherrensis*	LWG - 61626	Madhya-maheshwar	3335 m. asl	30°63'89" N	79°22'86" E

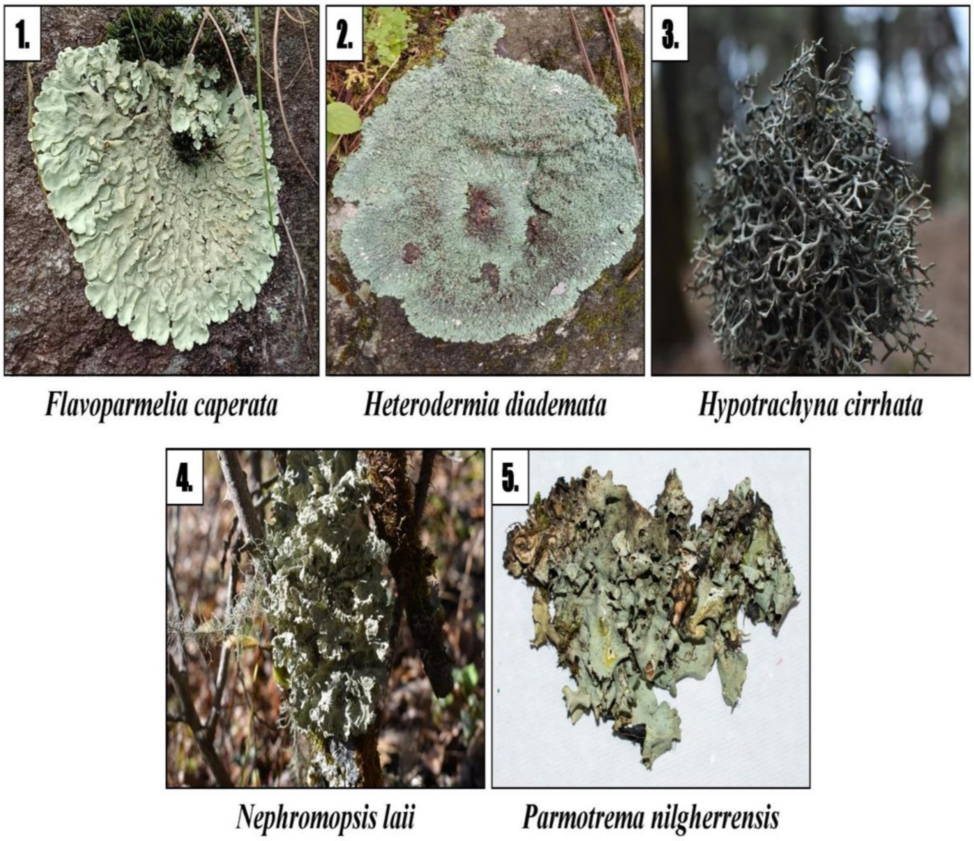

Fig. 1. Lichen selected for phytochemical analysis.

species employing the methods established by Rashmi and Rajkumar (2014).

RESULTS AND DISCUSSION

The analysis of phytochemicals in various lichens (*Flavoparmelia caperata*, *Heterodermia diademata*, *Hypotrachyna cirrhata*, *Nephromopsis laii* and *Parmotrema nilgherrensis*) using different solvents (Chloroform, Ethyl acetate, Ethanol and Methanol) reveals differences in the presence of key phytochemicals (Table 2 and Fig. 3).

Flavonoids were detected in *Flavoparmelia caperata* using methanolic extracts and in *Heterodermia diademata* using both chloroform and methanolic extracts. *Hypotrachyna cirrhata* contained flavonoids in all types of extracts, while no flavonoids were found in the extracts of *Nephromopsis laii* and *Parmotrema nilgherrensis*. Glycosides were present in the chloroform and ethyl acetate extracts of *Flavoparmelia caperata*, and in the chloroform, ethyl acetate and methanolic extracts of *Heterodermia diademata*. Ethyl acetate and ethanolic extracts of *Nephromopsis laii* also contained glycosides, while *Parmotrema nilgherrensis* displayed glycosides only in ethyl acetate extracts. No glycosides were observed in *Hypotrachyna cirrhata*. Saponins were identified in *Hypotrachyna cirrhata* and *Parmotrema nilgherrensis* in ethanolic and methanolic extracts, while *Flavoparmelia caperata* showed saponins only in ethanolic extracts. No saponins were found in the extracts of *Heterodermia diademata* and *Nephromopsis laii*. Steroids were exclusively found in *Hypotrachyna cirrhata* in the chloroform extract, with no presence in the other lichens. Tannins were detected in *Nephromopsis laii* in ethanolic and methanolic extracts, in *Hypotrachyna cirrhata* and *Flavoparmelia caperata*

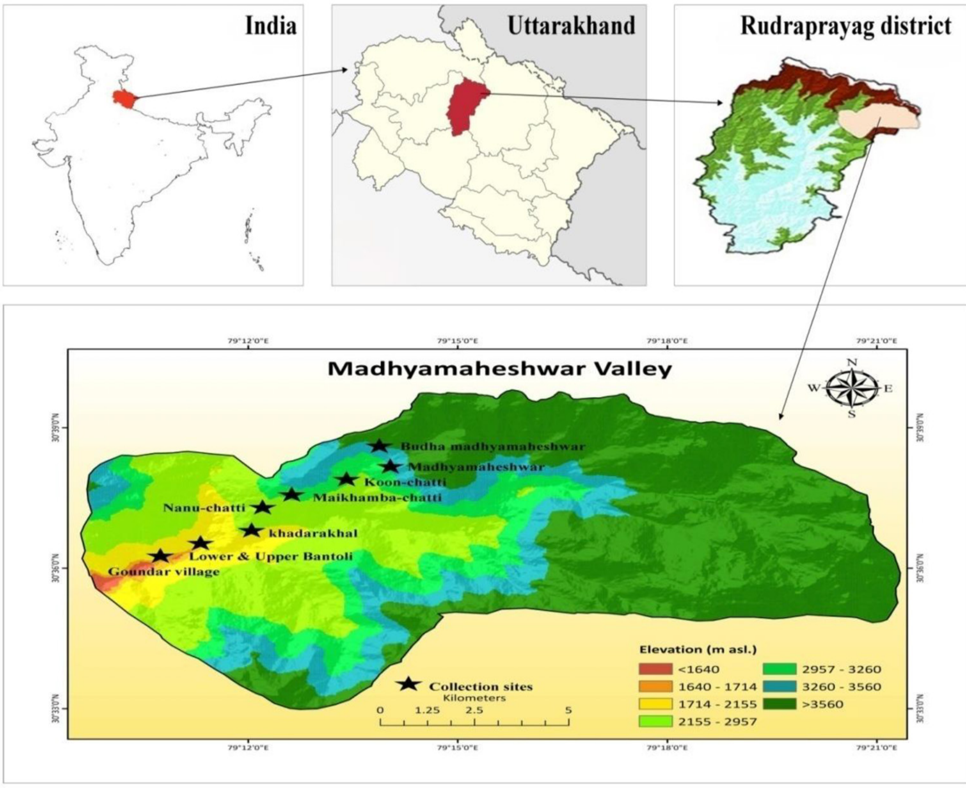

Fig. 2. Location and geographical map of the sampling area.

in ethanolic extracts and in *Parmotrema nilgherrensis* and *Heterodermia diademata* in methanolic extracts. Phenols were present in *Nephromopsis laii* in ethanolic and methanolic extracts, in *Hypotrachyna cirrhata*

Table 2. Phytochemical profiles of lichen species extracted with different solvents. [+] – Presence, [-] – Absence.

Sl. No.	Phytochemicals	*Flavoparmelia caperata*				*Heterodermia diademata*				*Hypotrachyna cirrhata*				*Nephromopsis laii*				*Parmotrema nilgherrensis*			
		Solvent used in extraction																			
		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	Flavonoids	-	-	-	+	+	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-
2	Glycosides	+	+	-	-	+	+	-	+	-	-	-	-	-	+	+	-	-	+	+	-
3	Saponins	-	-	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+	+
4	Steroids	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
5	Tannins	-	-	+	-	-	-	-	+	-	-	+	-	-	-	+	+	-	-	-	+
6	Phenols	-	-	+	-	-	-	-	+	-	-	+	-	-	-	+	+	-	-	-	+
7	Terpenoids	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	Alkaloids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

and *Flavoparmelia caperata* in ethanolic extracts, and in *Parmotrema nilgherrensis* and *Heterodermia diademata* in methanolic extracts. Terpenoids were only identified in *Heterodermia diademata* in chloroform extracts, with no terpenoids found in the other lichens. No alkaloids were detected in any of the lichen species across the different extraction solvents.

The previous research by Rashmi and Rajkumar (2014) identified tannins, flavonoids, proteins, triterpenes, carbohydrates and steroids in *Flavoparmelia caperata*. In contrast, our current study detected flavonoids, glycosides, saponins, tannins and phenols in *Flavoparmelia caperata*. Reddy *et al.* (2017) reported tannins, alkaloids, glycosides and triterpenoids in *Heterodermia diademata*, as well as alkaloids, glycosides, tannins and triterpenoids in *Parmotrema nilgherrense*. In our study, we found flavonoids, glycosides, tannins, phenols and terpenoids in *Heterodermia diademata*, and glycosides, tannins

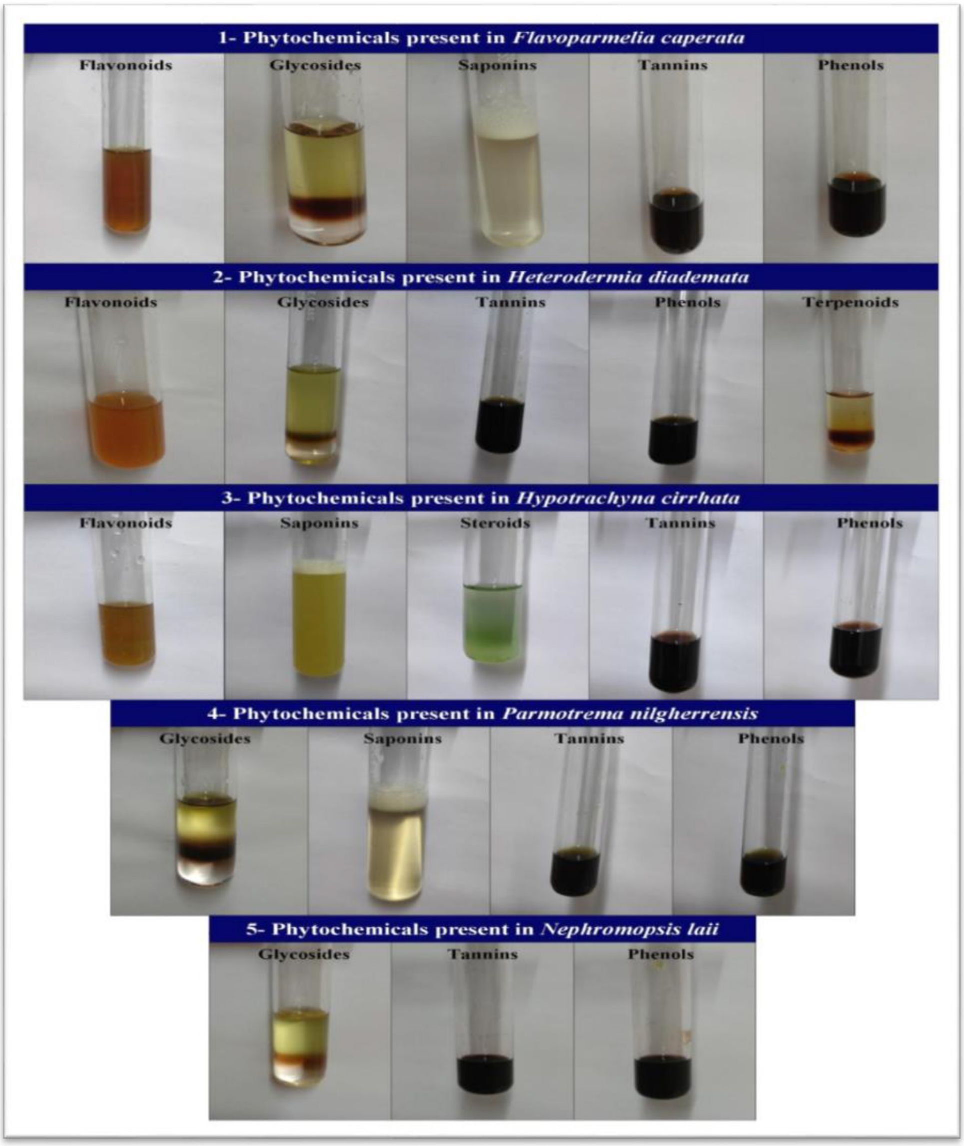

Fig. 3. Presence of diverse phytochemicals in selected lichens.

Table 3. Traditional knowledge and biological activities of selected lichen species.

Sl. No.	Lichen species	Traditional knowledge	Biological activities	References
1	*Flavoparmelia caperata*	Decoction known to expel intestinal worms	Antioxidant, antimicrobial, antiproliferative, cytotoxic, antibacterial, antimycobacterial, amylase inhibitory	Nayaka *et al.* (2010), Mitrović *et al.* (2011), Fernández *et al.* (2016), Dieu *et al.* (2020), Gupta *et al.* (2007), Thadhani and Karunaratne (2017),
2	*Heterodermia diademata*	Paste of thalli used as a plaster for cuts and injuries, serves as an antiseptic	Anti-lipoxygenase, radical scavenging, antimicrobial, antifungal	Behera *et al*. (2016), Balasubramanian and Nirmala (2014)
3	*Hypotrachyna cirrhata*	Used as spice, food and medicine, crude drug 'Chharila' treats various ailments, including wounds blood disorders, and respiratory issues	Antioxidative, cardioprotective, anticancer, antifungal, cytotoxic, anti-obesity (pancreatic-lipase inhibitory), antimicrobial, anti-helminthic, insecticidal, anti-mycobacterial, antibacterial, amylase inhibitory	Jain (2016), Pol *et al*. (2017), Prashith Kekuda *et al. (*2012), Anil *et al*.(2011), Swathi *et al.* (2010), Gupta *et al*. (2007), Sati and Joshi (2011), Nayaka *et al*. (2010), Thadhani and Karunaratne (2017), Prabhakar *et al*. (2025)
4	*Nephromopsis laii*	Not reported	Antioxidant	Singh *et al.* (2020)
5	*Parmotrema nilgherrensis*	Used as spice, food, and medicine, crude drug 'Chharila' treats various ailments, similar to *Hypotrachyna cirrhata*	Antioxidative, cardioprotective, anticancer, antifungal, cytotoxic, anti-obesity (pancreatic lipase inhibitory), antimicrobial, anti-helminthic, insecticidal, anti-mycobacterial, antibacterial, amylase inhibitory	Jain (2016), Pol *et al*.(2017), Prashith Kekuda *et al*. (2012), Anil *et al.* (2011), Swathi *et al*. (2010), Gupta *et al*. (2007), Sati and Joshi (2011), Nayaka *et al*. (2010), Thadhani and Karunaratne (2017)

and phenols in *Parmotrema nilgherrensis*. Swathi *et al.* (2010) identified alkaloids, saponins, tannins and terpenoids in *Everniastrum cirrhatum*. Conversely, our research detected flavonoids, saponins, steroids, tannins and phenols in *Hypotrachyna cirrhata*. Additionally, previous research by Singh *et al.* (2020) reported the presence of phenols and flavonoids compounds in *Nephromopsis laii*. Our study reports the presence of glycosides, tannins and phenols in *Nephromopsis laii*.

Ethnobotanical significance lichens

In traditional medicine, lichens are commonly employed to address various health issues, including wound healing, skin disorders and respiratory conditions. They are also utilized for gynecological and obstetric concerns. Their natural properties make them a valuable resource in herbal remedies providing therapeutic benefits that have been recognized across different cultures for generations (Crawford 2019, Saklani and Upreti 1992, Shah 2014). In several temperate regions across Asia, Africa, Europe and the USA, lichens have been the subject of extensive research. In particular ethnobotanical studies of lichens are well documented in Asian countries such as India, China, Nepal and Tibet, showcasing their significance in traditional practices and local medicinal knowledge (Wang *et al.* 2001, Upreti *et al.* 2005, Kunwar *et al.* 2010, Devkota *et al.* 2017). Lichens have been utilized as traditional foods, medicines, and in sacred fires known as 'HAVAN' or 'HOMA' for thousands of years. They are essential for ecosystem health and human well-being. Throughout the middle ages medicinal practitioners relied heavily on lichens for their healing properties (Devkota *et al.* 2017). We have presented the ethnobotanical uses and significance of five selected lichen species namely, *Flavoparmelia caperata*, *Heterodermia diademata*, *Hypotrachyna cirrhata*, *Nephromopsis laii* and *Parmotrema nilgherrensis* in Table 3.

CONCLUSION

These findings underscore the rich and varied chemical compositions of the species studied, highlighting their potential as valuable sources for future research. The distinct presence and absence of key phytochemicals across different species and extraction methods suggest diverse biological activities and therapeutic possibilities. This variability provides a compelling basis for further investigation into their pharmacological applications and the development of novel natural products, potentially leading to significant advancements in drug discovery and therapeutic development.

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