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Phytochemical Profiling and Antibacterial Activity of *Cladonia fenestralis* from Garhwal Himalaya, Uttarakhand, India

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Abstract: Lichens are known for their bioactive secondary metabolites with diverse pharmacological properties. This study investigated the phytochemical composition and antibacterial activity of *Cladonia fenestralis* collected from Nandi Kund Lake, Uttarakhand. Methanolic extracts were screened for secondary metabolites, revealing flavonoids, glycosides, phenols, saponins, steroids, tannins, and terpenoids, while alkaloids were absent. Antibacterial activity was evaluated against *Escherichia coli* and *Staphylococcus aureus* using the agar well diffusion method, showing concentration-dependent inhibition, with higher efficacy against Gram-positive bacteria. These results highlight *C. fenestralis* as a potential source of bioactive compounds for pharmaceutical applications and warrant further metabolite characterization.

Keywords: Lichen • *Cladonia fenestralis* • Phytochemicals • Antibacterial activity • Bioactive compounds.

Introduction

Lichens are formed through a mutual association between a fungal component (mycobiont) and a photosynthetic component (photobiont), which is typically represented by either a green alga or a cyanobacterium (Prabhakar et al., 2024a; Prabhakar et al., 2026a, 2026b, 2026c). On a global scale, nearly 19,387 lichen species have been recorded to date (Lücking et al., 2017). Since ancient times, lichens have been utilized by humans for a variety of purposes, including their consumption as food and their use in producing essential oils, mineral-based compounds, fermented drinks, confectionery items, cosmetic formulations, and aromatic products (Elkhateeb et al., 2017). Bioactive compounds produced by lichens have gained growing attention in pharmaceutical research due to their potential medicinal value in the treatment of a wide range of diseases (Huneck, 1999). Differences in the presence of key phytochemical constituents across lichen species and extraction methods suggest a wide diversity of biological activities and medicinal applications (Aoussar et al., 2020; Prabhakar et al., 2025b; Kant et al., 2025). Extracts derived from lichens exhibit a wide spectrum of biological activities, including antibacterial, antiviral, antiprotozoal, anticancer, cytotoxic, antipyretic, antidiabetic, analgesic, antioxidant, anti-inflammatory, anti-HIV, antiulcer, hepatoprotective, and antigenotoxic effects.

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Among these activities, antimicrobial, antioxidant, anti-inflammatory, and cytotoxic actions are the most frequently reported and extensively studied (Jha et al., 2017; Solárová et al., 2020; Prabhakar et al., 2024b; Kumar et al., 2025; Prabhakar et al., 2025a; Kant et al., 2026). Lichens synthesize bioactive substances, including usnic acid, that demonstrate inhibitory effects against microbial pathogens as well as antiviral activity (Renjini et al., 2024).

Traditional medicinal uses and bioactive properties of *Cladonia fenestralis*: *Cladonia fenestralis* has been traditionally used in various cultures (Table 1), yet its pharmaceutical potential remains largely unexplored.

Table 1: Traditional uses and bioactive activities of *Cladonia fenestralis*.

Lichen species	Traditional uses	Bioactive activities	Reference
***Cladonia fenestralis* Nuno**	Traditionally employed to treat bacterial skin infections, dizziness, nosebleeds, contagious dermatological conditions, and eye inflammation (conjunctivitis).	Not reported	Wang and Qian (2013)

With rising bacterial infections and antimicrobial resistance, discovering natural antibacterial agents is crucial. Methanolic extracts of *C. fenestralis* contained flavonoids, glycosides, phenols, saponins, steroids, tannins, and terpenoids, known for antimicrobial and pharmacological effects. The extract showed concentration-dependent inhibition of *Escherichia coli* and *Staphylococcus aureus*, with higher efficacy against Gram-positive bacteria. This is the first report on its phytochemical composition and antibacterial activity, highlighting its potential as a source of bioactive compounds.

Materials and Methods

Lichen specimen collection and identification:
A specimen of *Cladonia fenestralis* Nuno (Fig 1) was collected from the vicinity of Nandi Kund Lake in the Madhyamaheshwar Valley of Kedarnath Wildlife Sanctuary, Rudraprayag district, Uttarakhand (30°36′15″ N, 79°19′56″ E) at an altitude of 4600 m a.s.l. (Fig. 2). Its identification was based on morphological, anatomical, and chemical features, following standard references (Awasthi, 2007), at the Forest Ecology Laboratory, HNB Garhwal University, and subsequently verified at the Lichenology Laboratory, CSIR-NBRI, Lucknow. A voucher specimen was deposited in the herbarium under accession number LWG-69623.

Preparation of extract

The collected lichen sample was air-dried and ground into a fine powder. A 10 g portion of the powdered material was weighed and placed in a thimble for Soxhlet extraction using methanol at a ratio of 10 g/100 ml. After extraction, the solution was filtered through Whatman No. 1 filter paper. The filtrate was used for phytochemical analysis, while the solvent was evaporated to obtain a crude methanolic extract, which was re-dissolved in methanol and employed for antibacterial assays (Prabhakar et al., 2025a).

Preliminary phytochemical analysis

The methanol extract of the lichen sample was subjected to preliminary phytochemical screening to identify secondary metabolites, following the procedure of Rashmi and Rajkumar (2014).

Test microorganisms

For antibacterial assays, two human pathogenic bacterial strains were employed: *Staphylococcus aureus* (MTCC 1144, Gram-positive) and *Escherichia coli* (MTCC 68, Gram-negative). Both microbial cultures were procured from the Microbiology Research Laboratory, Department of Botany and Microbiology, Hemvati Nandan

Bahuguna Garhwal University (A Central University), Srinagar, Garhwal, Uttarakhand.

Fig. 1: Lichen species selected for phytochemical and antibacterial studies.

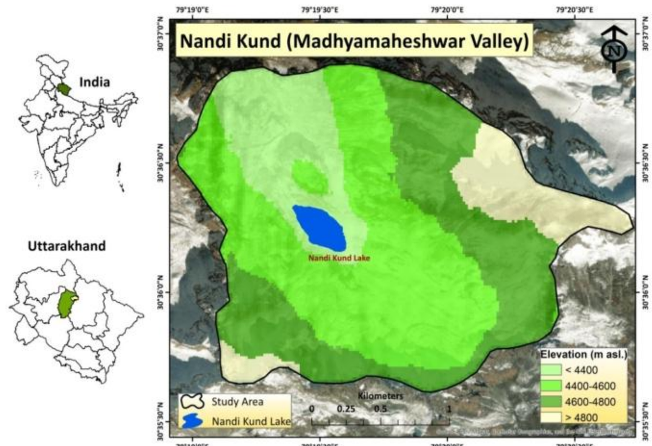

Fig. 2: Geographical location of the sampling site**.**

Agar well diffusion assay for antibacterial activity

The antimicrobial activity of *Cladonia fenestralis* extract was evaluated using the agar well diffusion method (Perez, 1990). For antibacterial testing, Mueller-Hinton Agar (MHA) was poured into sterile Petri dishes and allowed to solidify. A 100 µL suspension of bacterial culture, adjusted to the 0.5 McFarland standard, was spread uniformly on the agar surface. Wells of 6 mm diameter were made using a sterile cork borer, and each was filled with 100 µL of lichen extract at two concentrations (5 mg/well and 10 mg/well). Ampicillin (25 µg/disc) and Chloramphenicol (10 µg/disc) were used as positive antibacterial controls, while the solvent served as the negative control. Plates inoculated with bacterial strains were incubated at 37 ± 2 °C for 24 hours. Antibacterial activity was assessed by measuring the diameter of inhibition zones (mm) around the wells, excluding the well diameter. All experiments were performed in triplicate to ensure reproducibility and accuracy.

Results

Phytochemicals in *Cladonia fenestralis*

Phytochemical analysis of *Cladonia fenestralis* using methanol as a solvent indicates the presence of key bioactive compounds (Table 2). The methanol extract of *Cladonia fenestralis* revealed the presence of several important phytochemicals, including flavonoids, glycosides, phenols, saponins, steroids, tannins, and terpenoids. Alkaloids were not detected in this extract.

Table 2: Qualitative phytochemical profile of *Cladonia fenestralis* (methanolic extract).

Lichen species	Phytochemicals	Presence (+) / Absence (–)
***Cladonia fenestralis* Nuno**	Alkaloids	-
	Flavonoids	+
	Glycosides	+
	Phenols	+
	Saponins	+
	Steroids	+
	Tannins	+
	Terpenoids	+

Antibacterial activity of methanolic extract of *Cladonia fenestralis*

The antibacterial activity of the methanolic extract of *Cladonia fenestralis* was evaluated against *Escherichia coli* and *Staphylococcus aureus* using the agar well diffusion method ((Fig. 3). The extract showed a concentration-dependent inhibitory effect. At 5 mg/well, the zones of inhibition (ZOI) measured 3.07 ± 0.12 mm against *E. coli* and 8.13 ± 0.12 mm against *S. aureus*. Increasing the concentration to 10 mg/well enhanced the activity, producing ZOI values of 5.20 ± 0.20 mm and 11.23 ± 0.21 mm, respectively. No inhibition was observed in the negative control (solvent), confirming that the activity was solely due to the lichen extract. Among the positive controls, Ampicillin (25 µg/disc) produced inhibition zones of 11.10 ± 0.17 mm against *E. coli* and 10.00 ± 0.00 mm against *S. aureus*, while Chloramphenicol (10 µg/disc) exhibited the highest activity *E. coli*: 17.07 ± 0.12 mm; *S. aureus*: 19.03 ± 0.06 mm (Table 3 and Fig. 4). Overall, the methanolic extract exhibited distinct antibacterial activity, with greater efficacy against *S. aureus* than *E. coli*.

Fig. 3: Antibacterial activity of the methanolic extract of *Cladonia fenestralis* against the human pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus*, assessed by the well diffusion method.

Table 3: Antibacterial activity (zone of inhibition, ZOI, mm ± SD) of *Cladonia fenestralis* methanol extract against *Escherichia coli* and *Staphylococcus aureus*.

Lichen sample	Extract type	Concentration (mg)	Bacterial strain	
			Escherichia coli (ZOI, mm)	*Staphylococcus aureus* (ZOI, mm)
Cladonia fenestralis	Methanol	5 mg	3.07 ± 0.12	8.13 ± 0.12
		10 mg	5.20 ± 0.20	11.23 ± 0.21
Negative control (-)	Solvent	-	0.00 ± 0.00	0.00 ± 0.00
Positive control (+)	Ampicillin	25 µg	11.10 ± 0.17	10.0 ± 0.0
	Chloramphenicol	10 µg	17.07 ± 0.12	19.03 ± 0.06

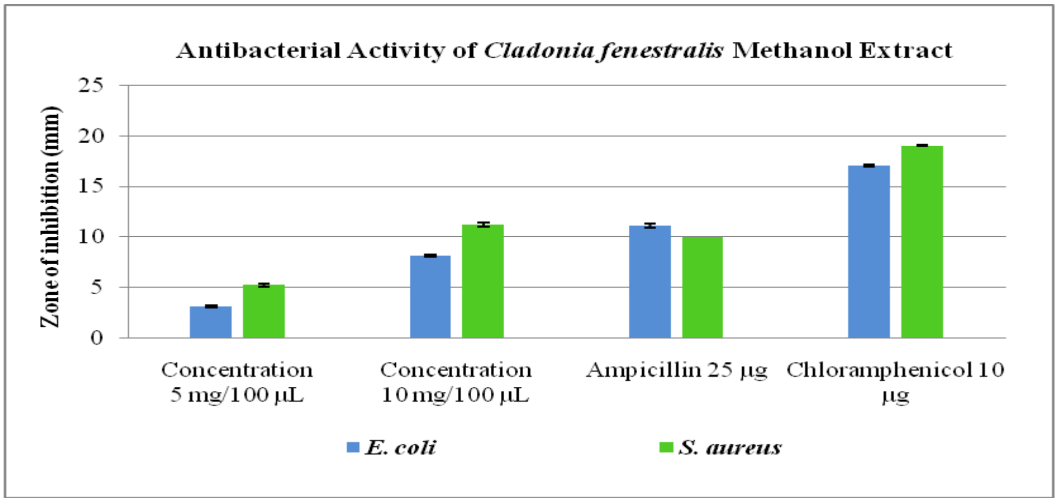

Fig. 4: Antibacterial activity of *Cladonia fenestralis* methanol extract against *Escherichia coli* and *Staphylococcus aureus*.

Discussion

The methanolic extract of *Cladonia fenestralis* was found to contain several key secondary metabolites, including flavonoids, glycosides, phenols, saponins, steroids, tannins, and terpenoids, while alkaloids were absent. These phytochemicals are well known for their antimicrobial and pharmacological properties, suggesting their possible role in the bioactivity of the extract.

The antibacterial assay demonstrated that the extract exerted a clear concentration-dependent inhibitory effect against both *Escherichia coli* and *Staphylococcus aureus*, with greater activity observed against the Gram-positive bacterium. This trend aligns with earlier findings in other lichens, where Gram-positive bacteria are generally more susceptible to lichen-derived compounds due to cell wall differences. Although the activity was lower than that of the standard antibiotics, the results are noteworthy as they establish *C. fenestralis* as a potential source of antibacterial agents.

To the best of our knowledge, this is the first report on the phytochemical composition and antibacterial activity of *Cladonia fenestralis*. These findings provide a foundation for further studies aimed at isolating and characterizing the bioactive metabolites responsible for the observed activity.

Conclusion

The present study demonstrates that the methanolic extract of *Cladonia fenestralis* contains key secondary metabolites, including flavonoids, glycosides, phenols, saponins, steroids, tannins, and terpenoids, which likely contribute to its antimicrobial properties. The extract exhibited a clear, concentration-dependent inhibitory effect against both *Escherichia coli* and *Staphylococcus aureus*, showing greater efficacy against the Gram-positive bacterium. Although less potent than standard antibiotics, these findings highlight *C. fenestralis* as a promising source of antibacterial agents. To the best of our knowledge, this is the first report on its phytochemical composition and antibacterial activity, providing a basis for future isolation and characterization of bioactive compounds.

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