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Exploring Antibacterial Potential: *Dolichousnea longissima* and *Hypotrachyna nepalensis* Lichens from Madhyamaheshwar Valley, Garhwal Himalaya, Uttarakhand

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Abstract: This study investigates the antibacterial potential of extracts from *Dolichousnea longissima* and *Hypotrachyna nepalensis* against *Escherichia coli* and *Staphylococcus aureus*. Lichens, which are symbiotic associations between an alga (photobiont) and a fungus (mycobiont), are known for synthesizing a diverse range of secondary compounds with various biological activities. The lichen extracts were prepared using chloroform, ethyl acetate, ethanol and methanol, and their antibacterial efficacy was assessed via the agar-well diffusion method. The results revealed significant antibacterial effects, particularly with the ethyl acetate extract from *Dolichousnea longissima* and the chloroform extract from *Hypotrachyna nepalensis*, both of which exhibited substantial inhibition zones against the bacterial strains. These findings underscore the potential of lichen extracts as sources of natural antibacterial agents and suggest their promise for future antimicrobial development. Further research aimed at isolating and characterizing specific bioactive compounds could uncover novel therapeutic applications in medicine and pharmaceuticals.

Keywords: Lichens • *Dolichousnea longissima* • *Hypotrachyna nepalensis* • Antibacterial activity • *Escherichia coli* • *Staphylococcus aureus*.

Introduction

Every type of terrestrial habitat, including the most severe ones, harbors cryptogams known as lichens. They are a special kind of living form, a symbiotic relationship between a cyanobacterium (photobiont) and a fungus (mycobiont). They typically form epiphytes on trees and leaves, as well as on rocks and infertile soils (Stamenković et al. 2011; Chahra et al. 2016). Lichens are regarded as important natural resources, serving various purposes such as medicines, food, animal feed, dyes, perfumes, spices and other miscellaneous uses (Hegnauer, 1962). Lichens and their derivatives have been used globally for treating ailments since ancient times. Many studies have evaluated the biological activity of lichen secondary metabolites against harmful pathogens (Fournet et al. 1997). Throughout the centuries, lichens have played an important role in sustaining the nutrition of various animals and humans during times of famine. China commonly prepares several types of edible lichens, such as *Dermatocarpon miniatum, Lobaria pulmonaria, Umbilicaria esculenta* and *Alectoria asiatica*, as dishes. They also serve as antiseptics, spices, textile colors, perfume industry odorants and medications for liver, kidney, and respiratory ailments (Mitrović et al. 2011; Aoussar et al. 2021; Muthu et al. 2021; Zhao et al. 2021). Lichen-derived compounds have been shown to possess antimicrobial properties (Boustie et al. 2011; Podterob, 2008; Shrestha and St. Clair, 2013a; Shukla et al. 2010). Lichens synthesize

several secondary compounds that serve as defensive protection against various pathogenic microbes (Lawrey, 1986). These metabolites play a critical role in the bioactivity of lichen extracts, which is important for current pharmacy and medical research. Recognized biological properties of some lichens include antibacterial, antiviral, anticancer, analgesic, antipyretic, antiproliferative and antiprotozoal properties (Kosanic et al. 2011; Mitrović et al. 2011 Edible lichens like *Everniastrum cirrhatum*, *E. nepalense* and *Parmotrema cetratum* are believed to treat various ailments. Lichens have been used in traditional medicine for conditions such as stomach disorders, diabetes, whooping cough, tuberculosis, cancer and skin diseases. Lichen extracts inhibit *Bacillus subtilis*, a bacterium causing nausea and diarrhea, making them a useful remedy. Recently, many plants have gained attention as potential antibiotic sources (Basile et al. 2000).

Materials and Methods

Collection of lichen samples: Two distinct lichen species, *Dolichousnea longissima* and *Hypotrachyna nepalensis* (Fig 1), were collected from Budha Madhyamaheshwar in the Madhyamaheshwar Valley. This location is situated at latitude 30°63'37" N and longitude 79°21'07" E, with an elevation of 3400 meters above sea level (Table 1 and Fig 2). Specimens were collected from both rocks and the outer layer of tree bark. The lichen specimens were washed with running tap water to remove debris and air-dried at room temperature. Sample were identified by determine their morphological, anatomical and chemical characteristics using existing literature as a reference (Awasthi 2007). The identification of the lichen samples was carried out at the Forest Ecology Laboratory in the Department of Botany & Microbiology at H.N.B. Garhwal University and was subsequently verified and authenticated at the Lichenology Laboratory of the CSIR - National Botanical Research Institute in Lucknow. The following accession numbers were assigned to the lichen samples: *Hypotrachyna nepalensis* (LWG-61633), *Dolichousnea longissima* (LWG-61632).

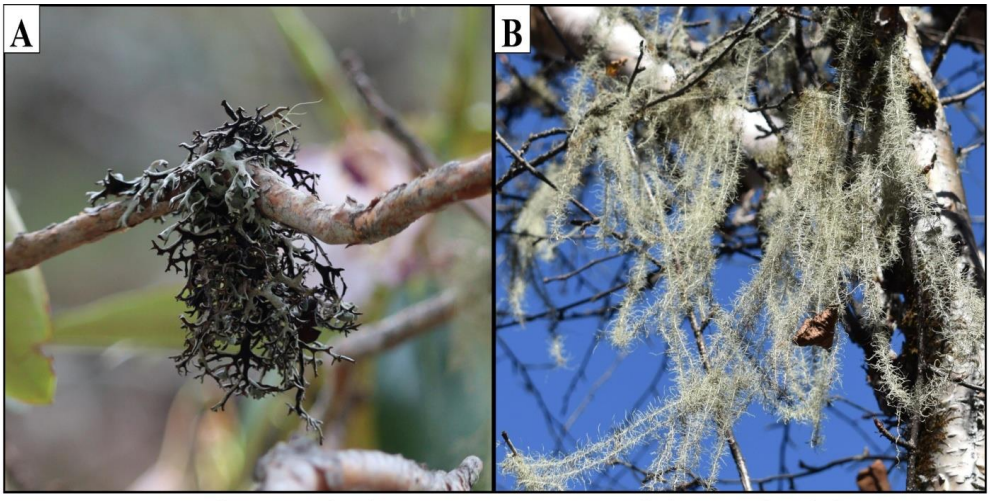

Fig 1: Lichen species selected for their antibacterial properties

Table 1: Location and geographical coordinates of selected lichen species

Lichen species	Accession number	Location of sampling	Latitude	Longitude	Elevation
Dolichousnea longissima	(LWG-61633)	Budha Madhyamaheshwar	30°63'37" N	79°21'07" E	3400 m ASL
Hypotrachyna nepalensis	(LWG-61632)				

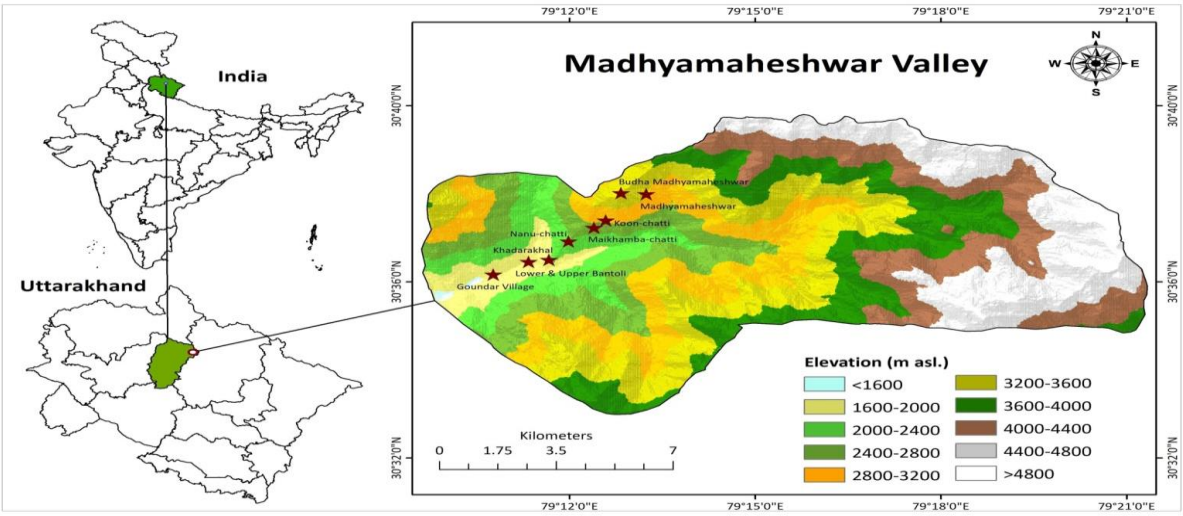

Fig 2: Geographical map of the study area

Preparation of sample extracts

For extraction, the air-dried lichen samples were finely ground into a powder using a mixer grinder. Each sample weighing 10 grams was placed into individual thimbles. Extraction was performed using a Soxhlet apparatus with four different solvents: chloroform, ethyl acetate, ethanol and methanol). Each solvent was used in a ratio of 10 grams of lichen per 100 milliliters of solvent. After extraction, the respective extracts were filtered using Whatman No. 1 filter paper to remove solid residues. The filtrates were then evaporated to concentrate the extracts, yielding crude extracts of chloroform, ethyl acetate, ethanol and methanol. These crude extracts were subsequently dissolved again in their respective solvents to prepare stock solutions for assessing antibacterial activity. The antibacterial activity of each extract was evaluated using standardized methods to determine their effectiveness against specific bacterial strains.

Antibacterial Activity

Microorganisms: Two strains of human pathogenic bacteria, including Gram-positive *Staphylococcus aureus* MTCC 1144 and Gram-negative *Escherichia coli* MTCC 68, were obtained from the microbiological research laboratory at the Department of Botany & Microbiology, HNB Garhwal University (A Central University), Srinagar Garhwal, Uttarakhand.

Agar well diffusion assay for antibacterial screening: The antibacterial screening of selected microorganisms was conducted using the agar well diffusion method (Perez et al. 1990). Sterile Petri dishes were filled with Mueller Hinton Agar and allowed to solidify. Subsequently, 100 µl of fresh culture of the test bacteria at a concentration equivalent to 0.5 McFarland standards was spread evenly on each

plate. Wells were then created using a sterile cork borer with a diameter of 7 mm into each well, 100 µl of lichen extract at two different concentrations (5 mg and 15 mg per well) was carefully added. As a positive control, 15 mg of erythromycin was used, while the respective solvents of the lichen extracts served as negative controls. The plates were then incubated at 37°C for 24 hours, allowing time for antibacterial activity to occur. Following incubation, the diameters of the zones of inhibition around each well were measured in millimeters to assess the effectiveness of the extracts against the test bacteria.

Results

Antibacterial activity of lichen extracts: This study investigated the antibacterial efficacy of extracts from *Dolichousnea longissima* and *Hypotrachyna nepalensis* against *Escherichia coli* and *Staphylococcus aureus* using the agar well diffusion method. The results of antibacterial activity obtained from *Dolichousnea longissima* extracts. The chloroform extract showed no inhibition zones against both bacterial strain at both 5 mg and 15 mg concentrations. In contrast, the ethyl acetate extract exhibited minimum to maximum activity with inhibition zones ranging from 6.1 mm to 10.5 mm against *Escherichia coli* and 10.5 to 14.1 mm against *Staphylococcus aureus*. The ethanol extract showed no activity at a concentration of 5 mg. However, at 15 mg concentration, it exhibited an inhibition zone of 10.5 mm against *Escherichia coli*. Against *Staphylococcus aureus*, the ethanol extract showed activity at both concentrations, with inhibition zones ranging from 7.5 mm to 11.1 mm. The methanol extract showed no activity at a concentration of 5 mg; however, at 15 mg, it exhibited an inhibition zone of 11.5 mm against *Escherichia coli*. Against *Staphylococcus aureus*, the methanol extract displayed activity at both concentrations, with inhibition zones ranging from 7.5 mm to 9.3 mm (Table - 2 and Fig - 3).

Table 2: Antibacterial activity results of *Dolichousnea longissima* with different concentrations and different solvent extracts

Lichen sample	**Extract type**	**Concentration (mg)**	**Bacterial strain**	
			***Escherichia coli* (ZOI in mm)**	***Staphylococcus aureus* (ZOI in mm)**
Dolichousnea longissima	Chloroform	5 mg	0 mm	0 mm
		15 mg	0 mm	0 mm
	Ethyl acetate	5 mg	6.1 mm	10.5 mm
		15 mg	10.5 mm	14.1 mm
	Ethanol	5 mg	0 mm	7.5 mm
		15 mg	10.5 mm	11.1 mm
	Methanol	5 mg	0 mm	7.5 mm
		15 mg	11.5 mm	9.3 mm
	Negative control (-)	Solvent	0 mm	0 mm
	Positive control (+)	15 mg Erythromycin	0 mm	24.5 mm

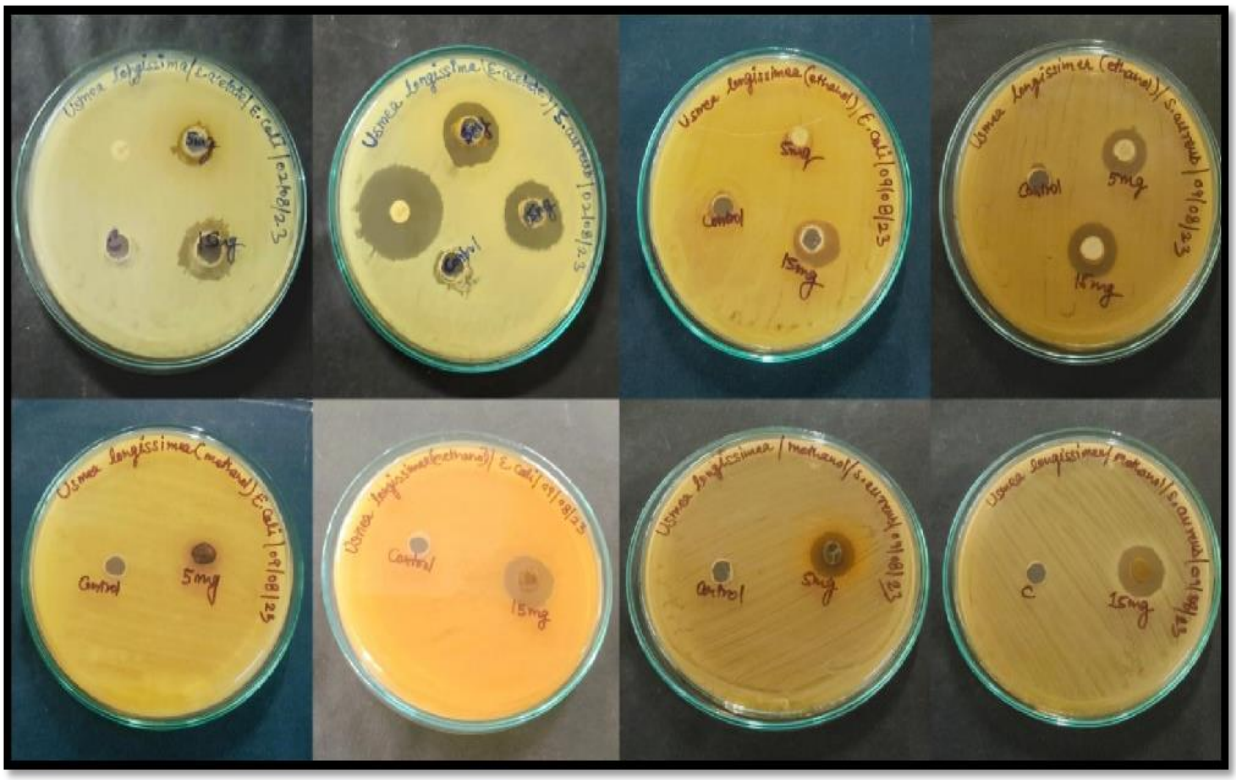

Fig 3: Antibacterial activity of *Dolichousnea longissima* extracts against human pathogens *Escherichia coli* and *Staphylococcus aureus*

The antibacterial activity results of *Hypotrachyna nepalensis* extracts. The chloroform extract exhibited minimum to maximum activity with inhibition zones of 12.8 mm and 21.1 mm against *Escherichia coli* at 5 mg and 15 mg concentrations, respectively, the chloroform extract exhibited minimum to maximum activity with inhibition zones of 8.5 to 13.5 mm against *Staphylococcus aureus* at 5 mg and 15 mg concentrations. The ethyl acetate extract demonstrated no activity against *Escherichia coli* at 5 mg and 15 mg concentrations. Respectively, ethyl acetate extract demonstrated minimum to maximum activity with inhibition zones 7.8 to 11.3 mm against *Staphylococcus aureus* at 5 mg and 15 mg concentrations. The ethanol extract demonstrated no activity against *Escherichia coli* at both concentrations, respectively; the ethanol extract exhibited activity with inhibition zones of 3.8 to 6.8 mm against *Staphylococcus aureus* at 5 mg and 15 mg concentrations. The methanol extracts demonstrated inhibition zones of 4.1 to 7.1 mm against *Escherichia coli* and 4.5 to 8.1 mm against *Staphylococcus aureus*. The negative control (solvent) did not inhibit bacterial growth, whereas the positive control (erythromycin) consistently exhibited strong antibacterial effects with inhibition zones of 24.5 mm against only *Staphylococcus aureus* bacterial strains (Table 3 and Fig 4).

Table 3: Antibacterial activity results of *Hypotrachyna nepalensis* with different concentrations and different solvent extracts

Lichen sample	Extract type	Concentration (mg)	Bacterial strain	
			***Escherichia coli* (ZOI in mm)**	***Staphylococcus aureus* (ZOI in mm)**
Hypotrachyna nepalensis	Chloroform	5 mg	12.8 mm	8.5 mm
		15 mg	21.1 mm	13.5 mm
	Ethyl acetate	5 mg	0 mm	7.8 mm
		15 mg	0 mm	11.3 mm
	Ethanol	5 mg	0 mm	3.8 mm
		15 mg	0 mm	6.8 mm
	Methanol	5 mg	4.1 mm	4.5 mm
		15 mg	7.1 mm	8.1 mm
	Negative control (-)	Solvent	0 mm	0 mm
	Positive control (+)	15 mg Erythromycin	0 mm	24.5 mm

These results indicate that both *Dolichousnea longissima* and *Hypotrachyna nepalensis* extracts possess varying degrees of antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. The ethyl acetate extract from *Dolichousnea longissima* and the chloroform extract from *Hypotrachyna nepalensis* demonstrated the most promising antibacterial activity. Further investigation is warranted to identify and isolate the specific bioactive compounds responsible for these effects. Understanding the mechanisms underlying their antibacterial properties could pave the way for their potential application in developing new antimicrobial agents.

Discussion

Previous research by Kumar et al. (2017) reported that ethyl acetate extracts of *usnea longissima* demonstrated a zone of inhibition (ZOI) of 13.0 ± 0.5 mm against *Escherichia coli*. Similarly, research conducted by Thippeswamy et al. (2011) reported that ethanol extracts of *usnea longissima* showed no activity against *Escherichia coli*. However, ethanol extracts produced a ZOI of 26. ± 0.5 mm against *Staphylococcus aureus*. A subsequent study by Devashree et al. (2019) reported that ethyl acetate extracts of *usnea longissima* demonstrated a zone of inhibition (ZOI) of 28.0 ± 0.7 mm against *Escherichia coli* and 27.0 ± 0.7 against *Staphylococcus aureus*. Ethanol extracts showed a ZOI of 32.0 ± 1.4 mm against *Escherichia coli* and 30.0 ± 1.4 against *Staphylococcus aureus*, while methanol extracts exhibited a ZOI of 34.0 ± 0.7 mm against *Escherichia coli* and 32.0 ± 0.7 against *Staphylococcus aureus*. In the present study reported antibacterial activity obtained from *Dolichousnea longissima* extracts. The chloroform extract showed no inhibition zones against both bacterial strain at both 5 mg and 15 mg concentrations. In contrast, the ethyl acetate extract exhibited minimum to maximum activity with inhibition zones ranging from 6.1 mm to 10.5 mm against *Escherichia coli* and 10.5 to 14.1 mm against *Staphylococcus aureus*. The ethanol extract showed no activity at a concentration of 5 mg. However, at 15 mg concentration, it exhibited an inhibition zone of 10.5 mm against *Escherichia coli*. Against *Staphylococcus aureus*, the ethanol extract showed activity at both concentrations, with

inhibition zones ranging from 7.5 mm to 11.1 mm. The methanol extract showed no activity at a concentration of 5 mg; however, at 15 mg, it exhibited an inhibition zone of 11.5 mm against *Escherichia coli*. Against *Staphylococcus aureus*, the methanol extract displayed activity at both concentrations, with inhibition zones ranging from 7.5 mm to 9.3 mm.

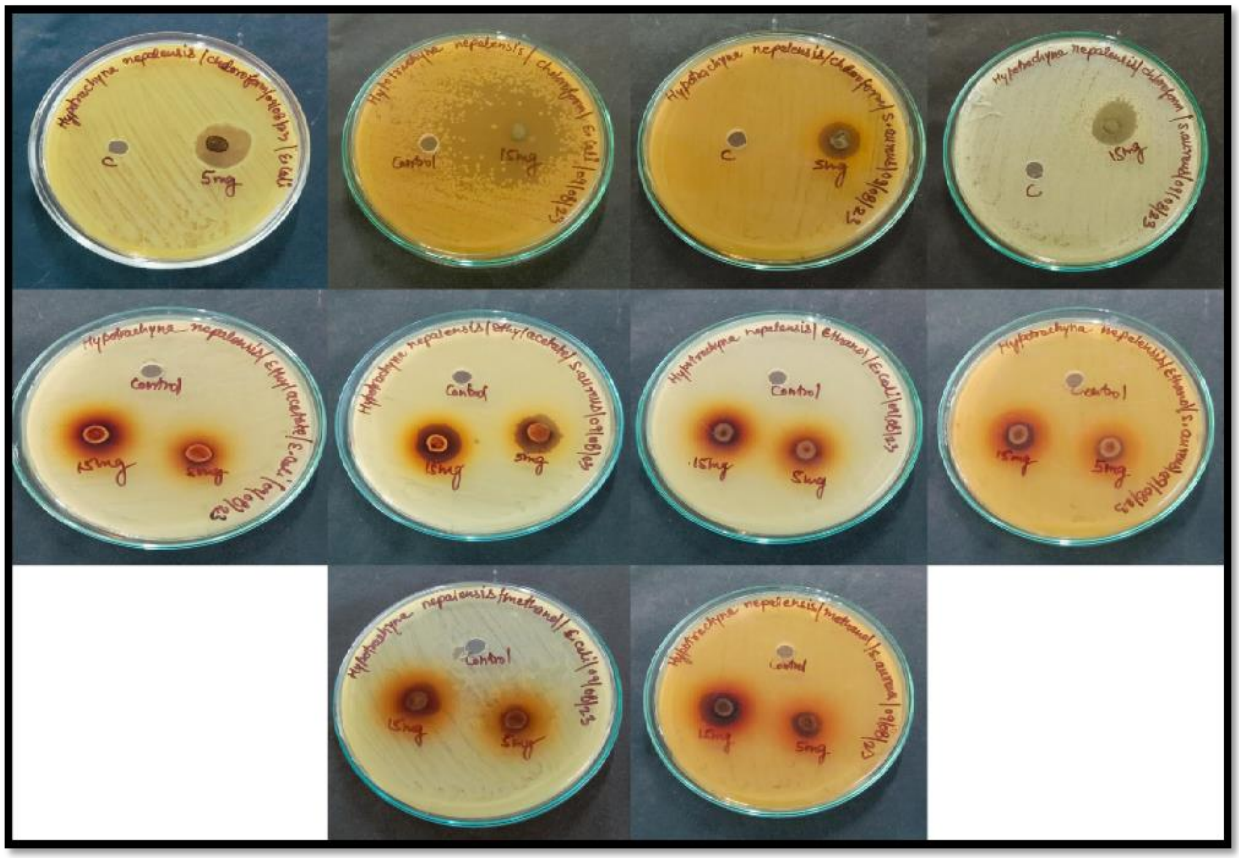

Fig 4: Antibacterial activity of *Hypotrachyna nepalensis* extracts against human pathogens *Escherichia coli* and *Staphylococcus aureus*

Earlier research by Sinha and Biswas (2011) reported that methanolic extracts of *Everniastrum nepalense* demonstrated a zone of inhibition (ZOI) of 35 mm against *Escherichia coli* and 38 mm against *Staphylococcus aureus*. Similarly, research conducted by Yonghang et al. (2019) reported that methanolic extracts of *Everniastrum nepalense* showed no activity against *Escherichia coli*. However, these extracts produced a ZOI of 12 to 13 mm against *Staphylococcus aureus*.

In the present study, chloroform extracts of *Hypotrachyna nepalensis* exhibited varying activity, with inhibition zones ranging from 12.8 mm to 21.1 mm against *Escherichia coli*. The chloroform extract also showed inhibition zones of 8.5 mm to 13.5 mm against *Staphylococcus aureus*. Ethyl acetate extracts demonstrated no activity against *Escherichia coli*, but showed inhibition zones of 7.8 mm to 11.3 mm against *Staphylococcus aureus*. Ethanol extracts exhibited no activity against *Escherichia coli* and showed inhibition zones of 3.8 mm to 6.8 mm against *Staphylococcus aureus*. Methanol extracts demonstrated inhibition zones of 4.1

mm to 7.1 mm against *Escherichia coli* and 4.5 mm to 8.1 mm against *Staphylococcus aureus*.

Conclusion

This study underscores that the extracts from *Dolichousnea longissima* and *Hypotrachyna nepalensis* exhibited notable antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, with varying potency depending on the solvent and concentration used. Particularly, the ethyl acetate extract from *Dolichousnea longissima* and the chloroform extract from *Hypotrachyna nepalensis* showed significant inhibition against both bacterial strains. These findings highlight the potential of lichen extracts as sources of natural antibacterial agents and suggest avenues for future research aimed at identifying and harnessing their bioactive components for the development of new antimicrobial therapies.

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References

Aoussar N, Achmit M, Es-Sadeqy Y, Vasiljević P, Rhallabi N, Ait Mhand R and Mellouki F (2021). Phytochemical constituents, antioxidant and antistaphylococcal activities of Evernia prunastri (L.) Ach., Pseudevernia furfuracea (L.) Zopf. and Ramalina farinacea (L.) Ach. from Morocco. *Archives of Microbiology*, *203*, 2887-2894.

Awasthi D.D (2007). A Compendium of the Macrolichens from India, Nepal and Sri Lanka. Bishen Singh Mahendra Pal Singh, Dehradun, India.

Basile A, Sorbo S, Giordano S, Ricciardi L, Ferrara S, Montesano D, Cobianchi RC, Vuotto ML and Ferrara L (2000). Antibacterial and allelopathic activity of extract from Castanea sativa leaves. Fitoterapia 71: 110-116.

Boustie J, Tomasi S, Grube M. (2011). Bioactive lichen metabolites: Alpine habitats as an untapped source. Phytochem Rev 10:287–307.Burkholder PR, Evans AW, Mcveigh I, Thornton HK. (1944). Antibioticactivity of lichens. Proc Natl Acad Sci USA 30:250–5.

Chahra D, Ramdani M, Lograda T, Chalard P, Figueredo G (2016). Chemical composition and antimicrobial activity of Evernia prunastri and Ramalina farinacea from Algeria. *Issues in Biological Sciences and Pharmaceutical Research*, 4(5), 35-42.

Devashree A P, Dikshit A and Nayaka S (2019). Antimicrobial role of Usnea longissima against pathogenic microorganisms. *International Journal of Scientific. Engineering Research* (10), *5*, 157-167.

Fournet A, Ferreira ME, de Arias AR, de Ortiz ST, Inchausti A, Yalaff G, Quilhot W, Fernandez E, Hidalgo ME. Activity of compounds isolated from chilean lichens against experimental cutaneous leishmaniasis. Comp Biochem Physiol, 1997; 116(1):51–4.

Hegnauer R (1962). Chemotaxonomie der Pfl anzen; eine Dbersicht iiber die Verbreitung und die Systematische Bedeutung der Pfl anzenstoffe. Birkhauser, Basel.

Kosanic M, Ranković B, Vukonevic J (2011). Antioxidant properties of some lichen

species. *Association of Food Scientists &Technologists*, 48(5), 584-590.

Kumar V, Tripathi M, Mathela C S and Joshi Y (2017). In vitro antibacterial activity of Himalayan lichenized Fungi. *Journal of Pharmacognosy and Natural Products*, *3*(128), 2472-0992.

Lawrey J D (1986). "Biological role of lichen substances," *Bryologist*, vol. 89, issue 2, pp. 111-122

Mitrovic T, Stamenkovic V, Tosic S, Stankovic M, Radojevic I, Stefanovic O, Comic L, Curcic M, Markovic S (2011). Antioxidant, antimicrobial and antiproliferative activities of five lichen species. *International Journal of Molecular Sciences*, 12, 5428-5448.

Muthu S, Murugan M, Rajendran K, Ponmurugan P (2021). An assessment of proximate composition, antioxidant activities and LC/MS based phytochemical profiling of some lichen species collected from Western Ghats of southern part of India. *Jordan Journal of Biological Sciences*, 14(4): 647-661.

Perez C, Pauli M and Bazerque P (1990) An Antibiotic Assay by Agar Well Diffusion Method. *Acta Biologiae Medicinae Experimentalis*, 15, 113-115.

Podterob AP. (2008). Chemical composition of lichens and their medicalapplications. Pharm Chem J 42:582–8.

Shrestha G, St. Clair LL. (2013b). Antimicrobial activity of extracts from two lichens Ramalina menziesii and Usnea lapponica. Bull Cali LichSoc 20:5–10.

Shukla V, Joshi G, Rawat MSM. (2010). Lichens as a potential natural source of bioactive compounds: A review. Phytochem Rev 9:303–14.

Sinha S N and Mrinal Biswas (2011). Evaluation of antibacterial activity of some lichen from Ravangla, Sikkim, India. *International Journal of Pharma and Bio Sciences* 2(4): 23-28

Stanković SM (2011). Total phenolic content, flavonoid concentration andantioxidant activity of Marrubium peregrinum L. extracts. *Kragujevac Journal of Science*, 33, 63-72.

Thippeswamy B, Naveenkumar K J, Bodharthi J G and Shivaprasad S R (2011). Antimicrobial activity of ethanolic extract of Usnea longissima. *Journal of Experimental Sciences*, *2*(12), 01-03.

Yonghang S, Rai S and Sah S N (2019). Antibacterial property of extract of *Everniastrum* nepalense (Edible Lichen) collected from hilly regions of Eastern Nepal. *Tribhuvan University Journal of Microbiology*, *6*, 51-58.

Zhao Y, Wang M, Xu B (2021). A comprehensive review on secondary metabolites and health-promoting effects of edible lichen. *Journal of Functional Foods*, 80, 1-17.