Applied Biological Research 27(3): 379-389; (2025)
DOI: 10.48165/abr.2025.27.01.37

PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF THREE LICHENS [*Heterodermia boryi, H. diademata* and *Lobaria retigera*] FROM INDIA

Kiran Pant[1], Pushpa Joshi[1], Dalip Kumar Upreti[2], Manoj Dhuni[1*], Sanjeeva Nayaka[2] and Jyoti Darmwal[1]

[1]*Department of Chemistry, D.S.B. Campus, Kumaun University, Nainital - 263 002, Uttarakhand (India)*
[2]*Lichenology Laboratory, CSIR-National Botanical Research Institute, Rana Pratap Marg, Lucknow - 226 001, Uttar Pradesh (India)*
*e-mail: *manojdhuni@gmail.com*

(Received 17 March, 2025; accepted 25 August, 2025)

ABSTRACT

Among natural products the phytochemicals are emerging as an important area of research, wherein lichens have attracted immense attention for their exceptional potency to cure various diseases. To explore the phytochemical and medicinal importance of lichens from India *Heterodermia boryi, H. diademata* and *Lobaria retigera* were analysed through gas chromatography–mass spectrometry. Hexane-, chloroform- and methanolic-extracts of each lichen were assessed for antimicrobial and antioxidant properties. The methanolic extracts of lichens showed the presence of 68 phytochemicals like hydroxychalcone, normethadol, pinostilbene, pipemidic acid, iodohiistidine, pyridine, pheophorbide a, chromone, isoorientin, dimethoxyflavone, perchloryl fluoride, sulforaphane and carvacrol. Methanol extracts of all the three species showed highest zone of inhibition ranging from 10.7±0.06 to 26.8±0.06 mm. All the extracts of three species exhibited potent antimicrobial activity against *S. typhi* followed by *K. pneumoriae.* Of the three extracts methanol extracts of both *H. boryi* and *H. diademata* showed the highest antioxidant activity with 0.899 and 0.62 mg g^{-1} ascorbic acid equivalent, respectively. While hexane and chloroform extracts of *H. boryi* showed highest radical scavenging activity followed by hexane and chloroform extract of *H. diademata* and hexane extract of *L. retigera*.

Keywords: Bioprospecting, gas-chromatography, lichenized fungi, minimum inhibitory concentration, phytochemicals

INTRODUCTION

Lichens are symbiotic association of two major microorganisms (alga and fungi) together with other microorganisms such as viruses, bacteria, and saccharomyceae fungi, forming a microecosystem (Hawksworth and Grube, 2020). They include a diverse species that thrive globally in varied and often extreme environments. This intrinsic resistance of lichens is primarily attributed to the presence of a diverse spectrum of secondary metabolites (Elix *et al*., 2008). These metabolites possess potential therapeutic significance, particularly in the treatment of wounds, external burns, asthma, tuberculosis, colds, gastritis, and other human ailments. Many of these compounds have been characterized for their distinct biological activities, including cytotoxic, fungicidal, antimicrobial, antioxidant, and anti-inflammatory effects (Kosanić and Ranković, 2019). Increasing attention has been focussed on lichens as natural sources of antioxidants and antimicrobial agents (Güvenç *et al.,* 2012). Since synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been reported to be toxic (Witschi, 1986), the search for natural antioxidants has intensified. However, the

antioxidant activities of lichens and their secondary metabolites are still not well understood, and only limited studies have provided insights into this field. The biological aspects of lichen metabolites also remain underexplored, even though their several metabolites are known to serve defensive functions. Therefore, further research focusing on *in vitro* antioxidant and antimicrobial properties of lichen extracts is highly warranted (Shrestha and St Clair, 2013).

Lichens produce over 1500 secondary metabolites through three biosynthetic pathways: the polyketide pathway (usnic acid, depsidones, depsides, xanthone), the shikimic acid pathway (pulvinic acid derivatives, cyclopeptides), and the mevalonic acid pathway (triterpenes, diterpenes, steroids) (Huneck and Yoshimura, 1996; Huneck, 2013). Kumar *et al.* (2014) highlighted the role of lichens in treating many diseases, including cancer, by interfering with disease development and progression pathways. Payal *et al*. (2016) carried out GC-MS analysis and evaluated the biological activities of medicinally important lichens *Parmelia perlata* (= *Parmotrema perlatum*) from India.

The natural compounds derived from lichens have attracted researchers because of their lower toxicity compared with available chemotherapeutic drugs (Demain and Vaishnav, 2011). The crude extracts of lichens or their isolated compounds exhibit wide range of biological activities, and the medicinal potential of any lichen is directly related to the variety of secondary metabolites it contains (Boustie and Grube, 2005). Hence, identifying potent compounds and exploiting their bioactive secondary metabolites are of great importance. Senthil *et al*. (2019) synthesized ecofriendly silver nanoparticles from methanolic extracts of lichen *Heterodermia boryi* and observed significant antibacterial potential against various bacterial pathogens like *Acinetobacter baumannii* and *Staphylococcus aureus*. This indicates that novel drugs can be designed against drug-resistant pathogens and lichen extracts hold promise in several biomedical applications. *Heterodermia diademata* (Taylor) D.D. Awasthi exhibited cytotoxic activity by inducing cell death at low doses (Norouzi *et al*., 2020) and showed anticancer properties against breast cancer cell lines (Jijo, 2019). The species of *Lobaria*, known as "lungwort," often possess cyanobacteria as symbionts and are traditionally used to treat lung diseases. Their extracts have demonstrated potential activity against hepatocellular carcinoma, breast, and lung cancers (Emsen *et al*., 2018). Suleyman *et al*. (2003) reported that *Lobaria* species extracts have moderate anti-inflammatory and strong anti-ulcerative effects in rats. *Lobaria retigera* (Bory) Trevis. extracts exhibit promising potential in nanomaterial applications, particularly as anticancer agents (Zhou *et al*., 2022). The crude acetone extract of *L. retigera* showed significant anticancer activity by decreasing spheroid formation and suppressing stemness in cancer cells through inhibition of Hedgehog and Notch signaling pathways (Deduke *et al*., 2012). These findings imply that *Lobaria* lichens have enormous potential for the development of novel therapeutic agents. India, being a megadiverse country, has rich plant diversity, including lichens, represented by more than 3000 species out of the approximately 20,000 species known worldwide (Upreti *et al.,* 2005). More than 160 lichen species in India are known to possess medicinal properties, as evidenced by traditional knowledge and preliminary biological screening (Nayaka *et al*., 2010). The present study was aimed to analyze the phytochemicals of three common macrolichens, namely *Lobaria retigera* (a cyanolichen producing thelephoric acid and triterpenoids), *Heterodermia boryi*, and *H. diademata* (both green algal lichens producing zeorin as the major metabolite), and to screen their antimicrobial and antioxidant potential.

MATERIALS AND METHODS

Lichen collection and identification

Three lichen species, *Heterodermia boryi* (HB), *H. diademata* (HD), and *Lobaria retigera* (LR), growing abundantly in the Western Himalayan region of India (altitudes 1500-3000 m masl) were selected (Fig. 1). Samples were collected from Bantar Forest, Pithoragarh district, Uttarakhand (India), at an altitude of 1650 m masl in May 2022 - June 2023. *H. boryi* and *H. diademata* were primarily found on bark,

Heterodermia boryi *Heterodermia diademata* *Lobaria retigera*

Fig. 1: The lichens explored for phytochemical analysis, antimicrobial and antioxidant studies

whereas *L. retigera* preferred moist, shady locations on both bark and soil. Additionally, *H. diademata* grew luxuriantly on rocks in damp habitats. The lichens were examined morphologically, anatomically, and chemically following Nayaka (2014) to confirm their identity. Species-level identification was performed with reference to Awasthi (2007). Thin hand-cut sections were mounted in 5% KOH and observed under a compound microscope to study thallus and reproductive structures. Chemical characterization used standard reagent-K (10% KOH), C (calcium hypochlorite), and P (paraphenylenediamine), and thin-layer chromatography (solvent system A: toluene: dioxane: acetic acid, 180: 60: 8) as per Orange *et al.* (2001). Voucher specimens of lichens were preserved in the Herbarium LWG of CSIR-National Botanical Research Institute, Lucknow, India, under accession numbers *H. boryi* LWG 67193, *H. diademata* LWG 67193/B, and *L. retigera* LWG 67194.

Preparation of extracts

The dried lichens were finely ground in an electric grinder and the ground lichens (10 g each) were extracted twice by soaking with 100 mL methanol for 48 h at room temperature. The separated extracts were filtered through Whatman No. 1 filter paper and the methanol filtrates were condensed on a rotary evaporator at 40˚C which yielded approximately 15-20% methanol extracts (Senthil *et al*., 2019).

GC-MS analysis

The GC-MS analysis was carried out using Thermo GC-Trace Ultra Ver: 5.0, Thermo MS DSQ II with column: DB 35-MS capillary standard non-polar column possessing dimension: 30 m, ID: 0.25 mm, film: 0.25 µm. The instrument was set to an initial temperature of 110°C and maintained at this temperature for 2 min. Then oven temperature was raised to 260°C @ 6°C min^{-1} and maintained for 9 min. Injection port temperature was maintained at 250°C and helium flow rate was 1 mL min^{-1}. An injection of 1 µL sample was used for analysis. The ionization voltage was 70 eV and the samples were injected in split mode as 10:1. Mass spectral scan range was set at 45-450 (MHz). The chemical constituents were identified by GC-MS. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using National Institute of Standards and Technology Mass Spectral database (NIST-MS) (NIST SRD 1A, 2023 Edition). The relative peak area of each component was calculated from the chromatogram. Interpretation of mass spectrum of GC-MS was done by using the database of NIST having more than 62,000 patterns. The spectra of unknown components were compared with the spectra of known components stored in NIST library. The name, molecular weight and structure of components of test materials were ascertained. The obtained list of compounds was checked in PubChem for having reported biological properties. Methanol extracts of all the three lichen samples were prepared separately for GC-MS analysis.

Antimicrobial studies

The antimicrobial activity of lichen extracts was evaluated using both the agar well diffusion method and microdilution method to determine the minimum inhibitory concentration (MIC). The bacterial strains *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, and *Klebsiella pneumoniae*, and the

fungal strain *Candida albicans* were used as test organisms. Fresh cultures were standardized prior to inoculation (Senthil *et al*., 2019).

Agar well diffusion assay: For the agar well diffusion assay, 100 µL of each lichen extract (HB-H: *H. boryi* hexane; HB-C: *H. boryi* chloroform; HB-M: *H. boryi* methanol; HD-H: *H. diademata* hexane; HD-C: *H. diademata* chloroform; HD-M: *H. diademata* methanol; LR-H: *L. retigera* hexane; LR-C: *L. retigera* chloroform; LR-M: *L. retigera* methanol) at 1 mg mL^{-1} was added into wells on agar plates inoculated with test microorganisms. Ampicillin (for bacteria; Ampicillin tablets, HiMedia Laboratories, India) and fluconazole (for fungi; Diflucan®, Pfizer, India) were used as positive controls, while DMSO served as a negative control. Plates were incubated at 37°C for bacteria and 28°C for fungi for 24-48 h, and zones of inhibition (ZOI) were measured (Senthil *et al*., 2019).

Minimum inhibitory concentration (MIC): The MIC of lichen extracts was determined by the micro-dilution method in nutrient broth for bacteria and PDA broth for fungi, following the CLSI guidelines (2012). Test concentrations of 1, 0.5, 0.25, 0.125, and 0.062 mg mL^{-1} were prepared in DMSO. Then 100 µL of each extract was mixed with 5 mL broth and inoculated with 20 µL of standardized microbial suspension. Tubes were incubated for 48 h, and MIC was recorded as the lowest concentration showing no visible growth (Senthil *et al*., 2019).

Antioxidant activities

The samples were subjected to two assays to measure their antioxidant potential.

Phosphomolybdenum method (Total antioxidant capacity-TAC): An aliquot of 0.5 mL sample solution was combined with 4.5 mL reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). In case of blank, 0.5 mL DMSO was used in place of sample. The samples added were HB-H, HB-C, HB-M, HD-H, HD-C, HD-M and LR-H, LR-C, LR-M at concentration of 1 mg mL^{-1}. The tubes were incubated in a boiling water bath at 95°C for 90 min, cooled to room temperature and then the absorbance of aqueous solution of each sample at 695 nm against blank in UV-2450 spectrophotometer (Shimadzu, Japan). Total antioxidant capacity of extract was expressed as ascorbic acid equivalent (AAE).

Hydrogen peroxide radical scavenging test: Hydrogen peroxide (H_2O_2) scavenging activity of natural antioxidants present in lichen extracts was determined by measuring decrement of H_2O_2 in an incubation system containing H_2O_2 and the scavenger using the classical UV-method (Ruch *et al*., 1989). The main disadvantage of this method is the possible interference from secondary metabolites present in plants which absorb in UV region. Phosphate buffer (pH 7.4) was added to all samples containing 0.6 mL hydrogen peroxide. After incubation for 10 min, absorbance was measured at 330 nm against a blank solution with phosphate buffer. The samples added were HB-H, HB-C, HB-M, HD-H, HD-C, HD-M and LR-H, LR-C and LR-M at concentration of 1 mg mL^{-1}. The percentage of inhibition of H_2O_2 was calculated as follows:

Percentage (%) of H_2O_2 radical scavenging assay = [(A0-A1)/A0] × 100, where A0 is control, A1 test sample.

Statistical analysis

All experiments were performed in triplicatein a completely randomized design, and data presented as mean ± standard deviation (SD). Percent inhibition for antioxidant assays were calculated and differences among extracts or species were analysed (Brand-Williams *et al*., 1995).

RESULTS AND DISCUSSION

The GC-MS analysis of methanolic extracts of *Heterodermia boryi* (HB), *H. diademata* (HD), and

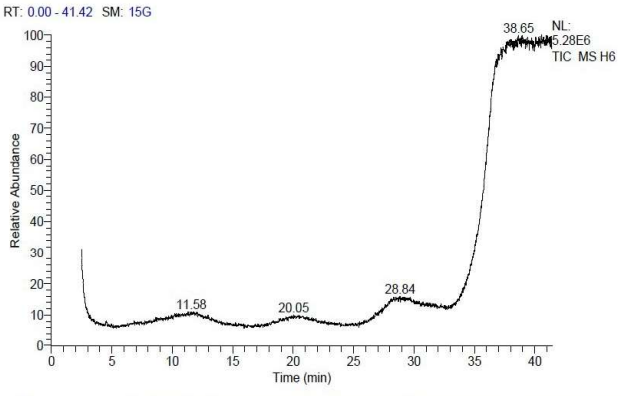

Fig. 2: GC-MS spectrum of the methanolic extract of *Heterodermia boryi*

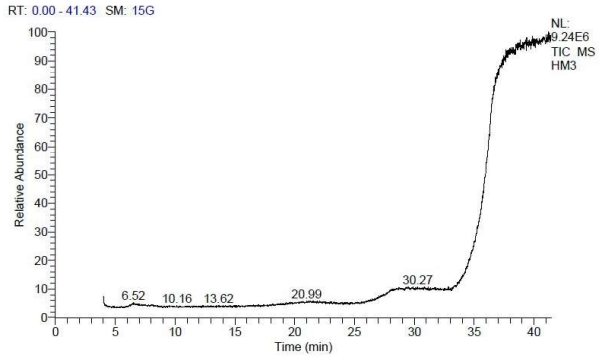

Fig. 3: GC-MS spectrum of the methanolic extract of *Heterodermia diademata*

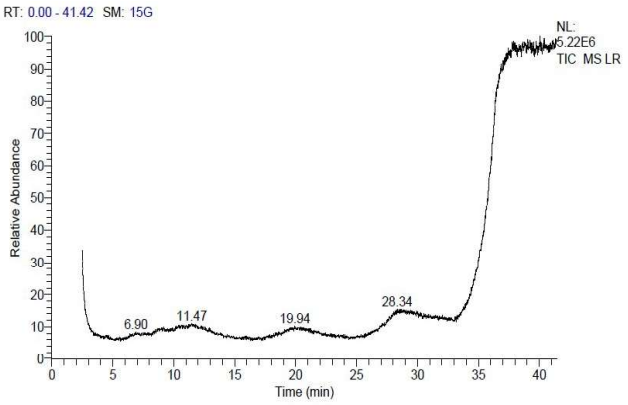

Fig. 4: GC-MS spectrum of the methanolic extract of *Lobaria retigera*

Lobaria retigera (LR) revealed diverse phytochemical profile. HB and HD gave 28 and 23 compounds, respectively, whereas LR showed fewer compounds (Fig. 2-4; Tables 1-3). Identified compounds included thiomorpholide, pinostilbene, glafenin, iodohistidine, pyridine, silane, hexanal, and thymol in HB; perchloryl fluoride, sulforaphene, angiogenin, chromone, and carvacrol in HD; and methanone, triazole, dimethoxyflavone, benzyl-sulfanyl-acetic acid, isoorientin, pipemidic acid, and torcetrapib in LR. Notably, only 3 phytochemicals were common in all test lichens, while chromone was present in HB and HD, and isoorientin and pipemidic acid in HB and LR. These results suggest that each lichen species produces a unique chemical profile, which could explain differences in their biological activities. Previous studies have similarly reported that lichen secondary metabolites vary among species and are influenced by their ecological habitats (Kosanić and Ranković, 2019; Shrestha and St. Clair, 2013). The presence of bioactive compounds such as thymol, carvacrol, and chromone, which are known for antimicrobial and antioxidant properties, supports the potential pharmacological signify-cance of these lichens (Güvenç *et al.,* 2012; Emsen *et al*., 2018).

All lichen extracts exhibited anti-microbial activity against *Bacillus subtilis*, *Candida albicans*, *Klebsiella pneumoniae*, *Salmonella typhi*, and *Staphylococcus aureus* (Tables 4 and 5). Methanolic extracts showed the highest activity (ZOI: 10.7 ± 0.06 to 26.8 ± 0.06 mm), followed by chloroform and hexane extracts. *S. typhi* was the most susceptible organism, with MIC values as low as 0.125 mg for methanolic and chloroform extracts of HB and methanolic extracts of HD and LR. The superior activity of methanolic extracts may be attributed to their higher content of polar bioactive compounds, including phenolics and flavonoids, enzymatic activity (Shrestha and St. Clair, 2013; Süleyman *et al.,* 2003). The differences in which are known to disrupt microbial cell walls and inhibit antimicrobial efficacy among extracts and species are consistent with previous reports that lichen chemical composition governs antimicrobial potential (Prabhu and Sudha, 2016).

Table 1: The compounds of pharmaceutical importance identified in *Heterodermia boryi*

S. No.	RT (min)	Compound name	Molecular formula	Mol. weight	Area (%)	Biological activity
1 .	3.50	Thiomorpholide	$C_{15}H_{19}ClN_2S_2$	326	0.29	Fungicides, herbicides
2	3.67	Hydroxychalcone	$C_{15}H_{12}O_2$	224	0.20	Antioxidant, anti-inflammatory
3	3.99	Normethadol	$C_{20}H_{27}NO$	297	0.21	Antioxidant, antimicrobial & anticancer
4	4.97	Pinostilbene	$C_{15}H_{14}O_3$	242	0.42	Antimicrobial, antidiabetic, anti-obesity
5	5.12	Zinecarbothioamide	$C_{14}H_{14}N_4O_3S_2$	350	0.27	Antimicrobial
6	5.41	Pipemidic acid	$C_{14}H_{17}N_5O_3$	303	0.41	Antimicrobial
7	5.70	Glafenin	$C_{19}H_{17}ClN_2O_4$	372	0.12	anti-inflammatory
8	5.79	Diatrizoic acid	$C_{11}H_9I_3N_2O_4$	614	0.13	Gastrointestinal studies
9	7.30	Iodohiistidine	$C_6H_8IN_3O_2$	281	0.25	Antibacterial
10	7.64	Pyridine	$C_{20}H_{15}F_3N_{2O}$	356	0.07	Antimicrobial, antidiabetic, anti-obesity
11	11.92	Methoxyamphetamine	$C_{10}H_{15}NO$	165	0.23	Antimicrobial
12	12.97	Pheophorbide a	$C_{35}H_{36}N_4O_5$	592	0.31	Antioxidant, anti-inflammatory
13	15.28	Silane	$C_{14}H_{24}OSi$	236	0.36	Antimicrobial and antiviral
14	18.96	Buflomedil	$C_{17}H_{25}NO_4$	307	0.40	Antiplatelet
15	21.55	Furosemide	$C_{18}H_{27}ClN_2O_5SSi_2$	474	0.35	Diuretic
16	21.55	Clocortolonepivalate	$C_{27}H_{36}ClFO_5$	494	0.35	Corticosteroid
17	21.55	Pentafluoropropionate	$C_{22}H_{19}F_5O_7$	490	0.35	Anti-inflammatory
18	23.88	Hexanal	$C_{12}H_{16}N_4O_4$	280	0.20	Antimicrobial
19	25.40	Androstane	$C_{29}H_{43}NO_3Si$	481	0.76	Anti-inflammatory
20	25.69	Chromone	$C_{14}H_{16}O_6$	280	0.19	Antioxidant, Anti-inflammatory
21	27.92	Heptasiloxane	$C_{14}H_{44}O_6Si_7$	504	0.50	Lubrication
22	27.92	Hexasiloxane	$C_{12}H_{38}O_5Si_6$	430	0.50	Lubrication
23	27.92	Octasiloxane	$C_{16}H_{50}O_7Si_8$	578	0.50	Lubrication
24	32.25	Isoorientin	$C_{21}H_{20}O_{11}$	448	0.54	Antioxidant
25	32.36	Thymol	$C_{16}H_{28}OSi$	264	0.38	Antimicrobial
26	33.11	Gibberellin	$C_{23}H_{32}O_5Si$	416	0.17	Growth regulator
27	33.11	Parathyroid Hormone	$C_{125}H_{199}N_{39}O_{33}S$	2805	0.17	Calcium regulator
28	34.96	W-18	$C_{19}H_{20}ClN_3O_4S$	421	0.71	Analgesic

Table 2: The compounds of pharmaceutical importance found in *Heterodermia diademata*

S. No.	RT (min)	Compound name	Molecular formula	Mol. weight	Area (%)	Biological activity
1	4.09	Sucralose	$C_{12}H_{19}Cl_3O_8$	396	0.08	Sweetener
2	4.30	Trichloromethane	$CHCl_3$	118	0.08	Anaesthetic
3	4.79	Difluorophosphoric acid	F_2HO_2P	102	0.55	Anticancer
4	6.28	Perchloryl fluoride	$ClFO_3$	102	0.26	Anti-oxidant
5	6.68	Thionyl chloride	Cl_2OS	118	0.11	Pesticidal
6	7.38	Cannabinol	$C_{21}H_{26}O_2$	310	0.21	Cannabinoid
7	7.38	Orcinol	$C_7H_8O_2$	124	0.21	Precursor
8	7.50	Sulforaphene	$C_6H_9NOS_2$	175	0.19	Antioxidant
9	10.21	Dichlormid	$C_8H_{11}Cl_2NO$	207	0.20	Herbicide
10	12.37	Apraclonidine	$C_9H_{10}Cl_2N_4$	244	0.15	Neuroprotective
11	16.54	Methylsterigmatocystin	$C_{19}H_{14}O_6$	338	0.29	Mycotoxin
12	17.40	Binapacryl	$C_{15}H_{18}N_2O_6$	322	0.30	Pesticide
13	21.64	Strophanthidol	$C_{23}H_{34}O_6$	406	0.16	Cardiotonic
14	21.75	Hydroxycerivastatin	$C_{26}H_{34}FNO_6$	475	0.43	Cholesterol-lowering agent
15	22.95	Angiogenin	$C_{83}H_{132}N_{26}O_{24}$	1876	0.37	Anticancer
16	23.06	Triforine	$C_{10}H_{14}Cl_6N_4O_2$	432	0.45	Fungicide
17	23.52	Apraclonidine	$C_9H_{10}Cl_2N_4$	244	0.09	Lower intraocular pressure
18	23.52	Nitralin	$C_{13}H_{19}N_3O_6S$	345	0.09	Herbicide
19	25.24	Benzenamine	$C_7H_7N_3O_4$	197	0.14	Antipyretics, & antimalarial
20	25.64	Piperazine	$C_{10}H_{13}N_3O_2$	207	0.51	Antihelminthic
21	25.86	Chromone	$C_{14}H_{16}O_6$	280	0.45	Anti-inflammatory
22	26.09	Dithiocarbamate	$C_7H_{13}NOS_2$	191	0.14	Pesticide
23	29.71	Carvacrol	$C_{16}H_{28}OSi$	264	0.48	Antimicrobial

Table 3: The compounds of pharmaceutical importance identified in *Lobaria retigera*

S. No	RT (min)	Compound name	Molecular formula	Mol. weight	Area (%)	Biological activity
1	5.20	Methanone	$C_{16}H_{16}O$	224	0.13	Antioxidant
2	5.89	Nitrophthalhydrazide	$C_8H_5N_3O_4$	207	0.20	Herbicide
3	6.50	Lansoprazole	$C_{16}H_{14}F_3N_3O_2S$	369	0.34	Antacid
4	10.06	Aristolochic acid	$C_{17}H_{11}NO_7$	341	0.14	Nephrotoxic, Carcinogenic
5	12.43	Triazole	$C_{15}H_{14}N_4S$	282	0.46	Antifungal
6	12.43	Dimethoxyflavone	$C_{17}H_{14}O_4$	282	0.46	Antioxidant
7	12.69	Tecloftalam	$C_{14}H_5C_{16}NO_3$	445	0.36	Insecticide
8	12.78	Benzylsulfanylacetic acid	$C_{17}H_{15}NO_2S_2$	329	0.21	Antibacterial
9	13.06	Lysergic acid	$C_{20}H_{25}N_3O$	323	0.12	Hallucinogenic
10	13.06	Isodemecolcine	$C_{20}H_{23}NO_5$	357	0.12	Anticancer
11	14.15	Heptanal	$C_{13}H_{18}N_4O_4$	294	0.24	Odorant
12	14.72	Ethanethiol	$C_{14}H_{23}NO_4S_2$	333	0.27	Odorant
13	15.32	Isoorientin	$C_{21}H_{20}O_{11}$	448	0.08	Antioxidant
14	16.42	Pipemidic acid	$C_{14}H_{17}N_5O_3$	303	0.05	Antibacterial
15	18.15	Phenylethanethiol	$C_{11}H_{18}SSi$	210	0.37	Stimulant
16	18.39	Dextroamphetamine	$C_9H_{13}N$	135	0.28	Anthelmintic
17	18.39	Milbemycin	$C_{33}H_{47}ClO_7$	590	0.28	Lipid-modifying agent
18	18.67	Torcetrapib	$C_{26}H_{25}F_9N_2O_4$	600	0.63	Antifungal
19	21.85	Octadecadiynoic acid	$C_{19}H_{30}O_2$	290	0.37	Antifungal
20	24.50	Eicosanoic acid	$C_{27}H_{46}O_2$	402	0.05	Anti-inflammatory

Table 4: The zone of inhibition by *Heterodermia boryi* (HB), *H. diademata* (DD), *Lobaria retigera* (LR) extracts against *Bacillus subtilis, Candida albicans, Klebsiella pneumonia, Salmonella typhi,* and *Staphylococcus aureus* (n = 3)

Microbes	HB-HE		HB-CE		HB-ME		HD-HE		HD-CE	
	ZOI (mm)	EF	ZOI (mm)	EF	ZOI (mm)	EF	ZOI (mm)	EF	ZOI (mm)	EF
Heterodermia boryi										
S. aureus	8.9 ± 0.03	+	12.8 ± 0.03	++	24.3 ± 0.05	+++	13.4 ± 0.02	++	10.6 ± 0.12	++
B. subtilis	9.2 ± 0.12	+	10.9 ± 0.05	++	15.9 ± 0.08	++	8.9 ± 0.01	+	7.9 ± 0.03	+
S. typhi	11.2 ± 0.04	++	24.2 ± 0.03	+++	26.8 ± 0.06	+++	14.4 ± 0.07	++	14.1 ± 0.05	++
K. pneumonia	10.8 ± 0.08	+	21.1± 0.06	++	20.2 ± 0.07	++	12.3 ± 0.08	++	15.5 ± 0.08	++
C. albicans	9.3 ± 0.13	+	13.1 ± 0.14	++	14.9 ± 0.14	++	11.2 ± 0.09	+	10.9 ± 0.13	++

Table 4 continue

Microbes	HD-ME		LR-HE		LR-CE		LR-ME		Amphicillin/ Flucanazole	
	ZOI (mm)	EF	ZOI (mm)	EF	ZOI (mm)	EF	ZOI (mm)	EF	ZOI (mm)	EF
S. aureus	13.8 ± 0.04	++	09.8 ± 0.03	+	12.7 ± 0.08	++	15.7 ± 0.06	++	32.2 ± 0.04	+++
B. subtilis	10.7 ± 0.06	+	08.5 ± 0.05	+	10.3 ± 0.02	+	11.5 ± 0.08	++	28.8 ± 0.23	+++
S. typhi	25.9 ± 0.08	+++	12.7 ± 0.14	++	14.9 ± 0.03	++	22.3 ± 0.09	+++	35.1 ± 0.16	+++
K. pneumoniae	23.9 ± 0.14	+++	10.2 ± 0.03	+	12.4 ± 0.12	++	14.8 ± 0.12	++	33.9 ± 0.08	+++
C. albicans	13.9 ± 0.04	++	11.9 ± 0.06	++	13.1 ± 0.04	++	17.4 ± 0.03	++	22.6 ± 0.30	+++

+++ = excellent, ++ = good, + = moderate;
HE = Hexane extract, CE = Chloroform extract, ME = Methanol extract, ZOI = Zone of inhibition in mm, EF = Efficacy

Total antioxidant capacity and H_2O_2 scavenging activity were highest in methanolic extracts, with *Heterodermia boryi* (HB) showing the maximum antioxidant capacity (0.899 mg g^{-1} AAE) followed by *H. diademata* [HB] (0.62 mg g^{-1} AAE) (Table 6). Interestingly, H_2O_2 scavenging activity was highest in the chloroform extract of HB (91.62%), followed by hexane extract (90.41%). This suggests that different solvent extracts preferentially extract compounds with varying antioxidant properties. The higher radical scavenging activity of hexane extracts may be due to the presence of fatty acids and other lipophilic compounds capable of quenching reactive oxygen species (Deduke *et al.,* 2012). These results confirm that the antioxidant potential of lichen extracts correlates with the diversity of bioactive metabolites detected in GC–MS analysis. Similar observations have been reported in other studies, highlighting the role of secondary metabolites such as usnic acid, depsides, and flavonoids in scavenging free radicals (Kumar *et al*., 2014; Norouzi *et al*., 2020).

Table 5: MIC values of lichen extracts against *Bacillus subtilis, Candida albicans, Klebsiella pneumonia, Salmonella typhi* and *Staphylococcus aureus*

Microbes	*H. boryi* - Hexane (conc. in mg)						*H. boryi* - Chloroform (conc. in mg)						*H. boryi* - Methanol (conc. in mg)					
	1	0.5	0.25	0.125	0.062	MIC	1	0.5	0.25	0.125	0.062	MIC	1	0.5	0.25	0.125	0.062	MIC
S. aureus	CL	CL	TR	TR	TR	0.50	CL	CL	CL	TR	TR	0.25	CL	CL	CL	CL	TR	0.125
B. subtilis	CL	CL	TR	TR	TR	0.50	CL	CL	CL	TR	TR	0.25	CL	CL	CL	TR	TR	0.25
S. typhi	CL	CL	CL	TR	TR	0.25	CL	CL	CL	CL	TR	0.125	CL	CL	CL	CL	TR	0.125
K. pneumoniae	CL	CL	TR	TR	TR	0.50	CL	CL	CL	TR	TR	0.25	CL	CL	CL	TR	TR	0.25
C. albicans	CL	TR	TR	TR	TR	1.00	CL	CL	TR	TR	TR	0.50	CL	CL	TR	TR	TR	0.50

Microbes	*H. diademata* – Hexane						*H. diademata* - Chloroform						*H. diademata* - Methanol					
	1	0.5	0.25	0.125	0.062	MIC	1	0.5	0.25	0.125	0.062	MIC	1	0.5	0.25	0.125	0.062	MIC
S. aureus	CL	CL	CL	TR	TR	0.25	CL	CL	CL	TR	TR	0.25	CL	CL	CL	TR	TR	0.25
B. subtilis	CL	CL	TR	TR	TR	0.50	CL	CL	TR	TR	TR	0.50	CL	CL	TR	TR	TR	0.50
S. typhi	CL	CL	CL	TR	TR	0.25	CL	CL	CL	TR	TR	0.25	CL	CL	CL	CL	TR	0.125
K. pneumoniae	CL	CL	CL	TR	TR	0.25	CL	CL	CL	TR	TR	0.25	CL	CL	CL	CL	TR	0.125
C. albicans	CL	TR	TR	TR	TR	1.00	CL	TR	TR	TR	TR	1.00	CL	CL	TR	TR	TR	0.50

Microbes	*L. retigera* – Hexane						*L. retigera* - Chloroform						*L.retigera* – Methanol					
	1	0.5	0.25	0.125	0.062	MIC	1	0.5	0.25	0.125	0.062	MIC	1	0.5	0.25	0.125	0.062	MIC
S. aureus	CL	CL	TR	TR	TR	0.50	CL	CL	CL	TR	TR	0.25	CL	CL	CL	TR	TR	0.25
B. subtilis	CL	CL	TR	TR	TR	0.50	CL	CL	TR	TR	TR	0.50	CL	CL	CL	TR	TR	0.25
S. typhi	CL	CL	CL	TR	TR	0.25	CL	CL	CL	TR	TR	0.25	CL	CL	CL	CL	TR	0.125
K. pneumoniae	CL	CL	TR	TR	TR	0.50	CL	CL	CL	TR	TR	0.25	CL	CL	CL	TR	TR	0.25
C. albicans	CL	CL	TR	TR	TR	0.50	CL	CL	TR	TR	TR	0.50	CL	CL	TR	TR	TR	0.50

*CL = Clear, TR = Turbid, Conc. = Concentration

Table 6: Total antioxidant capacity and hydrogen peroxide radical scavenging capacity of *H. boryi, H. diademata* and *L. retigera* extracts through phosphomolybdenum method (n = 3, AAE = ascorbic acid equivalent)

S. No.	Plant extracts (1 mg mL^{-1})	Total antioxidant capacity		Hydrogen peroxide radical scavenging capacity	
		OD at 695 nm	AAE (mg g^{-1})	OD at 330 nm	Percentage
1.	*H. boryi* - Hexane	0.516 ± 0.14	0.497	0.491± 0.06	90.41
2.	*H. boryi* - Chloroform	0.498 ± 0.13	0.463	0.429 ± 0.03	91.62
3.	*H. boryi* - Methanol	0.729 ± 0.04	0.899	2.342 ± 0.02	54.25
4.	*H. diademata* - Hexane	0.564 ± 0.07	0.586	0.689 ± 0.04	86.54
5.	*H. diademata* - Chloroform	0.502 ± 0.13	0.469	0.999 ± 0.16	80.48
6.	*H. diademata* - Methanol	0.582 ± 0.07	0.62	2.982 ± 0.02	41.75
7.	*L. retigera* - Hexane	0.438 ± 0.04	0.349	1.010 ± 0.12	80.27
8.	*L. retigera* - Chloroform	0.341 ± 0.13	0.166	2.316 ± 0.07	54.76
9.	*L. retigera* - Methanol	0.405 ± 0.06	0.287	2.966 ± 0.08	42.07

The findings indicate that the tested lichens, particularly *H. boryi*, possess significant antimicrobial and antioxidant potential, supporting their prospective for use in pharmacological and nutraceutical formulations. The species-specific variation in metabolite composition highlights the importance of chemical profiling prior to bioprospecting. These extracts could also be explored in food preservation to prevent oxidative degradation and enhance nutritional quality. Overall, the study demonstrates that lichens are valuable sources of bioactive compounds, which can contribute to the development of novel therapeutic agents.

Conclusion: The present study validates the potential of lichens *Heterodermia boryi*, *H. diademata*, and *Lecanora retigera* as natural sources of antimicrobial and antioxidant agents. GC–MS analysis confirmed the presence of diverse secondary metabolites including hydroxychalcone, normethadol, pyridine, pinostilbene, pipemidic acid, iodohistidine, pheophorbide a, chromone, isoorientin, dimethoxyflavone, perchloryl fluoride, sulforaphene, and carvacrol. These compounds are known to possess antimicrobial, anti-inflammatory, cytotoxic, and antioxidant properties, highlighting the

pharmacological potential of the studied lichens. Among the tested species, *H. boryi* exhibited the strongest antimicrobial and antioxidant activities, followed by *H. diademata* and *L. retigera*. The methanolic extracts of all three lichens demonstrated the highest bioactivity compared to other solvent extracts, indicating that polar compounds may play a dominant role in their biological effects. Overall, the findings suggest that these lichens contain bioactive constituents of considerable biotechnological and pharmaceutical interest. Further *in vivo* investigations and detailed isolation and characterization of the identified compounds are warranted to elucidate their mechanisms of action and to explore their potential in drug development, nutraceuticals, and natural antioxidant formulations.

Acknowledgements: We are thankful to Head, Department of Chemistry, Kumaun University, Nainital and Director, CSIR-NBRI, Lucknow for providing the laboratory facilities.

REFERENCES

Awasthi, D.D. 2007. *A Compendium of the Macrolichens from India, Nepal and Sri Lanka*. Bishen Singh Mahendra Pal Singh, Dehra Dun, Uttarakhand, India.

Boustie, J. and Grube, M. 2005. Lichens - A promising source of bioactive secondary metabolites. *Plant Genetic Resources*, **3**: 273-287.

Brand-Williams, W., Cuvelier, M.E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, **28**(1)**:** 25-30.

Deduke, C., Timsina, B. and Piercey-Normore, M.D. 2012. Effect of environmental change on secondary metabolite production in lichen-forming fungi. pp. 197-230. **In:** *International Perspectives on Global Environmental Change* ((eds. S.S. Young and S.E. Silvern). InTech. Rijeka, Croatia.

Demain, A.L. and Vaishnav, P. 2010. Natural products for cancer chemotherapy. *Microbial Biotechnology*, **4**: 687-699.

Elix, J.A. and Stocker-Wörgötter, E. 2008. Biochemistry and secondary metabolites. pp. 104-133. **In:** *Lichen Biology* (ed. T.H. Nash), Cambridge University Press, Cambridge, UK.

Emsen, B., Aslan, A., Turkez, H., Taghizadehghalehjoughi, A. and Kaya, A. 2018. The anti-cancer efficacies of diffractaic, lobaric, and usnic acid. *Journal of Cancer Research and Therapeutics*, **14:** 941-951.

Francis, J. 2019. Anti-cancer properties of the foliose lichen, *Heterodermia diademata* (Taylor) Awasthi against breast cancer cell lines. (**In:** *13th World Cancer Congress*, 25-26 Feb., 2019, Paris, France). *Journal of Medical Oncology and Therapeutics* **4**: 25.

Güvenç, A., Akkol, E.K., Süntar, İ., Keleş, H., Yıldız, S. and Çalış, İ. 2012. Biological activities of *Pseudevernia furfuracea* (L.) Zopf extracts and isolation of the active compounds. *Journal of Ethnopharmacology*, **144**: 726-734.

Hawksworth, D.L. and Grube, M. 2020. Lichens redefined as complex ecosystems. *New Phytologist*, **227**: 1281-1283.

Huneck, S. 1999. The significance of lichens and their metabolites. *Naturwissenschaften*, **8**(6): 559-570.

Huneck, S. and Yoshimura, I. (1996). Data of lichen substances. pp. 493-883 **In:** *Identification of Lichen Substances* (eds. S. Huneck and I. Yoshimura), Springer. Berlin, Germany.

Kosanić, M. and Ranković, B. 2019. Lichen secondary metabolites as potential antibiotic agents. pp. 99-128. **In:** *Lichen Secondary Metabolites: Bioactive Properties and Pharmaceutical Potential* (ed. B. Ranković). Springer International Publishing, Cham, Switzerland.

Kumar, J., Dhar, P., Tayade, A.B., Gupta, D., Chaurasia, O.P., Upreti, D.K., *et al.,* 2014. Antioxidant capacities, phenolic profile and cytotoxic effects of saxicolous lichens from Trans-Himalayan

cold desert of Ladakh. *PLoS ONE*, **9**: e98696. [*https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0098696*].

National Institute of Standards and Technology (NIST). 2023. *NIST Standard Reference Database 1A: NIST/EPA/NIH Mass Spectral Library with Search Program,* Data version: NIST 23. [*https://www.nist.gov/srd/nist-standard-reference-database-1a-v14*].

Nayaka, S. 2014. Methods and techniques in collection, preservation and identification of lichens. pp. 101-118. **In:** *Plant Taxonomy and Biosystematics: Classical and Modern Methods* (eds. T.S. Rana, K.N. Nair, and D.K. Upreti). New India Publishing Agency, New Delhi, India.

Nayaka, S., Upreti, D.K. and Khare, R. 2010. Medicinal lichens of India. pp. 1-15. **In**: *Drugs from Plants* (Ed. P.C. Trivedi). Avishkar Publishers, Jaipur, India.

Norouzi, H., Azizi, A., Gholami, M., Sohrabi, M. and Boustie, J. 2020. Chemotype variations among lichen ecotypes of *Umbilicaria aprina* as revealed by LC-ESI-MS/MS: A survey of antioxidant phenolics. *Environmental Science and Pollution Research*, **27**: 40296-40308.

Orange, A., James, P.W. and White, F.J. 2001. *Microchemical Methods for the Identification of Lichens*. British Lichen Society, London, UK.

Payal, P. and Sharma, M.C. 2016. GC-MS analysis and biological activities of medicinally important lichen: *Parmelia perlata*. *International Journal of Pharmacognosy and Phytochemical Research*, **8**: 1975-1985.

Prabhu, S.S. and Sudha, S.S. 2016. *In vitro* study on anthelmintic activity of *Heterodermia boryi* macrolichen collected from the Nilgiris, Tamil Nadu, India. *International Journal of Current Microbiology and Applied Sciences*, **5**: 764-768.

Ruch, R.J., Cheng, S.J. and Klaunig, J.E. 1989. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, **10**(6): 1003-1008.

Shrestha, G. and St. Clair, L.L. 2013. Lichens: A promising source of antibiotic and anticancer drugs. *Phytochemistry Reviews*, **12**: 229-244.

Süleyman, H., Odabasoglu, F., Aslan, A., Cakir, A., Karagoz, Y., Gocer, F., *et al.,* 2003. Anti-inflammatory and antiulcerogenic effects of the aqueous extract of *Lobaria pulmonaria* (L.) Hoffm. *Phytomedicine*, **10**: 552-557.

Upreti, D.K., Upreti, S.R. and Negi, S.S. 2005. Lichens of India: Diversity and distribution. *Indian Journal of Forestry*, **28**: 1-12.

Witschi, H.P. 1986. Enhanced tumor development by butylated hydroxytoluene (BHT) in the liver, lung, and gastrointestinal tract. *Food and Chemical Toxicology,* **24**(10-11): 1127-1130.

Zhou, R., Nguyen, T.T., Kim, K.K., Hur, J.S. and Kim, H. 2022. Extracts from lichen *Lobaria retigera* decrease the stemness potential of colorectal cancer cells. *Materials Express*, **12**: 234-240.