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Antibacterial and Antioxidant Properties of the Lichens *Bulbothrix isidiza* (Nyl.) Hale and *Parmotrema reticulatum* (Taylor) M. Choisy

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Abstract

Lichens, a symbiotic partnership between fungi and algae, possess a remarkable array of biological properties. This study explored the antibacterial and antioxidant potential of extracts from two lichen species: *Bulbothrix isidiza* and *Parmotrema reticulatum*. The disc diffusion method revealed promising antibacterial activity in both lichen extracts against Gram-positive and Gram-negative bacteria. Notably, the ethyl acetate extract of *B. isidiza* and the ethanol extract of *P. reticulatum* exhibited the strongest inhibitory effects. The DPPH free radical scavenging assay confirmed antioxidant activity in both lichen extracts, with scavenging activity increasing with extract concentration. These findings suggest the potential for these lichens as sources of natural antimicrobials and antioxidants. Further research is necessary to identify the bioactive compounds responsible for these activities and assess their potential therapeutic applications.

Keywords: Lichens, Antimicrobials, Antioxidants, Natural products.

INTRODUCTION

Lichens, a testament to the wonders of symbiosis, arise from the remarkable partnership between a fungus and an alga (Grube and Muggia, 2010). The dominant fungal partner dictates the lichen's form, while the photosynthetic alga, either green or a cyanobacterium, nourishes the association (Nayaka, 2014). These resilient organisms thrive in diverse environments, colonising surfaces like tree bark, exposed rock and even contributing to biological soil crusts. Their intricate body, the thallus, is anchored by hair-like structures called rhizines (Grube and Muggia, 2010). Lichens exhibit a fascinating array of growth forms. Crustose lichens form a thin, crust-like covering tightly bound to the substrate, while foliose lichens are large and leaf-like. In contrast, fruticose lichens can be hanging or upright, taking on hair-like, cup-like, or shrubby appearances (Nayaka, 2014). The internal organisation of the thallus further distinguishes them. Homoeomerous thalli have a relatively even distribution of algal and fungal cells, while heteromerous thalli are dominated by fungal cells (Nash, 2008).

Beyond their aesthetic appeal, lichens play a crucial ecological role. The protective fungal partner allows water-dependent algae to survive in harsh environments, enduring dry and sunny climates with the help of occasional rain or flooding (George *et al*., 2014). Lichens not only facilitate algal survival but also contribute to oxygen production through photosynthesis, a vital process for all life forms. Additionally, they serve as ecological indicators, accumulating heavy metals and pollutants from the atmosphere. By analysing these accumulated compounds, scientists can gauge air quality. Interestingly, lichens produce metabolites with a wide range of biological properties, including antibacterial, antioxidant, and antitumour effects (Huneck and Yoshimura, 1996; George *et al.,* 2014). Usnic acid, for instance, demonstrates growth inhibition against various bacteria (George *et al.,* 2014).

This study focuses on the Parmeliaceae family, the largest among lichenised fungi, encompassing a staggering diversity of species. Notably, extracts from Parmeliaceae members exhibit antibacterial and other promising activities (Gómez-Serranillos *et al*., 2014). We delve into two specific genera within this family: *Bulbothrix*, characterised by its foliose thallus with bulbous structures along the lobe margins, and *Parmotrema*, recognised for its large, loosely attached

foliose thallus (Awasthi, 2007). This paper investigates *Bulbothrix isidiza*, a common species in Kerala, India (Biju *et al.,* 2014; Kumar, 2000; Purushothaman *et al.,* 2021) and *Parmotrema reticulatum*, a widely distributed species except for Antarctica (Awasthi, 2007). *P. reticulatum* is prevalent in high-altitude regions of Kerala (Kumar, 2000; Purushothaman *et al*., 2021). The present research aims to explore the antibacterial and antioxidant properties of these two lichen species, *B. isidiza* and *P. reticulatum*.

MATERIALS AND METHODS

Fresh lichen samples were collected from high-altitude regions of Kerala, with details like location, date, and host type documented. Morphological features were examined using a stereo zoom microscope (Leica S8APO), and standard spot tests (K, C and P) were performed to aid identification (Orange *et al.,* 2001). Thin Layer Chromatography (TLC) was used to identify lichen secondary metabolites. Acetone extracts were loaded onto silica gel plates and run with a Toluene: Dioxan: Acetic Acid solvent system. Fluorescent spots under UV light and colour development after sulphuric acid treatment were used for compound identification, with *P. reticulatum* (containing salazinic and atranorin) serving as a reference (Orange *et al.,* 2001). In addition, the lichen thallus was extracted with various solvents (isopropyl alcohol, ethanol, ethyl acetate and acetone) and tested against four bacterial strains (*Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*) using the disc diffusion method. The diameter of inhibition zones determined bacterial susceptibility (NCCLS, 2007).

The DPPH free radical scavenging assay was used to assess antioxidant activity. Lichen extracts were prepared using ethanol and compared to a standard ascorbic acid solution. The percentage inhibition of DPPH radical was calculated at various concentrations using a spectrophotometer at 517 nm (Blois, 1958).

RESULTS AND DISCUSSION

The disc diffusion method revealed promising antibacterial activity in extracts from both *B. isidiza* and *P. reticulatum* (Tables 1 & 2). In *B. isidiza*, the ethyl acetate extract exhibited the most potent activity against all tested bacteria, with the highest zone of inhibition observed against *E. coli* (Table 1). This suggests that ethyl acetate might be more suitable for extracting antibacterial compounds from *B. isidiza* than isopropyl alcohol, ethanol, and acetone. The present findings partially align with previous research by Rajan *et al.* (2015) who reported the antibacterial activity of ethanol and acetone extracts of *P. reticulatum* against similar bacterial strains. However, unlike the current study, Jain *et al.* (2016) did not observe any inhibitory effect from the acetone extract of *P. reticulatum* against the tested bacteria. These discrepancies might be attributed to variations in geographical location, lichen collection time or extraction procedures. The ethanol extract of *P. reticulatum* displayed strongest antibacterial activity, particularly against *P. aeruginosa* (Table 2). Like *B. isidiza*, ethanol appears to be a more effective solvent for extracting antibacterial compounds from *P. reticulatum* than the other tested solvents. The DPPH free radical scavenging assay confirmed antioxidant activity in *B. isidiza* and *P. reticulatum* (Tables 3 & 4). As expected, the percentage inhibition of DPPH radical increased with increasing extract concentration.

The ethanolic extract of *B. isidiza* demonstrated a concentration-dependent scavenging effect, with the highest activity (65% inhibition) observed at 130 µg/ml (Table 3). This result contrasts with Stanly *et al.* (2011) who reported the highest antioxidant activity in the acetone extract of *B. isidiza* using the beta-carotene bleaching assay. These differences highlight the potential influence of the chosen solvent and assay method on evaluating antioxidant activity. *P. reticulatum* extracts displayed significant DPPH scavenging activity at concentrations ranging from 50 µg/ml to 130 µg/ml, with the highest percentage inhibition (67%) observed at 130 µg/ml (Table 4). Various phytoconstituents in the lichen are likely responsible for its antioxidant properties.

Lichens have emerged as a rich and largely untapped reservoir of bioactive compounds with significant antibacterial and antioxidant properties. For centuries, various cultures have incorporated lichens into traditional medicine, but modern scientific inquiry is now rigorously validating and characterising these therapeutic potentials. The unique and harsh environments lichens colonise necessitate the production of a vast array of specialised metabolites, many of which are not found elsewhere in nature, serving as chemical defenses against microbial competitors and environmental stressors like UV radiation. The antibacterial prowess of lichens is primarily attributed to a suite of secondary metabolites, including usnic, salazinic, diffractaic, lobaric and protolichesterinic acids. Some lichen metabolites have shown efficacy against several bacteria and the mechanisms of action are diverse involve disruption of bacterial cell membranes and interference with vital metabolic processes (Karagöz and Öztürk Karagöz, 2022). Excitingly, certain compounds have demonstrated synergy with conventional antibiotics, offering a potential strategy to enhance the efficacy of existing drugs and combat the growing crisis of antimicrobial resistance.

Concurrently, lichens exhibit strong antioxidant activity, which is crucial for neutralising harmful free radicals implicated in oxidative stress-related diseases, such as neurodegeneration, cancer and aging. This activity stems from a different set of phytochemicals, including phenolic compounds like depsides and depsidones, as well as carotenoids. Lubis *et al.* (2025) reported that extracts from *P. hypotropum* have shown considerable antioxidant potential, with IC50 values

indicating their effectiveness. Compounds such as usnic acid, vanillic acid and various flavonoids contribute to this protective effect by scavenging free radicals and chelating metal ions. The presence of these potent antioxidants positions lichens as promising sources for natural additives in pharmaceutical, cosmetic, and food industries, aiming to combat oxidative deterioration and promote human health.

Table 1: Zone of inhibition of different solvent extracts of *B. isidiza* (mm)*

Solvent	*B. subtilis*	*E. coli*	*P. aeruginosa*	*S. aureus*
Acetone	7. 0 ± 0.33	9.0 ± 0.75	9.95 ± 0.74	18.05 ± 0.82
Ethyl acetate	8.0 ± 0.57	21 ± 1.63	12 ± 1.41	20 ± 1.47
Ethanol	5 ± 0.16	11.05 ± 0.42	15.5 ± 0.7	13.05 ± 0.74
Isopropyl alcohol	4.1 ± 0.26	13 ± 0.59	12 ± 0.75	9.05 ± 0.34

* Values are mean ± SD of 4 samples

Table 2: Zone of inhibition of different solvent extracts of *P. reticulatum*

Solvent	*B. subtilis*	*E. coli*	*P. aeruginosa*	*S. aureus*
Acetone	7.1 ± 0.53	7.12 ± 0.15	9.03 ± 0.88	8.02 ± 0.33
Ethyl acetate	6.0 ± 0.49	9.05 ± 0.31	9.0 ± 1.28	10.05 ± 0.90
Ethanol	11.08 ± 0.62	12.13 ± 0.75	16.25 ± 0.82	10.1 ± 0.81
Isopropyl alcohol	9.13 ± 0.15	9.25 ± 0.60	10.58 ± 0.81	9.0 ± 0.37

* Values are mean ± SD of 4 samples

Table 3: DPPH scavenging activity of standard ascorbic acid and sample *B. isidiza*

Concentration (µg)	Standard ascorbic acid (%)	*B. isidiza* (%)
10	15	6
30	37	16
50	54	29
70	63	41
90	66	49
110	76	60
130	85	65

Table 4: DPPH scavenging activity of standard ascorbic acid and sample *P. reticulatum*

Concentration (µg)	Standard ascorbic acid (%)	*P. reticulatum* (%)
0	15	-
30	37	-
50	54	28
70	66	41
90	69	49
110	79	58
130	84	67

CONCLUSION

This study investigated the antibacterial and antioxidant properties of extracts from two lichen species, *B. isidiza* and *P. reticulatum*. The disc diffusion method revealed promising antibacterial activity in both lichen extracts against a panel of Gram-positive and Gram-negative bacteria. Notably, the ethyl acetate extract of *B. isidiza* and the ethanol extract of *P. reticulatum* displayed the strongest activity, suggesting these solvents might be optimal for extracting antibacterial compounds from these specific lichens. The DPPH free radical scavenging assay confirmed antioxidant activity in both lichen extracts, with the percentage of free radical scavenging increasing with extract concentration. These findings suggest potential for the development of natural antioxidants from these lichen species. It is important to note that discrepancies were observed when comparing our results with previous research. These variations highlight the potential influence of factors like geographical location, lichen collection time, and extraction procedures on the biological activity of lichen extracts. In conclusion, this study demonstrates the potential of *B. isidiza* and *P. reticulatum* as sources of natural antibacterial and antioxidant agents. Further research is necessary to isolate and identify the specific bioactive compounds responsible for these activities. Additionally, *in vivo* studies are essential to evaluate the potential therapeutic applications of these lichen extracts.

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