Antimicrobial activity of *Heterodermia incana* (Stirt.) D.D. Awasthi

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Abstract

Objectives: Lichens represent one of the most successful symbiotic interactions and are formed from a photobiont and a mycobiont. The foliose lichen genus *Heterodermia* is one of the cosmopolitan lichen genera. The present study was conducted to investigate antibacterial and antifungal activity of *Heterodermia incana* (Stirt.) D.D. Awasthi, a foliose macrolichen belonging to the family *Physciaceae*. Materials and Methods: Extraction of dried and powdered lichen was carried out by maceration process. Antibacterial activity of the extract was evaluated against 2 Gram-positive and 2 Gram-negative bacteria by agar well diffusion assay. Antifungal activity of extract was determined against 3 seedborne fungi by poisoned food technique. Results: Extract was effective in inhibiting the growth of all test bacteria in a concentration dependent manner with marked activity against Gram-positive bacteria. *Bacillus cereus* (zone of inhibition 2.26 ± 0.05 cm) and *Pseudomonas aeruginosa* (zone of inhibition 1.76 ± 0.05 cm) were inhibited to higher extent among Gram-positive and Gram-negative bacteria, respectively, at 10 mg/ml extract concentration. The extract was effective in inhibiting the mycelial growth of test fungi in a concentration dependent manner. Among three fungi, the susceptibility to extract was in the order: *Fusarium* sp. > *Curvularia* sp. > *Alternaria* sp. At extract concentration 1 mg/ml, >60% inhibition of all test fungi was observed. Conclusion: The lichen *H. incana* is a promising resource of antimicrobial agents. The observed bioactivities could be attributed to the presence of secondary metabolites such as atranorin and zeorin present in the extract. In suitable form, the lichen can be used as anti-infective agent and in the management of seedborne fungal diseases.

Key words: Agar well diffusion, antimicrobial, *Heterodermia incana*, lichens, poisoned food technique

INTRODUCTION

Lichens are non-vascular cryptogams and comprise a self-supporting, symbiotic association between a photobiont (an alga or a cyanobacterium) and a mycobiont (an ascomycetes or basidiomycetes fungus). They represent one of the most stable and successful symbiotic interactions among organisms. Lichens are cosmopolitan and found distributed in almost every type of habitats on earth such as Arctic region, deserts, high mountains elevations, tropical and temperate forests, and others. Together with mosses, lichens form a dominant group of organisms covering over 8-10% of terrestrial habitats, especially at higher elevations. Lichens occur in any one of the three morphological forms such as crustose (spreading over surface of substratum), foliose (leafy and often loosely attached to substratum), and fruticose (bush like hanging and attached to substratum at a single point). Lichens are capable of growing on various substrates such as rock (saxicolous), bark (corticolous), soil (terricolous), plastic (plasticolous), and leaves (folicolous). Lichens are considered as one of the best indicators of air quality.[1-9] Since time immemorial, lichens have been used as sources of food, spice, medicine, and dye. Lichens have been considered to be a part of traditional medicine and are used to treat several human and veterinary ailments by various tribes of several countries.[10,11] Lichens produce a number of low molecular weight compounds (secondary metabolites, often termed as lichen substances) which do not occur in other organisms. Lichen extracts and the secondary metabolites of lichens are known to exhibit a range of bioactivities such as antimicrobial, antioxidant, insecticidal, anthelmintic,
antiviral, anthelmintic, antiproliferative, anti-inflammatory, analgesic, and enzyme inhibitory activities.[3,6,7,9,12-16]

The lichen genus *Heterodermia* belongs to *Physciaceae* and is one among the most common lichens in tropical regions. The genus *Heterodermia* is distinguished from other foliose lichen genera of *Physciaceae* as the genus is characterized by the presence of the prosoplectenchymatous upper cortex in combination with atranorin as a cortical substance. Moreover, many species are characterized by lacking a lower cortex and producing abundant marginal cilia that resembles rhizenes. Zeorin is one of the major lichen substances in *Heterodermia*. Some species of *Heterodermia* are known to have ethnomedical and traditional uses and are shown to exhibit bioactivities such as antimicrobial, antiviral, antioxidant, enzyme inhibitory, and anthelmintic activity.[11,17-25] *Heterodermia incana* is a corticolous (and rarely saxicolous) macrolichen. The lichen is reported from subtropical to lower temperate regions of India, Nepal and Sri Lanka and also found in China, Taiwan, and Thailand. Thallus is white to whitish gray on the upper side and the lower side is white, veined with marginal rhizenes. Thallus is about 6 cm across and branched with corticated on the upper side only. Lobes are spathulate and are apically 5 mm wide. Soredia and isidia are lacking. Apothecia are pedicellate with distinct margin (lecinulate) and about 8 mm in diameter.[19] The study of Behera et al.[9] has shown the anti-lipoxygenase, antimicrobial and antioxidant potential of *H. incana*. In this study, we evaluated antibacterial and antifungal potential of an extract of *H. incana*.

**MATERIALS AND METHODS**

**Collection and Identification of Lichen**

The corticolous foliose lichen *H. incana* [Figure 1] was collected at outskirts of Sagara, Shivamogga district, Karnataka, India, during February 2017. The collected lichen was identified on the basis of the result of morphological, anatomical, and color (K [potassium hydroxide], C [calcium hypochlorite], and Pd [paraphenylene diamine]) tests. Secondary metabolites in lichens were detected by thin layer chromatography (TLC) using solvent system A that comprised toluene, 1,4-dioxane and acetic acid.[26-28] A voucher specimen (KFGCS0756) was kept in the herbaria maintained in the Department of Botany, KFGC, Shikaripura, Karnataka, India.

**Extraction of Powdered Lichen Material**

The lichen was dried and powdered. Maceration process was used for extraction. In stoppered container, 10 g of powdered lichen material was left for 48 h in 100 ml of methanol, and the container was stirred occasionally. The content was filtered through Whatman No. 1 filter paper, and the filtrate was subjected for evaporation to get crude extract. The extract, thus, obtained was stored in the refrigerator.[29,30]

**Test Bacteria**

A total of 4 bacteria which included two Gram-positive bacteria (*Bacillus subtilis* NCIM 2063 and *Bacillus cereus* NCIM 2016) and two Gram-negative bacteria (*Escherichia coli* NCIM 2065 and *Pseudomonas aeruginosa* NCIM 2200) were used. The pure cultures of these bacteria were obtained from National Chemical Laboratory, Pune, India. The cultures were maintained on nutrient agar slants under refrigeration conditions.

**Antibacterial Activity of Lichen Extract**

The test bacteria were seeded into sterile nutrient broth tubes and incubated overnight at 37°C to obtain broth cultures. Antibacterial activity of lichen extracts was evaluated by agar well diffusion assay. Using sterile swabs, the broth cultures of test bacteria were inoculated all over the surface of sterile nutrient agar plates. Using a sterile cork borer, wells (8 mm diameter) were punched in the plates. Respective wells were filled with 100 µl of lichen extract (5 and 10 mg/ml of dimethyl sulfoxide [DMSO]), reference antibiotic (choloramphenicol, 1 mg/ml of sterile distilled water), and DMSO. The plates were incubated for 24 h at 37°C. Zones of inhibition formed were measured.[30,31]

**Test Fungi**

Three fungi, viz., *Alternaria* sp., *Fusarium* sp., and *Curvularia* sp., isolated previously from moldy grains of sorghum were used. The fungal cultures were maintained on Potato dextrose agar slants under refrigeration conditions.

**Antifungal Activity of Lichen Extract**

Poisoned food technique was carried out to investigate antifungal potential of lichen extract. The test fungi were allowed to grow on control (without extract) and poisoned...
Potato dextrose agar (0.5 and 1.0 mg extract/ml of medium) plates for 4 days at room temperature. After incubation, the diameter of fungal colonies was measured in mutual perpendicular directions. Antifungal potential of lichen extract, assessed in terms of inhibition of mycelial growth of test fungi, was determined using the formula:

Inhibition of mycelial growth (%) = \( \frac{(D_c - D_t)}{D_c} \times 100 \)

where “\( D_c \)” and “\( D_t \)” denotes the colony diameter of test fungi on control and poisoned plates, respectively.\(^{[8,30]}\)

**Statistical Analysis**

All experiments were done in triplicates. The results are represented as mean ± standard deviation of three trials.

**RESULTS AND DISCUSSION**

The details on morphological characteristics and result of color test and secondary metabolites are shown in Table 1. TLC showed the presence of a despide (atranorin) and a terpenoid (zeorin).

**Antibacterial Activity of H. incana**

Antibiotics have revolutionized the field of medicine as they have saved countless lives from infectious diseases caused by pathogenic bacteria. However, indiscriminate use of antibiotics and the ability of bacteria to transmit resistance trait to susceptible strains resulted in the emergence of antibiotic resistant bacteria. These resistant bacteria are of serious threat in both nosocomial and community settings. Treatment of diseases caused by resistant bacterial strains is often difficult. Hence, there is a great need for developing antibacterials from other sources. Natural products are shown to be one of the important alternatives for developing antimicrobial agents. Lichens and their metabolites are shown to possess antibacterial activity against various pathogenic bacteria including antibiotic resistant bacteria.\(^{[9,32-38]}\) In this study, we evaluated the potential of *H. incana* to inhibit Gram-positive and Gram-negative bacteria by agar well diffusion method. The presence of an inhibition zone around the well is an indication of antibacterial potential of extract. The result of antibacterial activity of lichen extract is shown in Table 2 and Figure 2. The extract exhibited inhibitory activity against test bacteria in a concentration dependent manner. Overall, extract showed marked inhibition of Gram-positive bacteria when compared to Gram-negative bacteria. *B. cereus* (zone of inhibition 2.36 ± 0.05 cm) and *P. aeruginosa* (zone of inhibition 1.76 ± 0.05 cm) were inhibited to a higher extent among Gram-positive and Gram-negative bacteria, respectively. Least inhibitory activity of extract was observed against *E. coli* (zone of inhibition

### Table 1: Morphological features, color test, and TLC of lichen

<table>
<thead>
<tr>
<th>Characteristics</th>
<th><em>H. incana</em></th>
</tr>
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<tbody>
<tr>
<td><strong>Morphological features</strong></td>
<td>Thallus (5-6 cm across) branched, upper side whitish gray, lower side is white, veined with marginal rhizenes, corticated on upper side only, lobes spathulate, soredia and isidia absent, apothecia pedicellate</td>
</tr>
<tr>
<td><strong>Color test</strong></td>
<td>Medulla K+ yellow; C -; P + yellow</td>
</tr>
<tr>
<td><strong>Secondary metabolites</strong></td>
<td>Atranorin, zeorin</td>
</tr>
</tbody>
</table>

*H. incana: Heterodermia incana, TLC: Thin layer chromatography*

### Table 2: Antibacterial activity of *H. incana*

<table>
<thead>
<tr>
<th>Test bacteria</th>
<th>Zone of inhibition in cm (mean±SD)</th>
<th>DMSO</th>
<th>Chloramphenicol</th>
<th>Lichen extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5 mg/ml</td>
<td>10 mg/ml</td>
<td>5 mg/ml</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>0.00±0.00</td>
<td>2.90±0.10</td>
<td>1.20±0.10</td>
<td>1.50±0.10</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>0.00±0.00</td>
<td>2.90±0.10</td>
<td>1.46±0.05</td>
<td>1.76±0.05</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>0.00±0.00</td>
<td>2.90±0.00</td>
<td>2.00±0.10</td>
<td>2.20±0.10</td>
</tr>
<tr>
<td><em>B. cereus</em></td>
<td>0.00±0.00</td>
<td>3.70±0.10</td>
<td>2.10±0.10</td>
<td>2.36±0.05</td>
</tr>
</tbody>
</table>

*E. coli: Escherichia coli, P. aeruginosa: Pseudomonas aeruginosa, B. subtilis: Bacillus subtilis, B. cereus: Bacillus cereus, H. incana: Heterodermia incana, DMSO: Dimethyl sulfoxide, SD: Standard deviation*
Reference antibiotic exhibited marked inhibitory activity against test bacteria when compared to lichen extract. No inhibitory activity against test bacteria was observed in case of DMSO. In an earlier study, Behera et al.\textsuperscript{[9]} showed the antibacterial effect of ethyl acetate extract of \textit{H. incana} against \textit{Streptococcus faecalis} with a minimum inhibitory concentration value of 1.624 mg/ml. Studies have shown the antibacterial potential of some species of \textit{Heterodermia} such as \textit{Heterodermia diademata},\textsuperscript{[9,36]} \textit{Heterodermia flabellata},\textsuperscript{[9]} \textit{Heterodermia pseudospeciosa},\textsuperscript{[9]} \textit{Heterodermia podocarpa},\textsuperscript{[9]} \textit{Heterodermia Obscurata},\textsuperscript{[9,40]} and \textit{Heterodermia boryi}.	extsuperscript{[15,41]}

### Antifungal Activity of \textit{H. incana}

Fungi are known to represent the dominant group of phytopathogenic organisms causing several diseases in crops. Fungi cause damage to crop in field conditions as well as during storage. Fungal diseases of plants result in decreased

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### Table 3: Colony diameter of test fungi in control and poisoned plates

<table>
<thead>
<tr>
<th>Concentration of extract</th>
<th>Colony diameter of test fungi (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>\textit{Alternaria sp.}</td>
</tr>
<tr>
<td>0.0 mg/ml (control)</td>
<td>4.26±0.05</td>
</tr>
<tr>
<td>0.5 mg/ml</td>
<td>2.30±0.10</td>
</tr>
<tr>
<td>1.0 mg/ml</td>
<td>1.33±0.05</td>
</tr>
</tbody>
</table>

SD: Standard deviation

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In this study, the TLC showed that the methanolic extract of H. incana exhibited antibacterial activity against the tested microorganisms. The results indicated that the extract effectively inhibited the growth of the test fungi, with percent inhibition varying among the species. The highest inhibition was observed against Curvularia sp. with 80.26% inhibition, followed by Alternaria sp. with 78.26% inhibition, and Fusarium sp. with 76.60% inhibition. These findings suggest that the secondary metabolites present in H. incana have potential for use in managing fungal diseases.

**CONCLUSIONS**

The result of this study clearly indicated the possible use of the foliose lichen H. incana as a resource of bioactive agents. The observed antibacterial and antifungal activity could be attributed to the presence of atranorin and zeorin, which were identified by TLC. The lichen can be used to treat infections and manage seedborne fungal diseases of plants. Further, studies concerned with the purification of lichen substances and their antimicrobial activity determination are to be carried out.

**REFERENCES**


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