Chemistry, ethnobotanical uses and biological activities of the lichen genus *Heterodermia* Trevis. (Physciaceae; Lecanorales; Ascomycota): A comprehensive review

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**ABSTRACT**

Lichens are composite organisms comprised of a photobiont (an alga or a cyanobacterium) and a mycobiont (an ascomycete or basidiomycete fungus) and represent a stable, ecologically obligate symbiotic association. The lichen genus *Heterodermia* Trevis (Physciaceae; Lecanorales; Ascomycota) is one of the lichen genera distributed worldwide. The thallus is foliose, dichotomously or irregularly branched and the genus *Heterodermia* differs from other foliose lichen genera in the family Physciaceae mainly on the basis of its prosoplectenchymatous upper cortex in combination with atranorin (a cortical lichen substance). In this review, an attempt is made to compile data (by referring books, journals and various search engines such as Google Scholar, PubMed, and ScienceDirect) available on the chemistry, traditional uses and biological activities of species of *Heterodermia*. Atranorin and zeorin are the major metabolites found in *Heterodermia* species. Besides these, salazinic acid and norstictic acid are also found in several *Heterodermia* species. *Heterodermia* species are used ethnobotanically as a flavoring agent, in preparation of perfumes and for treatment of wounds and infections. Literature survey revealed the potential of extracts and isolated constituents of *Heterodermia* species to exhibit biological activities such as antimicrobial, antioxidant, cytotoxic, antiinflammatory, insecticidal, immunomodulatory and anthelmintic activity.

**INTRODUCTION**

Lichens are composite organisms (holobionts) and represent a stable, self-supporting symbiotic relationship between a photosynthetic partner (also called photobiont, representing a microalga or a cyanobacterium) and a fungal partner (referred as mycobiont; represents the majority of the portion of lichens). They are known to be the first colonizers of the earth. Lichens are used traditionally as medicine (to cure dyspepsia, bleeding piles, bronchitis, scabies, stomach disorders, and many disorders), spice, flavoring agents (as an ingredient of garam masala, meat masala, and sambar masala) and sources of dyes and perfumes. Traditional systems of medicine such as Ayurveda and Unani make use of lichens (Upreti et al., 2005; Gupta et al., 2007; Nguyen et al., 2013; Shah, 2014; Behera et al., 2016). Lichens are distributed universally and occur in varied climatic conditions ranging from the poles to the tropics. They grow on various substrates such as rock (saxicolous), leaves (folicolous), soil (territicolous), bark (corticolous) and wood (lignicolous) and exhibit one of the different growth forms such as a) crustose-spreading rapidly over the surface b) foliose-leafy and loosely attached to the surface and c) fruticose-branched and shrubby, hanging from tree twigs or branches, with a single attachment (Sanders, 2001; Pinokiyo et al., 2006; Spribille et al., 2008; Kumar, 2009).

Lichens are shown to be the best indicators of air pollution as they are very sensitive to changes in the environment and usually disappear from the area in case of pollution (Gunathilaka et al., 2011; Rodriguez et al., 2016). Lichens produce a number of secondary metabolites which seldom occur in plants, animals, and other organisms. Most of these metabolites are produced by...
mycobiont and some of the metabolites are formed only in the lichenized state. These substances are often known by name lichen substances or lichen metabolites and are small molecules with complex structure. More than 1000 of such secondary metabolites are known and are derived from acetyl polymalonyl, mevalonic and shikimate pathways (Müller, 2001; Stocker-Wörgötter, 2008; Nguyen et al., 2013). Studies on lichens have shown that solvent extracts and purified compounds exhibit potent bioactivities such as antioxidant, hepatoprotective, analgesic, antimicrobial, antiviral, cytotoxic, insecticidal, antinociceptive, anthelmintic, neuroactive, anti-inflammatory, enzyme inhibitory, immunomodulatory and anticancer activity (Müller, 2001; Karunarathne et al., 2005; Oksanen, 2006; Verma et al., 2008a; Russo et al., 2012; Brisdelli et al., 2013; Córdova et al., 2013; White et al., 2014; Thadhani et al., 2015; Reddy et al., 2016).

THE GENUS HETERODERMIA

The lichen genus Heteroderma Trevis. (Physciaceae; Lecanorales; Ascomycota) is one of the most commonly found foliose macrolichens (Figure 1) found distributed in tropical and subtropical regions. Earlier, all species of Heteroderma were included in Anaptychia Körb. until thick-walled spores and the presence of atranorin were considered as useful characters for separating these two genera. The genus Heteroderma is distinguished from other foliose lichen genera in the family Physciaceae chiefly on the basis of its prosoplectenchymatous upper cortex in combination with atranorin (as a cortical lichen substance). Most Heteroderma species are also characterized by the production of abundant marginal cilia (that resembles rhizenes), lack of a lower cortex and the presence of norstictic and salazinic acids as the common medullary substances. Thallus of Heteroderma is foliose, adnate, suberect, rosulate to pendulous, irregularly or dichotomously branched, heteromerous and corticated on the upper side or both sides. The upper cortex is unevenly or uniformly thick. The photobiont is a green alga. Apothecia are laminal, sessile to pedicellate; asci 8-spored; ascospores 2-celled (Awasthi, 2007; Luckling et al., 2008; Wang et al., 2008; Wei et al., 2008). Some species of Heteroderma are used traditionally in certain countries as medicine and as spice and flavoring agent (Upreti et al., 2005; Rawat, 2016). In this review, we focus on traditional uses, chemistry and biological activities of Heteroderma species. A detailed and extensive literature survey was carried out on various aspects of the lichen genus Heteroderma by referring standard flora, journals, and
various search engines including Google Scholar, PubMed, and ScienceDirect.

ETHNOBOTANICAL USES OF HETERODERMIA SPECIES

Lichens have traditional uses worldwide. Some species of Heteroderma find potential use in the form of medicine and flavoring agents and in the preparation of perfumes. The ethnic group in Sikkim uses H. diademata traditionally and applies the thalli on the cuts for protecting from wetting and infection (Uperti et al., 2005). The ethnic communities in Madhya Pradesh, India make use of H. tremulans as spice and flavoring agent for meat and vegetables (Uperti et al., 2005). Together with Parmotrema, H. diademata is traditionally used as flavoring agent for meat and other food items in Karnataka, India. It is also used medicinally to heal cuts and wounds and is used as a plaster to protect the wound from infection (Vinayaka and Krishnamurthy, 2012). The traditional industries in Uttar Pradesh, India utilize Heteroderma species viz. H. diademata and H. boryi in the preparation of perfumes (Singh et al., 2015). The ethnic communities in Nepal use H. diademata for treatment of wound and to stop bleeding after the injury. The lichen is mixed with Artemisia vulgaris or Eupatorium odoratum and used to cure fresh wounds or cuts (Devkota et al., 2017). The indigenous Pankararu people in the semi-arid of Pernambuco State, Northeast of Brazil, utilize H. galactophylla for treating digestive system related problems such as diarrhea and vomiting and for treating epilepsy (Londoño-Castañeda et al., 2017).

COMMON LICHEN SUBSTANCES PRESENT IN HETERODERMIA SPECIES

Lichens are capable of producing a number of secondary metabolites which apparently do not occur in other organisms. Most of these metabolites are small molecules but are biologically active and exhibit myriad of biological activities. Besides, these metabolites are also useful in the taxonomy of lichens. Thin layer chromatography is one of the most widely used bioanalytical techniques employed to detect lichen substances. Besides, other techniques such as HPLC, column chromatography, liquid chromatography-mass spectrometry and nuclear magnetic resonance spectroscopic methods have been widely employed to detect and to elucidate the structures of lichen substances. Compounds viz. atranorin and zeorin are known to be the signature compounds to be present in Heteroderma species. Besides these compounds, compounds such as norstictic acid and salazinic acid are also present in many of Heteroderma species (Awasthi, 2007; Fazio et al., 2007; Molnár and Farkas, 2010; Honda et al., 2010; Musharraf et al., 2015; Kekuda and Vinayaka, 2016). A list of major secondary metabolites detected in some Heteroderma species is shown in Table 1 and Figure 2 presents structures of some lichen metabolites.

BIOLOGICAL ACTIVITIES OF HETERODERMIA SPECIES

Lichens are shown to display a variety of pharmacological activities. Several studies have been carried out by researchers to investigate biological activities of crude solvent extracts and purified compounds from Heteroderma species. Literature survey revealed that species of Heteroderma exhibits a range of biological activities such as antimicrobial, antioxidiant, antinociceptive, anti-inflammatory, immunomodulatory, enzyme inhibitory, cytotoxic, insecticidal and anthelmintic activities.

Antibacterial activity of Heteroderma species

Studies have shown that solvent extracts and purified compounds from Heteroderma species exhibit antibacterial properties. Ethanol extract of H. leucomela was found to inhibit Mycobacterium tuberculosis strains (Gupta et al., 2007). The study carried out by Paudel et al. (2012) revealed the potential of methanol extract of Heteroderma sp. to inhibit Bacillus subtilis. The methanol extract of H. diademata was effective in inhibiting Staphylococcus aureus (isolates from burn), Streptococcus mutans (cariogenic isolates), urosepathogenic bacteria (Kambar et al., 2014). Extract of H. obscurata was effective against gram positive and gram negative bacteria (Kekuda et al., 2015). Solvent extracts of H. boryi were shown to inhibit gram positive and gram negative bacteria (Prabhu and Sudha, 2015). Kekuda and Vinayaka (2016) observed anticiaries activity exhibited by H. leucomela against Streptococcus mutans isolates. Dichloromethane extract of H. diademata and H. podocarpa was inhibitory to Klebsiella pneumoniae while dichloromethane extract of H. leucomelos, H. indica and H. speciosa was shown to inhibit the growth of Staphylococcus aureus and K. pneumoniae (Jha et al., 2017). Kekuda et al. (2017) revealed inhibition of gram positive and gram negative bacteria by an extract of H. incana. In another study by Hengameh and Rajkumar (2017), solvent extracts of H. leucomelos were effective in inhibiting gram-positive and gram-negative bacteria. Atrnorin and sekiakic acid, isolated from H. obscurata, were shown to display inhibitory activity against bacteria viz. E. coli, B. subtilis and S. typhi (Thadhani et al., 2012).

Antifungal activity of Heteroderma species

Extracts and isolated constituents from Heteroderma species were shown to display antifungal properties. The aqueous extract obtained from H. leucomela was effective in exhibiting antifungal activity against a number of molds including plant pathogenic fungi and dermatophytes in terms of inhibition of spore germination. At 80 µl/ml concentration, 100% inhibition of germination of spores of all test fungi was observed (Shahi et al., 2001). Acetone, methanol and chloroform extracts of H. diademata were shown to exhibit antifungal activity against phytopathogenic fungi such as Aspergillus flavus, A. fumigatus, Alternaria alternata, Fusarium roseum, F. oxysporum, F. solani, Penicillium citrinum (Tiwari et al., 2011). The methanol extract of H. diademata was effective in inhibiting Candida albicans, Cryptococcus neoformans, Colletotrichum capsici (Kambar et al., 2014). The methanol extract of H. obscurata displayed antifungal activity against C. capsici, F. oxysporum, A. alternata and A. flavus (Kekuda et al., 2015). Solvent extracts viz. acetone, methanol and chloroform extracts of H. leucomelos were shown to inhibit the growth of A. niger, A. flavus, F. oxysporum, F. solani, C. falcatus (Babiah et al., 2015). Extracts of H. comosa were effective against F. solani and F. oxysporum (Shivanna and Garampalli, 2016). The methanol extract of H. incana was shown to inhibit mycelial growth of seed-borne fungi (Kekuda et al., 2017). Candida albicans was susceptible to dichloromethane fraction of H. indica.
and *H. diademata* (Jha et al., 2017). Atranorin, isolated from hexane extract of *H. microphylla*, is shown to exhibit antifungal activity against *Colletotrichum gloeosporioides* and *C. musae*. The compound was effective in inhibition germination of spores of the fungi (Bombuwela et al., 2008). Methyl β-orcinol carboxylate, derived from atranorin (isolated from *H. obscurata*) was effective in inhibiting yeasts and molds (Thadhani et al., 2012). Solvent extracts of *H. boryi* displayed inhibitory activity against *Pestalotia foedans*, *Phomopsis leptostromiformis* var. *occidentalis*, *F. oxysporum*, *Paecilomyces variotii* (Balasubramanian and Nirmala, 2014a).

### Table 1: Major secondary metabolites in various *Heterodermia* species.

<table>
<thead>
<tr>
<th><em>Heterodermia</em> sp.</th>
<th>Compounds detected</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. squamulosa</em></td>
<td>Atranorin, zeorin</td>
<td>Wang et al. (2008)</td>
</tr>
<tr>
<td><em>H. microphylla</em></td>
<td>Atranorin, chloroatranorin and zeorin</td>
<td>Bombuwela et al. (2008)</td>
</tr>
<tr>
<td><em>H. queensberryi</em></td>
<td>Atranorin, zeorin</td>
<td>Weerakoon and Aptroot (2014)</td>
</tr>
<tr>
<td><em>H. japonica</em></td>
<td>Atranorin, zeorin, salazinic acid, norstictic acid</td>
<td>Din et al. (2010)</td>
</tr>
<tr>
<td><em>H. appendiculata</em></td>
<td>Atranorin, zeorin, salazinic acid, norstictic acid, chloratranorin</td>
<td>Din et al. (2010)</td>
</tr>
<tr>
<td><em>H. leucomela</em></td>
<td>Atranorin, zeorin, salazinic acid, glyceryl trilinolate, 6a-hydroxyhop-21βH-22(29)-en, and 3,6-dimethyl-2-hydroxy-4-methoxybenzoic acid</td>
<td>Devkota (2008), Kathirgamanathan et al. (2006)</td>
</tr>
<tr>
<td><em>H. upretii</em></td>
<td>Atranorin, teloschistin and 7-chloroemodin</td>
<td>Joshi et al. (2014)</td>
</tr>
<tr>
<td><em>H. diademata</em></td>
<td>Atranorin, chlorotranorin and zeorin</td>
<td>Behera et al. (2016)</td>
</tr>
<tr>
<td><em>H. incana</em></td>
<td>Atranorin and zeorin</td>
<td>Kekuda et al. (2017)</td>
</tr>
<tr>
<td><em>H. obscurata</em></td>
<td>Atranorin, chlorotranorin, zeorin, emodin, 7-chloroemodin</td>
<td>Din et al. (2010)</td>
</tr>
<tr>
<td><em>H. albicans</em></td>
<td>Atranorin, zeorin and salazinic acid</td>
<td>Behera et al. (2016)</td>
</tr>
<tr>
<td><em>H. angustiloba</em></td>
<td>Atranorin, zeorin, salazinic acid and norstictic acid</td>
<td>Behera et al. (2016)</td>
</tr>
<tr>
<td><em>H. cirvinalis</em></td>
<td>Atranorin, zeorin</td>
<td>Boom et al. (2007)</td>
</tr>
<tr>
<td><em>H. granulifera</em></td>
<td>Atranorin, chloroatranorin, salazinic acid, zeorin, hypoconstrictic acid, consalazinic acid, norstictic acid, 3-O-methylconsalazinic acid, norhypoconstrictic acid</td>
<td>Boom et al. (2007)</td>
</tr>
<tr>
<td><em>H. magellanica</em></td>
<td>Atranorin, zeorin</td>
<td>Boom et al. (2007)</td>
</tr>
<tr>
<td><em>H. antillarum</em></td>
<td>Atranorin, zeorin and salazinic acid</td>
<td>Behera et al. (2016)</td>
</tr>
<tr>
<td><em>H. flabellata</em></td>
<td>Atranorin, zeorin, emodin, 7-chloroemodin</td>
<td>Din et al. (2010); Behera et al. (2016)</td>
</tr>
<tr>
<td><em>H. isidiophora</em></td>
<td>Atranorin and zeorin</td>
<td>Behera et al. (2016)</td>
</tr>
<tr>
<td><em>H. pseudospectosa</em></td>
<td>Atranorin, zeorin, salazinic acid and norstictic acid</td>
<td>Behera et al. (2016)</td>
</tr>
<tr>
<td><em>H. punctifera</em></td>
<td>Atranorin, zeorin and norstictic acid</td>
<td>Devkota (2008)</td>
</tr>
<tr>
<td><em>H. speciosa</em></td>
<td>Atranorin, zeorin</td>
<td>Devkota (2008)</td>
</tr>
<tr>
<td><em>H. dissecta</em></td>
<td>Atranorin, zeorin, salazinic acid and norstictic acid</td>
<td>Devkota (2008)</td>
</tr>
<tr>
<td><em>H. dentrica</em></td>
<td>Atranorin, salazinic acid, norstictic acid</td>
<td>Awashti (2007)</td>
</tr>
<tr>
<td><em>H. hypocaesia</em>, <em>H. rubricosa</em></td>
<td>Atranorin, zeorin, salazinic acid</td>
<td>Awashti (2007)</td>
</tr>
</tbody>
</table>

### Cytotoxic activity of *Heterodermia* species

Methanol extracts of some *Heterodermia* sp. were investigated for cytotoxicity by brine shrimp lethality assay. Some species were effective in terms of mortality of shrimps with an IC<sub>50</sub> value of 100 and 200 µg/ml (Paudel et al., 2012). Recently, dichloromethane fraction from *H. indica*, *H. leucomela*, *H. diademata*, *H. punctifera*, *H. microphylla*, *H. podocarpa* and *H. speciosa* displayed strong cytotoxicity in brine shrimp assay with >80% mortality (Jha et al., 2017).

### Antioxidant activity of *Heterodermia* species

Many *Heterodermia* species have been investigated for antioxidant activity. Verma et al. (2008b) showed a dose-dependent inhibition of lipid peroxidation by an extract of *H. podocarpa*. Thadhani et al. (2011) isolated compounds viz. methyl orsellinate, methyl haematommate, methyl-β-orcinolcarboxylate, atranorin, and m-depside sekikic acid from *H. obscurata* and evaluated their antioxidant activity by in vitro superoxide radical, nitric oxide radical and DPPH radical scavenging assays. The isolated compounds displayed lower scavenging potential when compared to reference antioxidants. Methanol extracts from *Heterodermia* species were effective in scavenging DPPH and ABTS radicals with marked scavenging potential against DPPH radicals (Paudel et al., 2012). Balasubramanian and Nirmala (2014b) screened...
antioxidant potential of *H. boryi*. The lichen extract was effective in scavenging DPPH and ABTS radicals and caused inhibition of lipid peroxidation and DNA damage. The study of Behera *et al.* (2016) revealed the antioxidant potential of ethyl acetate extract of *Heterodermia* species by DPPH and TEAC (Trolox equivalent activity capacity) assays. None of the species except *H. pseudospeciosa* exhibited an inhibition of DPPH to >50%. The study of Jha *et al.* (2017) revealed the potential of *H. indica, H. leucomela, H. microphylla,* and *H. speciosa* to scavenge DPPH radicals.

![Fig. 2: Structures of some lichen substances.](image)

Table 2: Enzyme inhibitory potential of *Heterodermia* species.

<table>
<thead>
<tr>
<th><em>Heterodermia</em> species</th>
<th>Enzyme inhibited</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. leucomeles</em></td>
<td>Amylase</td>
<td>Karthic <em>et al.</em> (2011)</td>
</tr>
<tr>
<td><em>H. leucomeles</em></td>
<td>β-Glucosidase</td>
<td>Parizadeh and Garampalli (2016)</td>
</tr>
<tr>
<td><em>H. leucomeles</em></td>
<td>Amylase</td>
<td>Hengameh <em>et al.</em> (2016)</td>
</tr>
<tr>
<td><em>H. leucomeles</em></td>
<td>Pancreatic lipase</td>
<td>Shivanna <em>et al.</em> (2017)</td>
</tr>
<tr>
<td><em>Heterodermia</em> sp.</td>
<td>Acetyl and butyryl-cholinesterase, phosphodiesterase, β-glucuronidase</td>
<td>Thadhani <em>et al.</em> (2014)</td>
</tr>
<tr>
<td><em>H. podocarpa</em></td>
<td>Tyrosinase</td>
<td>Verma <em>et al.</em> (2008b)</td>
</tr>
</tbody>
</table>

**Enzyme inhibitory activity of *Heterodermia* species**

Studies have shown the potential of some *Heterodermia* species to inhibit certain enzymes of clinical importance such as amylase, lipase, tyrosinase, and glucosidase. A brief detail on the enzyme inhibitory potential of extracts and purified compounds of *Heterodermia* species is presented in Table 2.

**Antinociceptive and anti-inflammatory activity of *Heterodermia* species**

Glucomannan was obtained from successive aqueous and alkaline extraction of the thallus of the lichenized fungus *H. obscurata*. Intra-peritoneal administration of glucomannan resulted in a marked and dose-dependent inhibition of acetic acid-induced visceral pain with an ID<sub>50</sub> of 0.6 mg/kg and inhibition of 88 ± 4%. It also reduced leukocyte migration indicating the potential utilization of glucomannan against pain and inflammation (Pereira *et al.*, 2010). In another study, glucomannan from *H. obscurata*, was investigated for antinociceptive activity in behavioral models of acute and chronic pain in mice. In the partial sciatic nerve ligation model, the glucomannan was found to reduce the mechanical allodynia and the levels of interleukin 1-β (IL-1β) in spinal cord and nerve. In case of systemic treatment, the polysaccharide inhibited the nociception induced by intraplantar injection of glutamate and by intrathecal injection of N-methyl-d-aspartic acid, (±)-1-aminocyclopentane-trans-1,3-dicarboxylic acid, tumour necrosis factor α and IL-1β. It was concluded that the
glucomannan has significant antinociceptive effect in acute and chronic pain (Córdova et al., 2013).

Immunomodulatory activity of Heteroderma species

Lobaric acid, a compound isolated from Heteroderma sp., was investigated for immunomodulatory activity by Thadhani et al. (2014). The compound was found to exhibit potent oxidative burst inhibitory activity in human polymorphonuclear (PMN) cells. The compound suppressed both the myeloperoxidase dependent and myeloperoxidase independent reactive oxygen species production of PMNs. In another study, compounds viz. methyl orsellinate, methyl haematommate, methyl-β-orcinolcarboxylate, lobaric acid and atranorin isolated from H. obscura were tested for immunomodulatory effect on the basis of their effect on respiratory burst of human whole blood phagocytes, isolated human polymorphonuclear leukocytes and murine macrophages using luminol or lucigenin-based chemiluminescence probes (Thadhani et al., 2015). Compounds viz. methyl haematimate and methyl orsellinate displayed moderate effect on whole blood and intra-cellular ROS (reactive oxygen species), however, these compounds strongly inhibited extra-cellular ROS with IC$_{50}$ values 3.3 ± 0.1 µg/ml and 6.1 ± 1.0 µg/ml respectively. Lobaric acid was shown to suppress myeloperoxidase dependent and myeloperoxidase independent ROS production in PMNs.

Insecticidal activity of Heteroderma species

Karthik et al. (2011) determined the insecticidal activity of methanol extract of H. leucocoma in terms of its larvicidal effect against 2nd and 3rd instar larvae of Aedes aegypti. Among larvae, marked susceptibility was shown by 2nd instar larvae. In another study 3,6-dimethyl-2-hydroxy-4-methoxybenzoic acid, isolated from H. leucocoma, was shown to exhibit larvicidal effect against 2nd instar larvae of A. aegypti (Kathirgamanathar et al., 2006).

Anthelmintic activity of Heteroderma species

The study carried out by Prabhu and Sudha (2016) revealed the anthelmintic activity of various solvent extracts viz. aqueous, methanol, petroleum ether, acetone and chloroform extract of H. boryi. The extracts were effective in causing paralysis and death of adult Indian earthworm (Peretition posthuma) in a dose-dependent manner. Acetone and methanol extracts displayed significant anthelmintic activity at the highest concentration tested.

CONCLUSIONS

An extensive literature survey carried out resulted in potential biological properties of Heteroderma species. Compounds such as atranorin, zeorin, salazinic acid and norstictic acid are common in many species of Heteroderma. The presence of these secondary metabolites might be attributed to the various biological activities displayed by Heteroderma species. Isolation of mycobiont and their mass cultivation for the purpose of obtaining bioactive metabolites should be considered to exploit the lichens for commercial purpose.

SOURCES OF SUPPORT

None.

CONFLICTS OF INTEREST

Author declare there are no conflict of interest.

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