# Anthelmintic and Antioxidant efficacy of two Macrolichens of Ramalinaceae

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## ABSTRACT

Lichens and lichen products have been used in traditional medicines for centuries and still hold considerable interest as alternative treatments in various parts of the world. In this study, the anthelmintic and antioxidant efficacy of methanol extract of two macrolichens of Bhadra wildlife sanctuary namely *Ramalina hossei* H. Magn & G. Awasthi and *Ramalina conduplicans* Vain. were investigated. Extracts of both lichens exhibited concentration dependent anthelmintic activity as revealed by paralysis and death of adult Indian earth worms. Among lichen extracts, *R. hossei* showed potent anthelmintic activity as compared to *R. conduplicans*. The DPPH free radical scavening and $Fe^{+3}$reducing assay showed potent antioxidant activity of extracts in a dose dependant manner. *R. hossei* exhibited high free radical scavenging activity than *R. conduplicans*. Metabolites namely usnic acid, sekikaic acid, selanizic acid, tannins and others were detected in methanol extracts of the lichens. The results of the present study suggest that the lichen extracts could be used as natural antioxidants and could be used to control helminthic infections. Further studies are to be taken to isolate active constituents and determine the antioxidant and anthelmintic efficacy *in vitro* and *in vivo*.

**Keywords:** Macrolichens, Bhadra wildlife sanctuary, Ramalina hossei, Ramalina conduplicans, Antioxidant activity, Anthelmintic activity
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## INTRODUCTION

Helminthic infections are among the most common infections in man, affecting a large proportion of the world's population. Today, the principal mode for control of gastrointestinal parasites is based on the commercial anthelmintics. Because of the increasing anthelmintic resistance and the impact of conventional anthelmintics on the environment, it is important to look for alternative strategies against gastrointestinal nematodes. The traditional medicines hold a great promise as a source of easily available effective anthelmintic agents to the people, particularly in developing countries, including India. It is in the context that people consume several plants or plant derived preparations to cure helminthic infections (1). Free radicals are chemical species containing one or more unpaired electrons that makes them highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability (2). Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS (3, 4). In recent years much attention has been devoted to natural antioxidant and their association with health benefits (2). India is a rich center of lichen diversity, contributing nearly 15% of the 13,500 species of lichens so far recorded in the world (5). Lichens and lichen products have been used in traditional medicines for centuries and still hold considerable interest as alternative treatments in various parts of the world. They produce characteristic secondary metabolites that are unique with respect to those of higher plants (6). In various systems of traditional medicine worldwide, including the Indian system of medicine, these lichen species are said to effectively cure dyspepsia, bleeding piles, bronchitis, scabies, stomach disorders, and many disorders of blood and heart (7-9). Bhadra reserve area (75°15'-75°50' E and 13°25'-13°50' N latitude)

comprises the forests of Western Ghats and its fringes. Sanctuary being situated in the south interior Karnataka, with cool climate throughout the year and affords pleasant days during the hot months. *Ramalina hossei* H. Magn & G. Awasthi (Ramalinaceae) is a tufted, errect, fruticose lichen with thallus corticolous, tufted, errect, yellowish grey in colour, branched. *Ramalina conduplicans* Vain. (Ramalinaceae) is a fruticose lichen with thallus corticolous 3–5 cm long, decumbent, greenish grey colour and branched (10). The present study was conducted to evaluate the anthelmintic and antioxidant efficacy of above two macrolichens of Bhadra wildlife sanctuary.

## MATERIALS AND METHODS

### *Collection and Identification of lichens*

The lichens namely *R. hossei* (Voucher no. KSV/KU00905) and *R. conduplicans* (Voucher no. KSV/KU101073) growing on trees in sanctuary were collected and identified by morphological, anatomical, chemical tests (10). Secondary metabolites were identified by Thin layer chromatography (TLC) in solvent A (180 toluene: 60 1-4, dioxine: 8 acetic acid) (11, 12). The lichen specimens were preserved in Department of Botany for future reference.

### *Extraction of powdered lichen material*

For extraction, 20 g portions of each powdered lichen material was taken and added to 100 ml of methanol. The mixture was sonicated for 30 min, and then left at room temperature overnight. The extract was filtered over Whatman No 1 filter paper, and the filtrates were concentrated under reduced pressure to pasty mass (13). The extracts were tested for the presence of alkaloids, tannins, steroids, saponins, flavonoids and terpenoids (14). The condensed methanol extracts were used determining biological activities.

### *Anthelmintic activity of methanol extracts*

The assay was performed on adult Indian earthworm *Pheretima pasthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. Standard drug (Piperazine citrate, 5%) and methanol extracts (5, 10 and 20mg/ml) were prepared in 0.85% normal saline and poured into respective labeled petriplates (50 ml). A saline control was kept. Six worms of nearly equal size were introduced into each of the plates. Observations were made for the time taken to paralysis and death of individual worm. Paralysis was said to occur when the worms were not able to move even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colors (15). Death was also confirmed by dipping the worms in slightly warm water. The mortality of parasite was assumed to have occurred when all signs of movement had ceased (1).

### *Antioxidant activity of lichen extracts*
### *DPPH free radical scavenging assay*

DPPH free radical scavenging assay was performed to determine the antioxidant activity of lichen extracts and the standard Ascorbic acid (16, 17). The methanol extracts (0.25, 0.50 and 1.00mg/ml) and DPPH (0.002%) were prepared in methanol. Equal volume of different concentrations of methanol extracts and DPPH were mixed in clean and labeled test tubes separately and the tubes were incubated at room temperature in dark for 30 minutes. The optical density was measured at 517nm using UV-Vis Spectrophotometer. The degree of stable DPPH* decolorization to DPPHH (reduced form of DPPH) yellow indicated the scavenging efficiency of the extract. The scavenging activity of the extract against the stable DPPH* was calculated using the following equation.

$$\text{Scavenging activity (\%)} = A - B / A \times 100$$

Where A is absorbance of DPPH and B is absorbance of DPPH and extract combination.

### *$Fe^{+3}$ reducing power assay*

Different concentrations of Methanolic extracts (0.25, 0.50 and 1.00mg/ml) in 1ml of methanol were mixed with 2.5ml of phosphate buffer (200mM, pH 6.6) and 2.5ml of 1% potassium ferricyanide separately. The mixtures were placed in a water bath for 20 min at 50°C, cooled rapidly, mixed with 2.5ml of 10% trichloroacetic acid and 0.5ml of 0.1% Ferric chloride. The intensity of iron (II)-ferricyanide complex was determined by measuring the formation of Perl's Prussian blue at 700nm after 10min. The higher absorbance of the reaction mixture indicates increased reducing power (16, 18).

## RESULTS AND DISCUSSION

TLC in solvent A showed the presence of Usnic acid and Sekikaic acid in *R. hossei*. Metabolite namely Selanizic acid was detected in *R. conduplicans* in addition to Usnic acid and Sekikaic acid. Preliminary phytochemical analysis of methanolic extracts showed the presence of tannins in both the lichen extracts. In addition to tannins, terpenoids were detected in *R. hossei* and steroids were detected in *R. conduplicans* (Table-1).

In this study, extracts of both *R. hossei* and *R. conduplicans* exhibited marked anthelmintic activity in

**Table 1: Chemical constituents detected in TLC and Phytochemical screening of extracts of lichen species**

| Metabolite | R. hossei | R. conduplicans |
|---|---|---|
| Usnic acid | + | + |
| Selanizic acid | – | + |
| Sekikaic acid | + | + |
| Alkaloids | – | – |
| Terpenoids | + | – |
| Tannins | + | + |
| Saponins | – | – |
| Flavonoids | – | – |
| Steroids | – | + |

'+' Detected; '–' Not detected

terms of causing paralysis and death of worms (Table-2). The paralysis time and death time in 5% piperazine citrate was found to be 16 and 28 minutes respectively. The extracts exhibited dose dependent activity. Among lichen extracts, *R. hossei* showed potent anthelmintic activity by causing paralysis and death of worms in shorter time as compared to *R. conduplicans*.

The result of antioxidant activity of methanol extracts of lichen species by DPPH free radical scavenging assay is shown in Table-3. The crude solvent extracts exhibited marked antioxidant activity by scavenging DPPH* (free radical) and converting into DPPHH. The extracts have exhibited concentration dependent radical scavenging activity i.e., higher the concentration, more scavenging potential. Among the lichen extracts, *R. hossei* exhibited high free radical scavenging activity as compared to *R. conduplicans*. Neither extracts showed activity as potent as the standard (ascorbic acid). The result of reducing power of methanol extracts of selected lichen species and tannic acid is represented in Table-4. In this study, the absorbance was found to increase with the dose of methanolic extracts and standard which is suggestive of reducing power.

Lichen metabolites exert a wide variety of biological actions including antibiotic, antimycobacterial, antiviral, anti-inflammatory, analgesic, antipyretic, antiproliferative and cytotoxic effects. Even though these manifold activities of lichen metabolites have now been recognized, their therapeutic potential has not yet been fully explored and thus remains pharmaceutically

**Table 2: Anthelmintic activity of different concentrations of methanol extracts**

| Concentration (mg/ml) | Paralysis time (in min) | | Death time (in min) | |
|---|---|---|---|---|
| | *R. hossei* | *R. conduplicans* | *R. hossei* | *R. conduplicans* |
| 5 | 63 | 96 | 102 | 126 |
| 10 | 51 | 79 | 61 | 89 |
| 20 | 20 | 25 | 35 | 38 |

**Table 3: Antioxidant potential of different concentrations of methanol extracts and standard by DPPH assay**

| Concentration (mg/ml) | Scavenging activity in % | | |
|---|---|---|---|
| | *R. hossei* | *R. conduplicans* | Ascorbic acid |
| 0.250 | 56.11 | 48.04 | 92.52 |
| 0.500 | 61.53 | 59.01 | 95.12 |
| 1.000 | 79.05 | 72.63 | 97.33 |

**Table 4: Antioxidant potential of different concentrations of methanol extracts and standard by by $Fe^{+3}$ reducing power assay**

| Concentration (mg/ml) | Absorbance at 700nm | | |
|---|---|---|---|
| | *R. hossei* | *R. conduplicans* | Tannic acid |
| 0.250 | 0.196 | 0.188 | 0.331 |
| 0.500 | 0.310 | 0.293 | 0.469 |
| 1.000 | 0.625 | 0.601 | 1.045 |

unexploited (19). The utility of lichens is due of range of secondary compounds produced by them. A wide range of secondary metabolites of lichens were characterized. According to their chemical structure, most lichen substances are phenolic compounds, dibenzofuranes, Usnic acids, depsidones, depsones, lactones, quinines and pulvunic acid derivatives (20). In this study, secondary metabolites such as usnic acid, sekikaic acid, and Salazinic acid are reported to be present in the lichen extracts.

Helminth infections are among the most common infections in man, affecting a large proportion of the world's population. Parasitoses have been of concern to the medical field for centuries and the helminths still cause considerable problems for human beings and animals. During the past few decades, despite numerous advances made in understanding the mode of transmission and the treatment of these parasites, there are still no efficient products to control certain helminthes and the indiscriminate use of some drugs has generated several cases of resistance. Furthermore, it has been recognized recently that anthelmintic substances having considerable toxicity to human beings are present in foods derived from livestock, posing a serious threat to human health. Consequently, the discovery and development of new chemical substances for helminth control is greatly needed and has promoted studies of traditionally used anthelmintic plants, which are generally considered to be very important sources of bioactive substances (21). The results of this study have shown promising anthelmintic activity suggesting the possible use of lichens extracts in intestinal nematode control. The anthelmintic activity of methanol extracts could be due to the constituents present.

There are several methods available to assess antioxidant activity of compounds. An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 1,1, diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically. In presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases (22). The antioxidant activity of lichen extracts might be due to the presence of various groups of phytochemicals in them. In this study, the scavenging activity was found to be dose dependent i.e., higher the concentration, more was the scavenging activity. Though the DPPH radical scavenging abilities of the extracts were less than that of ascorbic acid, the study showed that the extracts have the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. For the measurements of reductive ability, we have investigated the $Fe^{3+}$ reducing power in the presence of methanol extracts of selected formulations. The reducing capacity of compound may serve as significant indicator of its potential antioxidant activity (23). An increase in the absorbance revealed the reducing power of extracts. In this study, the reducing power of methanolic extracts was found to increase with the dose. The antioxidant activities have been reported to be the concomitant development of reducing power (24).

The traditional medicines hold a great promise as a source of easily available effective anthelmintic and antioxidant agents to the people, particularly in developing countries, including India. Indigenous system of medicine reports a number of natural for their biological efficacy. However, their scientific evaluation as compared to commercial agents is limited. Helminthic worms constitute a major public health menace and have been positively related with various types of illness. Nowadays even there is risk of development of resistance by these parasites to the drugs. In light of this, the results of the present study suggest that the lichen extracts could be used in the control of helminthic infections namely Ascariasis etc as the worms used in the study are in resemblance with the intestinal parasitic worms such as *Ascaris lumbricoides*. The extracts have also showed promising results in terms of their antioxidant potential and thus could be exploited as natural antioxidants.

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