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Research Article

ASSESSMENT OF ANTIMICROBIAL ACTIVITY OF EXTRACTS OF FEW COMMON LICHENS OF DARJEELING HILLS

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ABSTRACT
Five common lichens (*Cladonia* sp., *Everniastrum* sp., *Parmelia* sp., *Stereocaulon* sp. *and Usnea* sp.) of Darjeeling hills were extracted from different solvents like ethanol, methanol, petroleum ether, chloroform and aqueous extracts and tested against four Gram positive and four Gram negative bacterial strains. Ethanol extracts exerted stronger inhibitory action followed by methanol extracts. Aqueous extracts manifested less activity to the tested microorganisms. Previous reports on the antimicrobial properties of lichens showed the resistance of Gram negative bacteria but in our investigation such group of bacteria found sensitive to four of the five lichens tested (except *Everniastrum*).

Key Words: *Antimicrobial Activity, Lichens, Different Solvent Extracts, Darjeeling*

INTRODUCTION

Lichens has been used in the folk medicines for centuries; their biological properties explored by Native Americans, Indians and Europeans, who used in their traditional medicines to treat a variety of animals. Lichen synthesize numerous metabolites called lichen substances including aliphatic, cycloaliphatic, aromatic and terpenic components. These metabolites exert a wide variety of biological actions including antibiotic, antimycobacterial, immunomodulatory, antioxidant, cytotoxic, antiherbivore, and antitumour effects (Chand *et. al.,* 2009).

Lichen forming fungi produce antimicrobial secondary metabolites that protect many animals from pathogenic microorganisms. The first study of antibiotic properties of lichens was carried out by Burkholder (1944). Vartia (1973) reported antimicrobial properties of several lichens and other researchers have since then studied the antimicrobial activity of several lichens against gram-positive, gram- negative bacteria as well as several fungi.

The search for novel natural bioactive compounds as a foundation to new drug discovery is receiving attention as previously reliable standard drugs become less effective against the emerging new strains of multiple drug resistant pathogens (Muller, 2001).

India is a rich centre of biodiversity contributing nearly 15 % of the 13500 species of lichens (Negi, 2000). Many lichen species of the Himalayan region are said to effectively cure dyspepsia, bleeding piles, bronchitis, scabies, stomach disorders and many disorders of blood and heart (Saklani and Upreti,1992; Negi and Kareem, 1996; Sochting, 1999). Even though manifold activities of lichen metabolites have now been recognized their therapeutic potential has yet not been fully explored and thus remains pharmaceutically unexploited (Turk *et. al.,* 2003).

Darjeeling Himalaya is situated between 87°59' - 88°53' E and 28°31'-27°13' N in the Eastern Himalayan region of India. The altitudinal range of this hilly region varies from 130 to 3660 m., due to this a wide array of climatic zones are available, which favour the luxuriant growth of diversified and rich vegetation. This region is also the abode of many endemic elements and a number of species which have become rare, threatened and endangered (Das, 1995). It is known that microorganisms have developed resistance to many antibiotics. This creates enormous problems in the treatment of infectious disease, and investigators therefore seek new antimicrobial substances from different sources such as higher plants and lichens (Mitscher *et.al.,* 1987; Crittenden and Porter, 1991; Karaman *et.al.,* 2003). Locally ethnobotanical uses of *Usnea* include its use as aromatic in health recipes (Rai *et. al.,* 1998). Secondary metabolites of

lichens contain many active components; the purpose of the present study is to investigate their antimicrobial activity. A few reports are available on the studies on antimicrobial properties of lichens from Darjeeling hills. Gupta and Paul (1995) studied antimicrobial properties of five lichens and *Usnea floria* was found to be promising against *Bacillus megaterium* and *Staphylococcus aureus*. Ray *et. al.*, (2003) reported the antimicrobial activity of the extracts of *Usnea articulata, Ramalina jamisii and Parmelia tinctorum* against both Gram-positive and Gram-negative bacteria. The extracts were also found to be the inhibitor of protein synthesis, energy metabolism and growth of studied bacteria.
In this present work we have investigated the antibacterial potential of five common lichens i.e., *Cladonia* sp., *Everniastrum* sp., *Parmelia* sp., *Stereocaulon* sp. *and Usnea* sp. of Darjeeling Hills.

MATERIALS AND METHODS

Lichen materials

Five lichen materials were collected from profusely grown sites of Darjeeling Hills. The specimens were provisionally identified as *Cladonia* sp., *Everniastrum* sp., *Parmelia* sp., *Stereocaulon* sp. *and Usnea* sp. following the relevant key and monographs (Sochting, 1999). Voucher specimens of the samples are stored at the Herbarium of Department of Botany, Darjeeling Govt. College.

Microorganisms

Eight bacteria listed below were obtained from the stock culture of Microbiology Research Laboratory, Postgraduate Department of Botany, Darjeeling Government College.
List of bacteria:Alcaligens faecalis, Bacillus subtilis, Bacillus megaterium, Escherichia coli, Enterobacter aerogenes, Pseudomonas aeruginosa, Streptococcus mutans and Staphylococcus aureus.

Preparation of lichen extracts

Each lichen sample was washed to remove debris, dried and ground to powder and was stored in a sterile glass bottle in the refrigerator. The 5 g portions of sieved powder was added to 50 ml of solvents (ethanol, methanol, chloroform and petroleum ether), sonicated for 30 min and left overnight at room temperature. The crude extract was prepared by decanting, followed by filtration through muslin cloth, and further filtered with Whatman No. 1 filter paper to obtain a clear filtrate. The filtrate was evaporated to obtain 10 ml of concentrated extract and sterilized by membrane filtration using 450 nm bacteriological filters. Such sterilized filtrate was stored in screwcapped airtight containers in the refrigerator.

Screening of antibacterial activity

This procedure is based on disc diffusion method of Baur *et. al.*,(1966). Overnight grown bacterial cultures (1.5 x 10^8 CFU/ml) were spreadplated on nutrient agar plates to achieve semiconfluent growth. Sterile filter paper discs were soaked in extracts, allowed to dry between the applications and placed on plates which were then incubated at 37°C for 24 hrs. Streptomycin (10µg/ml) and sterile distilled water were taken as positive and negative control respectively. Growth was evaluated and inhibition zone were measured. All the experiments were repeated twice and data presented are average of three independent readings.

RESULTS AND DISCUSSION

Cladonia sp. extracts inhibited all the tested bacteria. Aqueous extract was inhibitory only against *Alcaligens faecalis* whereas ethanolic extracts could inhibit all the tested bacteria except *B. subtilis* (Table 1). *Everniastrum* sp. was found to be inhibitory only against two of the Gram positive bacteria i.e., *B. subtilis* and *S. aureus* (Table 2). Aqueous extracts of *Parmelia* sp. inhibited only gram positive bacteria (*B. megaterium* and *S.aureus*, rest of the extracts were inhibitory against both Gram-positive and Gram-negative bacteria (Table 3). Ethanolic fraction of *Stereocaulon* sp. inhibited all the tested bacteria except *S. mutans* (Table 4) whereas *B. subtilis* was sensitive to all the solvent fractions. Aqueous fractions of *Usnea* sp. inhibited only *E. coli* and ethanolic extract could inhibit seven of the tested bacteria and *S. mutans* was not inhibited (Table 5).

Table 1: Antibacterial activity of *Cladonia* sp. against test organisms

Test organisms	Diameter of inhibition zone (mm)						
	* SDW	STR	AQE	ETE	MTE	PEE	CHE
E.coli	-	18	-	13	-	10	11
E. aerogenes	-	19	-	14	20	12	10
P. aeruginosa	-	19	-	10	16	12	15
A. faecalis	-	18	9	8	-	-	-
B. subtilis	-	19	-	15	10	8	12
B. megaterium	-	19	-	-	10	9	-
S. mutans	-	18	-	9	-	-	7
S. aureus	-	19	-	11	17	8	10

* SDW (Sterile distilled water), STR (Streptomycin -10µg/ml), AQE (Aqueous extract), ETE (Ethanolic extract), MTE (Methanolic extract), PEE (Petroleum ether extract), CHE (Chloroform extract)

Table 2: Antibacterial activity of *Everniastrum* sp. against test organisms

Test organisms	Diameter of inhibition zone (mm)						
	* SDW	STR	AQE	ETE	MTE	PEE	CHE
E.coli	-	18	-	-	-	-	-
E. aerogenes	-	19	-	-	-	-	-
P. aeruginosa	-	19	-	-	-	-	-
A. faecalis	-	18	-	-	-	-	-
B. subtilis	-	19	-	-	-	-	-
B. megaterium	-	19	-	10	10	-	15
S. mutans	-	18	-	-	-	-	-
S. aureus	-	19	12	12	10	8	8

* SDW (Sterile distilled water), STR (Streptomycin -10µg/ml), AQE (Aqueous extract), ETE (Ethanolic extract), MTE (Methanolic extract), PEE (Petroleum ether extract), CHE (Chloroform extract)

Table3: Antibacterial activity of *Parmelia* sp. against test organisms

Test organisms	Diameter of inhibition zone (mm)						
	* SDW	STR	AQE	ETE	MTE	PEE	CHE
E.coli	-	18	-	10	8	19	-
E. aerogenes	-	19	-	8	-	16	-
P. aeruginosa	-	19	-	11	20	9	8
A. faecalis	-	18	-	-	8	-	-
B. subtilis	-	19	-	-	8	-	8
B. megaterium	-	19	8	10	9	8	-
S. mutans	-	18	-	9	-	-	8
S. aureus	-	19	8	8	20	8	-

* SDW (Sterile distilled water), STR (Streptomycin -10µg/ml), AQE (Aqueous extract), ETE (Ethanolic extract), MTE (Methanolic extract), PEE (Petroleum ether extract), CHE (Chloroform extract)

Table 4: Antibacterial activity of *Stereocaulon* sp. against test organisms

Test organisms	Diameter of inhibition zone (mm)						
	* SDW	STR	AQE	ETE	MTE	PEE	CHE
E.coli	-	18	10	8	-	-	-
E. aerogenes	-	19	-	8	-	-	-
P. aeruginosa	-	19	-	8	9	-	-
A. faecalis	-	18	-	10	-	-	-
B. subtilis	-	19	17	9	9	12	14
B. megaterium	-	19	8	10	-	8	-
S. mutans	-	18	-	-	-	-	-
S. aureus	-	19	-	10	8	8	-

* SDW (Sterile distilled water), STR (Streptomycin -10µg/ml), AQE (Aqueous extract), ETE (Ethanolic extract), MTE (Methanolic extract), PEE (Petroleum ether extract), CHE (Chloroform extract)

Table 5: Antibacterial activity of *Usnea* sp. against test organisms

Test organisms	Diameter of inhibition zone (mm)						
	* SDW	STR	AQE	ETE	MTE	PEE	CHE
E.coli	-	18	8	8	12	17	15
E. aerogenes	-	19	-	10	-	13	-
P. aeruginosa	-	19	-	12	12	15	10
A. faecalis	-	18	-	14	10	23	17
B. subtilis	-	19	-	12	10	19	14
B. megaterium	-	19	-	10	15	19	14
S. mutans	-	18	-	-	-	-	-
S. aureus	-	19	-	11	17	-	8

* SDW (Sterile distilled water), STR (Streptomycin -10µg/ml), AQE (Aqueous extract), ETE (Ethanolic extract), MTE (Methanolic extract), PEE (Petroleum ether extract), CHE (Chloroform extract)

Methanolic extract of *Cladonia* sp. was found to be more potent than Streptomycin against *E. aerogenes* (Table 1) and similarly methanolic extract of *Parmelia* sp. also showed larger inhibition zone than standard drug Streptomycin against *Pseudomonas* and *Staphylococcus aureus* (Table 4).

Aqueous extracts of all investigated lichens were less inhibitory to the test bacteria. Earlier studies did not find any antibacterial properties of lichens extracts in water (Tay *et. al.,* 2004). *S. mutans* showed the greatest resistance to the investigated lichen extracts.

Extracts of all investigated lichens showed antibacterial activity. The petroleum ether extract of *Usnea s*p showed strongest antibacterial activity (inhibition zone dia 23 mm) against *A. faecalis*. *Cladonia* sp. and *Parmelia* sp. inhibited all the tested bacteria. The weakest activity was shown by extracts of *Everniastrum* sp., which inhibited only two of the tested bacteria *i. e., B. megaterium* and *S. aureus*. All gram negative bacteria were resistant to the extracts of this lichen.

Ethanol extracts exerted stronger inhibitory action followed by methanol extracts. In general, Gram-negative bacteria were more resistant than Gram-positive bacteria. Aqueous extracts manifested very little activity to the tested microorganisms but such extracts of *Stereocaulon* sp. and *Cladonia* sp. showed significant activity to *B. subtilis* and *S. aureus* respectively. The probable reason for this is that majority of active substances present in the lichen thalli are either insoluble or poorly soluble in water (Karthikaidevi *et. al.,* 2009).

There are reports of inactiveness of purified active components of *Cladonia* sp. against gram negative organisms in literature (Yilmaz *et.al*., 2004; Lauterwein *et al.*, 1995; Ingolfsdottir, 2002)) but our experiments showed inhibitory action against all such organisms tested.

These similarities and differences in the antimicrobial activity of extracts of different lichen species probably are a consequence of the presence of different components with antimicrobial activity. The results presented here indicate that the investigated extracts manifest strong but varying antimicrobial activity, which suggests that extracted components from various lichens may prove useful in treating many diseases caused by microorganisms.

This broad variation of antimicrobial activity may be attributable to the differently soluble wide variety of bioactive compounds, such as phenolics, flavones, carotenoids and tannins, present in the selected lichen specimens. Moreover, significant differences in antibacterial activity can be attributable to extraction methods, time of collecting samples, environment, and genetic differences between tested samples (Shan

et al., 2005). This difference in sensitivity can be ascribed to morphological differences between the microorganisms, and above all to differences in permeability of the cell wall (Nostro *et. al.*, 2000).

Previous reports on the antimicrobial properties of lichens showed the resistance of Gram negative bacteria but in our investigation such group of bacteria found sensitive to four of the five lichens tested (except *Everniastrum* sp.). As gram negative bacteria are the major pathogens of gastrointestinal diseases, a further study is needed to improve the efficacy of lichen extracts against the microbes tested.

The reason why few extracts did not show antimicrobial activity in the screening may be their low quantities, probably lower than their MICs. Hence, detailed studies on the role of individual phytochemicals involved in the antibacterial activity of specific lichens are required for their use in the pharmaceutical industry.

From the results obtained in this study further research needs to be carried out to determine the exact phytochemicals (and their nature) involved in the antibacterial activity of the lichens. When these facts are harnessed, the studied lichens will surely be useful in the development of some new drugs with broad spectrum of antimicrobial activity.

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REFERENCES

Bauer AW, Kirby WMM, Scherris JKC and Turk M (1966). Antibiotic susceptibility testing by a standardized single disc method. *American Journal of Clinical Pathology* **45(4)** 493-496.

Burkholder PR, Evans AW, McVeigh I and Thornton HK (1944). Antibiotic activity of lichens. Proceedings on National Academic Science, USA. **30(9)** 250-255.

Chand P, Singh M and Rai Mayank (2009). Antibacterial activity of some Indian Lichens. *Journal of Ecophysiology and Occupational Health* **9** 23-29.

Crittenden PD and Porter N (1991). Lichen-forming fungi: potential sources of novel metabolites. *Trends in Biotechnolology* **9** 409-14.

Das A.P (1995). Diversity of angiospermic flora of Darjeeling Hills. *In:Taxonomy and Biodiversity* by AK Pandey (ed.) pp 118-127

Gupta, SK and Paul AK(1995). Studies on antimicrobial substances from lichens of Darjeeling Hills. *Journal of Hill Research* **8(1)** 61-66

Ingolfsdottir K (2002). Usnic acid. *Phytochemistry* **61** 729-736.

Karaman I, Sanin F, Gulluce M, Ogtc H, Sengul M and Adiguzel A (2003). Antimicrobial activity of aqueous and methanol extracts of *Funiperus oxicedrus*. *Journal of Ethnopharmacology* **37** 1-5.

Karthikaidevi G, Thirumaran G, Manivannan, K, Anantharaman, P, Kathiresan, K and Balasubaramanian T (2009). Screening of the antibacterial properties of lichen *Rocella belangeriana* (Awasthi) from Pichavaram mangrove (*Rhizophora* sp.). *Advances in Biological Research* **3(3-4)** 127-131.

Lauterwein M, Oethinger M, Belsner K, Peters T, and Marre R (1995). *In vitro* activities of the lichen secondary metabolites vulpinic acid, (+)-usnic acid and (-)-usnic acid against aerobic and anaerobic microorganisms. *Antimicrobial Agents and Chemotherapy* **39** 2541-2543.

Mitscher L, Drake S, Gollapudi SR, Okwute SK (1987). A modern look at folkloric use of anti-infective agents. *Journal of Natural Products* **50** 1025-40.

Muller, K (2002). Pharmaceutically relevant metabolites from lichens. *Applied Microbiology and Biotechnology* **56** 9-16.

Negi, HR and Kareem, K (1996). Lichens: The unsung heroes. *Amrut* **1** 3-6

Negi, HR 2000. On the patterns of abundance and diversity of macrolichens of Chopta-Tunganath in the Garhwal Himalaya. *Journal of Bioscience* **25** 367-378.

Nostro MP, Germano V, D'Angelo A, Marino, Cannatelli MA (2000). Extraction methods and bioautography for evaluation of medical plant antimicrobial activity. *Letters in Applied Microbiology* **30** 379-84.

Rai PC, Sarkar A, Bhujel RB and Das AP (1998). Ethnomedicinal studies in some fringe areas of Sikkim and Darjeeling Himalaya. *Journal of Hill Research* **11** 12-21.

Ray S, Sinhababu A and Mandal NC (2003). Antibacterial activity of three lichen specimens viz., *Usnea articulate, Ramalina jamesii, Parmelia tinctorum* from Eastern Himalaya. *Journal of Hill Research* **16(2)** 66-69.

Saklani A and Upreti DK (1992). Folk uses of some lichens in Sikkim. *Journal of Ethnopharmacology* **37** 229-333.

Shan B, Cai YZ, Sun M and Corke H (2005). Antioxidant capacity of 26 species lichen extracts and characterization of their phenolic constituents. *Journal of Agriculture and Food Chemistry* **53** 7749-7759.

Sochting U (1999). *Lichens of Bhutan. Biodiversity and Use.* Copenhagen Botanical Institute. Department of Mycology, University of Copenhagen

Tay T, Turk AO, Yilmaz M and Kivac M (2004). Evaluation of antibacterial activity of the acetone extract of the lichen *Ramalina farinacea* and its (+)-usnic acid and procetraric acid constituents. *Verlag der Zeitschrift fur Naturforschung,* **59(C)** 384-388.

Turk AO, Yilmaz M, Kivank M and Turk H (2003). Antimicrobial activity of extracts of the lichen *Cetraria aculeata* and its protolichesterinic acid constitient. *Verlag der Zeitschrift fur Naturforschung* (C) **58 (11-12)** 850-854.

Vartia KO (1973). Antibiotics in lichens. In: Ahmadijan V., Hale M.E. (eds): The Lichens. Academic Press, New York, pp. 547-561.

Yilmaz M, Turk AO, Tay T and Kivanc M (2004). The Antimicrobial Activity of Extracts of the Lichen *Cladonia foliacea* and Its (-)-Usnic Acid, Atranorin, and Fumarprotocetraric Acid Constituents. *Z. Naturforsch* **59(C)** 249-254.