



RESEARCH ARTICLE

MICROBIOLOGY

EVALUATION OF ANTIBACTERIAL ACTIVITY OF SOME LICHEN FROM RAVANGLA, SIKKIM, INDIA.

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ABSTRACT

Antibacterial activity of acetone, methanol and aqueous extract prepared from some lichen species were evaluated against five bacterial strains viz. *Staphylococcus aureus*, *Escherichia coli*, *Vibrio cholerae*, *Shigella dysenteriae*, *Shigella flexneri*. The aqueous, methanol and acetone extracts exhibited a variable range of antibacterial activity to bacterial strains. The acetone and methanol extracts of all investigated lichens showed selective antibacterial activity. The methanol extracts were usually the most active against test organisms. An aqueous extracts of investigated lichens were found to be inactive against all tested bacteria.



KEY WORDS

lichen, antibacterial activity, Staphylococcus aureus, Escherichia coli, Vibrio cholerae, Shigella dysenteriae. Shigella flexneri

INTRODUCTION

Lichens have been used for medicinal purpose since time immemorial and are known to produce unique secondary metabolites exhibiting considerable biological activities such as antimicrobial, antimycobacterial, antiviral, antioxidant, analgesic, cytotoxic, fungicidal, anti herbivore, herbicidal and antibiotic properties.¹⁻⁵ They have attracted a great attention by a large number of investigators as new sources of bioactive substances due to significant or marked antimicrobial activities.⁶⁻⁹

Cladonia mitis has been well documented for its use as a remedy for cold, arthritis, fever, jaundice and other problems.¹⁰ Similarly, *Parmotrema reticulatum* was used for ring worm- like skin diseases.¹¹ Species of *Everniastrum* is used in wound healing, antiseptic and bronchitis¹². Specimen of *Usnea* has been used for long time in Native American medicine, European medicine and Chinese medicine. Because of its high medicinal value, there is a much demand of these lichens in the market. The fact that lichens play an important role for the treatment of many diseases in rural and backward communities is necessary for further verification of their activity against bacterial pathogen in order to establish scientific rational for its use as antibacterial agents.

Considering the facts, the present study was undertaken to assess the *in vitro* antibacterial activity of some lichen species growing in hill region of Ravangla, Sikkim, India.

MATERIALS AND METHODS

Lichen samples

Samples of lichens *Usnea baileyi* (Stirt) Zahlbr, *Parmotrema reticulatum* (Taylor) M.choisy, *Everniastrum nepalense* (Taylor) Hale exsipman, and *Cladonia mitis* (Sandst) Hustich were collected from Ravangla (Lat 27° 14' 22" N, Long 88° 18' 15" E), Sikkim, India in the year 2009. The collected lichen samples were identified by Dr. Kanad Das, Scientist, BSI, Sikkim, Gangtok, India. Samples were dried at room temperature. Herbarium was prepared and the voucher specimens (Voucher no. LKU002, LKU003, LKU004, LKU005) were deposited in the department of Botany, University of Kalyani, Kalyani, W.B, India for future reference.

Extract preparation

Ten grams of the air dried powdered lichen sample was extracted in 250ml of acetone, methanol and water. Extraction was done in a soxhlet extractor for 72hr at room temperature not exceeding boiling point of the solvent¹³. The extracts were concentrated at 40°C in vacuum with the help of rotary evaporator. The residues thus obtained were kept in a freezer at -80°C until antibacterial screening.

Bacteria tested

One Gram positive (*Staphylococcus aureus*) and four Gram negative bacteria (*E. coli*, *Vibrio cholerae*, *Shigella flexneri*, and *S. dysenteriae*) were used for the present study. All the bacteria used were isolates of ID and BG hospitals, Beliaghata, Kolkata, India. Bacterial cultures were maintained on Mueller- Hinton agar substrates. All cultures were stored and maintained at 4°C and subcultured every 15 days interval.

Antibacterial assay

The determination of the inhibitory effect of lichen extracts was carried out through agar-well Diffusion method by using Mueller and Hinton Agar. Wells measuring 6mm in diameter were cut into the agar and filled with extracts. All the Petri plates were then incubated at 37°C for 24hr. The antibacterial activity was evaluated by measuring the diameter of inhibition zone.¹⁴ Control was maintained with solvent alone.

RESULTS AND DISCUSSION

The distribution and medicinal values of different species of lichen selected for present study was depicted in table 1. The antibacterial activity of aqueous, acetone and methanol extracts of lichens *Usnea baileyi* (Stirt) Zahlbr., *Parmotrema reticulatum* (Taylor) M.choisy, *Everniastrum nepalense* (Tayler) Hale exsipman, and

Cladonia mitis (Sandst) Hustich against the tested bacteria was estimated on the basis of the presence or absence of inhibitory zone and their diameters. The results of screening these lichen extracts for antibacterial activity are summarized in table 2. It was found that all the extracts exhibited antibacterial activity against most of the bacteria. The acetone and methanol extract of *Everniastrum nepalense* inhibited all of the tested bacterial species except *Vibrio cholerae* and *Shigella flexneri*, which turned out to be resistant. The acetone extract of *Cladonia mitis* showed no activity in relation to the tested bacteria. The methanol extract showed maximum antibacterial activity against the bacteria tested. Among the four lichens tested *Usnea baileyi* and *Everniastrum nepalense* showed maximum activity. The aqueous extracts of all the lichen species tested had no antibacterial activity.

Table1
Distribution of lichen species and their medicinal value.

S.No.	Lichen Species	Family	Substratum	Altitude	Medicinal/Folk value
1.	<i>Usnea baileyi</i>	Parmeliaceae	<i>Pinus longifolia</i>	8000ft.	Cleans lungs, resolves phlegm, controls bleeding, removes toxin
2.	<i>Parmotrema reticulatum</i>	Parmeliaceae	<i>Michelia champaca</i>	8000ft.	Anthelminthic, antioxidant value.
3.	<i>Everniastrum nepalense</i>	Parmeliaceae	On dry branches of	8000ft.	Anthelminthic, astringent, laxative.
4.	<i>Cladonia mitis</i>	Cladoniaceae	<i>Araucaria</i> . Rock	8000ft.	Remedy from cold, arthritis, fever, jaundice

Table2
Antibacterial activities of different extracts of lichen.

Lichen species	Extract	Inhibition zone diameter (mm)				
		Sa	Ec	Vc	Sf	Sd
<i>Usnea baileyi</i>	Aq	Nd	Nd	Nd	Nd	Nd
	Ac	28	23	21	30	18
	Me	32	35	35	30	35
<i>Parmotrema reticulatum</i>	Aq	Nd	Nd	Nd	Nd	Nd
	Ac	10	Nd	23	27	20
	Me	Nd	30	20	32	30
<i>Everniastrum nepalense</i>	Aq	Nd	Nd	Nd	Nd	Nd
	Ac	35	33	Nd	Nd	35
	Me	38	35	Nd	Nd	36
<i>Cladonia mitis</i>	Aq	Nd	Nd	Nd	Nd	Nd
	Ac	Nd	Nd	Nd	Nd	Nd
	Me	14	12	13	12	10

Sa *Staphylococcus aureus*, Ec *Escherichia coli*, Vc *Vibrio cholerae*, Sf *Shigella flexneri*, Sd *Shigella dysenteriae*, Aq aqueous extract, Ac Acetone extract, Me Methanol extract, Nd Not detected.

Behera et.al (2005)¹⁵ reported that the acetone and methanol extracts of lichen *Usnea ghattensis* were effective against *S aureus*.

The results obtained in the present study indicated differences in antimicrobial activity between extracts depending on the species of lichen and as a function of the type of extracting solvent. Similar results were recorded by other authors¹⁶⁻¹⁸. These results might be due to the fact that bioactive components of medicinal plants have different solubility in different extracting solvents¹⁹.

Aqueous extracts manifest no activity in relation to the bacteria tested. The reason for weak or no activity of aqueous extracts is that active substances present in the thalli of lichens are insoluble or poorly soluble in water²⁰.

In the present study, the Gram positive bacterium *Staphylococcus aureus* was more

sensitive than Gram negative ones. This difference of sensitivity between Gram positive and Gram negative bacteria can be ascribed to morphological differences in permeability of the cell wall.²¹

Gulluce et.al (2006)²² showed that methanol extract of lichen had stronger antibacterial activity.

This is the first study about antibacterial activity with acetone, methanol and aqueous extracts of the lichens *Usnea baileyi* (Stirt) Zahlbr, *Parmotrema reticulatum* (Taylor) M.choisy, *Everniastrum nepalense* (Tayler) Hale exsipman, *Cladonia mitis* (Sandst) Hustich.

The differences in the antibacterial activity of extracts of different species of lichens are probably a consequence of the presence of different compounds with antibacterial activity. Thus, further study is necessary to characterize the chemical constituents of the extracts of lichen species tested possess compounds with antibacterial properties, which require further studies to determine antibacterial agents for therapy of many diseases in human.



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