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Antibacterial Activity of the Himalayan Lichen *Parmotrema nilgherrense* Extracts

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Research Article

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ABSTRACT

The antibacterial activity of the methanol, ethanol, chloroform and aqueous extracts of the lichen *Parmotrema nilgherrense* collected from Nainital, Kumaun Himalaya, has been investigated. The extracts were tested against five pathogenic bacteria (*Bacillus subtilis*, *Erwinia chrysanthemi, Escherichia coli*, *Agrobacterium tumefaciens* and *Xanthomonas phaseoli*) using agar-well method. All the extracts of *P. nilgherrense* were found effective by showing a mark zone of inhibition (ZOI) except aqueous extract. The chloroform extract exhibited potential antibacterial activity against the tested microorganisms (ZOI, 23-38mm) followed by ethanol and methanol extract (ZOI, 12-24 mm). Solvents treated wells were used as negative control and wells filled with standard antibiotic served as positive control in the experiment. Obtained results showed that *P. nilgherrense* extracts possess a broad-spectrum activity against a panel of bacteria responsible for the most common plants and animal diseases.

Keywords: Lichen, Parmotrema nilgherrense, Antibacterial activity, Kumaun Himalaya;

1. INTRODUCTION

Lichen 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 (Rankovic et al., 2007; Richardson, 1991). Burkholder et al. (1944) reported for the first time the presence of antibiotic substances in lichens. After the discovery of penicillin from a fungus, many lichens were also screened for antibacterial activity time to time (Vartia, 1973).

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Lichens are known to produce various secondary metabolites that are unique with respect to those of higher plants (Lawrey, 1986). These are Phenolic compounds, Debenzofuranes, Usnic acids, Depsidones, Deposones, Lactones, Quinines and Pulvinic acid derivatives (Boustie and Grube, 2005). Some of them are used in pharmaceutical sciences. Lichen extracts have been used for various remedies in folk medicine. Screening of lichens extracts have revealed the frequent occurrence of these metabolites with antibiotic, antimycobacterial, antiviral, antitumor, analgesic and antipyretic properties (Rankovic et al., 2007). Secondary metabolites in lichens are produced by the fungus alone and secreted onto the surface of lichens thallus in crystals form (Ozturk et al., 1999).

In various system of traditional medicine worldwide, including the Indian system of medicine, lichen species are said to be effective in curing of dyspepsia, bleeding piles, bronchitis, scabies, stomach disorders and many disorders of blood and heart (Lal and Upreti, 1995; Negi and Kareem, 1996; Saklani and Upreti, 1992; Sochting, 1999).

Parmotrema nilgherrense is commonly occurring Kumaun Himalayan lichen. It is well developed loboid, grayish in colour foliose lichen growing abundantly on the tree barks (corticolous). As evident from the available literature there is no previous record on the antibacterial activity of this lichen therefore, the present study was carried out to examine the antibacterial activity of chloroformic, methanolic, ethanolic and aqueous extracts of the lichen *P. nilgherrense* against five pathogenic bacteria.

2. MATERIALS AND METHODS

2.1 COLLECTION OF LICHEN MATERIALS

The lichen thalli of *Parmotrema nilgherrense* were collected from the tree bark at Hanumangrah, Nainital, Kumaun Himalaya, (India), during September 2008. The identification was made with the help of pertinent authority and parts of the thalli were also preserved as voucher specimen in the herbarium of the Botany Department, Kumaun University, Nainital.

2.2 EXTRACTION PROCEDURE

The lichen thalli were thoroughly washed, spread on paper sheet and dried at room temperature (20±2°C) in the lab. The dried material was powdered in an electric grinder. To prepare stock solution 10 g of this powder were taken and added to 100 ml of solvents of methanol, ethanol, chloroform and distill water (w/v, 10g/100ml). Each extract was passed through Whatman filter paper No. 1 and the final filtrate- the crude extract 10 % thus obtained was utilized for the experiment.

2.3 TEST ORGANISMS

A total of five bacteria (gram +ve and -ve) were used in this investigation. Microorganisms (*Bacillus subtilis* MTCC No. 121, *Escherichia coli* MTCC No.40, *Agrobacterium tumefaciens* MTCC No.609), borrowed from Institute of Microbial Technology, Chandigarh, India and *Xanthomonas phaseoli* and *Erwinia chrysanthemi* were obtained from Plant Pathology Department, G. B. Pant University, Pantnagar, India.

2.4 SCREENING OF ANTIMICROBIAL ACTIVITY

Antibacterial test of selected microorganism were carried out using Agar well diffusion method (Perez et al. 1990). Nutrient agar media was poured into the Petri plates. A small sterile cotton swab was dipped into the 24 hour old culture of bacteria. Then the dried surfaces of plates were inoculated by streaking the swab over the entire sterile agar surface. This process is repeated by streaking the swab 2 or more times rotating the plates approximately 60^0 each time to ensure even distribution of inoculum. After inoculation the plates were allowed to dry at room temperature (20±2°C) for 15minutes in laminar chamber for absorption to take place. Wells were made in agar plates using a cork borer (7mm diameter) and 200 µl of the extract was added into each well (Fig. 1). The plates were incubated at 37^0 C in dark and observed for inhibition zone (a circular zone formed due to killing effects of extracts,) after 24 hours. Standard antibacterial agent streptomycin (Nicholas Piramal India Ltd., Jaipur) at a concentration of 2 g/ml was also used on all the bacterial organisms as positive control and respective solvent was used as the negative control. The experiments were carried out in triplicate. The diameter of the inhibition zones were measured in millimeter (including well size 7mm) and expressed as mean value with standard error on means.

3. RESULTS AND DISCUSSION

3.1 RESULTS

Lichen *P*. *nilgherrense* belongs to the family Parmeliaceae, was collected from tree bark in the open places and it is a commonly growing species of the locality.

Agar-well diffusion assays of *P. nilgherrense* indicate that crude chloroform, ethanol and methanol extracts have good antibacterial activity against all pathogens tested, while crude aqueous extract exhibited no activity (Table1).

Table 1. Antibacterial activity of different extracts of *Parmotrema nilgherrense*

Microorganisms	Diameter of inhibition zone(mm)*				
	C	E	M	A	S
B .subtilis	35 ± 0.3	20 ± 2.8	12 ± 1.5	na	35 ± 0.3
A. tumefaciens	27 ± 1.5	13 ± 0.6	14 ± 3.0	na	35 ± 0.3
X. phaseoli	38 ± 0.8	13 ± 0.6	14 ± 0.8	na	34 ± 1.0
E. chrysanthemi	23 ± 2.4	24 ± 2.3	20 ± 1.7	na	36 ± 1.8
E. coli	29 ± 1.3	15 ± 0.3	19 ± 1.3	na	36 ± 1.0

** All the values are mean ± SEM of three determinations;*
C, E, M, A- *Chloroform, Ethanol, Methanol, Aqueous extracts,* ***S-*** *Streptomycin (+control),* ***na-*** *not active*

The chloroform extract showed highest antibacterial activity against all the bacterial strains (*Bacillus subtilis*, *Erwinia chrysanthemi, Escherichia coli*, *Agrobacterium tumefaciens* and *Xanthomonas phaseoli*) and it was close to the inhibition zone of commercially available antibiotic drug streptomycin (Fig. 1).

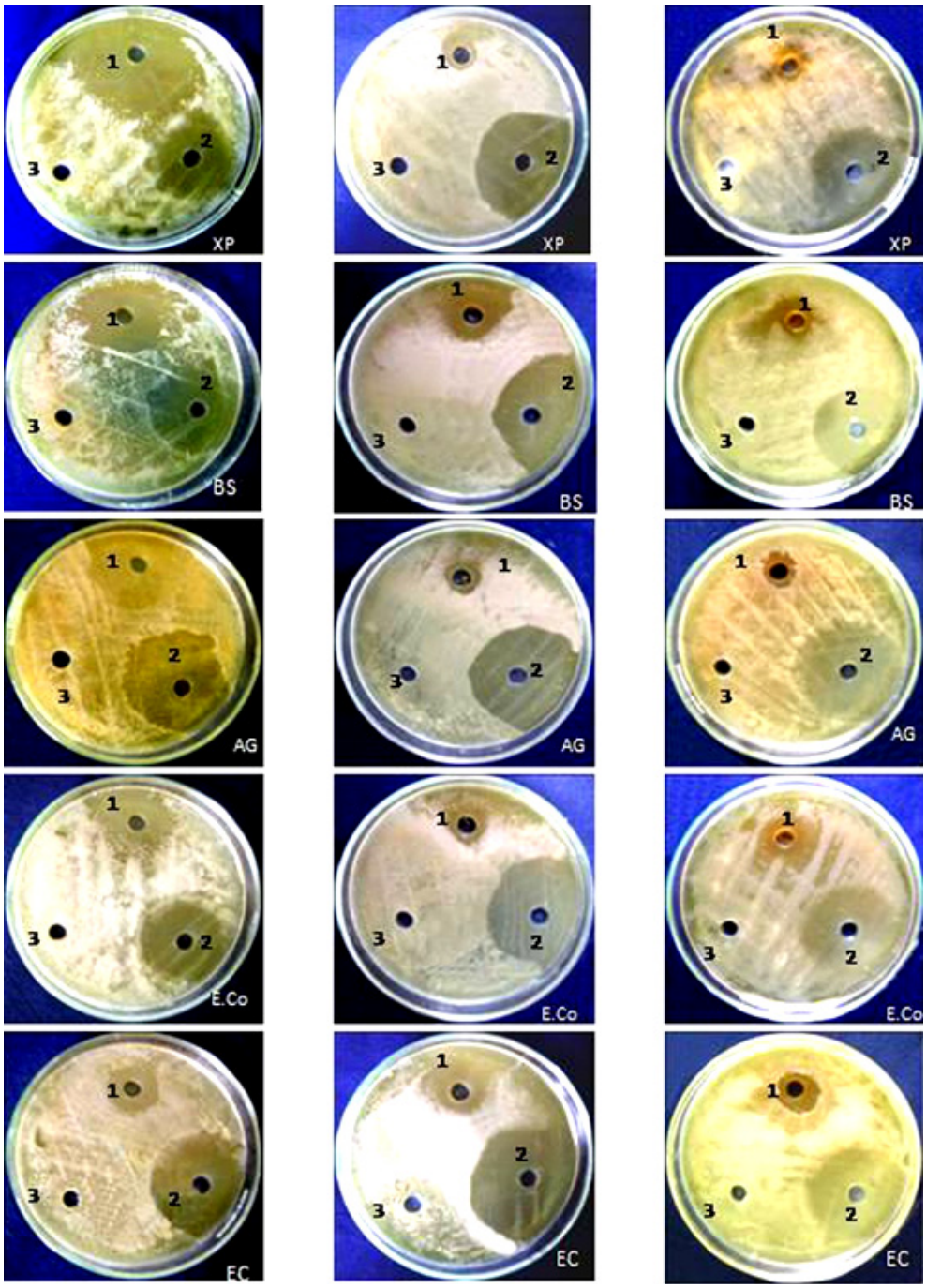

Fig. 1. Antimicrobial activity of *P. nilgherrense* thallus extracts against some pathogenic bacteria

[*(A) - Chloroform extract (B) - Ethanol extract (C) - Methanol extract; XP- Xanthomonas phaseoli; BS- Bacillus subtilis; AG- Agrobacterium tumefaciens; E. Co. - Escherichia coli; EC- Erwinia chrysanthemi;1- Extract, 2- positive control (streptomycin), 3- negative control (solvent)]*

It is interesting to note that this extract showed highest activity against *X. phaseoli* (38 ± 0.8mm) which was higher than the standard drug Streptomycin (34 ± 1.0mm). Ethanol and methanol extract of this lichen showed moderate activity against *Bacillus subtilis, Agrobacterium tumefaciens* and *Xanthomonas phaseoli* while both extracts showed maximum activity against *Erwinia chrysanthemi*, having inhibition zone of 24 ± 2.3mm and 20 ± 1.7mm respectively (Fig. 2). As aqueous extract were completely inactive against all the pathogenic bacteria tested therefore, aqueous extract bar did not appear in the graph.

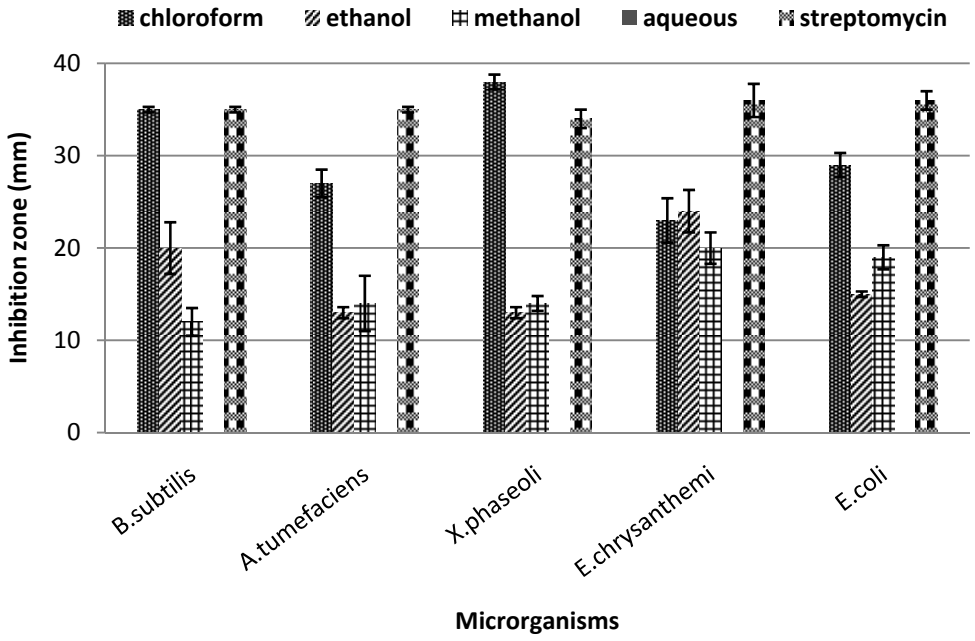

Fig. 2. Antibacterial activity of *P. nilgherrense* against some bacterial strains

3.2 DISCUSSION

The search for novel bioactive compounds from natural resources to improve pharmaceutical, cosmetic and agriculture applications is an ancient practice and currently it is regaining more rapid importance. The Lichen compounds are not an exception in this field. Currently the interest on the lichen secondary compound is increasing because of ineffectiveness of some known previously reliable drug (Huneek, 1999).

The present bioassay with crude extracts of *P. nilgherrense* against Gram positive and Gram negative bacteria show that the chloroform extract had highest activity against all the bacterial strains. This is one of the promising results of present investigation as in some instances zone of inhibition reached more than the inhibition caused by standard drug streptomycin (Fig. 2). This suggests that the chloroform, which is a weak polar solvent, can dissolve maximum bioactive compound of the tested lichen and supports the findings of other workers (Ozturk and Guvene, 1995; Dulger et al., 1998 and Ylmaz et al., 2004).

The polar solvent methanol and ethanol extracts showed comparatively less inhibitory effect against the tested bacteria and indicates that the used lichen possess less active methanol

and ethanol soluble compounds. On the other hand, the aqueous extract of *P. nilgherrense* was found not effective against all the pathogens tested and suggest that it does contain no any bioactive compounds to inhibit the tested strains of bacteria (Fig. 2).

In a study, Balaji et al. (2007) reported that methanol, acetone, hexane, dichloromethane extracts of *P. praesorediosum* showed inhibitory activity against some bacteria. However, Kharel et al. (2000) and Behra et al. (2008) isolated compounds like dehydrocollatolic acid from *P. nilgherrense* and salazinic acid from *P. tinctorum* respectively, and conducted antibacterial activity against some animal pathogenic bacteria. The present study also supports their finding, as the crude extracts possess all these active compounds, which are responsible for the inhibition of bacterial growth. Lowwhoff and Crisp (2002), have also recovered malanoprotocetraric acid from *P. conformatum,* but they did not test it for its antibacterial activity.

4. CONCLUSION

The results obtained in the present study can be concluded that *P. nilgherrense* have broad spectrum of antibacterial potentiality. All the organic solvent extract of this lichen *viz.,* chloroform, ethanol and methanol possess significant inhibitory activity against the plant and animal pathogenic bacteria. Hence, this lichen *P. nilgherrense* can be a potential source for evolving newer antibacterial compounds.
This study also provide a new leads as there is no previous record on the antibacterial activity of *P. nilgherrense* extracts against the plant pathogenic bacteria (*X. phaseoli, A. tumefaciens* and *E. chrysanthemi*) responsible for various plant diseases like crown gall, leaf blight, leaf spot and rot diseases.

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