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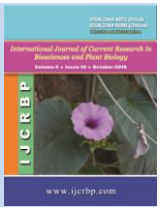

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Antifungal Activities of Foliose Lichen *Punctelia subrudecta* from Nainital, Uttarakhand, India

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ABSTRACT

Punctelia subrudecta (Nyl.) Krog is common foliose lichen collected from different locations of Nainital. In this study antifungal activity of methanol and acetone extracts of *P. subrudecta* beside of five plant pathogen fungi like *Aspergillus niger*, *Alternaria alternata, Fusarium solani, Albugo candida* and *Penicillium citrinum* has been selected for the study. The acetone extract were found more effective followed by the methanol extracts. The results indicate that the activity of lichen extract vary with the type of solvent used. *P. subrudecta* separates show an expansive range action against the tried plant pathogenic growths and could be of huge use as a potential antifungal.

Introduction

Lichens are symbiotic organisms composed of a fungus (mycobiont) and an alga (photobiont) and produce characteristic secondary metabolites which seldom occur in other organisms. Lichen metabolites exert a wide variety of biological actions including antibiotic, anti mycobacterial, antiviral, anti-inflammatory, analgesic, antipyretic, anti proliferative and cytotoxic effect. Even though of manifold activities only few active substances, however, are used by mankind because of the limited supply of natural lichens and their inability to grow under laboratory conditions. Lichen metabolites exert a wide variety of biological effects yet their therapeutic potential has not been fully explored. Since lichens are very slow growing organism exploitation for their secondary metabolites could threaten their survival. Lichen mycobiont grows much faster than natural thalli and produces the secondary metabolites in culture conditions, but it still grows slowly than other micro organisms. It is necessary to improve the growth rates of the cultured mycobiont in order to enlarge the access to lichen derived substances which possess possible application in pharmaceutical research. Lichens produce characteristic secondary metabolites that are unique

with respect to those of higher plants (Hale, 1983; Lawery, 1986; Richardson, 1991).

The lichen substances are extracellular products of relatively low molecular weight crystallized on the hyphal cell walls, they are usually insoluble in water and can be extracted into organic solvents (Ötzürk et al., 1999). Lichen metabolites known to have manifold biological activities as antiviral, antitumor, allergic, plant growth inhibitory, antiherbivore, ecological roles and enzyme inhibitory (Huneck, 1999; Dügler et al., 1997 and 1998). The antibiotic screenings of Hiamlayan lichens have been recently initiated. The lichens constitute the major biomass of lichens in the Himalayan forest and can be used for their screening as antifungal agents. Thus, the present investigation deal with the *in vitro* evaluation of the antifungal activity manifested by acetone and methanol extracts of *P. subrudecta* commonly found in Nainital district, collected from different locations of the district.

Materials and methods

Preparation of lichen sample

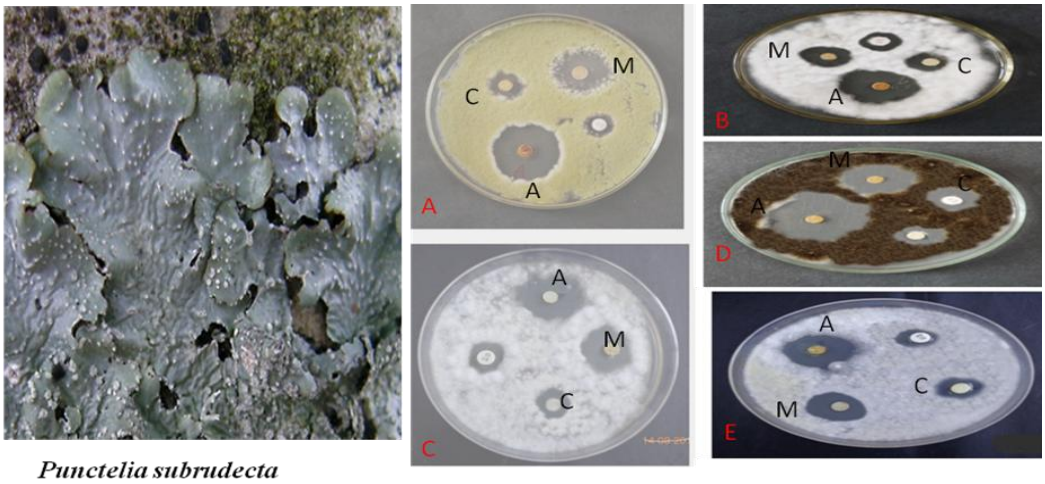
The lichen sample was collected and studied, morpho-anatomically, chemically and identified at the species level with the help of relevant keys (Awasthi, 2007) using a Labomed stereomicroscope and Leica DM 500 light microscope. The lichen substances were identified with the help of thin-layer chromatography (TLC) following the method of Elix et al. (1993) and Orange et al. (2001).

Extraction from lichen sample

Two different solvent systems such as Acetone and Methanol were used. The sample was sterilized and 10 g of dried lichen sample were taken and wrapped in 8-6 cm cylindrical thimble made up of Whatman filter paper grade 1 and kept inside the extractor of Soxhlet apparatus equipped with a reflux condenser. The solvent extraction was carried out at the specific boiling temperature of the solvents acetone 56°C, methanol 65°C and chloroform 61.2°C for 48 h for complete extraction of secondary compounds. The residues extracted were recovered from the solvents using Buchi^{TM} rotavapor.

Microorganisms and media

Ten fungal strains were procured from the mycological collection maintained by the Mycological Laboratory within the Department of Singhania University, Rajasthan. The fungi used as test organisms were: *Aspergillus niger*, *Alternaria alternata*, *Fusarium solani*, *Albugo candida* and *Penicillium citrinum*. Fungal cultures were maintained on Potato Dextrose agar (PDA) and Sabourad dextrose agar (Fig. 1).



Punctelia subrudecta

Fig. 1: Antifungal activity of lichen *Punctelia subrudecta* against A) *Aspergillus niger*, B) *Alternaria alternata*, C) *Fusarium solani*, D) *Albugo candida* and E) *Penicillium citrinum*.

Results and discussion

P. subrudecta contains lecanoric acid as its major lichen substance where as atranorin acid is produced as a minor substance. Gomes et al. (2002) reported the antifungal activity of lecanoric acid isolated from *Parmotrema tinctorum* against *Cladosporium sphaerospermum.* Disc diffusion assays of *P. subrudecta* indicate that the crude acetone and methanol extracts showed antifungal activity against all the test organisms while chloroform extract exhibited activity against (Table 1). The methanol extract exhibited highest antifungal activity against all the five tested fungi. The acetone extract was also effective and both the extracts exhibited zones of inhibition close to the commercially available antifungal Ketoconazole. It was observed that in case of fungi *Fusarium solani* Ketoconazole was found ineffective whereas the acetone extract showed zones of inhibition against *F. solani* (10.3 ± 0.3 mm). Similarly, methanol extract was also effective against *F. solani* and exhibited zones of inhibition of 17.6 ± 0.3 mm and 18 ± 0.5 mm diameter respectively.

Table 1. Antifungal activities in different solvent.

Plant pathogenic fungi	Diameter of inhibition zone (mm)*	
	Acetone extract	Methanol extract
Aspergillus niger	14.7 ± 0.3	19 ± 0.6
Alternaria alternata	8.3 ± 1.2	8.7 ± 0.3
Albugo candida	13 ± 0.6	18.3 ± 1.2
Fusarium solani	10.3 ± 0.3	17.6 ± 0.3
Penicillium citrinum	14.3 ± 0.6	24.3 ± 1.2

*Values are in Arithmetic mean ± Standard error

It was interesting to note that the activity of acetone (14.3 ± 0.6 mm) and methanol (24.3 ± 1.2 mm) extracts was better than Ketoconazole (11 ± 0.7 mm) whereas chloroform extract exhibited no activity against *P. subrudecta.* All the two solvents extracts were active against a particular tested fungi *Fusarium solani* and exhibited zones of inhibition better than commercially available antifungal Ketoconazole (11 ± 0.3 mm). The methanol extract showed highest activity of 33 ± 1.5 mm followed by acetone extract exhibiting inhibition zone of 28.3 ± 0.8 mm diameter, whereas chloroform extract showed zone of inhibition of diameter 14.7mm. Bioactive compounds in recent past are gaining edge over traditionally known drugs because of their improved effectiveness against pathogens, the lichen compounds are not an exception in this field (Huneck, 1999). Extracts of lichen thalli proved to have strong antifungal activity against various plant pathogenic fungi (Gulluce et al., 2006; Halama and Van Haluwin, 2004). Gomes et al. (2002) reported that the lecanoric acid isolated from *Parmotrema tinctorum* have good antifungal activity.

Conclusion

The result shows extracts of selected species against some well known plant pathogenic fungi. The selective antifungal effect of acetone and methanol extracts of test lichens can be attributed to the presence of different constituent secondary metabolites in lichen thalli (Goel et al., 2011; Halama and Van Haluwin, 2004). The effective results are found in the extracts against commercially available antifungal Ketoconazole against some (*Aspergillus niger, Penicillium citrinum* and *Albugo candida*) these pathogenic fungi suggested as potentials source for fungicides.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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