Mycopath (2013) 11(1): 15-17 **Research Article**

Antifungal efficacy of *Hyophila rosea* (Bryophyta: Pottiaceae)

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Abstract

In vitro antifungal activity of the ethanol, methanol and chloroform extracts of *Hyophila rosea* Williams were investigated against three fungal species namely *Aspergillus flavus* (Montagne) de Bary, *Alternaria alternata* (Fries) Keissler, and *Phytophthora infestans* (Montagne) de Bary using disc diffusion method. All the three extracts exhibited significant efficacy against fungi in comparison to the synthetic bifonazol. Chloroform extract showed maximum antifungal activity against *P. infestans* followed by *A. flavus* and *A. alternata*.

Key words: Antifungal activity, bryophyta, disc diffusion method, *Hyophila*, phytopathogens.

Introduction

Plants have been known as eventual natural source of medicine. They always provide specific bioactive compounds of medicinal use. Estimation of medicinal properties of higher plants is a straightforward and usual choice of the workers. However, even if with diverse medicinal value, the lower plants such as bryophytes, known for their antibacterial and antiviral activities are not assessed upto the larger level (Alam, 2012a). They are well recognized to have several secondary metabolites of antimicrobial activities by several workers (Asakawa, 2001; Banerjee, 2001; 2004; Frahm, 2004). They have been used as medicinal plants in traditional medicines for a long time. The antimicrobial activity is because of compounds like aromatic and phenolic substances, phenylquinone, oligosaccharides, polysaccharides, sugar alcohols, amino acids, fatty acids, aliphatic compounds provide protection against these microorganisms, and therefore, bryophytes have the potential for medical use (Ando and Matsuo, 1984). Their use against skin infection and in wound treatment had been revealed since long time. Beside these facts these amphibians of plant kingdom are usually neglected because of their selective habitat, identification and low biomass. Nevertheless, many taxa have been reported recently as potent antimicrobial agents worldwide (Lahlou *et al*., 2000; Illhans *et al*., 2006; Veljic *et al.*, 2010; Alam *et al.*, 2012b). The present work was, therefore, carried out to evaluate the *in vitro* antifungal activity of extracts of *H. rosea*, a commonly growing moss taxon in Rajasthan (India) against *A. flavus*, *A. alternata* and *P. infestans.*

Materials and Methods

Collection and identification of selected moss

H. rosea, growing abundantly in xeric regions of Rajasthan, India was collected from native habitat from Banasthali Vidyapith Campus, Tonk (Rajasthan) during rainy season (August-September, 2013). The identification was done morpho-anatomically using stereoscopic zoom microscope. Identification was finalized using relevant key and available monograph (Gangulee, 1969-1980). The specimens were deposited at the Banasthali Vidyapith Herbarium (BVH), Tonk (Rajasthan), India.

Extraction from plant

Fresh plants were treated with Tween 20 to remove surface microflora. Afterwards, samples were cleaned and shade dried prior to extraction. Three different solvent systems ethanol, methanol and chloroform were used. A dried sample (5 g) was powdered and extracted separately with 70% organic solvents for 24 h at 40 ^{o}C. The extract was filtered with cellulose acetate membrane paper. The filtrate was evaporated until dry with a rotary evaporator and then dry extract was dissolved in 1 mL dimethyl sulfoxide (Illhan *et al*., 2006).

Procurement of fungi

Three fungal species [*A. flavus* (MTCC 8834); *A. alternata* (MTCC 8459) and *P. infestans* (MTCC 48720)] were obtained from the Microbial

Type Culture Collection and Gene bank (MTCC) and maintained by Microbiology Laboratory, Department of Bioscience and Biotechnology, Banasthali Vidyapith, Tonk. Fungal culture was maintained on potato dextrose agar (PDA) and was transferred to sabouraud dextrose agar (SDA) for experimental use.

Assessment of antifungal activity by disc diffusion method

Antifungal activity of moss against the selected fungi was determined using disk diffusion method. Disc of the respective culture was placed in the centre of Petri dish. Sterilized filter paper discs were then individually impregnated with test concentrations (0.05-1 mg $disc^{-1}$), air dried under sterilized conditions and were placed on the inoculated Petri dishes with Bifonazol (50 µg mL^{-1}) as positive control. Petri dishes were incubated at 27 ^{o}C for 72 h. The antifungal activities of different extracts were evaluated by measuring the diameter of inhibition zone. Antifungal activities were expressed in terms of per cent inhibition (PI). Each extract was tested in triplicate and experiment was performed three times.

Percentage Inhibition (PI) = (X-Y/Z-Y) × 100

Where X is the inhibition zone diameter of control; Y is the inhibition zone diameter of solvent, and Z is the inhibition zone diameter of positive control.

Results and Discussion

In comparison to positive control, all the three plant extracts showed better inhibitory efficacies on the growth of selected fungal strains. However, chloroform extract exhibited the highest antifungal activity against all the three selected fungal strains. Whereas, *P. infestans* was inhibited the most significantly (PI=12.5) followed by *A. flavus* (PI= 6.6) and *A. alternata* (PI= 6.2) (Fig. 1).

The antifungal property of bryophytes is well documented against wide range of plant pathogens (Daouk *et al*., 1995; Veljic *et al*., 2010) and it was evident in *H. rosea* also. The possibly mode of action of these plant extracts include few cellular changes such as granulation of cytoplasm and deformities in cell wall structure, ultimately affects the overall growth of hyphae and subsequent mycelia (Sharma and Tripathi, 2008; Alam *et al*., 2011). Usually the methanolic extract are more potent but in present study chloroform emerged as a better option (Hanel and Raether, 1988). The nature of active components involved in each extract is not clear, though they are promising. This findings can form the foundation for further studies to plan an optimize preparation of the herbal extracts to further estimate them against a wider range of fungal strains. The possible antifungal constituents can be isolated at mass scale by using advance techniques like tissue culture so that they can be use in economical and eco-friendly manner to control these phytopathogens.

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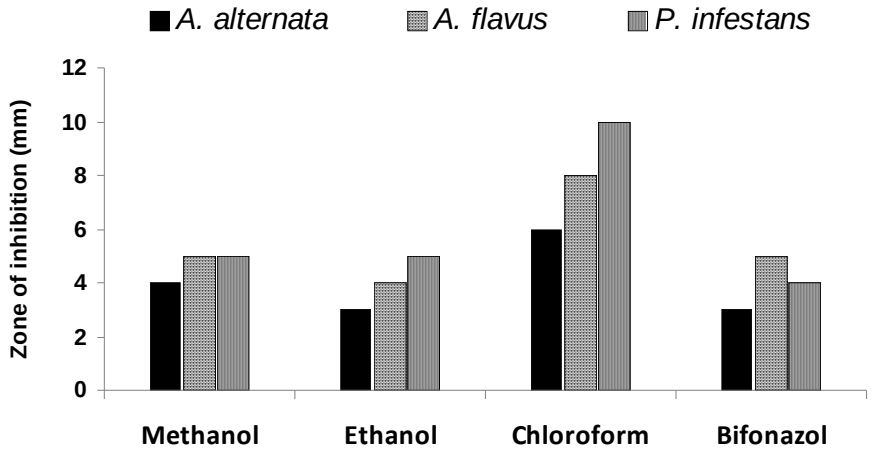

Fig. 1: Comparative antifungal activity of different extracts of *Hyophila rosea*

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