Mycopath (2015) 13(2): 89-92 **Research Article**

Comparative assessment of antifungal efficacy of *Anoectangium clarum* Mitt. and *Hyophila spathulata* (Harv.) A. Jaeger

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Abstract

In this study, the antifungal activity of *Anoectangium clarum* Mitt. and *Hyophila spathulata* (Harv.) A. Jaeger. was tested against three different fungal species viz. *Aspergillus niger* van Tieghem, *Fusarium solani* (Mart.) Sacc. and *Trichoderma viride* Pers. Five extraction solvents were used, i.e. acetone, chlororform, distilled water, ethanol and methanol. The activity was tested by the disc-diffusion method. Ethanol and methanol extracts at concentrations of 4% and 5% of the selected mosses showed significant antifungal activity against the fungal strains. In more specific and comparative manner, it is concluded here that *Hyophila spathulata* was even more effective antifungal agent than *Anoectangium clarum*.
Keywords: Antifungal, *Anoectangium clarum*, *Hyophila spathulata*, moss, solvents.

Introduction

Plants have been the important medicinal source for humans since the inception of civilisation. They are always the usual and the first choice of the medicine men of ancient time and contemporary scientists too. The medicinal properties of the plants have considered specific, reliable and at times novel source of bioactive compounds (Gibbons, 2004).

Today, various environmental problems have occurred due to the frequent usage of synthetic fungicides in agriculture and horticulture. Hence, scientists now very actively working in this direction to restrict the usage of hazardous chemicals and are eagerly looking for the natural and plant derived substitute (Rani *et al*., 2009). Initially, angiosperms were the first choice due to multifaceted ease, but in recent years, bryophytes have drawn remarkable attention because of their antimicrobial properties (Sharma and Verma, 1991; Sharma, 1992; Bishop and Thorton, 1997; Asakawa *et al.*, 2000). Bryophytes, being the first land plants and at many times used for treating various skin problems and wound healing purposes because of the conventional belief that they protect the skin and open wounds from pathogenic attacks (Flowers, 1957).

Bryophytes are used unadventurously in various communities for 400 years, for instance *Polytrichum* and *Fissidens* species as diuretics and hair stimulating drugs (Asakawa, 1990) and species like *Bryum*, *Mnium* and *Philonotis* are also very common among North American Indians for burns and bruises (Ilhan *et al.*, 2006). Many different species of bryophytes exhibit antimicrobial effects against various strains of fungi and bacteria (Subhisha and Subramoniam, 2005; Sabovljevic *et al.*, 2006; Bodade *et al.*, 2008; Dülger *et al.*, 2009; Alam *et al.*, 2011; 2012). The phytochemistry of different species of bryophytes has been investigated by scientists and have confirmed the presence of many secondary metabolites known for antifungal and antibacterial properties (Asakawa, 2001; Frahm, 2004). All these antimicrobial features are due to the presence of phenolic and aromatic compounds like phenylquinone, oligosaccharides, polysaccharides, sugar alcohols, amino acids, fatty acids, etc., and hence are medicinally used (Ando and Matsuo, 1984). In spite of all mentioned importance these 'amphibians of the plant kingdom' are not thoroughly studied and exploited yet (Alam, 2012).

In this study, the antifungal effect of different extracts of two most commonly grown moss species (*Anoectangium clarum* and *Hyophila spathulata*) in Rajasthan (India) was investigated against *Aspergillus niger* van Tieghem, *Fusarium solani* (Mart.) Sacc. and *Trichoderma viride* Pers.

Materials and Methods

Plant material

Anoectangium clarum Mitt. was collected from Mt. Abu (Western Rajasthan) and *Hyophila spathulata* (Harv.) A. Jaeger. from Banasthali

Vidyapith Campus, Tonk (Rajasthan) in the month of August and September 2015. The identification was confirmed using relevant key and published monographs (Gangulee, 1969-1980). The specimens were then deposited at the Banasthali University Rajasthan, India (BURI) Herbarium.

Preparation of extracts

The samples were washed first with tap water and then distilled water and were air dried in the shade. The material was further ground into fine powder and about 5 g of this powder was exhaustively extracted with 50 mL of 95% alcohol for over 24 h. The liquid was then filtered and centrifuged at 4000 rpm for 10 min and then the obtained filtrate was consecutively separated in different solvents (acetone, chloroform, distilled water, ethanol and methanol). The homogenous surface liquid of the crude solvent extracts was used to impregnate the diffusion discs with antifungal activity.

Fungal Strains

Three fungal species *Aspergillus niger* van Tieghem (An; MTCC282), *Fusarium solani* (Mart.) Sacc. (Fs; MTCC350) and *Trichoderma viride* Pers. (Tv; MTCC2417) were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh. All fungal cultures were maintained on potato dextrose agar (PDA) and their spore suspension was made by adding 3-5 mL sterile distilled water to the agar tubes. These were well shaken in order to release the spores and then this suspension was used as a base layer in petri plates.

Disc diffusion method

The antifungal efficacy of the selected moss species was determined using disc diffusion method (Gould and Bowie, 1952; Anna King and Brown, 2001). Discs of the respective plant samples with concentrations varying from 1-5% were placed in petri plates with a lower base layer of PDA and upper base layer of fungal spore suspension. Fluconazol (10 µg mL^{-1}) disc was placed in the centre of the petri plate as positive control. The petri plates were then incubated for 24 h at 37 °C. The activity was measured in terms of zone of inhibition (ZOI) in mm.

Results and Discussion

In this experiment, we tested the resistance of two bryophyte species, i.e. *Anoectangium clarum* and *Hyophila spathulata* against three fungal strains viz. *Aspergillus niger*, *Fusarium solani* and *Trichoderma viride*. In order to compare their activity, a synthetic fungicide fluconazole was used as positive control. According to the obtained results, it is very much evident that these 2 species of bryophytes showed significant antifungal activities at different concentrations. In fact, *Anoectangiun* and *Hyophila* at concentrations of 4% and 5% of all extracts showed better results than the fluconazole itself. If we compare both plant species, they both exhibited a great noticeable activity against the test organisms at all concentrations. However, most significantly at 4% and 5% concentrations of acetone, choloroform, ethanol and methanol. Comparatively it was observed that *Hyophila spathulata* shows even more differentiated resistance against the selected fungal strains at 3%, 4% and 5% conc. of ethanol and methanol than *Anoectangium clarum* (Fig. 1-3).

On the basis of this study, it can be concluded that both plant species have considerable antifungal properties even against those fungus which are known as resistant species (Castaldo *et al.*, 1988; Alam *et al.*, 2011; Alam, 2013). This study helped us to find the natural antifungal agents in the form of these two important moss species which can be used in the control of fungal attacks in eco-friendly way.

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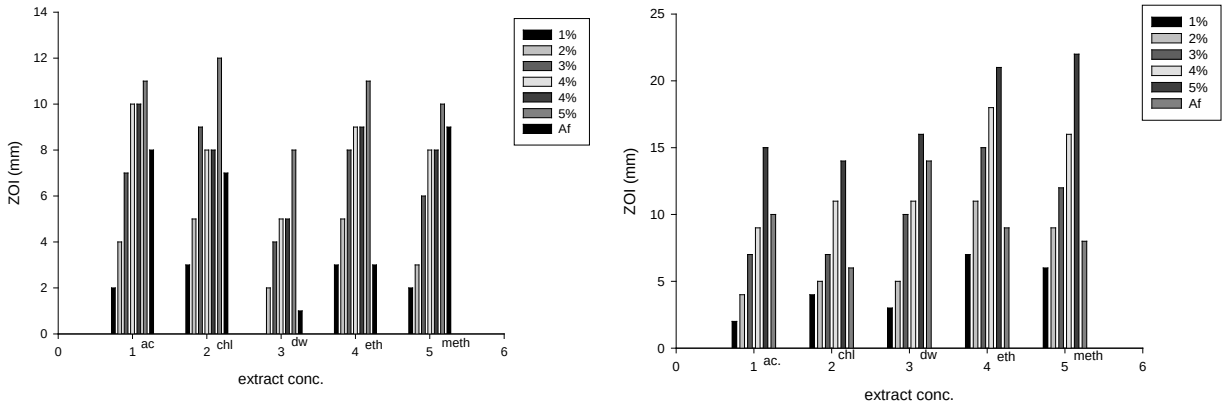

Fig. 1: Antifungal activity of *Anoectangium clarum* (left) and *Hyophila spathulata* (right) extracts at different concentrations (x-axis) of solvents against *Aspergillus niger*.

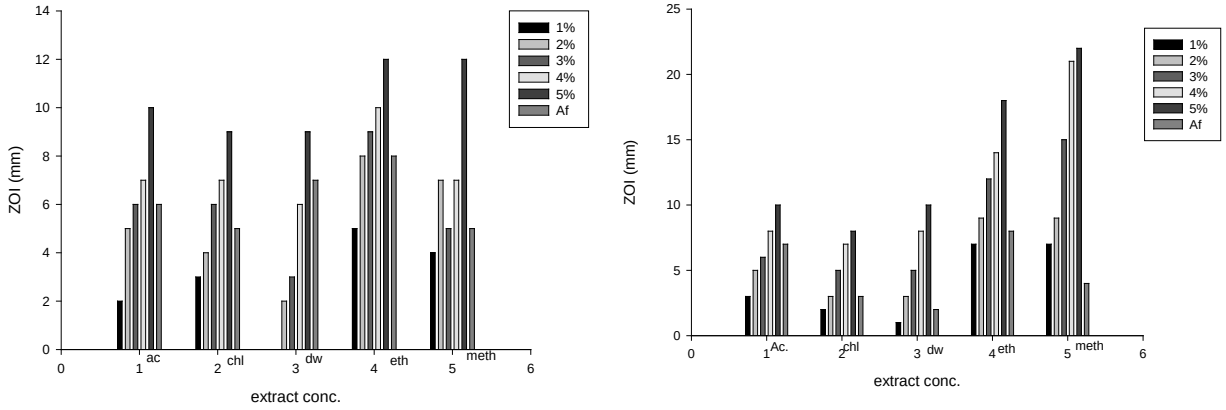

Fig. 2: Antifungal activity of *Anoectangium clarum* (left) and *Hyophila spathulata* (right) extracts at different concentrations (x-axis) of solvents against *Fusarium solani.*

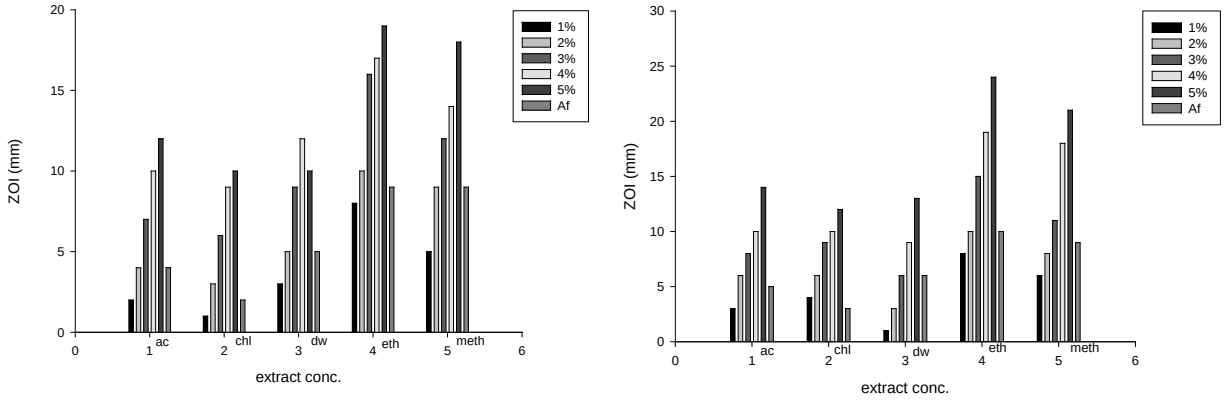

Fig. 3: Antifungal activity of *Anoectangium clarum* (left) and *Hyophila spathulata* (right) extracts at different concentrations of solvents against *Trichoderma viride.*

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