Antifungal activity of *Plagiochasma rupestre* (Forst.) Steph. extracts

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Abstract: Methanol extracts of the thalloid liverwort *Plagiochasma rupestre* (Frost.) Steph. was evaluated against three plant pathogenic fungi: *Alternaria alternata*, *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma viridae*, *Phytophthora infestans*, *Fusarium oxysporium* f. sp. *gladioli*, *Penicillium expansum*. *In vitro* antifungal activity was assessed by micro dilution method, which exhibit total or strong inhibition on *Trichoderma viridae, Aspergillus niger* and *Phytophthora infestans* growth. These results confirm the antifungal activities in liverwort extracts.
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Key words: Bryophyte; Liverwort; *Plagiochasma rupestre*; Plant pathogenic fungi.

1. Introduction

Since ancient time bryophytes has been used as medicinal plants in customary medicines. They are used for treatment of various skin problems and wounds (Flowers, 1957). Various kinds of biological activities are reported so far from bryophytes (Asakawa, 2001; 2004). Conventional medical use of bryophytes in China initiated more than 400 years ago. For example, *Polytrichum* and *Fissidens* species were used as diuretic and hair growth stimulating drugs in China (Asakawa, 1990). Moreover, North American Indians used *Polytrichum juniperinum*, *Bryum, Mnium* and *Philonotis* mosses to heal burns, bruises and wounds (Ilhan et al., 2006).

Many other bryophytes also exhibit antimicrobial effects against fungi and bacteria (Basile et al, 1998a; 1998b; 1999; Banerjee, 2001; Frahm and Kirchhoff, 2002; Scher et al., 2004; Subhisha and Subramoniam, 2005; Sabovljevic et al., 2006; Bodade et al., 2008; Dülger et al., 2009). Liverwort like *Marchantia tosana* exhibited antifungal, antibacterial and antitumour activity (Lahlou et al., 2000). It has also been shown that *Ptilidium pulcherrimum* have antibacterial and antifungal activity (Veljic et al., 2010).

Almost all species of bryophytes are not damaged by fungi, bacteria, insect larvae (Asakawa, 2001) because, biological compounds like phenylquinone, aromatic and phenolic substances, oligosaccharides, polysaccharides, sugar alcohols, amino acids, fatty acids, aliphatic compounds in bryophytes provide protection aganist these organisms, therefore, bryophytes have the potential for medical use ((Asakawa, 1990; 1981; 1984; Askawa et al., 2000).

At present, several environmental problems are caused by the rigorous use of synthetic fungicides in agriculture. Natural plant-derived products for agriculture have less shock on the environment. Usually, the secondary metabolites produced by bryophytes are known for antifungal and antibacterial properties. However, various studies have confirmed that extracts and bryophytes play various biological roles and appear to function as allelopathic agents in nature.

In this study, we investigated the liverwort *Plagiochasma rupestre* (Figure 1) which is a terricolous taxa, it grows on exposed rocks and soil surfaces. This species is well known from all the bryo-geographical regions of the world. The aim of this work was to determine the antifungal effects of this liverwort and to make a contribution to the pharmaceutical botany studies to be done in the future in India.

2. Materials and Methods

Plant material:

Plant materials of this study were collected from the Mount Abu, western Rajasthan, at an altitude of 1600m, 24°31' to 24°43'N and 72°38' to 72°53' E, in August 2011. Specimens (BHBV 78604-BHBV 78620/2011; Legit.: A. Alam and S. C. Sharma; Det.: A. Alam) are deposited in the Bryophyte Herbarium of Banasthali Vidyapith (BHBV), Rajasthan (India).

Pytopathogenic Fungi:

The phytopathogenic fungi were obtained from the culture collection of Plant Pathology Lab, Department of Bioscience and Biotechnology, Banasthali University. The fungal species used in the experiment were *Alternaria alternata* BVPPL 08 (Aa), *Aspergillus niger* BVPPL 17 (An), *Aspergillus flavus* BVPPL 28 (Af), *Trichoderma viridae* BVPPL 44 (Tv), *Phytopthora infestans* BVPPL 53 (Pi), *Fusarium oxysporium* f. sp. *gladioli* BVPPL 33 (Fog), *Penicillium expansum* BVPPL 58 (Pe). The culture of the phytopathogenic organisms were maintained on the PDA at 4^{o}C.

Preparation of the extracts:

A Sample (10 g) was treated with 0.8% Tween 80 aqueous solution to remove epiphytic hosts found on the plant surface. Then, the samples were washed in tap and distilled water, and dried on filter paper. The samples were extracted with methanol (100 x 2ml) for 24 h at 40°C. the extract was filtered with a cellulose-acetate membrane. The filterate was evaporated until dry with a rotatory evaporator and 100 mg of dry extract was then dissolved with 1 ml dimethyl sulfoxide (Ilhan et al., 2006).

Determination of antimicrobial activity:

Micro dilution method: The modified micro dilution method was also used to obtain quantitative data for the compounds under study (Hanel and Raether, 1998; Daouk et al., 1995). The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v) and adjusted with sterile saline to a concentration of 1.0×10^5 in a final volume of 100 µl/ml. the inocula were stored at 4°C for further use. Dilutions of the inocula were cultured on solid MA to verify the absence of contamination and to check the validity of the inoculum.

Minimum inhibitory concentration (MIC) determination was performed by serial dilution technique. The investigated compounds were dissolved in broth medium with inoculum to obtain the required concentration (0.05-20 mg/ml). microplates were incubated at 28°C for 72 h. The minimum fungicidal concentration (MFCs) was determined by serial sub cultivation of 2 µl into microtitre plates containing 100 µl of broth per well and further incubation at 28°C for 72 h. The lowest concentration with no visible was defined as MFC, indicating 99% killing of original inoculum. DMSO was used as control and Bifonazol was used as positive control.

3. Results and Discussion

In the present study, the antifungal activity of the methanol extract of *Plagiochasma rupestre* was tested against six fungal species. For comparison of antifungal activity a synthetic fungicide Bifonazol was used. According to obtained results it is evident that the extract of *Plagiochasma rupestre* at a concentration of 20mg/ml has significant antifungal activity. In case of *Alternaria alternata*, *Trichoderma viridae*, *Penicillium expansum* and *Phytophthora infestans* this concentration showed better inhibition than the Bifonazol. The concentration of 2.5 mg/ml or higher exhibit a more significant antifungal activity against the susceptible fungal species (Table 1).

4. Conclusion

On the basis of the present study it can be concluded that the extracts of *Plagiochasma rupestre* show considerable effect as antifungal agent. Moreover, fungus like *Trichoderma viridae* and *Phytophthora infestans*, which are known as resistant species were also sensitive to this extract. The present study also showed that selected liverwort has significant antifungal activity against four out of seven selected fungi. The results obtained are similar to some researchers' report that extracts from mosses and liverworts exhibit antifungal activities (Castaldo et al., 1998; Alam et al., 2011). This study helps in the establishment of bryophytes as bio-control agents against infectious diseases caused by these fugal species.

Table 1. Antifungal activity of *Plagiochasma rupestre* methanol extract assessed by micro dilution method

Concentration of plant extract and Bifonazol (mg/ml)

	P. rupestre		Bifonazol	
Fungal Spp.	MIC	MFC	MIC	MFC
Aa	0.5	2.5	1.0	1.0
Af	0.5	2.5	0.1	0.1
An	2.5	5.0	0.1	0.1
Fog	0.5	2.5	0.5	1.0
Pi	0.5	2.5	1.0	1.0
Tv	0.5	2.5	1.0	1.0
Pe	0.5	2.5	1.0	1.0

Figure 1: A patch of *Plagiochasma rupestre* (Frost.) Steph. (BHBV 78620/2011)

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