

ANTIFUNGAL POTENTIAL OF *BRYUM CELLULARE* AGAINST SOME COMMON DISEASES OF MAIZE

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ABSTRACT

Present study was carried out to evaluate the antifungal properties of *Bryum cellulare* (moss) extracts on fungus *Drechslera maydis*, the causal organism of southern corn leaf blight using hanging drop method. Ethanol, methanol and aqueous extracts were used against selected test fungi for antimicrobial assay. Phytochemical screening of the extracts was also carried out to determine the active antifungal substances. The results showed that all the extracts possess significant antifungal activity but to varying degrees. The highest inhibition in spore germination per cent was observed in ethanolic extract of *Bryum cellulare*. The percentage of spore germination was 66.9 to 21.63 from 10 to 100 per cent concentrations of the extract.

KEYWORDS: Bryophytes, *Bryum cellulare*, Antifungal Potential, Phytochemical Screening

INTRODUCTION

Bryophytes are the second largest group of terrestrial plants with an estimated number of 20,000 to 28,000 species worldwide [1]. The small size and biomass of these plants have caused them to be neglected for wider use. One of the features that helped bryophytes to survive and maintain their place in today's is their content of biologically active compounds. Although bryophytes are very familiar, their medicinal importance is not exploited completely. They are used in pharmaceutical products, horticulture, household purposes and are also ecologically important as good indicators of environmental conditions [2,3]. The search for plants with antimicrobial activity grown in importance in recent years, due to a growing concern about increase in the rate of infection caused by antibiotic-resistant microorganism. Asakawa [4,5] has analysed approximately 1000 bryophyte species from the world total of 27000. However, few studies have been carried out about the antimicrobial properties of bryophytes. In literature, reports have been found about antibacterial activity of 23 bryophytes species [6]. Donald and Bishop [7] evaluate that green plants possess antimicrobial substance which inhibit microbial growth. Banerjee and Sen [8] reported that bryophytes also possess anticancer and antimicrobial activity due to their unique chemical constituents.

Mekuria *et al.*, [9] studied the effect of moss extracts against phytopathogenic fungi and showed that alcoholic extract of mosses was active against *Candida albicans*. Keyhanian *et al.* [10] studied the effect of several fungicidal and insecticidal seed treatment to control rapeseed seedling damaging plants and observed that fungicidal and insecticidal compounds were active against tested pathogen. Subhisha and Subramonian [11] screened the extracts of *Pallavicinia leyelli* and evaluated that it possesses antifungal activity. Iwashina [12] reported that flavanoid compounds are widely distributed in bryophytes and possess many biological activities against plants, fungi and other microorganism.

Ilham *et al*, [13] studied acetic and methanolic extract of *Palustriella comutata* against 11 bacteria, 1 yeast and 8 moulds. Deora *et al*, [14] studied three bryophytes *Plagiochasma articulatum*, *Anthoceros longii*, *Fissidend bryoides*; liverwort, hornwort and moss respectively for their antibiotic effect on *Agrobacterium tumefaciens*. The result showed that mosses are highly antibiotic in nature followed by hornwort and liverworts. The aim of the present study was to find out the antifungal activity of *Bryum cellulare* against phytopathogenic fungi *Drechslera maydis*. Phytochemical screening of different extract will also be carried out to detect the presence of secondary metabolites.

MATERIAL AND METHODS

Plant Material and Extract Preparation

The moss *Bryum cellulare* was collected in rainy season (2013) from Mt. Abu, Distt. Sirohi (Raj.) around Nakki Lake, Guru Shikhar and Sunset point in both vegetative and sporophytic phases. Both plants were washed with distilled water to remove soil particles, attached litter, dead material. For ethanolic extract preparation, plant material weighted was grinded in mortar and pestle with equal amount of ethanol till the formation of fine paste, then it was centrifuged and filtered. This filtrate was used as (100%) crude extract then it was serially diluted by double distilled water to prepare various concentrations from 10-100 per cent. The same method was adopted for methanolic and aqueous extract preparation except grinding the plant material with methanol and water instead of ethanol.

Test Organism

The pure culture of test fungi *Drechslera maydis* was obtained from the Department of Pathology RCA, (Udaipur, Rajasthan) India. This test organism was sub-cultured in laboratory at 25°C temperature to obtain its pure isolates.

Screening of Antifungal Activity

Fungal spores of the test fungi were bioassayed against the extracts on cavity slides by hanging drop method. Hyphal length was measured after 8 hrs. of inoculation using Ocular-micrometer under Compound Microscope. Percentage of spore germination was counted under light microscope after 24 hrs of incubation.

Phytochemical Analysis

Phytochemical analysis of moss *Bryum cellulare* extract was done by the methods of Trease and Evans [15] to detect the presence or absence of certain bioactive compounds.

Table 1: Phytochemical Screening of the *Bryum cellulare* Extracts

Compound	Ethanolic Extract	Methanolic Extract	Aqueous Extract
Alkaloids	-	-	-
Anthoquinones	-	-	-
Cardic Glycosides	+	+	+
Flavanoids	+	+	+
Saponins	-	-	-
Sterols	+	+	+
Terpenoids	+	+	+

(+) = Phytoconstituents Present

(-) = Phytoconstituents Absent

RESULTS AND DISCUSSIONS

Antimicrobial activity of selected bryophyte extract in different solvents on test microorganism *Drechslera maydis* are represented in Table (2,3,4). The percentage of spore germination and hyphal length of *Drechslera maydis* was examined using different concentrations of ethanolic, methanolic and boiled water extract of *Bryum cellulare*. The observations on the spore germination indicated the adverse effect of ethanolic extract. The spore germination was decreased from lower to higher concentrations, only 21.63 per cent spore germination reported at 100 per cent concentration while it was 66.9 per cent at 10 per cent. Hyphal length also decreased from 67.46 μm to 14.93 at 10 to 100 per cent concentration (Table 2, Figure 1, Figure 2). Efficacy of methanolic extract on spore germination and hyphal length was significant. 69.96 per cent spore germination and 82.83 μm hyphal length were observed at 10 per cent while 30.20 per cent and 22.30 μm were noticed in 100 per cent, respectively (Table 3, Figure 3, Figure 4). Percentage of spore germination was 84.70 and 40.26 at 10 to 100 per cent concentration and 109.13 μm and 35.33 μm were observed at 10 to 100 per cent concentration of boiled water extract (Table 4, Figure 5, Figure 6).

Extract were tested for the presence of flavanoids, terpenoids, sterols, alkaloids etc. however terpenoids, sterols, flavanoids, cardiac glycosides were detected in extracts of *Bryum cellulare* (Table 1). The bryophyte extracts prepared in different solvents were found effective in reducing fungal growth as they possess various secondary metabolites which acts as antifungal agent. The activity of different solvent extracts was in order of ethanolic>methanolic>boiled water as the bioactive compounds are more soluble in organic solvents. The present results showed similarity with the results of Deora *et al.*, [15] who determined the antifungal activity of a moss against certain phytopathogenic fungi. Deora and Suhalka [16] studied the effect of liverwort *R.gangetica* against *F.moniliforme* and found cold water extract was more effective than boiled water extract. Bodade *et al.*, [17] evaluate the antimicrobial effect of *Plagiochasma appendiculatum*, *Thuidium cymbifolium*, *Bryum cellulare*, *Bryum argentium* and *Racomitrium crispulum* on 12 microorganism. Solubility data and antibiotic spectra of the active plants indicated the occurrence of the variety of antibiotic substances among bryophytes.

Table 2: Effect of Ethanolic Crude Extract of *Bryum cellulare* on *Drechslera maydis*

S. No	Extract Concentration (%)	Spore Germination (%)		Hyphal Length (μm)	
		Mean	SD	Mean	SD
1	Control	92.2333	0.3193	137.5000	0.1000
2	10	66.9000	0.7934	67.4667	0.4938
3	20	56.0000	0.5000	55.1667	0.7504
4	40	49.6000	0.3603	45.2333	0.5132
5	60	33.2000	0.3606	33.3333	0.4617
6	80	29.4000	0.5292	20.7000	0.5292
7	100	21.6333	0.1526	14.9333	0.1528
	GM	49.8524	23.2945	53.4762	39.2934
	Se	0.2714		0.2757	
	CD5%	0.8232		0.8364	
	CD1%	1.1433		1.1616	
	CV	0.94		0.89	

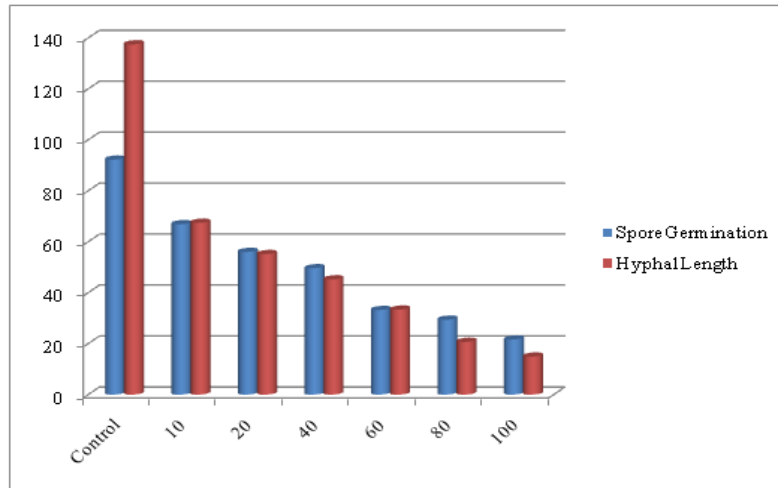


Figure 1



Figure 2: Spore Germination and Hyphal Length of *Drechslera maydis* in Control (A); 10 (B), 20 (C), 40 (D), 60 (E), 80 (F) and 100 (G) Percent Concentrations of *Bryum cellular* Ethanolic Extract Photoplate 2

Table 3: Effect of Methanolic Crude Extract of *Bryum cellulare* on *Drechslera maydis*

S. No	Extract Concentration (%)	Spore Germination (%)		Hyphal Length (µm)	
		Mean	SD	Mean	SD
1	Control	92.3333	0.3055	138.9667	0.3482
2	10	69.9667	0.3064	82.8300	0.4583
3	20	65.5667	0.4613	68.1000	0.4006
4	40	58.4000	0.4585	57.0333	0.2508
5	60	49.2333	0.3058	34.2000	0.6244
6	80	34.4333	0.4724	25.1000	0.4358
7	100	30.2000	0.3001	22.3000	0.4358

Table 3: Contd.,

	GM	57.1619	20.4214	59.7429	38.2853
	Se	0.2200		0.2513	
	CD5%	0.6674		0.7624	
	CD1%	0.9269		1.0588	
	CV	0.67		0.73	

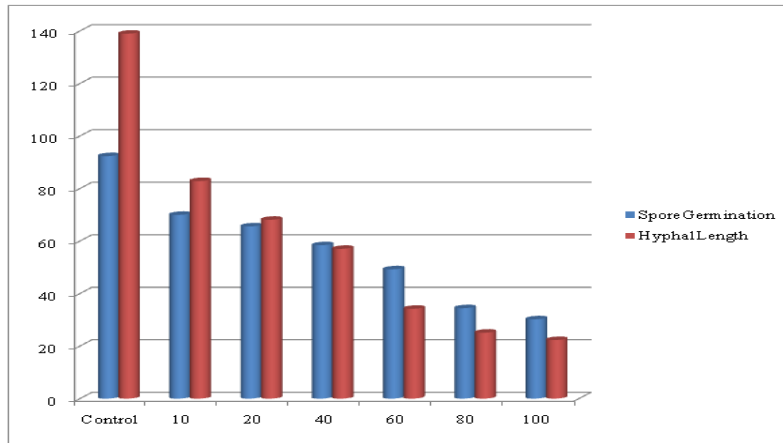


Figure 3

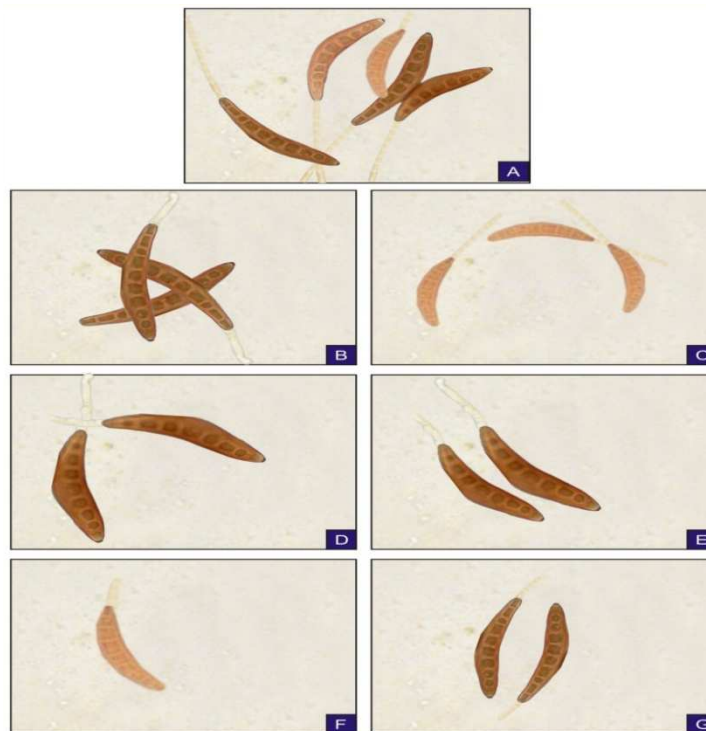


Figure 4: Spore Germination and Hyphal Length of *Drechslera maydis* in Control (A); 10 (B), 20 (C), 40 (D), 60 (E), 80 (F) and 100 (G) per cent Concentrations of *Bryum cellulare* Methanolic Extract Photoplate 3

Table 4: Effect of Boiled Water Extract of *Bryum cellulare* on *Drechslera Maydis*

S. No	Extract Concentration (%)	Spore Germination (%)		Hyphal Length (μm)	
		Mean	SD	Mean	SD
1	Control	95.1667	0.2082	143.4667	0.3180
2	10	84.7000	0.3596	109.1333	0.6000

Table 4 : Contd.,

3	20	72.1667	0.3055	79.6667	0.2082
4	40	69.1667	0.2321	67.3000	0.6083
5	60	55.1333	0.3222	52.0667	0.1528
6	80	49.9000	0.2006	42.3000	0.3470
7	100	40.2667	0.2894	35.3333	0.4617
	GM	71.6429	14.6476	87.7333	36.4095
	Se	0.1613		0.2423	
	CD5%	0.4894		0.7351	
	CD1%	0.6797		1.0209	
	CV	0.39		0.48	

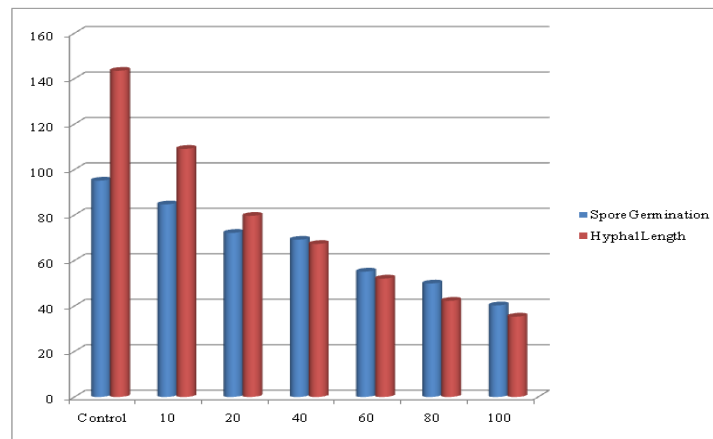


Figure 5

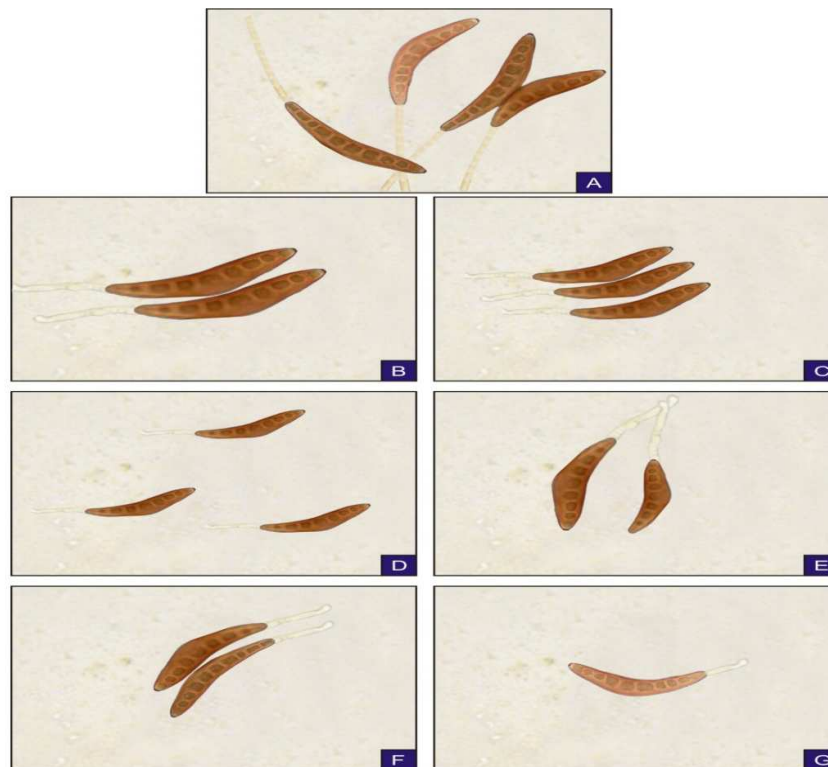


Figure 6: Spore Germination and Hyphal Length of *Drechslera maydis* in Control (A); 10 (B), 20 (C), 40 (D), 60 (E), 80 (F) and 100 (G) per cent Concentrations of *Bryum cellular* Boiled Water Extract Photoplate 4

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