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PHYTOCHEMICAL SCREENING AND ANTIFUNGAL ACTIVITY OF CERTAIN BRYOPHYTES FROM RAJASTHAN

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The present study was undertaken to evaluate the effect of different fractions of aqueous, ethanolic and methanolic extracts of certain bryophytes such as *Plagiochasma appendiculatum* (Liverwort) *Anthoceros longii* (Hornwort), *Fissidens bryoides* and *Entodon prorepens* (Mosses) against phytopathogenic fungi *Alternaria solani*, *Curvularia lunata* and *Fusarium moniliforme* using poisoned food technique. The results revealed that the radial growth in form of colony diameter, fresh weight of test fungi drastically reduced in response to all concentrations ranged from 10 to 100 per cent especially in the plant material extracted with methanol showed strong antifungal activity with significance inhibition on percent inhibition and fungal hyphal length of all test fungi. Among plants *P.appendiculatum* extract showed potent antifungal activity followed by *A. longii*, *F. bryoides* and *E. prorepens*. Fungus *F. moniliforme* exhibited most sensitivity in methanolic extract of *P. appendiculatum* followed by *C. lunata* and *A. solani.* Findings of this study confirmed that all four bryophytes have some potent bioactive phytochemicals which showed antifungal activity resulting in the suppression of fungal growth therefore, after further analysis of extracts and confirmation of phytochemicals, extracts of these, plants can be used as natural and ecofriendly fungicides to control phytopathogenic fungi to reduce the pressure of use of synthetic fungicides.

Key words: Antifungal activity, Bryophytes, Phytochemical screening, Poisoned food technique, Synthetic fungicide

Introduction

Fungi ranks second only to insects as a cause of plant diseases, which results in heavy loss of plant products. Pathogenic fungi alone cause about 20 per cent reduction in the yield of major food and cash crops[1]. One third agricultural production is reportedly destroyed each year by different pests and diseases[2].

No doubt the use of chemicals has been found very effective in controlling plant fungal diseases but some major problems threaten to limit the continued use of synthetic fungicides. Firstly, fungi have developed resistance to chemicals. This necessitates higher dosage or the development of new chemicals to replace those to which fungi are resistant. Secondly,

some fungicides are not readily biodegradable and tend to persist for years in the detrimental effects of chemicals on organisms other than target fungi[3]. Because of these problems associated with the use of chemicals, researchers are now trying to use ecofriendly and safe alternative methods of fungal control. Plant extracts have been used as efficient fungicides inhibiting the growth of many fungal pathogens.

The occurrence of antibiotic substances in bryophytes have been well documented by botanists and microbiologists[4].They possess compounds such as alkaloids, flavonoids, terpenoids but only few species have been thoroughly studied for antifungal property. Therefore, bryophytes can be a promising source of many new biologically active compounds in nature. The chemistry of bryophytes are poorly known and the results on are very scattered. The reason for this is the difficulty for identification and small amount of availability for analysis, usually by sophisticated methods. Therefore, bryophytes are indicated as source of chemically new and unknown compounds[5].

Bioactive guided isolation of antifungal compounds from liverwort *Bazzania trilobata* was studied. For this purpose thin layer chromatography was used to isolate six antifungal sesquiterpenes and their structure was investigated using extensive NMR spectral evidence[6.] An aqueous, methanolic and ethanolic crude extracts of *Bryum cellulare* was potent to inhibit spore germination and hyphal length of fungus *Curvularia lunata*, a causal organism of Zea mays[7]. Phytochemical quercetin was estimated from moss *Philonotis revoluta* by HPLC method and its antifungal activity of an aqueous, ethanolic and methanolic extracts were screened against colony diameter, fresh weight and per cent inhibition of mycelial growth of fungus *Helminthosporium turcicum* a causal organism of northern leaf blight of corn[8]. The object of the present study was to analyze phytochemically extracts of selected bryophytes and to screen it for antifungal potential against test fungi and also compare with the antifungal activity of commercially available fungicides such as zineb and mancozeb.

Materials and Methods

Collection of plant materials

Plants selected for phytochemical analysis and evaluating antifungal activity were *Plagiochasma appendiculatum* (Kash.), *Anthoceros longii* (Kash.), *Fissidens bryoides* (Hedw.) and *Entodon prorepens* (Mitt.) Jaeg. These plants were collected during rainy season from different localities of Rajasthan in both vegetative and sporophytic stages.

Test organisms

Fungal test organisms selected for present study were *Alternaria solani* Souer, a causal organism of potato and tomato; *Fusarium moniliforme* Scheld a causal organism of ear rot of corn and *Curvularia lunata* (Wakker) Boedin, a causal organism of leaf spot of maize and gram. *C. lunata* and *F. moniliforme* were obtained from Institute of Microbial Technology, Chandigarh whereas *A. solani* was procured from Maharana Pratap University of Agriculture and Technology Udaipur (Rajasthan). These test organisms were sub-cultured in the laboratory as per prescribed suggestions from MTECH Chandigarh and MPUAT Udaipur to obtain pure culture for further use.

Preparation of extracts

The plant materials were collected and brought to the laboratory than thoroughly washed with running tap water followed by double distilled water (DDW). Washed plant materials were dried in an oven at 50 ^{0}C for 48 hrs. Completely dried plant materials

were ground into fine powder with the help of pastel and mortar. This powdered material was stored in air tight jars in refrigerator at 4 ^{o}C. Three solvents of aqueous, methanolic and ethanolic were prepared from selected plant parts for antifungal assay.

For aqueous extract preparation 25 g of powdered plant material was dissolved in double distilled water (DDW) to make 100 ml (25% w/v).Than this mixture was kept in as such position for 24 hrs. at room temperature in a sterile flask covered with aluminum foil to avoid evaporation then this extract was filtered through Whatmann filter paper no. 1. After filtration, the extract was evaporated in water bath till 25 ml extract was left. For methanolic extract preparation 25 g powdered plant material was dissolved in 100 ml methanol (25% w/v). For ethanolic extract preparation, the method was same instead of using methanol, ethanol was used. All extracts were immediately used for antifungal assay for fungi[9].

Preparation of fungal inoculum

The stock solutions of selected three fungal isolates were standardized to 10^6 spores /ml by spectrophotometrically at 530 nm and were adjusted to 80 to 85% transmittance. The fungal inoculum was also determined by plate count on PDA followed by incubation at 25^0C for seven days and observations were taken for visible growth of fungi at regular interval during the incubation period[10-11].

Antifungal activity assay by poisoned food technique

Antifungal activity of all four plant extracts was evaluated against three selected test fungi by using poisoned food technique. In this technique all the selected fungi were inoculated on PDA Petri plates at 25^0C for 7 days to obtain young actively growing colonies of fungus. 100 µl of plant extract was mixed with 15 ml cooled (45^0C) molten PDA medium, then poured on the plates and allowed to solidify at room temperature for 30 minutes. A mycelial disc of 6 mm diameter cut from periphery of 7 days old cultures was aseptically inoculated on the agar plates containing the extract. PDA plates without 100 µl of solvent were used as control[12]. These incubated plates were inoculated at 25^0C. The different fractions were bioassayed by colony diameter, fresh weight, percent inhibition and spore hyphal length. Colony diameter was measured after 72 hrs; fresh weight of the colony was measured by harvesting the colonies from molted medium in mm. The presence of inhibition zone indicated the antifungal activity of the extract. The zone of inhibition was measured in millimeters (mm) calculated as per formula of given by Vincent[13].

Fungal spores of the test fungi were bioassayed against the extracts on cavity slides by hanging drop methods[14]. Hyphal length was measured after 8 hrs. of incubation using Ocular micrometer[15].

Phytochemical screening

Quantitative phytochemical analysis of *P. appendiculatum, A. longii, F. bryoides and Entodon prorepens* extracts was done by the standard methods of Trease and Evans[16].

Results and Discussion

Preliminary quantitative phytochemical screening of methanolic extracts of *P. appendiculatum*, *A. longii, F. bryoides* and *E. prorepens* by common methods revealed the presence of alkaloid, phenols, flavonoids, phytosterols, terpenoids and glycosides (Table,1).

Antifungal activity aqueous, ethanolic and methanolic extracts of *P. appendiculatum, A. longii, F. bryoides and E. prorepens* was assayed and data on the effect of plant extracts on the growth of test fungi is presented in table, 2,3 and 4. Results showed the reduction in the growth

of all three test organisms in respect of all the plant extracts tested. Further it was observed that Methanolic extracts of all four plants was more potent to inhibit fungal growth. In *A. solani*, *C. lunata* and *F. moniliforme* colony diameter was 27.2240, 27.4200 and 26.3220 mm whereas fresh weight was 1.0000, 1.1230 and 0.6210 gm in 10 per cent concentration respectively. Further gradual reduction in colony diameter and fresh weight was reported from lower to higher concentrations and it was 4.2342, 4.2400 and 4.2120 mm colony diameter in *A. solani, C. lunata* and *F. moniliforme* respectively whereas fresh weight was 0.1110, 0.2210 and 1100 gm in *A. solani, C. lunata* and *F. moniliforme* respectively at 100 per cent concentrations of *P. appendiculatum* in comparison to the control. Similar results were obtained in cash of percentage inhibition and hyphal length of test fungi.

It was also observed that the higher concentrations inhibited growth of all test fungi but among the all three test fungi studied *F. moniliforme* exhibited most sensitivity against methanolic extract of *P.appendiculatum* followed by *C. lunata* and *A. solani* in *A. longii, F. bryoides* and *E. prorepens.* The results of this work corresponded to the earlier findings. Extracts of certain bryophytes such as *Plagiochasma articulatum, Anthoceros longii* and *Fissidens bryoides* showed antibiotic activity against *Agrobacterium tumifaciens*[17] (Deora and Bhati 2007). Alcoholic extract of a moss was active against Candida albicans[18]. Similar observations were also reported by Banerjee and Sen[4] .

The methanolic extract of a moss *Philonotis revoluta* exhibited a superior effect against spore germination of fungus *Helminthosporium turcicum* and malformation such as stunting of growth, curling and dying of tip of fungal growth was reporeted[19.] Cold water of extract of *Riccia gangetica* (a liverwort) was more active against the growth of fungus *Fusarium moniliforme* than the boiled water extract[20.]

Table 1- Preliminary phytochemical profile of selected bryophytes.

Active compounds	Phytochemical tests	Observations	Results (Intensity)			
			Pa	Al	Fb	Ep
Alkaloids	Mayer's test	Precipitation formation	++	+	+	+
	Hager's test	Precipitation formation	++	+	+	+
Anthroquinin	Born raggers test	No layer formation	_	_	_	_
Cardiac glycosides	Killer Killan test	Brown ring formation	+ +	+	+	+
Flavonoids ferric chloride	Ferric chloride test	Green colour	+++	++	++	+
	Lead acetate test	Yellow precipitation	+++	++	++	+
	Alkaline reagent test	Yellow florescent colour	+++	++	++	+
	Sodium hydrochloride test	Yellow colour	++	+	+	+
Saponins	Froth test	No froth formation	_	_	_	_
Phytosterols	Salkowiski test	Reddish brown colour	++	+	++	+
	Liebermann-Burchardt test	Brown ring	++	++	+	+
Terpenoids	Salkowiski test	Lower layer turn yellow	+++	++	++	+
	Liebermann-Burchardt test	Deep red colour	+++	++	++	+
Phenols	Ferric chloride test	Green colour	+++	++	++	++
	Lead acetate test	Yellow precipitation	+++	++	++	++

Note: Pa = *Plagiochasma appendiculatum*, Al = *Anthoceros longii*, Fb = *Fissidens bryoides*, Ep = *Entodon prorepens*

Table 2. Antifungal activity of certain bryophytes against selected test fungi in aqueous extracts.

Plants	Test fungi	Extract concentrations	Mean Colony diameter (mm)	Fresh weight (gm)	Percent inhibition	Hyphal length (µm)
Pa	As	Control	36.0000	1.8652	0.0000	163.2230
		10 min.	30.8300	1.2800	34.59	112.6840
		100 max.	7.5624	0.2640	81.7543	23.1232
	Cl	Control	38.733	2.1233	0.00	162.6780
		10 min	32.4000	1.8533	30.0000	112.4630
		100 max.	7.9667	0.3567	81.6667	28.2262
	Fm	Control	35.2333	1.8000	0.00	142.1360
		10 min	29.3667	0.9600	28.8700	87.2600
		100 max.	6.9667	0.1367	79.9267	19.4640
Al	As	Control	38.1333	2.3000	0.0000	167.4420
		10 min.	31.0333	1.9033	28.1333	126.2640
		100 max.	7.9000	0.7667	75.1200	43.4440
	Cl	Control	39.3667	2.8700	0.0000	170.0200
		10 min.	34.5000	1.8933	25.5500	130.2420
		100 max.	8.7330	0.6267	68.0067	50.4020
	Fm	Control	35.6667	2.1267	0.0000	172.4040
		10 min.	30.5000	1.9200	23.1533	124.6200
		100 max.	7.3000	0.9600	64.8967	43.2400
Fb	As	Control	38.2667	1.8467	0.0000	173.2320
		10 min.	32.7333	1.0667	22.3533	138.4433
		100 max.	8.2330	0.5600	73.4500	56.2380
	Cl	Control	39.9500	2.9200	0.0000	174.2430
		10 min.	35.1200	1.9800	19.5800	136.2460
		100 max.	8.9500	0.7200	71.3000	59.2440
	Fm	Control	35.9864	2.6864	0.0000	174.2380
Ep		10 min.	32.4621	1.9860	16.8900	124.3280
		100 max	7.8649	0.9800	69.6890	44.3080
	As	Control	38.9864	2.1000	0.0000	175.6060
		10	33.2145	1.6800	64.4680	136.4200
		100	8.8670	0.7800	16.2000	59.2620
	Cl	Control	40.6480	2.8900	0.0000	174.4200
		10	35.8640	1.9000	60.4260	137.4200
		100	9.4840	0.7640	14.8000	59.2040
	Fm	Control	41.9860	2.8900	0.0000	175.2460
		10	32.8460	1.9880	60.9000	137.3280
		100	8.9800	0.7800	14.2010	59.2600

Table 3. Antifungal activity of certain bryophytes against selected test fungi in ethanolic extracts.

Plants	Test fungi	Extract concentrations	Mean Colony diameter (mm)	Fresh weight (gm)	Percent inhibition	Hyphal length (µm)
Pa	As	Control	34.4800	1.4650	0.0000	160.4650
		10 min.	28.8420	1.2000	32.6890	110.1200
		100 max.	6.4624	0.2110	78.8540	20.2040
	Cl	Control	36.2280	2.0000	0.0000	160.3230
		10 min	32.8400	1.4530	28.6000	110.2080
		100 max.	6.8400	0.3200	77.4660	26.2140
	Fm	Control	33.4233	1.6000	0.0000	141.2000
		10 min	27.6660	0.9200	24.8200	85.4200
		100 max.	6.4260	0.1300	77.8262	17.4330
Al	As	Control	36.6332	2.1000	0.0000	165.4220
		10 min.	29.4330	1.6890	26.130	123.4660
		100 max.	7.4080	0.7430	78.1244	41.4400
	Cl	Control	37.6660	2.4400	0.0000	168.2080
		10 min.	32.4000	1.6930	23.8800	127.5230
		100 max.	8.0000	0.5860	66.4467	48.2630
	Fm	Control	33.4400	2.1000	0.0000	169.2040
		10 min.	28.6000	1.6200	21.6533	127.2310
		100 max.	6.9000	0.8700	68.8960	48.4220
Fb	As	Control	36.8660	1.6460	0.0000	169.2330
		10 min.	30.8330	1.0000	20.66534	136.2330
		100 max.	8.9330	0.5200	71.8300	54.4330
	Cl	Control	37.6400	2.60000	0.0000	170.2430
		10 min.	33.8260	1.6460	17.3400	134.5460
		100 max.	7.6500	0.6100	68.000	57.2200
	Fm	Control	33.4824	2.4460	0.0000	171.3240
		10 min.	30.6620	1.6840	14.2200	122.3200
		100 max	6.9640	0.6800	67.3420	56.4000
Ep	As	Control	36.4860	1.9000	0.0000	173.6000
		10	31.6146	1.2400	62.4280	134.4200
		100	6.9680	0.6500	14.0000	57.4440
	Cl	Control	38.8046	2.4480	0.0000	170.3200
		10	33.6040	1.6040	58.4460	135.2400
		100	8.8840	0.5680	12.6000	57.4080

Table 4. Antifungal activity of certain bryophytes against selected test fungi in methanolic extracts.

Plants	Test fungi	Extract concentrations	Mean Colony diameter (mm)	Fresh weight (gm)	Percent inhibition	Hyphal length (µm)
Pa	As	Control	30.2200	1.1200	0.0000	157.4660
		10 min.	27.2240	1.0000	30.6890	107.1000
		100 max.	4.2342	0.1210	75.8660	18.2000
	Cl	Control	31.0480	1.6000	0.0000	157.6230
		10 min	27.4200	1.1230	26.6800	108.4040
		100 max.	4.2400	0.2100	74.6680	24.2140
	Fm	Control	30.2030	1.2000	0.0000	139.4080
		10 min	26.3220	0.6210	26.8600	82.8260
		100 max.	4.2120	0.1100	74.8480	15.2360
Al	As	Control	32.2230	1.7100	0.0000	162.4000
		10 min.	26.2300	1.2120	24.2300	120.4220
		100 max.	5.2020	0.3420	75.1240	39.2400
	Cl	Control	32.2020	1.8800	0.0000	164.2040
		10 min.	26.2080	1.1000	21.8600	123.2030
		100 max.	6.0000	0.3460	63.6460	46.2420
	Fm	Control	31.2600	1.8900	0.0000	165.4010
		10 min.	25.8200	1.3200	19.6230	123.4310
		100 max.	4.4020	0.5600	65.4460	46.4260
Fb	As	Control	32.8220	1.3260	0.0000	165.4680
		10 min.	27.4220	0.8000	18.2250	128.2000
		100 max.	2.6320	0.3100	68.2300	51.2320
	Cl	Control	33.2430	1.9000	0.0000	164.4030
		10 min.	28.4240	1.2060	15.2100	129.6440
		100 max.	5.2400	0.4100	65.0000	52.4260
	Fm	Control	29.2220	1.9220	0.0000	167.2260
		10 min.	26.2320	1.6840	12.1120	120.2400
		100 max	4.6240	0.3620	65.1220	52.8020
Ep	As	Control	32.3460	1.2040	0.0000	169.2020
		10	28.2140	0.9400	59.4020	130.2200
		100	5.6620	0.3700	12.0020	52.6480
	Cl	Control	34.4042	1.9300	0.0000	168.2200
		10	29.2060	1.6920	55.2280	131.4200
		100	6.4240	0.4220	10.2030	52.8820

Note: Results based on mean of three replicates

1. Agrios JM 2000, Plant Pathology, 2 535-568.
2. Maqbool MA, Hashmi S and Ghafar A 1988, Problem of root knot nematodes in Pakistan and strategy for their control, *Proc. U.S, Pakistan Intern. Workshop on plant nematodes*. 229-240.
3. Brady NC 1984, Thenature and properties of soil, MacMillon Publishing Company, New York, 528.
4. Banerjee RD and Sen S.P. 1979, Antibiotic activity of bryophytes, *The bryologists*, 82 2 141-153.
5. Klavinja L, Bikavens O, Stenberga I, Maksimova V and Eglite L 2012, Characterization of chemical composition of some bryophytes common in Latvia, *Environmental and Experimental Biology*, 10 27-34.
6. Scher JM, Speakerman JB, Zapp J and Becker H 2004, Bioactivity guided isolation of antifungal components from the liverwort *Bazzania trilobata* (L.) S.F. GRAY. *Phytochemistry*, 65 18 2583-2588.
7. Deora GS and Guhil N 2016, Studies on antifungal potential of *Bryum cellulare* (A moss) crude extracts against spore germination of fungus *Curvularia lunata. IJPSR*, 7 1 353-357.
8. Deora GS and Suhalka D 2017, Estimation of Quercetin by HPLC and antifungal activity of *Philonotis revoluta*, *IJPSR*, 8 1 294-300.
9. Barreto M, Critchley AT and Straker CJ 2002, Exracts from sea weeds can promote fungal growth, *J. Basic Microbiol*. 42 302-310.
10. Rassoli I and Abynek 2004, Inhibitory effect of thyme oil on growth and aflatoxin production by *Aspergillus paraciticus*, *Food Control,* 15 479-483.
11. Pundir RK and Jain Pranay 2010, Comparative studies on the antimicrobial activity of Black papper (Piper nigrum) and Turmeric (Curcuma longa) extracts. *International Journal of Applied Biology and Pharmaceutical Technology,* 1 2 492-501.
12. Grover RJ and Moore JD 1962, Toximetric studies of fungicides against the brown rot organisms, *Sclerotinia fructicola* and *S. laxa, Phytopathology,* 52 876-880.
13. Vincent JM 1927, Distoration of fungal hyphae in the presence of certain inhibitors, Nature, 59 850.
14. Gerald M, and Lampen JO 1962, Inhibition by antibiotics of the growth of bacterial and yeast protoplasts, 84 508-512.
15. Aneja KR 2003, Experiments in microbiology, plant pathology and biotechnology. New Age International Publisher New Dehli.
16. Trease GE and Evans WC 2002, Pharmacognosy book, Edinberg, New York WB Sauders Publisher 3rd Edition, 23-67.
17. Deora GS and Bhati D 2007, Antibiotic effects of certain bryophytes on Agrobacterium tumifaciens, *Pure and Applied Micro.,* 15 1 215-219
18. Mekuria T, Blaener PS, and Frahm, JP 1999, Effect of moss extracts against phytopathogenic fungi in W.laux (Ed.) 51 Deutsche Pflanzenschutz- Tagung 5-8 Halle/Saale Mitt BBA 357 167-168.
19. Deora GS and Suhalka D 2016, Phytochemical composition and fungicidal potential of moss Philonotis revoluta against spore germination process of fungus Helminthosporium turcicum, *J Pharm and Bio Sci*, 11 6 38-43.
20. Deora GS and Suhalka D 2010, Effect of Riccia gangetica (a liverwort) extract against *Fusarium moniliforme*, *Current Sciences*, 15 1 87-99.