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Potential of *Plagiochasma appendiculatum* on Inhibition of Certain Economically Important Plant Pathogens

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ABSTRACT: Present study was carried out to evaluate the antimicrobial properties of *Plagiochasma appendiculatum* (liverwort) extract used against selected test fungus and bacteria for antimicrobial assay. Effect of aqueous and different organic extracts *viz*. petroleum ether, ethanol, chloroform, acetone, methanol and aqueous extract of *Plagiochasma appendiculatum,* at different concentrations (1000, 500, 200 and 40 µg ml^{-1} and at different time intervals i.e. 24, 48 and 72 hrs) were examined for their bioactivity against certain important fungal species (*Rhizoctonia solani, Sclerotium rolfsii, Fusarium oxysporum* and *Tilletia indica)* and some important bacterial pathogens (*Xanthomonas oryzae* pv. *oryzae, Salmonella enterica, Pasteurella multocida,* and *Melissococcus plutonius*). All organic extracts of *P. appendiculatum* failed to inhibit growth of *Xanthomonas oryzae* and *S. enterica*. Most of the extracts showed maximum activity against the *R. solani* with MIC of 2.50 and MFC value of 5.00 in petroleum ether extract. MBC value was found maximum in *M. plutonius* with value of 5.00 extracted in petroleum ether.

Key Words: *Plagiochasma appendiculatum,* liverwort, antimicrobial properties, Plant Pathogen.

INTRODUCTION

All bryophytes contain cocktails of different active compounds, mainly terpenoids to protect themselves against infections of fungi and bacteria, with which they live in direct contact. These effects of bryoflora were thus not developed by chance but are a necessity for their survival. The anti-microbial effects of bryophytes go back on an inhibition of cell division, not only of bacteria and fungi but also of mammal cells. Other effects include anti-feeding effects against beetles, slug and snail or fish killing effects. Several insect anti-feedents have now been found in diverse bryophytes making this group of plants a useful source of insecticides and insect repellant.

Mekuria *et al.* (1999) studied the effect of moss extracts against phytopathogenic fungi and showed that alcoholic extract of mosss was active against *candida albicans*. Keyhanian *et al*. (2002) 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 (2005) screened the extracts of *Pallavicinia leyelli* and evaluated that it posses antifunagal activity. Iwashina (2003) reported that flavanoid compounds are widely distributed in bryophytes and posses many biological activities against plants, fungi and other microorganism.

In particular, liverworts contain pinguisane-type sesquiterpenoids, sacculatane-type diterpenoids and bis (bi-benzyl) aromatic compounds which are not found in higher plants (Asakawa, 1995; Nagashima *et al.*, 2002). They show higher antifungal properties than the mosses. It is due to the presence of lunularic acid, an aging hormone found in liverworts but not in mosses (Pryce, 1972). Degree of antibiotic activity in a given species may depend upon the age of the gametophyte, season of collection and other such ecological parameters (Banerjee and Sen, 1979).

Plagiochasma appendiculatum is one of the important Indian liverwort belongs to the order Marchantiales under family Aytoniaceae. *Plagiochasma* is a thalloid liverwort represented by 30 species (Bischler, 1978) but in India only 10 species have been reported.

Plagiochasma appendiculatum is widely distributed in western, eastern Himalayas, central India and south India and generally grow to an altitude upto 3000-8000 ft from sea level. This species in known from the east part of the Central and South African continent Eritrea, Ethiopia, Kenya, Tanzania to Rhodesia, Zimbabwe and South Africa (Perold, 1999; Wigginton, 2002). *Plagiochasma appendiculatum* is significant taxon which possesses antimicrobial property (Banerjee, 2000). In India, it is used by Gaddi tribes in Himachal Pradesh for the treatment of cuts, wounds and burns (Kumar *et al.,* 2001; Singh *et al.,* 2006).

Keeping this view, present work will provide a comparative study of sensitivity of bacteria and fungi against *P. appendiculatum* which indicates that the liverworts are rich store house of antibacterial and antifungal substances. The observations of this work will play a key role for biological control of plants pathogens.

MATERIAL AND METHODS

A. Collection of bryophytes

Bryophytes were collected from walls, roofs and natural rocks where nearly no overhanging vegetation or tree canopy was present. They were collected only from over approx. 1.5 m above ground level, to avoid road-water splashes and areas rich with domestic wastes. The survey was made in urban, sub-urban and rural sites of Kumaon hills. For the present study, only mature plants were collected and brownish or pale yellow and dried plants were rejected.

B. Identification and Taxonomy

Collection of bryophytes was made during 2009-2010 from different parts of Kumaon hills. They were brought to the laboratory in plastic bags and identified on the basis of morphological examination and with the help of various available literatures (Chopra, 1975; Smith, 1978; Haji, 1984; Gangulee, 1969; Saxena et al., 2008).

C. Preparation of plant extract for antimicrobial activity

The plant material was carefully cleaned from attached litter and dead material, under running tap water and finally with sterile distilled water, shade-dried and then finely powdered (100 g) with the help of a grinder. The powdered plant material was then Soxhlet extracted with 500 ml of different organic solvents in different solvents such as petroleum ether, chloroform, acetone, ethanol, methanol. The extracts were filtered through muslin cloth and kept at room temperature till complete evaporation. Different concentrations of crude extract (40, 200, 500 and 1 000 µg/mL) were prepared and used for further study.

D. Antimicrobial assay

Disc diffusion assay was used for evaluation of antimicrobial activity (Basri and Fan, 2005). In assay for antibacterial activity, the nutrient agar plates of bacteria treated with organic extracts (40 µL into each disc) of different concentrations were incubated at (37 ± 2°C) for 24 h. Antibacterial activity of the plant extracts was determined by measuring the zone of inhibition (ZI) in mm against all bacteria. The antibiotics as positive controls (streptomycin and ampicillin) were used for comparison with the extracts regarding antibacterial activity; fungicides (Chloramine T for *F. oxysporum* f. sp. *lycopersci, T. indica, S. rolfsii* and *R. solanii*) were used as positive control for antifungal activity, and respective solvents as negative control. In assay for antifungal activity, potato dextrose agar was poured aseptically in the plates and kept for solidification at (28 ± 2) °C for 72 h. Four discs, two treated with plant extracts and two controls along with the test fungus were kept in same Petri plate. Percentage (%) Inhibition of fungal growth was calculated by the following formula:

% Inhibition = Mycelial growth (control) - Mycelial growth (treatment)/Mycelial growth (control) × 100

where, mycelial growth was determined by measuring the diameter of the fungus both in control and treatment.

E. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) of organic extracts

Micro broth dilution assay was done to determine both inhibitory and bactericidal/fungicidal concentration of organic extracts (Janovská et al., 2003). Freshly prepared nutrient broth for bacteria and potato dextrose broth for fungi were used as diluents. Fresh and revived culture of test microorganisms were diluted 100 folds in broth (100 µL of microorganism in 10 mL broth). For inoculation of culture, CFU was determined and was found to be 1×10^6 CFU/mL for bacteria, while it was 1×10^9 CFU/mL for fungi by taking optical density at 620 nm using UV-visible spectrophotometer. Decreasing concentrations of the plant extract (1000 to 0.98 µg/mL) in two fold dilution series were added to the test tubes containing the fresh microorganism cultures. All tubes with bacterial and fungal organisms were incubated at 37°C for 24 h and 28°C for 72 h, respectively.

Visible turbidity and optical density of cultures were determined at 620 nm by using UV visible spectrophotometer. The lowest concentration that inhibited visible growth of tested organisms was recorded as MIC, and that caused no visible microbial growth was considered as MBC.

F. Data Analysis

The statistical evaluation of complete data was done and all analyses were performed based on three replicates. Values were expressed as mean ± SE. ANOVA revealed level of significance at $P<0.05$ among different microorganisms and different extracts by using (JMP 5.0, SAS Institute, Cary, NC, USA) to analyze the effect of each treatment separately. The treatment means were separated using Tukey HSD test.

RESULTS AND DISCUSSION

The results obtained showed that most of the test microorganisms were sensitive to the organic extracts of *P. appendiculatum* in dose dependent manner.

All organic extracts of *P. appendiculatum* showed inhibition at different conc. (1000, 500, 200 and 40 µg ml^{-1}) and at different time intervals i.e. 24, 48 and 72 hrs. Percent inhibition in petroleum ether extract ranged from 10.64 minimum at 72 hrs in 40 µg ml^{-1} conc. to 28.25 at 24 hrs in 1000 µg ml^{-1} conc. In addition, methanol extract ranged from 8.47 to 23.48 with minimum at 72 hrs in 40 µg ml^{-1} conc. and maximum at 24 hrs in 1000 µg ml^{-1} conc. (Table 1).

Table 1: Percent inhibition in the growth of *F. oxysporum* f. sp. *lycopersici* with different extract of *P. appendiculatum*

Nature of extract	Concentration (µg ml^{-1})											
	Time (hrs.)											
	1000			500			200			40		
	24	48	72	24	48	72	24	48	72	24	48	72
Petroleum ether	28.25^{b} ± 0.28	26.23^{b} ± 0.27	24.45^{b} ± 0.40	26.41^{b} ± 0.42	23.61^{b} ± 0.23	20.57^{b} ± 0.39	20.31^{c} ± 0.32	18.75^{c} ± 0.34	14.73^{b} ± 0.31	15.46^{d} ± 0.38	13.90^{d} ± 0.30	10.64^{b} ± 0.42
Methanol	23.48^{d} ± 0.41	20.12^{d} ± 0.24	18.43^{c} ± 0.32	20.43^{d} ± 0.40	18.38^{d} ± 0.45	15.27^{d} ± 0.30	16.46^{e} ± 0.41	12.65^{f} ± 0.46	10.79^{d} ± 0.38	13.83^{e} ± 0.31	10.82^{e} ± 0.22	8.47^{c} ± 0.36
Chloroform	21.90^{e} ± 0.12	19.43^{d} ± 0.41	18.49^{c} ± 0.43	18.12^{e} ± 0.17	15.72^{e} ± 0.28	13.53^{e} ± 0.42	16.81^{e} ± 0.37	14.21^{e} ± 0.22	12.79^{c} ± 0.30	13.92^{e} ± 0.36	10.87^{e} ± 0.49	8.67^{c} ± 0.53
Ethanol	33.30^{a} ± 0.49	30.51^{a} ± 0.44	27.48^{a} ± 0.40	28.49^{a} ± 0.43	25.44^{a} ± 0.39	23.33^{a} ± 0.33	26.46^{a} ± 0.41	23.47^{a} ± 0.48	19.39^{a} ± 0.30	20.89^{a} ± 0.80	17.77^{a} ± 0.28	13.85^{a} ± 0.25
Acetone	25.84^{c} ± 0.24	23.50^{c} ± 0.47	24.46^{b} ± 0.43	20.37^{d} ± 0.43	18.47^{d} ± 0.41	17.42^{c} ± 0.38	18.39^{d} ± 0.50	16.18^{d} ± 0.41	14.45^{b} ± 0.27	16.61^{c} ± 0.16	14.77^{c} ± 0.39	10.84^{b} ± 0.36
Chloramine T	22.14^{e} ± 0.65	25.26^{b} ± 0.43	26.49^{a} ± 0.35	23.45^{c} ± 0.41	21.48^{c} ± 0.36	24.28^{a} ± 0.31	22.50^{b} ± 0.39	20.40^{b} ± 0.35	18.51^{a} ± 0.58	19.46^{b} ± 0.34	16.72^{b} ± 0.48	13.22^{a} ± 0.28

* Values are represented as mean ± SD.

*The treatment means were separated using Tukey HSD at 0.05% probability level

*Level not connected by same letter are significantly different in vertical columns.

Out of the five extracts (organic) of *P. appndiculatum*, the maximum percent inhibition was observed at 24 hrs (33.30) at 1000 µg ml^{-1} conc. in ethanol extract and minimum at 72 hrs (8.47) in methanol at 40 µg ml^{-1} concentration when used against *Fusarium oxysporum* (Table 1). Inaddition, *Tilletia indica*, organic extracts of *P. appendiculatum*, and the maximum percent inhibition was observed at 24 hrs (28.71) in petroleum ether extract at 1000 µg ml^{-1} concentration and minimum at 72 hrs (8.60) in chloroform at 40 µg ml^{-1} concentration (Table 2). As compared to standard antifungal petroleum ether extract shows good activity. Whereas, the extracts of *P. appendiculatum* showed the maximum percent inhibition at 24 hrs (22.73) at 1000 µg ml^{-1} concentration and minimum at 72 hrs (5.36) in methanol at 40 µg ml^{-1} concentration against *Sclerotium rolfsii*. (Table 3).

Table 2: Percent inhibition in the growth of *T. indica* Mitra with different extract of *P. appendiculatum*.

Nature of extract	Concentration (µg ml^{-1})											
	Time (hrs.)											
	1000			500			200			40		
	24	48	72	24	48	72	24	48	72	24	48	72
Petroleum ether	28.71^{a} ± 0.43	24.41^{b} ± 0.44	20.55^{c} ± 0.43	26.46a ± 0.43	23.80^{a} ± 0.46	17.92^{c} ± 0.20	23.45^{a} ± 0.40	20.38^{b} ± 0.37	18.46^{b} ± 0.36	19.15^{a} ± 0.13	14.43^{c} ± 0.43	11.42^{b} ± 0.37
Methanol	23.21^{c} ± 0.18	20.65^{c} ± 0.45	16.28^{e} ± 0.38	22.38^{bc} ± 0.47	18.68c ± 0.31	14.72^{d} ± 0.38	19.78^{c} ± 0.19	15.81^{d} ± 0.19	12.51^{c} ± 0.49	17.83^{b} ± 0.22	13.50^{c} ± 0.45	10.43^{c} ± 0.34
Chloroform	22.40^{c} ± 0.94	25.43^{b} ± 0.37	18.34^{d} ± 0.30	18.38^{d} ± 0.45	17.75^{c} ± 0.36	12.87^{e} ± 0.28	15.81^{d} ± 0.40	13.46^{e} ± 0.27	10.49^{d} ± 0.38	12.47^{d} ± 0.43	13.48^{c} ± 0.48	8.60^{d} ± 0.55
Ethanol	25.65^{b} ± 0.38	23.46^{b} ± 0.34	20.48^{c} ± 0.32	23.45^{b} ± 44	20.77^{b} ± 0.28	17.51^{c} ± 0.36	16.52^{d} ± 0.49	13.51^{e} ± 0.49	10.82^{d} ± 0.23	13.71^{d} ± 0.30	10.43^{d} ± 0.40	8.79^{d} ± 019
Acetone	25.85^{b} ± 0.34	24.70^{b} ± 0.22	26.64^{a} ± 0.45	23.38^{b} ± 0.43	24.75^{a} ± 0.57	23.54^{b} ± 0.42	21.44^{b} ± 0.31	23.49^{a} ± 0.35	20.35^{a} ± 0.26	15.82^{c} ± 0.87	18.45^{a} ± 0.27	14.59^{a} ± 0.34
Chloramine T	25.33^{b} ± 0.33	28.43^{a} ± 0.30	23.42^{b} ± 0.48	22.46^{c} ± 0.35	20.81^{b} ± 0.38	24.40^{a} ± 0.30	21.47^{b} ± 0.37	17.39^{c} ± 0.24	19.50^{a} ± 0.38	20.39^{a} ± 0.34	16.34^{b} ± 0.33	14.34^{a} ± 0.72

*Values are represented as mean ± SD.
*The treatment means were separated using Tukey HSD at 0.05% probability level.
*Level not connected by same letter are significantly different in vertical columns.

Table 3: Percent inhibition in the growth of *S. rolfsii* with different extract of *P. appendiculatum*.

Nature of extract	Concentration (µg ml^{-1})											
	Time (hrs.)											
	1000			500			200			40		
	24	48	72	24	48	72	24	48	72	24	48	72
Petroleum ether	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Methanol	22.73^{a} ± 0.36	14.42^{c} ± 0.34	11.42^{bc} ± 0.49	21.64^{a} ± 0.22	13.79^{b} ± 0.43	10.22^{d} ± 0.50	15.63^{a} ± 0.34	14.29^{a} ± 0.65	12.23^{ab} ± 0.97	11.15^{b} ± 0.66	10.36^{bc} ± 0.80	5.36^{c} ± 0.88
Chloroform	20.82^{b} ± 0.33	16.50^{b} ± 0.44	10.58^{c} ± 0.49	20.79^{b} ± 0.44	9.83^{c} ± 0.49	12.78^{b} ± 0.44	14.81^{ab} ± 0.74	12.67^{b} ± 0.69	12.69^{ab} ± 0.46	12.61^{a} ± 0.31	11.48^{b} ± 0.49	7.94^{b} ± 0.20
Ethanol	19.02^{c} ± 0.13	16.46^{b} ± 0.43	12.41^{b} ± 0.32	16.66^{c} ± 0.41	14.29^{b} ± 0.55	11.43^{c} ± 0.43	13.44^{c} ± 0.36	12.18^{b} ± 0.55	11.07^{b} ± 0.83	10.94^{b} ± 0.37	9.72^{c} ± 0.54	9.30^{b} ± 0.76
Acetone	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chloramine T	19.79^{d} ± 0.39	21.49^{a} ± 0.16	18.61^{a} ± 0.55	16.29^{c} ± 0.21	18.67^{a} ± 0.62	15.31^{a} ± 0.56	14.45b^{c} ± 0.47	15.13^{a} ± 0.41	13.27^{a} ± 0.83	12.42^{a} ± 0.18	14.41^{a} ± 0.44	11.30^{a} ± 0.47

*Values are represented as mean ± SD.
*The treatment means were separated using Tukey HSD at 0.05% probability level.
*Level not connected by same letter are significantly different in vertical columns.

Table 4: Percent inhibition in the growth of *R. solanii* with different extract of *P. appendiculatum*.

Nature of extract	Concentration (µg ml^{-1})											
	Time (hrs.)											
	1000			500			200			40		
	24	48	72	24	48	72	24	48	72	24	48	72
Petroleum ether	6.49^{f} ± 0.46	5.37^{d} ± 0.35	3.04^{f} ± 0.19	7.31^{d} ± 0.47	7.33^{c} ± 0.43	2.43^{e} ± 0.40	5.28^{e} ± 0.34	9.14^{c} ± 0.36	2.45^{d} ± 0.48	6.31^{e} ± 0.31	3.32^{d} ± 0.43	0.72^{d} ± 0.25
Methanol	15.14^{c} ± 0.31	17.60^{a} ± 0.51	15.23^{d} ± 0.43	17.34^{a} ± 0.37	14.09^{b} ± 0.18	12.33^{b} ± 0.18	19.30^{a} ± 0.36	8.47^{cd} ± 0.32	7.25^{c} ± 0.33	10.32^{c} ± 0.27	11.38^{a} ± 0.36	10.37^{b} ± 0.40
Chloroform	16.24^{b} ± 0.45	17.07^{a} ± 0.21	16.17^{c} ± 0.27	16.97^{a} ± 0.30	14.52^{b} ± 0.36	12.38^{b} ± 0.52	14.37^{b} ± 0.37	12.42^{a} ± 0.32	10.74^{ab} ± 0.24	12.68^{b} ± 0.40	9.54^{b} ± 0.49	6.28^{c} ± 0.35
Ethanol	11.06^{e} ± 0.10	10.71^{c} ± 0.46	14.18^{e} ± 0.12	13.61^{c} ± 0.32	7.94^{c} ± 0.12	5.63^{d} ± 0.30	10.25^{d} ± 0.68	7.49^{d} ± 0.43	3.39^{d} ± 0.52	8.29 ^{d} ± 0.31	2.36^{e} ± 0.30	0.65^{d} ± 0.29
Acetone	17.38 ^{a} ± 0.47	14.23^{b} ± 0.25	17.74^{b} ± 0.44	15.36^{b} ± 0.27	14.45^{b} ± 0.43	10.70^{c} ± 0.50	12.30^{c} ± 0.37	11.32^{b} ± 0.54	9.66^{b} ± 0.48	10.80^{c} ± 0.42	8.31^{c} ± 0.43	6.97^{c} ± 0.30
Chloramine T	12.30^{d} ± 0.43	14.10^{b} ± 0.28	19.07^{a} ± 0.13	15.97^{b} ± 0.36	16.95^{a} ± 0.23	14.28^{a} ± 0.52	15.23^{b} ± 0.46	13.19^{a} ± 0.33	11.21^{a} ± 0.48	14.34^{a} ± 0.24	12.17^{a} ± 0.16	13.27^{a} ± 0.34

*Values are represented as mean ± SD.
*The treatment means were separated using Tukey HSD at 0.05% probability level.
*Level not connected by same letter are significantly different in vertical columns.

Table 5: Activity Index of the growth of *M. plutonius* (MP) *and P. multocida* (PM) with different extract of *P. appendiculatum*.

Nature of extract	Concentration (µg ml^{-1})							
	1000		500		200		40	
	MP	PM	MP	PM	MP	PM	MP	PM
Petroleum ether	8.33^{d} ±0.28	10.17^{c} ±0.24	6.19^{d} ±0.29	9.18^{d} ±0.25	0.0	7.21^{d} ±0.27	0.0	5.23^{d} ±0.37
Methanol	10.26^{c} ±0.31	13.22^{b} ±0.33	9.25^{c} ±0.27	11.24^{c} ±0.28	8.37^{c} ±0.22	9.19^{c} ±0.27	6.28^{c} ±0.28	6.27^{c} ±0.34
Chloroform	15.23^{a} ±0.29	15.24^{a} ±0.29	13.24^{a} ±0.28	13.21^{a} ±0.32	11.22^{a} ±0.24	11.18^{a} ±0.25	9.16^{a} ±0.25	9.20^{a} ±0.24
Ethanol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Acetone	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ampicillin	15.23^{a} ±0.29	15.20^{a} ±0.28	13.22^{a} ±0.29	13.56^{a} ±0.52	11.22^{a} ±0.26	11.24^{a} ±0.28	9.20^{a} ±0.28	9.20^{a} ±.23
Streptomycin	13.22^{b} ±0.22	15.27^{a} ±0.30	11.23^{b} ±0.36	12.22^{b} ±0.35	10.23^{b} ±0.35	10.24^{b} ±0.28	8.22^{b} ±0.34	8.19^{b} ±0.28

*Values are represented as mean ± SD.
*The treatment means were separated using Tukey HSD at 0.05% probability level.
*Level not connected by same letter are significantly different invertical columns.

For *Rhizoctonia solani*, out of all the organic extracts of *P. appendiculatum*, the maximum percent inhibition was observed at 72 hrs (17.74) in acetone extract at 1000 µg ml^{-1} conc. and minimum at 72 hrs (0.65) in ethanol at 40 µg ml^{-1} concentration (Table 4).

All the organic extracts of *P. appndiculatum* showed strong and broad spectrum inhibition against *F. oxysporum*, *T. indica*, *S. rolfsii*, *R. solanii*, *M. plutonius* and *P. multocida*. Earlier reports also suggested good antimicrobial activity in organic extracts of liverwort. Wolter (1964) studied antifungal activities of 18 species of bryophytes. Out of which, *Diplophyllum albicans*, *Plagiothecium denticulatun* and *Pogonatum aloides* showed remarkable antifungal activity. Number of studies were carried out in past many year. Dubey *et al.* (2002) screened acetone extract of *Conocephalum conicum*, *Marchantia polymorpha* and *P. appendiculatum* against two fungi (*Aspergillus niger* and *Candida albicans*) and two Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*) and found that acetone soluble extract of all the bryophytes showed inhibitory effect against the pathogens. In the present study only the acetone extract of *P. appendiculatum* showed potent activity against the fungus *Rhizoctonia solani.* Later on, Singh *et al.*, (2006) reported that the alcoholic and aqueous extract of *P. appendiculatum* used ethno medically by Gaddi tribe in Kangra valley for treating skin diseases and showed significant antibacterial and antifungal activity with MIC value of 2.5 µg/disc. Two year later, Bodade *et al.*, (2008) tested the antimicrobial activity of *P. appendiculatum* and some other bryophytes and showed that among the different tested fungi *A. niger*, *Fusarium monili*, *Rhizoctonia bataticola* and *Fusarium moniliforme* were most sensitive to the ethanolic extract of *P. appendiculatum.* For *Melissococcus plutonius*, out of the five organic extracts of *P. appendiculatum*, the activity was maximum 15.23 with chloroform extract and minimum 6.19 with petroleum ether extract (Table 5). On comparing chloroform with ampicillin (positive control) non significant values were obtained at all concentrations.

All the extracts of *P. appendiculatum* failed to inhibit growth of *Xanthomonas oryzae* and *S. enterica*. In case of *Pasteurella multocida* out of the five extracts of *P. appendiculatum*, the activity was maximum 15.24 with chloroform extract and minimum 5.23 in petroleum ether extract (Table 5).

Non significant values were observed in chloroform at all concentrations over to both the positive controls i.e. ampicillin and streptomycin. In the present finding, *M. plutonius* was found to be most sensitive bacteria while *F. oxysporum f. sp. lycopersici* and *T. indica* Mitra were most sensitive fungi. This broad spectrum antimicrobial activity of the liverwort extract is because of the presence of phinolics, flavonoids, and other antimicrobial substances.

Bodade *et al.* (2008) indicated that the extracts of different bryophytes were found to be active against different microorganisms. Ethanolic, acetone and chloroform extracts were found to be more effective on *Escherichia coli* and *Staphylococcus aureus.* Among the fungi *Aspergillus niger* was most sensitive to the ethanolic extract of *Plagiochasma appendiculatum* and *Bryum argentium.* In the present study, ethanol extract was also most effective against the *Salmonella enterica*; acetone extract was most effective against the *Melissococcus plutonius* and chloroform extract was most effective against the *Xanthomonas oryzae*. The MBC and MFC values were found to be highest for acetone extracts than their MIC value (Table 6). This may be due to impure form of bioactive compounds. In some cases, the MIC and MBC/MFC values were same which may be due to the presence of specific group of compounds as also indicated in other studies.

The MIC values were greater than their respective MFC/MBC values. This may be due to impure form of the bioactive compounds while in some cases these values were equal to their respective MFC/MBC values which may be due to presence of a specific group of compounds. Mewari *et al.*, (2008) also found that the MBC/MFC values were higher in case of methanol extract than their MIC values. While free flavonoid extract showed the same MBC/MFC and MIC values.

An overview of bioactivity data obtained from the present investigation indicates higher antifungal activity of methanol, acetone and chloroform extracts of *P. appediculatum* of liverwort than the other extracts. A perusal of different extracts of organic, from *P. appediculatum* with different concentrations (1000, 500, 200 and 40 µg ml^{-1}) and at different time intervals (24, 48 and 72 hrs) indicated maximum inhibition on the growth with ethanol extract of *P. appendiculatum at* 1000 µg ml^{-1} concentrations.

Table 6: MIC, MFC and MBC of different extract of *P. appendiculatum* different pathogens (µg/ml).

Pathogen	**Petroleum ether**		**Methanol**		**Chloroform**		**Etahnol**		**Acetone**		**STANDARDS**
Fungi	**MIC**	**MBC/ MFC**	**MIC**	**MBC/ MFC**	**MIC**	**MBC/ MFC**	**MIC**	**MBC/ MFC**	**MIC**	**MBC/ MFC**	**MIC (MBC/MFC)**
S. rolfsii	-	-	1.25	1.75	1.25	1.75	1.25	1.75	-	-	0.50 (0.80)
R. solani	2.50	5.00	1.25	1.75	2.50	2.50	2.50	4.50	2.50	2.50	1.00 (1.25)
T. indica	1.25	1.85	0.65	1.00	1.25	2.00	1.75	2.00	0.65	1.25	0.50 (0.60)
F. oxysporium	1.25	1.75	1.25	1.85	1.25	1.75	0.65	0.65	1.25	1.25	0.50 (0.60)
Bacteria											
P. multocida	1.25	1.65	1.25	2.50	0.65	1.25	-	-	-	-	0.50 (0.70)
S. enterica	-	-	-	-	-	-	-	-	-	-	0.25 (0.25)
M. plutonius	2.50	5.00	1.25	2.50	0.65	1.25	-	-	-	-	0.65 (0.80)
X. oryzae	-	-	-	-	-	-	-	-	-	-	0.65 (0.75)

Chloramines T and Ampicillin are used as standards for fungi and bacteria respectively.

CONCLUSION

The present investigation clearly indicates that bryophytes are rich storehouse of unknown botanicals, justifying a more extensive and critical analysis of bioactivity for this group. From this study, it may be concluded that *P. appendiculatum* have a vast potential as botanical pesticides and can serve as new source of fungicide and antibiotics in near future.

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