

## Research Article



## Phytochemical Screening and Antibacterial Studies of Certain Bryophytes against Some Phytopathogenic Bacteria

G.S. Deora\*

Centre of advanced study, Department of Botany, Jai Narain Vyas University, Jodhpur, India.

\*Corresponding author's E-mail: [gsdbiotech04@yahoo.co.in](mailto:gsdbiotech04@yahoo.co.in)

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### ABSTRACT

The present work was carried out to evaluate antimicrobial potential of three bryophytes collected from different localities in Rajasthan in rainy season. Crude aqueous extract of *Plagiochasma appendiculatum*, *P.intermedium* and *Fissidens bryoides* were prepared to analyze the presence of various biological active compounds such as flavonoids, terpenoids, alkaloids, glycosides, saponins, anthoquinins and sterols and their reverse effect against the growth of *Xanthomonas citri* and *Pseudomonas syringae* using spread plate and agar well diffusion methods for colony counting and zone of inhibition respectively. In present study it was noticed that all selected bryophytes possess antimicrobial potential against the growth of *X.citri* and *P.syringae* due to presence of flavonoids, terpenoids, glycosides and sterols in high amount. Among the extracts, *P.appendiculatum* was most effective followed by *P.intermedium* and *F. bryoides*. Two commercially available antibiotic drugs, streptomycin and amoxillin were used as positive control to compare antimicrobial effect of these bryophytes against selected plant pathogenic bacteria.

**Keywords:** Phytochemical screening, antimicrobial activity, bryophytes spread plate, agar well diffusion method phytopathogen.

### INTRODUCTION

Bryophytes are rich store house of biologically active compounds such as terpenoids, flavonoids, alkaloids, glycosides, saponins, anthoquinins and sterols and other aromatic chemicals. They not only defend themselves against the plant competition, drought resistance and freezing but also function against microbial attack and insect or animal predation<sup>1</sup>. Extract of many bryophytes have been shown to possess varying levels of antimicrobial potential and many chemicals were isolated from them which inhibited the growth of phytopathogenic microorganisms<sup>2-7</sup>.

The antibiotic activity of 52 species of bryophytes tested against 12 microorganisms<sup>8</sup>. Several terpenoids and aromatic compounds were isolated from various liverworts (Hepaticeae) and antimicrobial activities and inhibitory activities of 5-lipoxygenase and calmodilin studied<sup>9</sup>. The antimicrobial activity of liverwort, *Lunularia cruciata* was studied and evaluated the action of its acetone extracts against 13 bacterial strains. Inhibition of bacterial growth was compared with that of nacetoxine, benzyl penicillin and tetracycline<sup>10</sup>

### MATERIALS AND METHODS

The plant materials of *P.appendiculatum*, *P.intermedium* and *F.bryoides* were collected from various localities of Mt.Abu and Udaipur district of Rajasthan in rainy season both in vegetative and sporophytic phase. All collected plant materials were washed with tap water to remove adherent soil particles, and then plant materials were dried on blotting paper in laboratory at room temperature to remove extra moisture. After removing moisture the materials were grinded in pastel and

mortar. Sun light exposure was avoided to prevent loss of active aromatic compounds.

**Extract preparation:** For aqueous extract preparation, 100 gm. plant material was grinded in mortar and pastel with equal amount double distilled water (DDW) till the formation of fine paste, and then the smooth paste was filtered through filter paper and was centrifuged at 600 rpm for 20 minutes. After centrifugation the supernatant was filtered through Whatman filter paper no. 1 and stored in conical flask for further study.

**Preparation different concentrations of extracts:** The filtrate was used as 100 per cent concentration of the crude extract. It was then serially diluted adding double distilled water (DDW) to prepare various concentrations from 10 to 100 per cent. Thus total 10 concentrations were obtained (10-100) from each solvent extraction for all the selected bryophytes. Commercial antibiotics

**Commercial antibiotics:** Two commercially available antibiotics, streptomycin and amoxillin were used as positive control to compare the antimicrobial effect of bryophyte extract against selected phytopathogenic bacteria. Standard solutions were made by dissolving both the antibiotics separately in DDW (1 gram drug/10 ml DDW). The standard solutions were treated as 100 per cent concentration which was then diluted using DDW to prepare different strength of this solution from 10 to 100 per cent.

**Test organisms:** Test organism, *X.citri* and *P. syringae* was on nutrient agar and broth in laboratory collected from diseased plant. The pure culture was subcultured and serially diluted to obtain single colony. Pure culture of *P.syringae* (MTCC1604) was obtained from IMTECH Chandigarh (India).



**Phytochemical screening:** The aqueous extracts of all three selected bryophytes were subjected to various phytochemical tests to detect the presence of certain bioactive compounds<sup>11,12</sup>.

### Bioassay of antimicrobial activity

#### Spread plate assay for colony count experiment

For spread plate assay serial dilution of bacterial cultures was done. For this 1ml. of 24 hr old bacterial broth culture was taken and diluted up to 10<sup>-2</sup> dilutions. 10 µl of this dilution was then spread evenly over the surface of solidified agar medium containing plant extracts and antibiotic solutions of different concentrations in each Petri plate in a ratio of 1:1 to compare antibacterial effect of plant extracts and antibiotic drugs. All the Petri plates were sealed with Para film and incubated at 30 °C for 24 hrs after which bacterial colonies were counted in each case. The tests were carried out in triplicate with negative control, DDW for each case.

#### Agar well diffusion assay for inhibition zone experiments.

Solidified medium was punched with a three millimeters diameter well using a sterile cork borer and then inoculated with 100 µL of 24 hrs. old broth culture of test microorganisms.

The bacterial solution was evenly spread on the medium surface than the well was filled with 100 µL of the plant extract /antibiotic solutions of different concentrations.

The plates were sealed with Para film and incubated at 30 °C for 24 hrs.

The antibacterial activity was calculated by measuring the diameter of inhibition zone appeared after incubation period. The tests were carried out in triplicate with a negative control for each set.

## RESULTS AND DISCUSSION

### Phytochemical Screening

Results on phytochemical screening of selected bryophytes showed that terpenoids, flavonoids, sterols and glycosides were present in all three tested bryophytes where as alkaloids, saponins and anthoquinin were not reported. (Table-1).

### Evaluation of antibacterial activity

Aqueous extracts showed positive effect against both the selected bacterial strains. Further it was observed that crude aqueous extract of *P. appendiculatum* was comparatively more effective in case of *X. citri* with minimum (16.0) number of colonies followed by *P. intermedium* 21.1 and *F. bryoides* 24.1 in 100 per cent concentrations in comparison to the control. Similar pattern of results were obtained in case of zone of inhibition in different concentrations of extracts of all three bryophytes. Zone of inhibition was maximum (49.6) in *P. appendiculatum* extract followed by *P. intermedium* (42.9) and *F. bryoides* (36.9) in 100 per cent concentration in comparison to its control (Table-2, 3). Table-2 Number of colonies of *Xanthomonas citri* in aqueous crude extracts of *Plagiochasma appendiculatum*, *P. intermedium* and *Fissidens broides* after 24 hrs. of incubation at 30<sup>o</sup>C.

In case of *P. syringae* it was reported that extracts of all three bryophytes were effective against bacterial growth but slightly less effective as in *X. citri*. Number of colonies of *P. syringae* was 26.2 followed by 27.3 and 29.3 in *P. appendiculatum*, *P. intermedium* and *F. bryoides* respectively in 100 per cent aqueous extract concentrations. Similarly zone of inhibition was maximum (34.5) in *P. appendiculatum* followed by *P. intermedium* (30.5) and *F. bryoides* (25.0).

**Table 1:** Phytochemical profile of selected bryophytes

Active compounds	Phytochemical tests	Observations	Results		
			<i>Plagiochasma appendiculatum</i>	<i>Plagiochasma intermedium</i>	<i>Fissidens bryoides</i>
Alkaloids	Mayers test	No precipitation	-	-	-
	Hagers test	No precipitation	-	-	-
Anthroquinin	Borntragers test	No layer formation	-	-	-
Cardia glycosides	Keller Killeni test	Brown ring	++	++	+
Flavanoids	Ferric chloride test	Green colour	++	+	++
	Lead acetate	Yellow precipitate	++	++	+
	Alkaline reagent test	Yellow florescent test	++	++	+
	Sodium hydrochloride test	Yellow colour	++	+	+
Saponins	Froth test	No froth formation	-	-	-
Sterols	Salkowaski test	Reddish	++	++	+
	Liebermann-Burchardt test	brown colour	++	++	++
	Burchardt test	Brown ring	++	++	++
Terpenoids	Salkowaski test	Lower layer turned yellow	++	+	++
	Liebermann-Burchardt test	Deep red colour	+	++	++
	Burchardt test	Deep red colour	+	++	++

Note: Results based on mean of three replicates.



**Table 2:** Number of colonies of *Xanthomonas citri* in aqueous crude extracts of *Plagiochasma appendiculatum*, *P. intermedium* and *Fissidens broides* after 24 hrs. of incubation at 30°C.

S. No.	Concentration %	Streptomycin	Amaxillin	P. appendiculatum	P. intermedium	F. bryoides
1	Control	97.0	97.0	97.0	97.0	97.0
2	10	78.3	79.2	89.1	90.0	92.3
3	20	67.1	69.4	77.0	82.0	85.1
4	30	58.4	61.7	68.2	76.1	70.5
5	40	47.6	50.1	56.5	61.4	65.6
6	50	33.4	34.4	42.3	52.6	54.9
7	60	24.3	26.6	36.6	43.5	45.2
8	70	18.2	20.3	27.8	37.3	39.7
9	80	13.3	14.5	22.1	29.2	32.3
10	90	9.1	10.6	18.9	26.0	28.7
11	100	6.2	8.0	16.0	21.1	24.1

Note: Results based on mean of three replicates

**Table 3:** Zone of inhibition of *Xanthomonas citri* in aqueous crude extract of *Plagiochasma appendiculatum*, *P. intermedium* and *Fissidens broides* after 24 hrs. of incubation at 30°C

S. No.	Concentration %	Streptomycin	Amaxillin	P. appendiculatum	P. intermedium	F. bryoides
1	Control	00	00	00	00	00
2	10	36.2	12.6	10.1	9.0	7.0
3	20	37.3	14.6	12.0	10.8	7.3
4	30	40.0	16.1	14.1	12.1	10.6
5	40	41.2	24.9	16.2	14.4	11.2
6	50	43.1	30.7	17.9	15.3	12.0
7	60	46.0	40.3	19.1	17.5	14.7
8	70	57.2	51.4	25.6	22.7	16.8
9	80	68.3	62.6	31.7	24.4	20.2
10	90	77.6	72.3	40.1	30.2	24.3
11	100	89.0	81.1	49.6	42.9	36.6

Note: Results based on three replicates.

**Table 4:** Number of colonies of *P.syringae* in aqueous crude extracts of *Plagiochasma appendiculatum*, *P.intermedium*, and *Fissidens broides* after 24 hrs. of incubation at 30 °C.

S. No.	Concentrations %	Streptomycin	Amoxillin	Pa	Pi	Fb
1	Control	96	96	96	96	96
2	10	77.2	88.3	90.0	92.1	93.0
3	20	58.3	71.1	82.2	88.4	88.6
4	30	47.7	68.3	88.3	87.5	88.9
5	40	34.5	57.0	71.4	73.3	74.1
6	50	25.1	45.6	61.3	62.6	63.0
7	60	20.0	32.1	51.2	53.4	54.1
8	70	15.4	25.0	42.0	43.1	44.1
9	80	12.6	24.1	36.4	40.0	41.6
10	90	10.4	22.7	32.1	34.3	37.2
11	100	8.0	18.3	26.2	27.3	29.3

Note: Results based on mean of three replicates.



**Table 5:** Zone of inhibition (in mm) of *Pseudomonas syringae* in aqueous crude extract of *P.appendiculatum*, *P.intermedium* and *F.bryoides*.

S.No.	% Concentrations	Streptomycin	Amoxillin	Pa	Pi	Fb
1	Control	00	00	00	00	00
2	10	38.2	13.5	9.2	9.0	7.6
3	20	39.7	14.1	10.1	9.6	8.1
4	30	40.3	15.3	11.3	11.0	9.2
5	40	43.1	22.4	11.6	12.0	10.1
6	50	46.3	37.0	14.0	13.1	11.0
7	60	50.0	37.0	17.3	15.5	13.0
8	70	57.1	47.2	20.4	18.4	15.2
9	80	66.0	59.3	26.1	22.0	18.6
10	90	77.1	71.5	30.0	26.0	22.4
11	100	90.0	76.6	34.5	30.5	25.0

Note: Results based on mean of three replicates.

Various concentrations of commercially available antibiotics, streptomycin and amoxillin tested against both the bacterial strains were quite potent than the extracts of plants.

Among the antibiotics streptomycin was more effective than the amoxillin in both the cases.

Bryophytes are the potent source of various bioactive compounds and show antimicrobial activity against phytopathogenic microbes and suggested that these plants may be a potent tool to control different plant diseases caused by microbes instead of using synthetic chemicals<sup>13,14</sup>.

Antimicrobial activity of liverwort *Lunularia cruciata* extracts against 13 bacterial strains.

Inhibition of bacterial growth was compared with the nacefotixine, benxyl penicillin and tetracycline and reported similar results<sup>15</sup>.

## CONCLUSION

Results of this study indicated the presence of active biological compounds such as terpenoids, flavonoids, sterols and glycosides.

Further these results suggest that these chemical compounds could be potent antimicrobial agents to treat plant associated diseases such as Citrus canker and Bean blight caused by *Xanthomonas citri* and *Pseudomonas syringae* respectively.

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