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Antimicrobial Activity of Certain Bryophytes

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The antibacterial effect of Plagiochasma articulatum (A. liverwort) and Fissidens bryoides (A. moss) was evaluated on some bacterial strains such as Agrobacterium tumifaciens, Streptomyces scabies and Xanthmonas citri. The solvent used for the extract preparation was water. The in vitro antibacterial activity was performed by streak plate disc diffusion method (Spdd). P. articulatum extract was more potent than F. bryoides against all selected test microorganisms. Further it was concluded that S. scabies showed maximum growth inhibitory activity followed by X. citri and A.tumifaciens. The results obtained in the present work suggest that both the bryophytes can be used for the treatment of plant diseases caused by these test microorganisms.

Key words: Antimicrobial activity, Bryophytes, *Plagiochasma articulatum*, *Fissidens bryoides*, microorganisms.

Antibiotic activity of bryophytes has drawn the attention of botanists and microbiologists in past few years because their life cycle involve alternation between a diploid sporophyte and a dominant free living haploid gametophyte generation. An interesting feature of bryophytes is that they are relatively free from attack by parasitic microorganisms. This may be due to their immunological properties or antimicrobial activity.

Hayes (1947) found that the aqueous extracts of *Conocephalum conicum* to be active against certain microorganisms. Madsen and Pates (1952) and Pates and Madsen (1955) studied eight bryophytes, of which *C. conicum, Dumortiera hirtuta, Sphagnum portorecense* and *S. strictum* were active. The former two were active against *Candida albicans* and the species of *Sphagnum* inhibited *Staphylococcus aureus* and *Pseudomonas acreiginosa.* 52 plant species were extracted and tested against 12 microorganisms by Benerjee and Sen (1979). Solubility data and antibiotic spectra of the active plants indicated the occurrence of variety of antibiotic substrates among bryophytes.

The antibacterial activity of the liverwort *Lunularia crutiata* studied by Basil *et al.,* (1998). They evaluated the action of its acetone extract against 13 bacterial strains. Inhibition of bacterial growth was compared with that of nacefotaxine, benzyl penicillin and tetracycline.

Wakuli *et al.,* (2003) studied that the extracted pigments of bryophytes exhibited antibiotic properties against gram positive bacteria (*Aureobacterium liquefaciens, Arthrobacter globiformis, Bacillus brevis, B. cirulans, B. subtilis* and *Curlobacterium plantanum*). Catenarin also inhibited the growth of fungi accompanying *P. tritici-repentis* during the saprophtic phase of development. The most sensitive species was *Epicouim nigrum,* whose growth was inhibited upto 90 per cent.

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Opelt and Berg (2004) investigated that very little is known about the interaction of bryophytes with bacteria. Analysis was carried out using bacteria associated with three bryophyte species, *Tortula muralis, Aulacomnium palustre* and *Sphagnum rupellum,* which represent typical moss species of three nutrient poor plant communities at the Southern baltic sea cost in Germany. By using of two cultivation independent techniques, denaturing gradient gel electrophoresis and single strand configuration polymorphism analysis of the 16s ribosomal DNA, a high degree of moss specificity was found for associated bacterial communities.

Plant pathogenic bacterial species occur in genera *Xanthomonas, Agrobacterium, Streptomyces, Corynebacterium* etc. These pathogenic bacteria were widely distributed throughout the world and cause several plant diseases including hypertrophy with rots, blights, galls etc. The bacterial canker caused by *X. citri* is one of the most serious disease of acid line prevalent all over the country. The disease is highly infectious spread through tree to tree through water splashes and affects all the aerial parts of plant. Due to the presence of cankerious tissue the fruit has poor appeal to the consumers.

Here is dearth of information regarding antibacterial activities and antibacterial substances in bryophytes. The bryophytes are diverse group of land plants that usually colonize habitates with most or extremely variable conditions.

Keeping this view, present work will provide a comparative study of sensitivity of bacteria against bryophytes which indicates that the bryophytes are rich store house of antibacterial substances. The observations of this work will play a key role for biological control of microorganism against plants.

MATERIALS AND METHODS

Collection and storage of plants

The plant materials were collected from the natural habitat from Mt. Abu. Since the bryophytes usually grow in closed association with each other so homogenous patches were looked for during collection. The fresh plants *i.e. Plagiochasma articulatum* and *Fissidens bryoides* with proper reproductive organs and devoid of parts of other plants and soil adhered to rhizoids were preferred. The plant materials were packed in polyethylene bags and brought to the laboratory. In the laboratory the plant materials were washed with water till all the debris and dust particles were removed and they were washed with distill water. The materials were then dried in between blotting paper to remove extra moisture. 100 gm of plant materials were taken in pestle and mortar to prepare aqueous crude extracts and rest of the material wrapped in filter paper and packed in polyethylene bags and stored in freeze to prevent them from drying and decaying.

Preparation of extracts

100 gm of *Plagiochasma articulatum* and *Fissidens bryoides* were grinded in the pestle and mortar with 200ml of distill water to prepare a smooth pulp which was kept over night so that all the antibacterial ingredients of bryophytes dissolve in the water. The extract was then filtered through Whatman filter paper, extract so prepared was 50 per cent concentration. Different concentrations of extracts were prepared by this extracts and autoclaved.

Glassware

Borosil make conical flasks and petri plates were used as culture vessels for microbial studies. Measuring cylinders were used for media preparation and for other purposes.

Washing

The glasswares were first rinsed with tap water followed by washing with liquid detergent (non-abrasive). After removal of detergent with tap water, the glasswares were first rinsed with chromic acid and then with single distilled water. The rinsed glasswares were allowed to dry in hot air oven at 120°C for 24 hrs. The plastic ware were cleaned only with abrasive liquid detergent or hot water and washed with tap water prior to rinsing with single distilled water. They were then dried in dust free cabinets.

Medium used for maintenance of test organisms

Broth Agar basal medium and beef peptone agar basal medium of different pH *i.e.* 4, 5, 6, 6.5, 7, 7.3, 7.5 pH were prepared to check the growth of test organism, *Xanthmonas citri, Agrobacterium tumifaciens* and *Streptomyces scabies*. The pH of both the beef peptone and broth liquid basal media were adjusted at 7.3. This range

of pH was selected for microbial growth.

Experimental set up

The solid media of broth nutrient agar and beef peptone agar of different pH were prepared in sterilized conical flask, fitted with cotton plugs and then autoclaved. The experiments were set up in the petri plates in aseptic condition on laminar airflow bench. The petri plates for both the plants with both the media of different pH were prepared alongwith extracts of different percentage concentrations. When the medium alongwith extract was solidified than bacterium *Xanthomonas citri* was inoculated in all the petri plates by streak plate disc diffusion method (Spdd) method and petri plates were covered and wrapped in the aluminum foil. These petri plates were placed in the incubator for 24 hours at 37°C. Results were observed after 24 hours and photographs were taken. In both the media, the growth of bacteria was minimum in medium of pH 4. As the pH of medium raised the, bacterial growth also increased. The growth was maximum at pH 7.3 in the liquid medium. The beef peptone and broth basal medium of 7.3 pH were prepared in the sterilized conical flask and autoclaved. The experiments with both liquid media were set up in the sterilized conical flask fitted with cotton plug on the laminar airflow bench. The extracts of different concentrations were put in conical flask containing 100ml medium and the bacteria was inoculated. The conical flasks were tightly closed with cotton plug and were placed in the culture racks for 24 hours. Optical density was obtained with the help of spectrophotometer. Biomass was obtained by centrifugation of the medium with plant extracts and bacteria. All experiments were repeated for thrice. The results were shown in table 1-3 based on mean of three replicates.

RESULTS AND DISCUSSION

The liquid media of beef peptone (BPBM) and broth agar (BBM) having pH of 7.3 were prepared and experiments were set up adding different concentrations of aqueous crude extract of *Plagiochasma articulatum* and *Fissidens bryoides*. The test bacteria were inoculated in them. The effects of crude extracts of different concentrations of both the plants were observed after 24 hours. The table 1, 2 and 3 showed estimation of biomass in mg on dry weight basis and optical density in µm/ml of *Xanthomonas citri, Agrobacterium tumifacience* and *Streptomyces scabies* respectively. The growth rate of various bacteria was determined by biomass production and optical density.

Effect of *Particulatum* crude extract

Effect of *P. articulatum* crude extract on *Xanthomonas citri*

The crude extract of *P. articulatum* played an important role against bacterial growth in both the BPBM and BBM. The maximum biomass and optical density were observed in the control of both media. In BPBM it was 0.001mg and 1.0990

Table 1. Showing the antibacterial activity of *Plagiochasma articulatum* and *Fissidens bryoides* extracts on *Xanthomonas citri*

Concentration%	*Plagiochasma articulatum* extracts				*Fissidens bryoides* extracts			
	BPBM		BBM		BPBM		BBM	
	Biomass	O.D.	Biomass	O.D.	Biomass	O.D.	Biomass	O.D.
Control	0.0011	1.0990	0.0014	2.0914	0.0059	2.9961	0.0044	2.9980
1.0	0.0011	1.0971	0.0013	2.0896	0.0054	2.8963	0.0044	2.8751
10.0	0.0009	1.0696	0.0009	1.8887	0.0027	2.7543	0.0031	2.8613
20.0	0.0008	0.9102	0.0009	0.9986	0.0019	1.9865	0.0023	1.9964
30.0	0.0005	0.8170	0.0006	0.8691	0.0014	1.7524	0.0016	1.8424
40.0	0.0003	0.5021	0.0005	0.5164	0.0010	0.9687	0.0013	1.2194
50.0	0.0001	0.3012	0.0002	0.3542	0.0006	0.6454	0.0009	0.9476
60.0-100.0	–	–	–	–	–	–	–	–

Note:- Results based on mean of three replicates.
BPBM – Beef peptone basal medium
BBM - Broth Basal Medium
O.D. - Optical Density

µ/ml whereas, it was 0.0014mg and 2.0914 µ/ml in BBM. Biomass and optical density were gradually decreased along with the increased concentrations of the extract. At one per cent concentration biomass in both the media was 0.011 and 0.0013mg. Optical density reported on the same concentration was 1.0971 and 2.0896 µ / ml. As concentration increased from one to 50 per cent bacterial growth was decreased suggesting that higher concentrations showed inhibitory effect resulted low biomass and optical density (Table 1).

Effect of *P. articulatum* extract on *Agrobacterium tumifaciens*

Maximum growth of *A. tumifaciens* was observed in the extract free media (control) followed by one to 10 per cent concentrations of the extract. Biomass at the control, one to 10 per cent was 0.0043 and 0.0035mg respectively. Optical density in the same concentrations was 2.9990, 2.9990 and 2.5696 µ/ml in BPBM. Biomass in BBM was 0.0054, 0.0054 and 0.0045mg in the control, one to 10 per cent concentrations of the extract whereas, optical density was 3.4814 and 3.0617 µ/ml in the concentrations of extract in BBM (Table 2). After 10 per cent as concentrations of extract increased biomass and optical density decreased in both media suggested that *P. articulatum* extract showed inhibitory effect for the growth of *A. tumifaciens* up to 70 per cent. Beyond this concentration no bacterial growth was observed.

Effect of *P. articulatum* crude extract on *Streptomyces scabies*

It was observed that *P. articulatum* crude extract adversely affected the bacterial growth. The maximum (0.0016mg) biomass was reported in the control followed by 10 per cent (0.0014mg). Gradual decrease was noticed in the higher concentrations in BPBM. Optical density was maximum (1.6230) in the control and one per cent. It was 1.3170 mg at 10 per cent concentration. At 80 per cent and above this concentration no bacterial growth was reported. Similarly in BBM maximum (0.0016mg) biomass was reported in the control followed by 0.0015 and 0.0013mg at one and 10 percent concentrations of extract. Higher concentrations showed inhibitory effect. Optical density was 1.6232, 1.6231 and 1.5421 µ /ml in the control one and 10 per cent concentrations respectively. Again gradual decreased in O.D. was reported in higher concentrations of the extract (Table 3).

Table 2. Showing the antibacterial activity of *Plagiochasma articulatum* and *Fissidens bryoides* extracts on *Agrobacterium tumifaciens*

Concentration%	*Plagiochasma articulatum* extracts				*Fissidens bryoides* extracts			
	BPBM		BBM		BPBM		BBM	
	Biomass	O.D.	Biomass	O.D.	Biomass	O.D.	Biomass	O.D.
Control	0.0043	2.9990	0.0054	3.4814	0.0071	3.9984	0.0071	3.9984
1.0	0.0043	2.9990	0.0054	3.4814	0.0076	3.9982	0.0071	3.9983
10.0	0.0035	2.5696	0.0045	3.0617	0.0068	3.8765	0.0065	3.4534
20.0	0.0027	1.9102	0.0038	2.9617	0.0066	3.6637	0.0065	3.6334
30.0	0.0015	1.4170	0.0029	2.6017	0.0055	3.5986	0.0051	3.3763
40.0	0.0010	0.8021	0.0024	1.8420	0.0045	3.0978	0.0040	3.1498
50.0	0.0007	0.6121	0.0018	1.6324	0.0037	2.9789	0.0035	2.7356
60.0	0.0003	0.2312	0.0014	1.0917	0.0029	1.7302	0.0029	1.7302
70.0	0.0001	0.0116	0.0012	0.9068	0.0024	0.9864	0.0021	0.8640
80.0	0.0000	0.0000	0.0008	0.5421	0.0020	0.7953	0.0007	0.6167
90.0	-	-	-	-	0.0009	0.6324	0.0004	0.3154
100.00	-	-	-	-	0.0000	0.0000	0.0000	0.0000

Note:- Results based on mean of three replicates.
BPBM – Beef peptone basal medium
BBM - Broth Basal Medium
O.D. - Optical Density

Effect of *Fissidens bryoides* crude extract

Effect of *F. bryoides* crude extract on *Xanthomonas citri*

It was observed that plant extract enriched BPBM and BBM showed comparatively more bacterial growth at lower concentrations but it was less than the control. Maximum (0.0059 mg) biomass was reported in the control followed by 0.0054 and 0.0027 mg at one and 10 per cent concentrations of the extract in the BPBM. Optical density in the same medium at same concentrations was 2.9921, 2.8963 and 2.7543 µ /ml. Beyond this concentrations gradual decrease in both the parameters was noticed. Above 50 per cent concentration no bacterial growth was reported. In BBM 0.0044, 0.0044 and 0.0031 mg biomass was observed in the control, one and 10 per cent concentrations of the extract respectively. Optical density was 2.9980, 2.8751 and 2.8613 µ /ml in the control, one and 10 per cent concentrations respectively. Bacterial growth was observed only up to 50 per cent concentrations (Table 1).

Effect of *F. bryoides* crude extract on *Agrobacterium tumifaciens*

In the BPBM with plant extract, maximum (0.0071 mg) biomass was reported in the control followed by one (0.0076 mg) and 10 per cent (0.0068 mg) concentrations. Optical density was 3.9984, 3.9982 and 3.8765 µ /ml in the control, one and 10 per cent concentrations respectively. Biomass and optical density of *A. tumifaciens* against *F. bryoides* were also reported in BBM. Biomass was 0.0071, 0.0071 and 0.0065 mg in the control, one and 10 per cent concentrations. Optical density was 3.9984, 3.9983 and 3.4534 µ /ml in the same concentrations of the extract. A gradual decrease was observed along with the increased concentrations but it was much less than the control and lower concentrations (Table 2).

Effect of *F. bryoides* crude extract on *Streptomyces scabies*

Inhibitory activity of *F. bryoides* crude extract against *S. scabies* was reported in both the BPBM and BBM. In the BPBM extract free and extract enriched experiments, biomass was 0.0021, 0.0021 and 0.0020 mg in the control, one and 10 per cent concentrations. Optical density was 1.6891, 1.6890 and 1.6120 µ/ml in the control, one and 10 per cent concentrations of the extract. In the BBM biomass was 0.0021, 0.0020 and 0.0019 mg in the control, one and 10 per cent concentrations. Optical density was 1.6890, 1.6890 and 1.6891 µ /ml in the same concentrations of same medium. Biomass and optical density of *S. scabies* also decreased

Table 3. Showing the antibacterial activity of *Plagiochasma articulatum* and *Fissidens bryoides* extracts on *Streptomyces scabies*

Concentration%	*Plagiochasma articulatum* extracts				*Fissidens bryoides* extracts			
	BPBM		BBM		BPBM		BBM	
	Biomass	O.D.	Biomass	O.D.	Biomass	O.D.	Biomass	O.D.
Control	0.0016	1.6230	0.0016	1.6232	0.0021	1.6891	0.0021	1.6890
1.0	0.0016	1.6230	0.0015	1.6231	0.0021	1.6890	0.0020	1.6890
10.0	0.0014	1.3170	0.0013	1.5421	0.0020	1.6120	0.0020	1.6891
20.0	0.0012	0.9162	0.0013	1.5421	0.0020	1.6120	0.0019	1.5410
30.0	0.0009	0.7814	0.0011	1.9121	0.0018	1.2130	0.0019	1.5410
40.0	0.0008	0.5632	0.0011	1.9121	0.0017	0.9887	0.0016	0.9787
50.0	0.0007	0.5414	0.0009	0.2431	0.0014	0.7543	0.0015	0.8624
60.0	0.0005	0.3421	0.0004	0.9142	0.0014	0.7543	0.0013	0.7341
70.0	0.0001	0.0102	0.0002	0.4327	0.0012	0.5471	0.0008	0.4932
80.0	0.0000	0.0000	0.0000	0.0000	0.0008	0.3183	0.0006	0.3089
90.0	-	-	-	-	0.0004	0.1720	0.0003	0.1210
100.00	-	-	-	-	0.0000	0.0000	0.0000	0.0000

Note:- Results based on mean of three replicates.
BPBM – Beef peptone basal medium
BBM - Broth Basal Medium
O.D. - Optical Density

with increased concentrations of the extract of *F. bryoides* (Table-3).

On the basis of results in table 1, 2 and 3 it can be concluded that extract of *Plagiochasma articulatum* and *Fissidens bryoides* showed antibacterial activity against all selected test microbes in both the media.

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