JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2007. Vol. **1**(2), p. 215-219

Antibiotic Effects of certain Bryophytes on *Agrobacterium tumifacians*

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(Received: 20 August 2007; accepted: 30 September 2007)

Phytotoxic effects of crude aquous extracts of three bryophytes, *Plagiochasma articulatum* (Kash.) a liverwort, *Anthoceros longii* (Steph.) a hornwort and *Fissidens bryoides* a moss was carried out on *Agrobacterium tumifacians* to unlock their antibiotic activity. The aquous crude extracts of different ppm were prepared. The number of colonies in pour plate method was evaluated. It was observed that the inhibition of bacterial growth was maximum in *P. articulatum* extract followed by *A.longii* and *F.bryoides* .

Keywords: Phytotoxins, bryophytes, crude extract, *Agrobacterium tumifacians*, liverwort, hornwort, moss.

Studies on antibiotic substances in green plants have been conducted by number of investigators in our country and abroad. Their results indicate the frequent occurrence of antimicrobial substances in bryophytes due to which they are free from microbial attack which may be due to their immunological properties or antimicrobial activity. Madsen and Pates (1952)[1] studied eight bryophytes of which *Conocephallum conicum* , *Dumortiera hirtuta* , *Sphagnum portorecense* and *S. strictum* were active against one or more pathogens . The former two were active against *Candida albicans* and the species of *Sphagnum* inhibited *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The antibiotic activity of 52 species of bryophytes tested against 12 microorganisms by Benerjee and Sen (1979)[2]. Solubility data and antibiotic spectra of the active plants indicated the occurrence of variety of antibiotic substances among bryophytes. Opelt and Berg (2004)[3] investigated that very little is known about the interaction of bryophytes with bacteria. Analysis was carried out using bacteria associated with three bryophytes species, *Tortula muralis, Aulacomium palustre* and *Sphagnum rupellum* which represent typical moss species of three nutrient poor plant communities at the southern Baltic sea coast of Germany.

Antibacterial potency of methanol extracts of three green lower plants, *Pneumatopteries afra, Platycerium bifurcatum* and *Nephrolepsis bisserata* was determined using agar clinical strains of *Escherichia coli*, *Staphylococcus aureus*, *Kbebsiella spp*. by Ojo, Ajayi, and Anibijuwon (2007)[4].

Bryophytes contain some active antibiotic substance and such type of studies have a great significance to explore their antimicrobial activity which leads to Biocontrol Programme (B.C.P.) of several country to overcome overdosed chemical pesticide problem. Further dearth of information regarding antibacterial activity and antibacterial substances in bryophytes, this study was undertaken. The bryophytes are diverse group

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of land plants that usually colonize habitates with moist or extremely variable conditions. Keeping in view the present work will provide comparative study of sensitivity of bacteria against bryophytes which indicate that the bryophytes are rich store house of antibacterial substances. The observations of this work will play a key role of biological control of microorganism against plant.

MATERIAL AND METHODS

Collection and storage of plants

The plant materials were collected from the natural habitats of Mt. Abu. Since the bryophytes usually grow in closed association with each other so homogenous patches were looked for during collection. The fresh material of *P.articulatum*, *A. longii* and *F. 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 were brought to the laboratory. In the laboratory plant materials were washed with water till all the debris and dust particles were removed and they were than washed with distilled water repeatedly. The materials were than dried in between blotting paper to remove extra moisture. The required plant materials for extract preparation were taken in pestle and mortar and rest of the materials were wrapped in blotting paper and packed in polyethylene bags and stored in freeze to prevent from drying.

Test organism

Agrobacterium tumifacians was used as a test organism. It is rod shaped bacteria which cause crown gall disease in plants. The test organism was brought from the IMTECH (Chandigarh) in powder form and was cultured in nutrient agar tubes and broth in the laboratory. The pure culture was subcultured and than serial dilution of pure culture was done in order to obtain single colony. Experiments were set up taking bacteria in microlitres.

Extract preparation

1000mg of each of the plant materials i.e. *P. articulatum, A. longii* and *F. bryoides* were taken in the pestle and mortar and were grinded with 1000ml distill water to prepare smooth pulp which was than kept overnight so that all the water soluble antibiotic ingredients of bryophytes dissolve in the water. The extracts were than filtered through Whatsman filter paper and the extracts prepared were of 100000ppm. From these extracts different concentration (10000ppm to90000ppm) were prepared and stored in volumetric flask in freeze. Experiments were set taking extracts in microlitres.

Preparation of medium

Nutrient agar medium and YEM medium of different pH ranging from 3 to 8.5 pH were prepared and autoclaved. The bacteria were then cultured on them in Petri dishes and it was observed that *Agrobacterium tumifacians* grew best on 7.3 pH of nutrient agar. It was observed that growth was maximum in nutrient agar in comparison to YEM medium. Therefore nutrient agar of 7.3 pH was selected for further experimental studies.

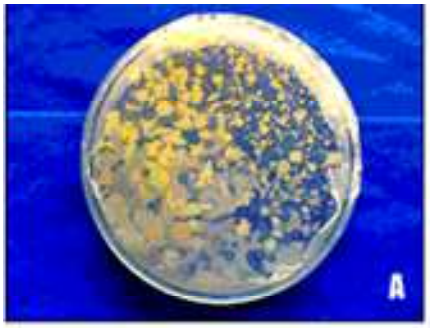
Analysis of antibacterial activities

The experiments were set in completely aseptic condition on laminar air flow bench. Two methods, well and pour plate methods were adopted to assess the microbial growth under the influence of extracts of *P. articulatum, A. longii* and *F. bryoides*. In well method 10 µl of bacteria was spread over the agar film in Petri dish and well were made in it. In each well 25µl of extract of different ppm was poured and inhibition zone of growth was measured after every 24 hrs and 48 hrs. In pour plate 10 µl bacterial culture, 25 µl extract of different ppm and medium were poured in Petri dishes. All these Petri dishes were sealed with Para film and placed in incubator at 28°C for 24 hrs and 48 hrs.Number of colonies were counted after every 24 hrs and 48 hrs by colony counter. Photographs were also taken after 24 hrs and 48 hrs at the time of study.

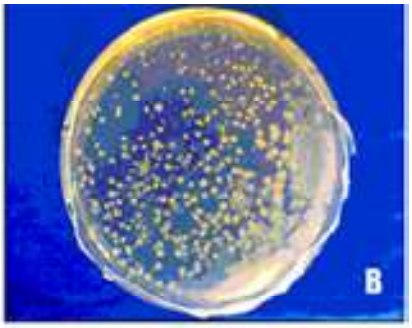
RESULTS AND DISCUSSION

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

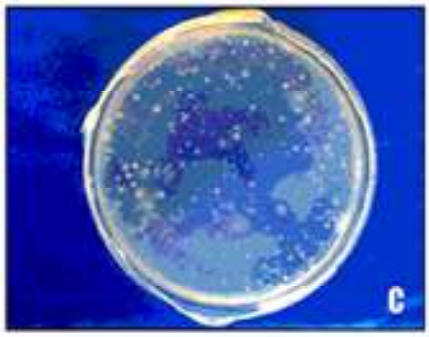
The perusal of data (Fig. 1) indicated that inhibition zone of bacterial growth was minimum (1mm) in 10000ppm and maximum (8.5) in 100000ppm after 24 hours whereas, It was 1.3mm and 9mmin10000ppm and 100000ppm extract respectively after 48 hours (Fig. 1). Effect of aquous crude extract of *P. articulatum* (Fig. 2)



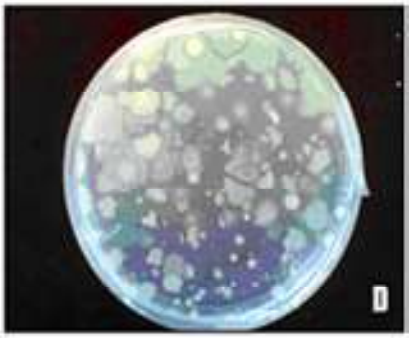
A. Number of colonies in control.



B. Number of colonies in 10000ppm extract of *P. articulatum*.

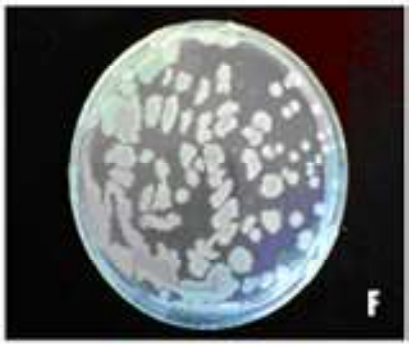


C. Number of colonies in 100000ppm extract of *P.articulatum*.

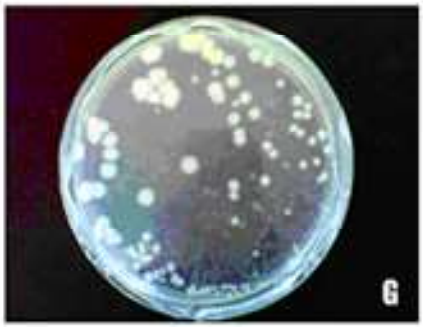


D. Number of colonies in 10000ppm extract of *A.longii.*

E. Number of colonies in 100000ppm extract of *A.longii.*



F. Number of colonies in 10000ppm extract of *F.bryoides*.



G. Number of colonies in 100000ppm extract of *F.bryoides*.

Photo Plate 1

colonies were maximum (321 and 327) in 10000ppm and minimum (272 and 277) in 100000ppm extract after 24 and 48 hours respectively.

Effect of *A .longii* extract on *Agrobacterium tumifacians*

It was observed that the inhibition zone was minimum (1 and 1.3mm) in 10000ppm

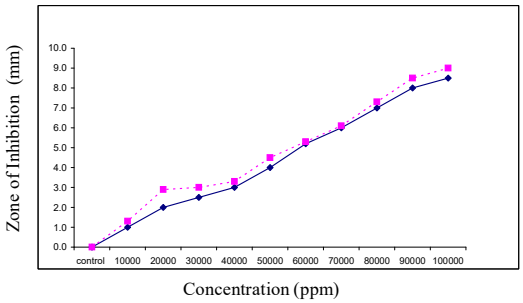


Fig.1. Effect of aquous crude extract of *P.articulatum* on *Agrobacterium tumifacians* in well method.

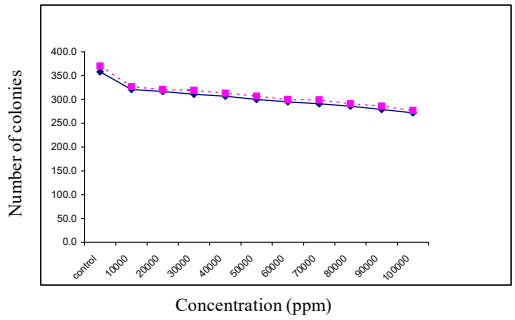


Fig. 2. Effect of aquous crude extract of .*articulatum* on *Agrobacterium tumifacians* in pour plate method.

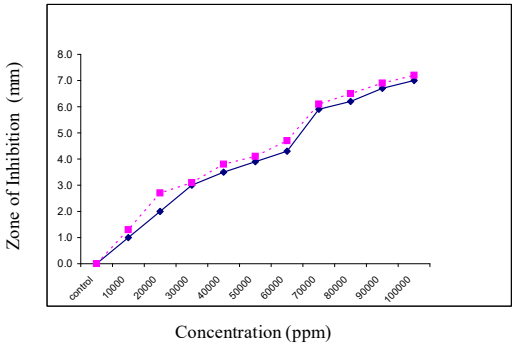


Fig.3. Effect of aquous crude extract of *A. longii* on *Agrobacterium tumifacians* in well method.

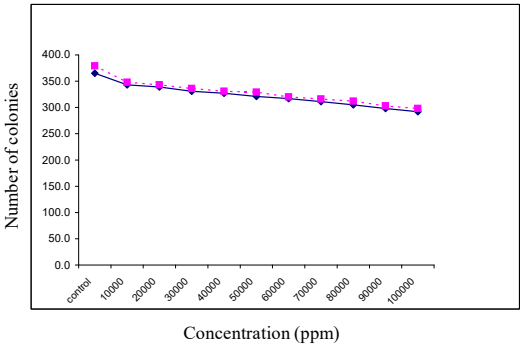


Fig.4. Effect of aquous crude extract of *A.longii* on *Agrobacterium tumifacians* in pour plate method.

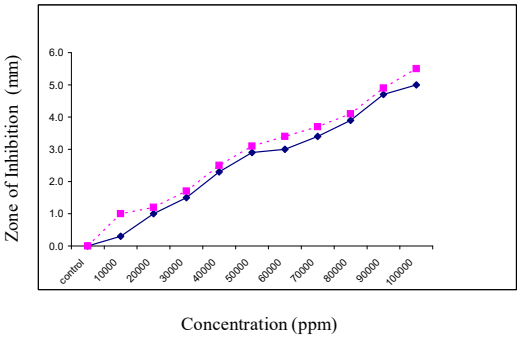


Fig.5. Effect of aquous crude extract of *F. bryoides* on *Agrobacterium tumifacians* in well method.

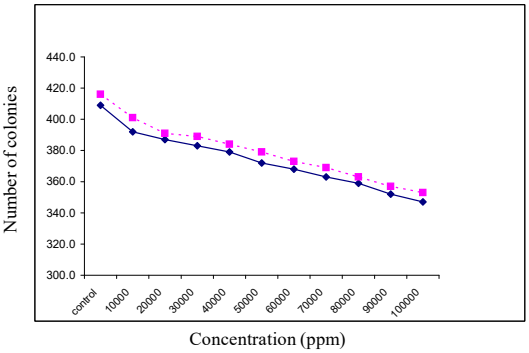


Fig.6. Effect of aquous crude extract of *F.bryoides* on *Agrobacterium tumifacians* in pour plate method.

showed that number of whereas, it was maximum (7.0 and 7.2mm) in 100000ppm extract of *A. longii* after 24 and 48 hours.(Fig. 3). It was also reported (Fig. 4) that the number of colonies were maximum (343 and 348) in 10000ppm concentration of extract and minimum (292 and 298) in 100000ppm extract after 24 and 48 hours.

Effect of *F. bryoides* extract on *Agrobacterium tumifacians*

The minimum inhibition of bacterial growth (.3 and 1mm) was reported in 10000ppm extract. It was maximum (5 and 5.5) in 100000ppm extract of *F .bryoides.* (Fig.5). The crude extract of *F. bryoides* (Fig.6) showed the inhibitory effect .on the bacterial growth and it was found that the number of colonies were maximum (392 and 401) in 10000ppm whereas, it was minimum (347 and 353) in 100000ppm extract after 24 and 48 hours respectively.

The process of colony formation and number were comparatively more in *F.bryoides* and *A.longii* extract than that of *P .articulatum*. Therefore it was revealed that the antimicrobial activity was maximum in order of liverworts > hornworts > mosses. Madsen and Pates (1952)[1] studied eight bryophytes of which *C.conicum* and *Dumortiera hirtuta* were active against *Candida albicans* while *Sphagnum* sps. inhibited *Staphylococcus aureus* and *Pseudomonas aereiginosa* .

The present study suggested that these three bryophytes are active against test organism *Agrobacterium tumifacians* as evaluated in Figures 1 to 6. Benerjee and Sen (1979) *[2]; Opelt and Berg (2004) *[3] found similar observations and suggested that liverworts are more active than the mosses against certain selected microorganisms.

REFERENCES

1. Madsen, G.C. and Pates, A. L. Occurrence of antimicrobial substances in chlorophyllous plants growing in Florida. *Bot. Gaz.* 1952; **113**: 293-300.
2. Banerjee, R.D. and Sen, S. P. Antibiotic activity of bryophytes. *The bryologist.* 1979; **82**(2): 141-153.
3. Opelt, K. and Gerg, G. Diversity and antagonistic potential of bacteria associated with bryophytes from nutrient poor habitats of the Baltic Sea coast. *Applied and Environmental Microbiology.* 2004; **70**(11): 6569-6579.
4. Ojo, O.O., Ajayi, A. O. and Anibijuwon, I. I. Antibacterial potency of methanol extracts of lower plants. *J. Zhejiang Univ . Sci.* B. 2007; **8**(3): 189 -191.