Screening of Antifungal Activity of *Asterella angusta* (Steph.) Against *Aspergillus nidulans*

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Antifungal potency of *Asterella angusta* (Steph.) was determined on *Aspergillus nidulans*, using pour plate method. Results based on colony diameter and biomass revealed decline in vegetative growth of colony and spore formation in all concentrations. Minimum inhibition was recorded at 10 per cent while maximum inhibition was at 100 per cent extract concentration suggested that *Asterella angusta* (Steph.) – A bryophyte can be used to control fungal growth.

Key words: Asterella angusta, Aspergillus nidulans, Antifungal activity, Inhibition.

The most infectious diseases are microbial in origin. As resistant strains of pathogens are increasing day by day demand of antimicrobial therapeutics is rising correspondingly. Bryophyte plants posses biologically active compounds therefore they are used throughout the world as drugs for various illnesses (Bodade, 2008).

In search of herbal antibiotic substances, bryophytes are proved to possess tremendous potential against pathogenic organisms. Bryophytes are closely linked with civilization, culture, beliefs, and ethics of humankind. These are used by different cultural groups for wounds and skin diseases suggesting that they protect the skin and open wounds from microbial pathogens. They are used in Chinese, European, North American and Indian medicines to treat cardiovascular diseases, tonsillitis, bronchitis, cuts and burns (Banerjee and Sen, 1979). They are also free from microbial attack due to their immunological properties. This group of plants is now well known for their rich source of potential antimicrobial substances such as isoflavonoides, flavonoides, biflvonoides, terpenoides and phenolic compounds (Xie *et a*l, 2008).

Phytotoxic effects of crude aqueous extracts of three bryophytes, Plagiochasma articulatum (Kash.) a liverwort, *Anthoceros longii* (Steph.) a hornwort and Fissidens *bryoides* a moss was carried out on *Agrobaeterium tumifacians*. It was observed that the inhibition of bacterial growth was maximum in P. *articulatum* extract followed by *A .longii* and *F .bryoides* (Deora, Bhati and Jain, 2007).

Some species of *Fissidens* and *Polytrichum* were utilized as diuretic and hair growth stimulating drug in china more than 400 years ago (Samara *et al*, 2006). Extracts of many bryophytes have been shown to possess varying levels of antibacterial and anticancer activities *in vitro* (Subhisha and Subramoniam, 2005). Bryophytes like *Lunularia cruciata* have also

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been reported for antioxidant activity (Umachigi and Kumar, 2007).

Antibacterial potency of methanol extracts of three green lower plants, *Pneumatopteris afra, Platycerium bifurcatum* and *Nephrolepsis bisserata* was determined on clinical strains of *Escherichia coli, Staphylococcus aureus, Klebsiella* spp. and *Salmomelia typhi*, Antibacterial activities were observed at concentrations of 12.5, 25.0, 50.0 and 100.0 µg/ml. (Ojo, 2007).

Studies on Asterella angusta showed its antibacterial activity against Pseudomonas aeruginosa and Basillus subtilis (Chaudhary et al, 2007). Present investigation deals with the study on antifungal activity of Asterella angusta against Aspergillus nidulans. This will add a step further in understanding antimicrobial properties of liverworts.

MATERIALAND METHODS

Thalli of Asterella angusta in vegetative as well as sporophytic phages were collected from Mount Abu, Dist. Sirohi (Rajasthan) in rainy season from the lime coated walls of Achleshwar Temple and near Nakki Lake. The collected material was washed using distilled water thoroughly to remove dirt, soil and lime. It was then pressed between filter papers to remove excess water. Fresh weight was taken and then kept in refrigerator till extract formation. An equal amount of double distilled water was added in the plant material and grinded to form a fine paste using a mixture grinder and left over night so that all water soluble substances get dissolved in water. It was then filtered. The filtered was centrifuged at 6000 rpm for 20 minutes. The supernatant was collected and was filtered using Whatsman

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filter paper no. 1. The pure extract thus prepared was serially diluted using double distilled water, to form extracts of different concentrations from 10 - 100 per cent. All extract solutions were autoclaved and stored in refrigerator. Ten days old pure culture of Aspergillus nidulans were used for each experiment. Two parameters namely colony diameter and colony fresh weight were studied to determine the effect of extract using pour plate method. Three replicates of each extract concentration were set. All the experiments were set in completely aseptic condition on laminar air flow bench. PDA and extract (1: 1) poured in pre sterilized petri dishes were used to inoculated fungal colonies of 3 mm. diameter, at four places in each petri plate. The control was also established each time. The petri dishes were then kept at room temperature for 9 days. Data for colony diameter and weight were recorded on 9th day.

RESULTS AND DISCUSSION

The mean values of colony diameters and fresh weight for different extract concentrations are plotted in Fig. 1. Results showed that maximum colony diameter was 530 mm. recorded in the control, followed by 520 mm in 10 per cent aqueous crude extract. Minimum colony diameter was 140 mm recorded in 100 per cent extract concentration.

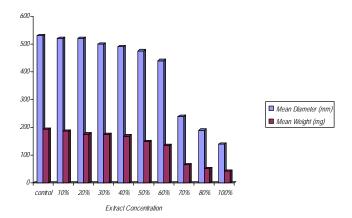
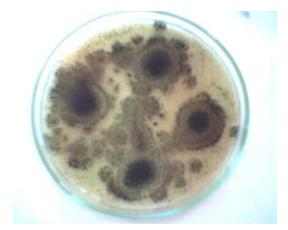


Fig. 1. Effect of aqueous crude extract of Asterella angusta on Aspergillus nidulans

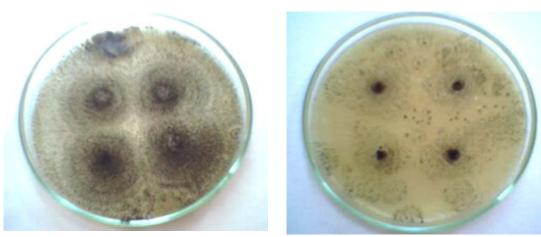
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A) Control





C) - 40

D) - 60

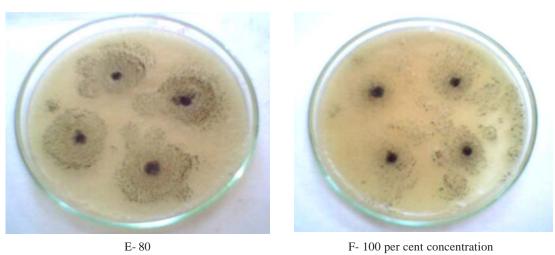


Plate 1. Growth of *Aspergillus nidulans* on different concentrations of aqueous crude extract of *Asterella angusta*

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Similarly fresh weight was maximum, 194 mg. in the control, followed by 186 mg. in 10 per cent extract concentration and was minimum, and 43 mg. in 100 per cent extract concentration. A comparative study of colony diameters and colony fresh weight in different concentrations showed that lower concentrations were less toxic but higher concentrations showed significant toxicity against fungal growth (Photo plate I). Both hyphal growth and conidial development were suppressed by concentrated extracts resulting in decreased colony diameter and biomass. Results of the present experiment completely agree all earlier studies. Antifungal compounds were isolated from New Zealand liverwort Plagiochilla faciculata (lorimer, 1994).

Study reports of Subhisha et al (2007) on antifungal activity of Pallavicinia lyellii, a liverwort against four test fungi A. niger, A. fumigates, F. oxysporum and C. albicans showed broad spectrum activity of this bryophyte. The alcohol extracts were found to be more active than water and hexane extracts. A. fumigates was found to be most susceptible. The drug inhibits the germination of spores as well as the multiplication of mycelia just as observed in present study of ours suggesting that liverworts are active against certain selected microorganisms. Α dichloromethane and a methanol extract of the liverwort Bazzania trilobata showed antifungal activity against the phytopathogenic fungi Botrytis cinerea, Cladosporium cucumerinum, Phythophthora infestans, Pyricularia oryzae and Septoria tritici (Jochen, 2004). Benerjee and Sen (1979) gave reports of Broad spectrum antibiotic activities of moss Brachythecium procumbens and liverworts Asterella sanguine and Marchantia paleacea.

Asterella angusta contains four bis(bibenzyl)s, asterelin A (1), asterelin B (2), 11-O-dimethylmarchantin I (3) and dihydroptychantol A(4) (Qu J *et al*, 2007).

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Thus previous investigators found similar results that bryophytes have potent antimicrobial substances in them. From results of this experiment *Asterella angusta* appears to be promising material for development of novel antifungal drugs. Further research is being carried out for isolation of bioactive chemicals and their mode of action on fungal mycelium.

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