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RESEARCH ARTICLE

MICROBIOLOGY

IN VITRO STUDIES ON ANTIBACTERIAL ACTIVITY OF *PHYSCOMITRIUM JAPONICUM* HEDW. (FUNARIACEAE)

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ABSTRACT

The antibacterial activity of moss *Physcomitrium japonicum* (Hedw.) was studied against the various bacteria namely *Bacillus subtilis* (MTCC-441) , *Escherichia coli* (MTCC-41) *, Staphylococcus aureus* (MTCC-740)*, Pseudomonas aeruginosa* (MTCC-424) and *Agrobacterium tumefaciens* (MTCC-2250) by agar well diffusion technique. The extracts of aqueous and organic solvents were tested against the all bacterial culture. All the extracts showed antibacterial activity against all tested microorganism. The results suggest the potential of *P. japonicum* for developing a broad spectrum antibacterial formulation in future.

KEYWORDS

Antibacterial, moss, bacteria, phytochemical screening, Agar well assay, Zone of inhibition (ZI)

INTRODUCTION

Natural plant products have been widely used in the last few decades as additional markers, in plant systemic and taxonomy. About 800-900 liverworts and 200 mosses have been identified between 1980 and 1995 (Asakawa,1990a&b;Huneck,1983).For a long time bryophytes were neglected and little attention was paid on the chemical exploration of these plants,though,fragmentary researches were carried out. During the end of 1970s the screening of bryophytes for antimicrobial properties got momentum throughout the globe. A large numbers of the complex chemical compounds were reported from bryophytes which normally lack in other group of plants.

The Kumaun region with diversified micro-climate has favoured the growth of rich and varied bryophytic flora. For a long time bryophytes have been treated as the neglected group of plants because of their no use except academic interest and ecological significans.In recent years bryophytes have drawn attention of the biologists as they restore a substantial amount of medicinally important complex benzene ringed compounds. They contain high concentrations of terpenoids, sesquiterpenoids and other secondary metabolites that enable them to withstand against the attack of microorganisms such as bacteria, actinomycetes, snails, mollusks, etc.There are only records available on those bryophytes parasitized by bacteria and fungi or eaten by the insects, snails and other animals (McCleary and Walkington, 1966).A number of bryophytes have been used as medicinal plants in China, Europe and North America.

Gupta and Singh (1971) found high occurrence of antibacterial activity in extracts of *Barbula* species, reaching as high as 36.2%,whereas it was only half that in *Timmiella* species(18.8%).In 1982, Asakawa *et al*.(1982) isolated three prenyl bibenzyls from *Radula* spp. As demonstrated that these bibenzyls could inhibit growth of *Staphylococcus aureus* at concentrations of 20.3µg ml^{-1}. Out of more than 80 species tested, Ichikawa (1982) and Ichikawa coworkers (1983) found antimicrobial activity in nearly all. Acyclic acetyleinic fatty acid and cyclophentenonyl fatty acid extracts from the mosses completely inhibited the growth of the rice blast fungus *Pyricularia oryzae*.Belcik and Wiegner (1980) reported antimicrobial activity in extracts of the liverworts *Pallavicinia* and *Reboulia*, and Isoe (1983) reported it from *Porella.*

MATERIALS AND METHODS

Collection of plant material: *Physcomitrium japonicum* Hedw. was collected from College campus ,Udaipur and Sitamata wildlife sanctuary (Chittorgarh and Partapgarh district) Rajasthan, in the month of November 2006-2007.The plants were identified and voucher specimens have been deposited in Bryology Laboratory, Dept. of Botany, Univ. College of science, Udaipur for future reference.

Extraction procedure and phytochemical screening: The plant material was carefully cleaned from attached litter and dead material under running tap water and finally with sterile distilled water. Air-dried and powdered approximately 20 g plant material of *Physcomitrium japonicum* was extracted by cold percolation in petroleum ether, benzene, acetone, methanol, ethanol or about 200 ml autoclaved water. The extract was decanted, filtered with whatman No.1 filter paper and concentrated at reduced pressure below 40ºc through rota vapour and lyophilized (Buchi, Labconco, US) to obtain dry extract. 0.1mg

crude extracts were taken up for biological screening and also to observe the presence and absence of different phytochemical constituents.viz. alkaloids (Dragendorff's test), reducing sugars (Fehling's test), saponins (foam formation), flavonoids (using NaOH and dil.HCl), terpenes (Liebermann-Burchard's test), tannins and steroids (using acetic anhydride) according to standard methods (Sofowora, 1982, Trease and Evans, 1987).

Test microorganisms: Four test microorganisms were used in antibacterial sensitivity test and were procured from Microbial Type Culture Collection and Gene Bank (IMTECH, Chandigarh,India) the Gram positive bacteria *Bacillus subtilis* (MTCC-441), *Staphylococcus aureus* (MTCC-740) Gram negative bacteria *Escherichia coli* (MTCC-41), *Agrobacterium tumefaciens* (MTCC-2250) and *Pseudomonas aeruginosa* (MTCC-424).All the bacterial strains were maintained at 4^{o}C on nutrient agar slants and sub cultured as required.

Antibacterial activity: The agar well diffusion method (Murray *et al*., 1995) evaluates the antibacterial activity. Bacteria were cultured overnight at 37ºc in nutrient broth (Hi-media, Bombay) were used as inoculum. 20 ml nutrient agar medium was poured in sterilized Petri plates and allowed to solidify at room temperature. 24 h broth culture of test bacteria was used as inoculum under sterile condition. The freshly activated 100µl of organisms was set to 0.5 optical density spread with a sterile L shaped bent glass rod. Using cork borer several wells of 6mm diameter were punched. Each well 100 µl crude extract of various concentration (1000, 800, 500, 250, 125, 65µg/ml). DMSO (Dimethyl Sulphoxide) was used in making of extracts concentration and neutralized with 0.1N NaOH and 0.1N HCl. The plates were incubated under 37ºC temperature. Streptomycin and DMSO were used as positive and negative control respectively. The experiment was performed in triplicates and average results were recorded. Finally the diameter of zone of inhibition including laterally around the well was measured with antibiotic zone scale in mm.

Table 1
Phytochemical screening of Physcomitrium japonicum

Phytochemical Test	Results
Alkaloid	+
Flavonoid	+
Saponins	+
Reducing sugar	+
Tannins	-
Steroids	-
Terpenoids	+

(+)Presence (-) Absence

Table 2

Results of the antibacterial activity of the investigated plant Physcomitrium japonicum by Agar well assay method against some pathogenic bacterial strains

Microorganisms	Extracts	Different Concentration of the Plant extracts (in µg/ml)					
		65	125	250	500	800	1000
		Zone of Inhibition (mm)					
Bacillus subtilis	Pet. Ether	3	5	8	10	13	15
	Benzene	4	8	9	11	15	18
	Acetone	3	8	10	13	15	16
	Methanol	5	11	14	17	19	22
	Ethanol	8	10	12	15	17	18
	Aqueous	4	7	18	11	13	15
	Streptomycin	10	11	13	16	19	23
Staphylococcus aureus	Pet. Ether	10	11	14	18	19	20
	Benzene	8	11	13	16	19	20
	Acetone	7	10	12	15	19	21
	Methanol	9	13	16	19	20	21
	Ethanol	4	8	11	15	18	19
	Aqueous	3	6	8	12	15	16
	Streptomycin	7	10	10	15	19	22
Escherichia coli	Pet. Ether	4	6	9	11	13	16
	Benzene	3	4	7	9	11	12
	Acetone	6	8	11	14	17	19
	Methanol	4	7	10	12	15	17
	Ethanol	2	7	9	13	15	18
	Aqueous	2	4	9	12	14	16
	Streptomycin	13	17	19	23	27	30
Pseudomonas aeruginosa	Pet. Ether	5	7	8	10	13	15
	Benzene	2	4	5	8	11	13
	Acetone	8	11	14	16	19	21
	Methanol	12	16	14	19	19	20
	Ethanol	7	10	12	15	17	20
	Aqueous	6	8	8	12	15	18
	Streptomycin	11	14	17	22	25	28
Agrobacterium tumefaciens	Pet. Ether	8	10	13	16	19	22
	Benzene	13	14	14	19	26	27
	Acetone	7	9	12	15	17	20
	Methanol	10	14	17	20	22	23
	Ethanol	8	10	13	16	19	21
	Aqueous	10	14	17	18	19	25
	Streptomycin	14	17	20	24	27	30

Values include cup borer diameter (6.00mm) and are mean of three replicates

RESULTS AND DISCUSSION

Preliminary phytochemical screening of the plant showed the presence of alkaloids and flavonoids in *Physcomitrium japonicum* while tannins and steroids here absent (Table-1). The results of testing the antibacterial activity of crude extracts of moss *P. japonicum* are presented. These obtained by the Agar well diffusion method against various bacterial cultures. Streptomycin and DMSO were used as positive and negative control respectively. All the plant extract showed the activity against different microorganism but the maximum antibacterial activity approximately 27 mm zone of inhibition was observed in benzene extract of the plant against the *Agrobacterium tumefaciens.* The comparative antibacterial activities against various plant extracts are clearly evident in table-2. The results suggest the potential of *P. japonicum* for developing a broad spectrum antibacterial formulation in future.

Plant was extracted using both aqueous and organic solvents. Data obtained demonstrates the antibacterial activity of plant depends largely upon the types of solvent used for extraction. Data indicate that almost all organic extracts of the plant showed antibacterial activity against all bacterial culture. (Fig.1, 2, 3, 4, 5)

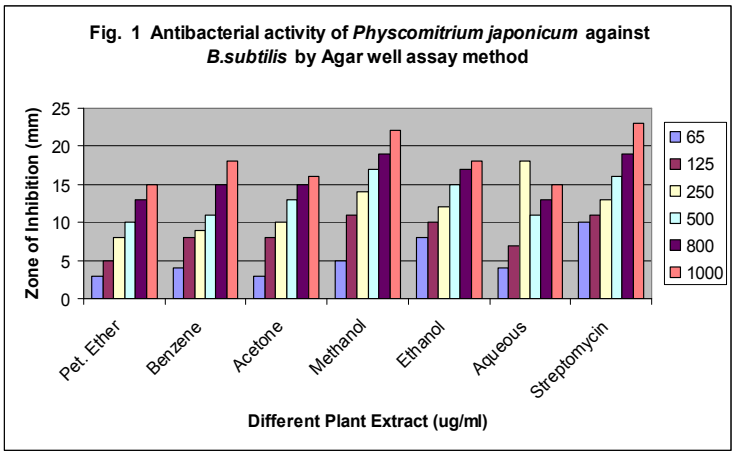


Fig. 1 Antibacterial activity of *Physcomitrium japonicum* against *B.subtilis* by Agar well assay method

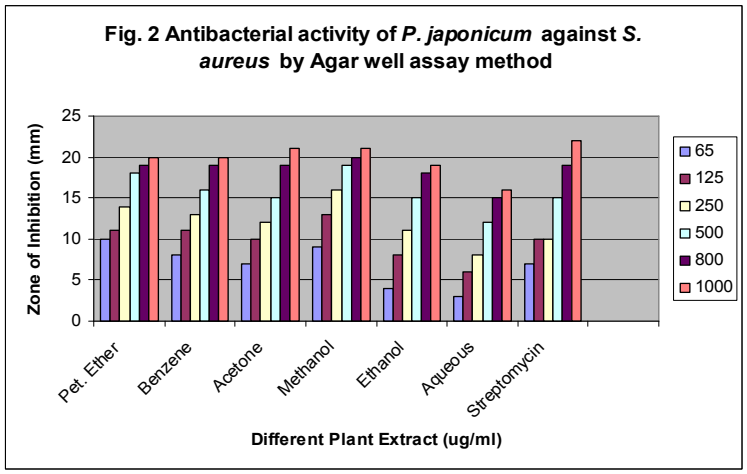


Fig. 2 Antibacterial activity of *P. japonicum* against *S. aureus* by Agar well assay method

Fig. 3 Antibacterial activity of *P.japonicum* against *E.coli* by Agar well assay method

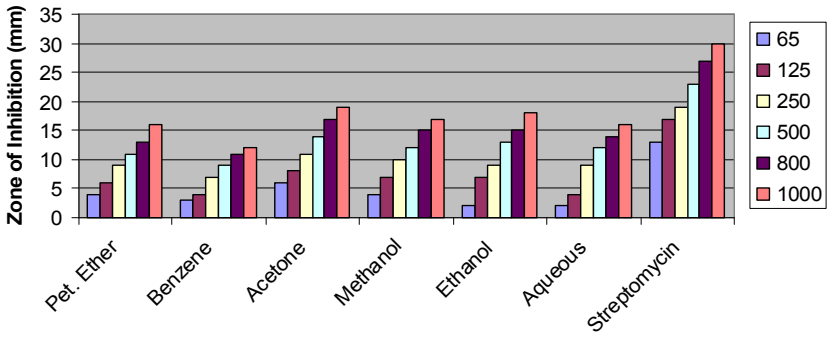


Fig. 4 Antibacterial activity of *P. japonicum* against *P.aeruginosa* by Agar well assay method

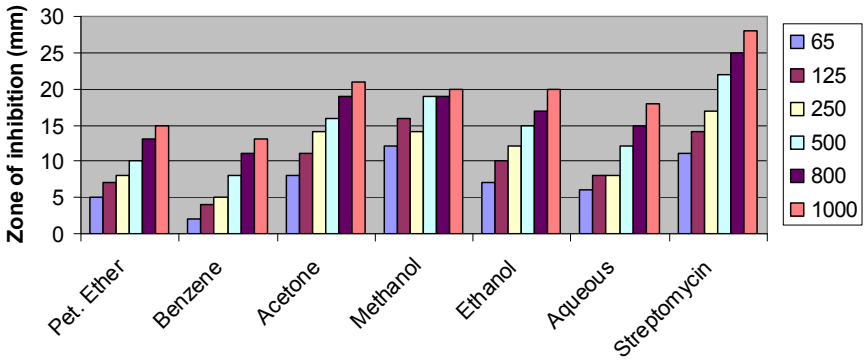
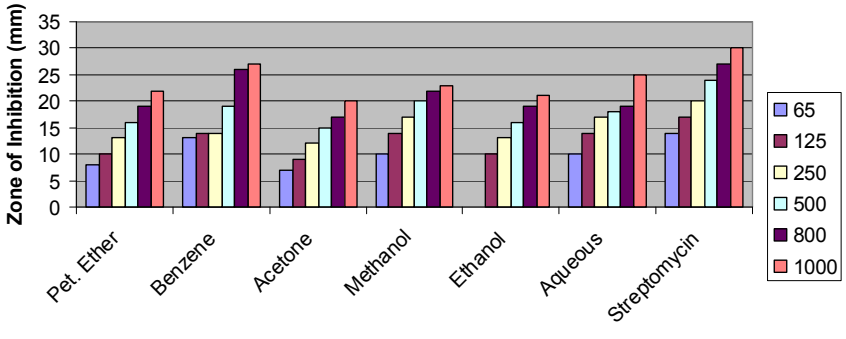


Fig. 5 Antibacterial activity of *P. japonicum* against *Agrobacterium tumefaciens* by Agar well assay method



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