RESEARCH ARTICLE

Antimicrobial Potential of the Moss *Brachymenium turgidum* Broth. ex. Dix. From Melghat Forest

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ABSTRACT

The plant *Brachymenium turgidum* is a small tufts of moss occurred on branches and trunks of trees with corticolous habitat as well as on moist forest ground surface. These plants found mostly in humid areas during rainy seasons where humidity is always higher. The plants generally slender, greenish brown tufts with erect stem. The turgid, sub-pendulous capsules are the key features of the thallus. Since time immemorial to man, the antimicrobial potential of the mosses known to the world especially against wounds, burns and skin infections. In the present investigations, certain preliminary phytochemical tests carried out to trace the presence of alkaloids, tannins, saponins, sterols, terpenoids, flavonoids, glycosides. The antibacterial sensitivity test was elicited out against about seven bacterial and three fungal pathogenic microorganisms. The plant powder extracted in polar and non-polar solvents like water, methanol, ethanol, petroleum ether, chloroform, and acetone to obtain different fractions. Antibacterial effect of these fractions was determined by disc diffusion method The results were compared with the standard antibiotic like, Tetracycline and Nystatin (10 µg/ml). The preliminary phytochemical analysis confirms the presence of alkaloids, flavonoids, glycosides and terpenoids as an important phyto constituent. The antimicrobial activity showed that most of the extract was sensitive to at least one microorganism by exhibiting significant zone of inhibition. Hence, potential antimicrobial activity recorded among the moss and can be found more pronounced in future advanced chemical characterization.

Key words: Moss, Phytochemistry and antimicrobial activity.

INTRODUCTION

Melghat is a prime biodiversity repository of Maharashtra state enriched with diverse forest cover of lower plants like bryophytes. Present study attempted to explore the bryophytic flora of Melghat region along with potential assessment of its *in vitro* antimicrobial screening with chemical analysis. They can grow as epiphytes on bark of trees (Corticolous), leaves (Folicolous), rocks (Rupicolous) on stones

and pebbles (Saxicolous), on fallen logs (Lignicolous), riverbanks and roadside cuts (Terricolous). Since water is inevitable for completing their life cycle, they are known as the "amphibians'' of the plant kingdom (Daniels and Kariyappa, 2007). Bryophytes make a significant contribution to the floral diversity of this "watery planet'' and since its inception constitute an important component of the forest ecosystem being the first colonizers on variety of habitats (Alam et al., 2011). Madsen and Pates (1952) reported first time antibiosis in moss *Sphagnum strictum* against *Staphylococcus aureus* and *Pseudomonas aeruginosa.* Belkin *et al.,* (1952) found that ethanolic extract of the moss *Polytrichum juniperum* possess antitumourogenic activity. McCleary and Walkington (1966) examined 50 species of mosses of which 18 showed strong antibacterial activity while 7 exhibited less but positive activity and rest 25 were inactive. Banerjee and Sen (1979) examined 52 species (40 genera) of the bryophyte for their antimicrobial activity. Out of those species, 29 were active against at least one of the test bacteria. The liverwort *Asterella sanguinea* and *Marchantia paleacea* and the moss *Brachythecium procumbens* showed broad spectrum of antimicrobial activity. Gupta and Singh (1971) have reported antibacterial activity of petroleum ether extracts of mosses *Barbula* and *Timella* against 33 species of bacterial pathogens. Singh *et al.,* (2007) reported antimicrobial activity of ethanolic extracts of 15 Indian mosses like *Sphagnum* sp., *Barbula* sp., *Brachythemium* sp., *Mnium* sp., *Entodon* sp. and found active against 12 micro-organisms. Bodade *et al.*, (2008) described *in-vitro* screening of bryophytes like *Plagiochasma* sp., *Thuidium* sp., *Bryum* sp. and *Rocomitrium* sp. for antimicrobial activity against 10 bacteria and 3 fungi. Ücüncü *et al.*, (2010) recorded antibacterial activity of Turkish moss *Tortula muralis* (Hedw.), *Homalothecium lutescens* (Hedw.), *Hypnum cupressiformae* (Hedw.) and *Pohlia nutans* (Hedw.) against 6 bacteria and 3 fungi. Elibol *et al.*, (2011) reported six Turkish acrocarpic mosses like *Syntrichia* sp., *Grimmia* sp., *Bryum* sp., *Tortella* sp., *Orthotrichum* sp. and *Pleurochaete* sp. showing antibacterial activity. Sharma *et al.,* (2013) reported the antimicrobial activity of the moss *Polytrichum commune* from the Solan region, Himachal Pradesh. against the microorganisms like *S. aureus* and *P. aeruginosa* with promising and significant results.

MATERIALS AND METHODS

The moss thalli collected during rainy season, cleaned carefully and washed under tap water followed by shade dried and powdered in blender. Using Soxhlet apparatus, the powdered samples of plant were extracted in ethanol, methanol, petroleum ether, chloroform and acetone and different solvent fractions were obtained. Dried extracts were stored in labeled sterile wide mouthed screw capped bottles at 4^0C and used for further study (Parekh and Chanda, 2008), (Banerjee and Sen, 1979), (Singh *et al.*, 2006).

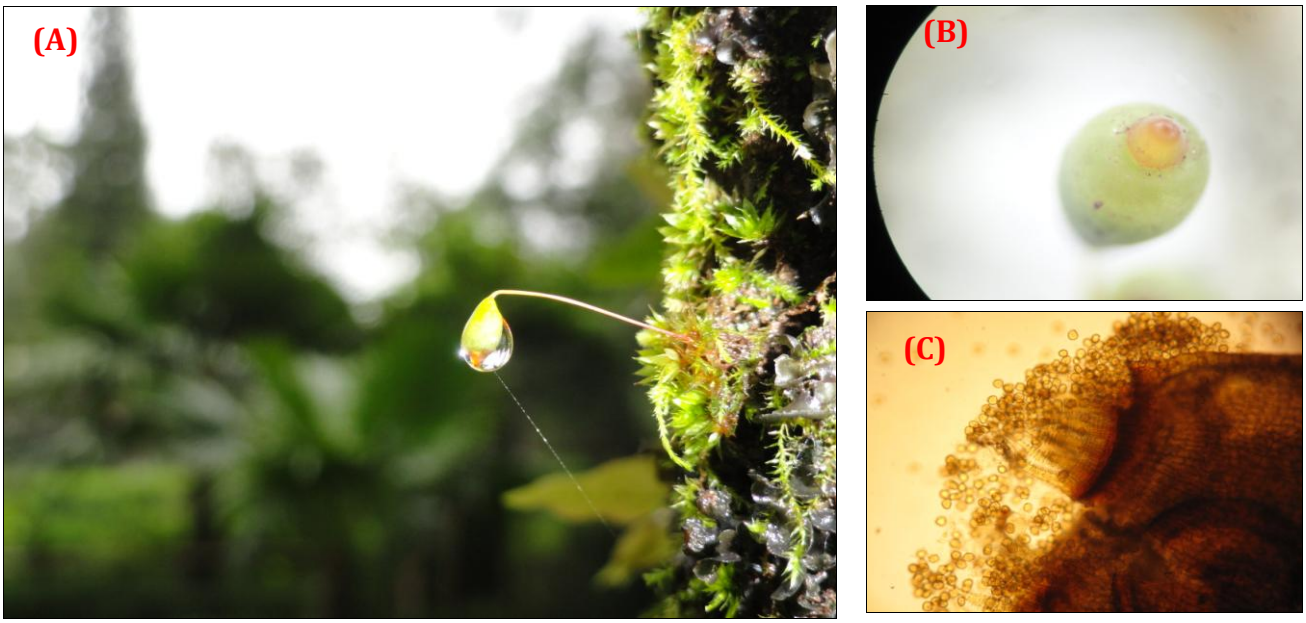

Fig.1: (A) *Brachymenium turgidum* with Sporophyte; **(B)** *B. turgidum* with operculum lid ; **(C)** *B. turgidum* with many spores

The standard pathogenic bacterial and fungal strain cultures procured from Microbial Type Culture Collection and Gene Bank (IMTECH), Chandigarh, India. The bacteria rejuvenated in nutrient broth (Hi-media laboratories, Mumbai, India) at 37^{0}C for 18 hrs and then stored at 4^{0}C on Nutrient agar. The fungal organisms were sub cultured on Sabaroud's dextrose agar. Subcultures were prepared from the stock for bioassay. About 10 different pathogenic microorganisms including gram positive *Staphylococcus aureus* MTCC -96, and gram negative *Escherichia coli* MTCC -729, *Salmonella typhi* MTCC-98, *Klebsiella pneumoniae* MTCC -661, *Proteus vulgar* MTCC - 744, *Pseudomonas aeruginosa* MTCC – 424, and *Shigella flexneri* MTCC- 1457 along with fungus *Aspergillus niger* -343, *Candida albicans*-183, *Rhizopus oryzae*-284 were used with disc diffusion method (NCCLS, 1990). The zone of inhibitions also measured as diameter in mm; the experiment were carried out in triplicate and the averages diameter of zone of inhibition was recorded (Lalitha *et al.,* 1997). The results compared with the standard antibiotic like tetracycline and nystatin (10 µg/ml)

RESULTS AND DISCUSSION

Brachymenium turgidum a moss, extracts obtained in different solvents and tested against various pathogens showing positive results (Table 1.)

The aqueous extract of the plant in distilled water showed least interaction against *E. coli* and *S. aureus* and no activity against other bacterial or fungal test pathogens. The petroleum ether extract showed null effect against all the pathogens under interaction However, the ethanolic extract of the plant was found highly interactive with most of pathogens under study and exhibited sensitivity against microorganisms like *E. coli, P. vulgaris, K. pneumoniae, S. flexneri, S. aureus, P. aeruginosa, S. typhimurium, A. niger* and *C. albicans.* No reaction noticed against the pathogenic fungi *R. oryzae.*

Table 1: Antimicrobial sensitivity test of *Brachymenium turgidum*

Plant Herbal Preparation	Solvent Extract	Zone of Inhibition [mm]									
		EC	PV	KP	SF	SA	PA	ST	AN	CA	RA
Brachymenium turgidum	Aqueous	03	0	0	0	03	0	0	0	0	0
	Petroleum Ether	0	0	0	0	0	0	0	0	0	0
	Ethanol	07	05	04	06	04	0	06	08	07	0
	Chloroform	05	04	06	0	06	0	0	07	06	0
	Methanol	04	05	03	0	06	04	0	04	05	0
	Acetone	0	0	04	03	0	05	06	07	06	0
	Tetracycline	27	24	23	21	22	26	27	-	-	-
	Nystatin	-	-	-	-	-	-	-	27	26	30

* Data represented in mean of three replicates.

***EC =** *Escherichia coli* [MTCC-729], **PV=** *Proteus vulgaris* [MTCC-744], **KP =** *Klebsiella pneumoniae* [MTCC-661], **SF =** *Shigella flexneri* [MTCC-1457], **SA=** *Staphylococcus aureus* [MTCC-96] **, PA=** *Pseudomonas aeruginosa* [MTCC-424], **ST =** *Salmonella typhimurium* [MTCC-98], **AN =** *Aspergillus niger* [MTCC-281] , **CA=** *Candida albicans* [MTCC-227], **RA=** *Rhizopus oryzae* [MTCC-554]

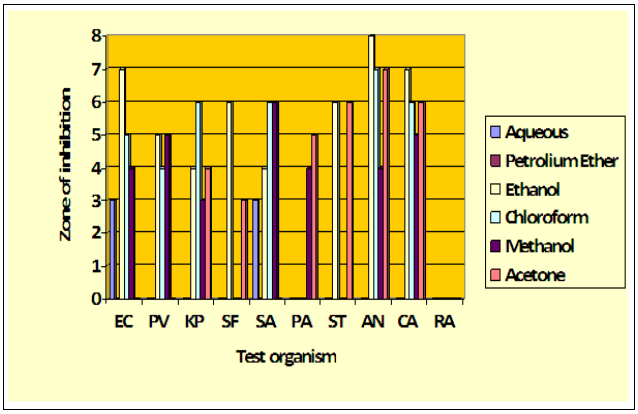

Fig 2: Analysis of antimicrobial sensitivity of *Brachymenium turgidum*

The dark green chloroform extract was found to be effective against the pathogens like *E. coli, P. vulgaris, K. pneumoniae, S. aureus A. niger* and *C. albicans* however, no interaction was noticed in pathogens like *S. flexneri, P. aeruginosa, S. typhimurium* and *R. oryzae.* Subsequently, the methanolic extract of the plant revealed promising results against microorganisms such as *E. coli, P. vulgaris, K. pneumoniae, S. aureus, P. aeruginosa, A. niger* and *C. albicans* while negative results were found against *S. flexneri, S. typhimurium* and *R. oryzae.* The acetone extract showed significant results against pathogen *K. pneumoniae, S. flexneri, P. aeruginosa, S. typhimurium, A. niger* and *C. albicans*

while no specific interaction was observed against bacteria *E coli, P. vulgaris, S. aureus* and the fungus *R. oryzae* Bodade *et al*., (2008) Singh *et al*., (2007).

It is interested and intended to put the results of antimicrobial activity on the canvas of present investigation that during course of activity the aqueous and acetone extracts were less interactive as compared to the other extract like ethanol, chloroform and methanol. The test organism *Rhizopus oryzae* remained negative against all the extracts. The highest zone of inhibition of 8 mm was found in ethanol extract against fungus *Aspergillus niger* and least in aqueous extract against *É. coli* and *C. aureus* with 3 mm and above same as in acetone extract against *S. flexneri*.

CONCLUSION

The phenomenon of antibiosis reported to occur in many bryophytes even though they are at a lower level of evolution as compared to the higher plants. Hence, the occurrence of antimicrobial substances in the thalli of several bryophytes is a key attribute of these novel plants to establish as well as to compete on this earth. During present investigations, *Brachymenium turgidum* found sensitive to various microorganisms in different extracts. The bacterial organisms like *E. coli, P. aeruginosa, K. pneumoniae* and *S. aureus* found most reactive against various extracts of bryophytes. Moreover, the fungal pathogens like *Candida albicans* and *Aspergillus niger* found most reactive against all the extracts which were studied. Hence, it is concluded that all the plant extracts reacted to most of the gram-negative bacteria than gram-positive bacteria. It is observed that all the conventional drugs available today reacts more with gram-positive bacterial strains than gram-negative bacteria. These findings will open new avenues and provide insight to the prospects of medicinal world.

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