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RESEARCH ARTICLE

In vitro assessment of antimicrobial activity of aqueous and alcoholic extracts of moss *Atrichum undulatum* (Hedw.) P. Beauv.

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Abstract Bryophytes, the shade loving plants, have tremendous medicinal properties. The aqueous and alcoholic extracts of *Atrichum undulatum* (Hedw.) P. Beauv. were analysed for antimicrobial properties against the fungi *Aspergillus fumigatus* and *Fusarium oxysporum* and the bacteria *Escherichia coli, Bacillus mycoides, Proteus mirabilis, Staphylococcus aureus* and *Salmonella typhi.* The study is an attempt to investigate the medicinal properties of *Atrichum undulatum* (Hedw.) P. Beauv. using disc-diffusion method. No inhibition was observed against *A. fumigatus* and *P. mirabilis*. For bacteria *S. typhi* and *E. coli* (20 and 15 mm), aqueous and alcoholic extracts of *Atrichum* showed significant inhibition. However, alcoholic extract was found remarkably effective against bacteria rather than aqueous extract.

Keywords *Atrichum undulatum* · Moss · Antimicrobial properties · Aqueous · Alcoholic extract

Introduction

'Amphibians of the plant kingdom' are well known for their various traits. They have advantages to deal with the stress conditions either mediated through non living (metals, air-precipitation) or living (pathogen) components. Bryophytes fight with various biotic and abiotic stresses by producing therapeutic potent secondary metabolites (Krzaczkowski et al. 2008; Üçüncü et al. 2010). These days, bryophytes including mosses and liverworts are used in biotechnology, biomonitoring and pharmacological applications (Decker et al. 2003; Singh et al. 2006). Owing to the antimicrobial properties of mosses, these tiny plants have been used as traditional therapeutic source in Indian culture (Frahm 2001). Although very little information is available, these plants have been found to produce various valuable medicinal compounds (Asakawa 2008).The potential of mosses as antioxidative (Dey and De 2011), antibiotic (Kang et al. 2007), anti-inflammatory and anti-ulcer (Nakagawara et al. 1992) plants were reported earlier and compounds obtained from mosses enable them as soft substitute of complex synthetic chemicals. Apparently these plants of small and simple morphology produce high potent biomolecules.

There are also the records of bryophytes as traditional medicine (Harris 2008). Not only the cost effectiveness but also almost nil side effects have gained importance over the past few years. As the synthetic chemicals possess hazardous toxic effects on environment and humans, the alternative natural compounds are required (Chandra et al. 2017). Plant extracts and phytochemicals were obtained for remedial purposes in microbe-generated diseases (Alam et al. 2015).

Since a long time, bryophytes remain least examined and underexplored for their medicinal properties among the non-vascular as well as vascular plants. The present study has an objective to explore the potential of *A. undulatum* in therapeutic remedies.

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Materials and methods

Plant harvesting

Atrichum undulatum (Hedw.) P. Beauv. was harvested for screening from its native habitat Jageshwar of Kumaon hills (Almora region 29°37.915; 79°50.650′) Uttarakhand, India (November 2009) (Fig. 1). The plant material was carefully cleaned from attached litter and dead material under running tap water and finally with sterile distilled water. Samples were identified in the Botany Department, Bareilly College, Bareilly (U.P., India) through identifying the key to the genera (Chopra 1975) and voucher specimens (200711000067, a–d; 200803000067, a–d; 200911000067, a–d) were made to deposit at the Cryptogrammic Herbarium in the Botany Department at Bareilly College, Bareilly. Diagram was elucidated by Camera Lucida. Taxonomic References used were Lal (2005) for mosses.

Pathogenic agents

Pathogenic bacterial cultures *Escherichia coli, Bacillus mycoides, Staphylococcus aureus, Proteus mirabilis, Salmonella typhi* and the fungi *Aspergillus fumigatus* and *Fusarium oxysporum*, were already procured vide receipt no.-MTCC/RECE/072685 Date 19 September 2007 from Microbial Type Culture Collection and Gene Bank (MTCC), CSIR, Chandigarh, India.

The bacterial and fungal cultures were maintained on nutrient Agar (pH 7.0 ± 0.2) and Czapek's Dox medium (pH 5.0 ± 0.2) at 4 °C temperature respectively. Media and growth conditions like temperature (37 °C for bacteria; 30 °C for fungi), and incubation period (24–48 h for bacteria; 48–72 h for fungi) used for culturing these strains were maintained in the laboratory.

Preparation of extract

Sample of plant was dried, powdered and weighed for 20 g with 99% ethyl alcohol and distilled water for alcoholic and aqueous extract respectively. Further it was extracted in Soxhlet assembly for 24 to 48 h then dried in Vaccuo rota evaporator and refrigerated for screening.

Antimicrobial assay

For antimicrobial assessment, disc diffusion method was used (Basri and Fan 2005). Bacterial and fungal strains were cultured with MHA (Muller-Hinton Agar) and SDA (Sabouraud Dextrose Agar). 1×10^8 CFU/mL inoculum for bacteria and 1×10^7 CFU/mL inoculum for fungi were poured in sterilized petri plates. Whatman filter paper disc of 6 mm were sterilized, kept overnight in 1000 µL of 250 mg/ml extracts (resuspended in alcohol) for 25 mg/ml then transferred to petri-plates. Incubation period for bacterial and fungal strains was given specifically (37 °C for 24 h and 27 °C for 48 h). Extract performance was measured in millimetre as zone of inhibition for bacterial and fungal strains by using the method of Snedecor and Cochran (1987). 0.1 ml of Ampicillin (10 µg/ml) for bacteria, 0.1 ml of Fluconazol (10 µg/ml) for fungus were used as standard for comparison.

Minimum inhibitory concentration (MIC) estimation

Extracts were allowed to suspend in alcohol and used as stock solution. Dilutions of stock solution were incorporated into a 96-well microtiter plate, having broth media (MH broth for bacteria and SD broth for fungi). Specific amount of inoculum suspension i.e. 1000 µL (1×10^8 CFU/mL for bacteria and 1×10^7 CFU/mL for fungi) was transferred to the wells of the microtiter plate. Control was performed applying standard drug solution (Ampicillin for bacteria, Fluconazole for fungi as positive controls; and cultures without extract and standard drug as negative control).The experiment set was tested in triplicate.

Results

The alcoholic and aqueous extracts of *A. undulatum* showed good inhibitory activity against most of the pathogens (Tables 1, 2). *S. typhi* was more potent for both of the extracts (Table 3). Alcoholic extract exhibited high antibacterial activity against *S. typhi* (25.4 mm), followed by *S. aureus* and *E. coli* (21.1 and 18.1 mm) (Table 1). *A. fumigatus* (14.8 mm) was more sensitive for alcoholic extracts while *F. oxysporum* showed (10 mm) inhibition zone for alcoholic extracts (Table 2).

Alcoholic extract showed maximum inhibition against *S. typhi* (25.4 mm) at 1000 µg/disc (Fig. 1d) while the minimum inhibition zone was against *F. oxysporum* i.e. 10 µg/disc.

The MIC of alcoholic extract against bacterial pathogens ranged 0.65 to 3.74 µg/ml while MIC of aqueous extract against bacterial pathogen ranged from 0.78 to 2.45 µg/ml (Table 3). *P. Mirabilis* was observed to be insensitive against both the extracts. The alcoholic extract inhibited growth of *A. fumigatus*, showing maximum and minimum inhibition zone 14.8 and 10.9 mm, 1000 µg/disc respectively. Inhibition zone of the extracts (alcoholic and aqueous) against all tested pathogens was less than

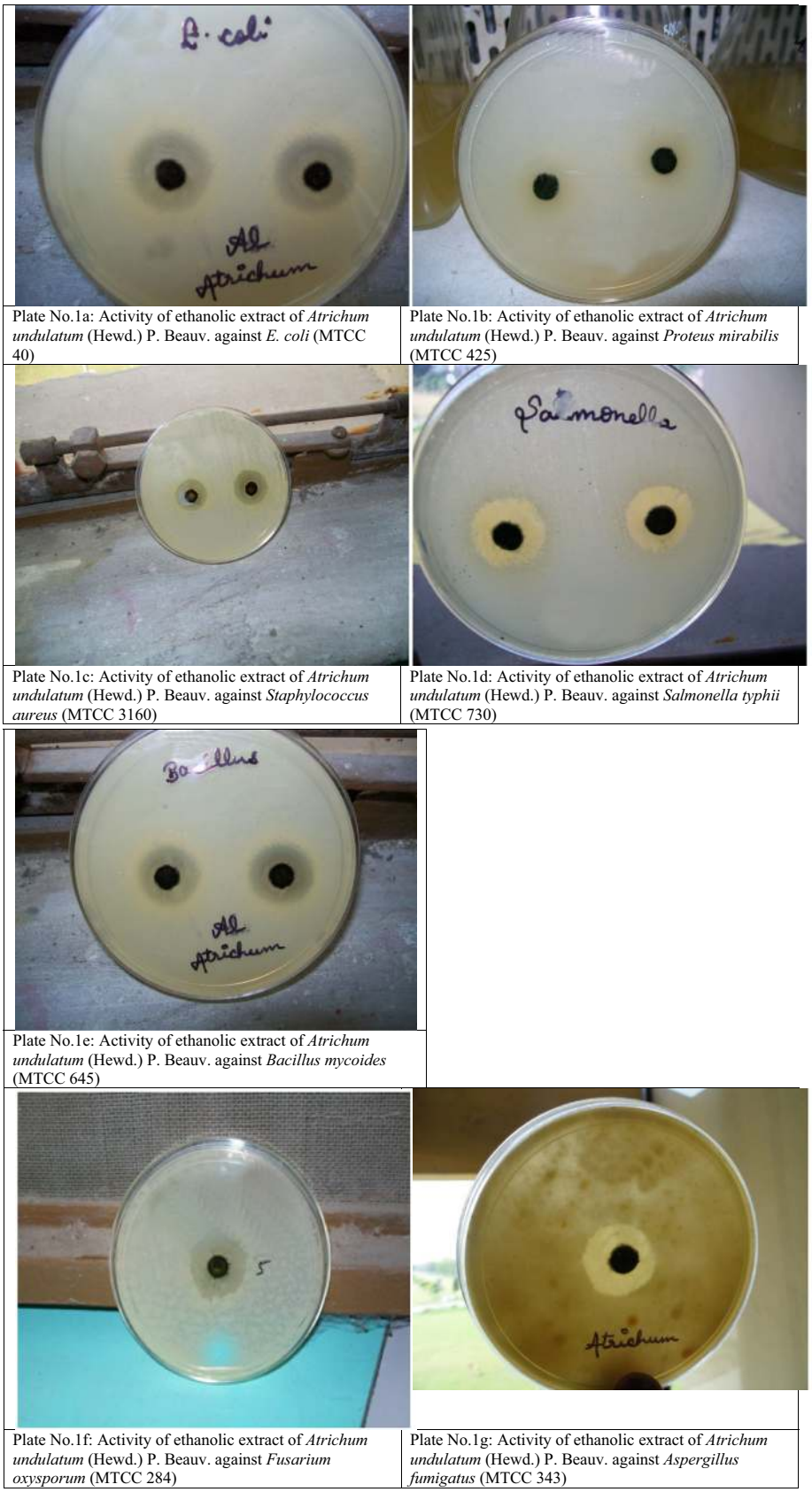

Plate No.1a: Activity of ethanolic extract of *Atrichum undulatum* (Hewd.) P. Beauv. against *E. coli* (MTCC 40)

Plate No.1b: Activity of ethanolic extract of *Atrichum undulatum* (Hewd.) P. Beauv. against *Proteus mirabilis* (MTCC 425)

Plate No.1c: Activity of ethanolic extract of *Atrichum undulatum* (Hewd.) P. Beauv. against *Staphylococcus aureus* (MTCC 3160)

Plate No.1d: Activity of ethanolic extract of *Atrichum undulatum* (Hewd.) P. Beauv. against *Salmonella typhii* (MTCC 730)

Plate No.1e: Activity of ethanolic extract of *Atrichum undulatum* (Hewd.) P. Beauv. against *Bacillus mycoides* (MTCC 645)

Plate No.1f: Activity of ethanolic extract of *Atrichum undulatum* (Hewd.) P. Beauv. against *Fusarium oxysporum* (MTCC 284)

Plate No.1g: Activity of ethanolic extract of *Atrichum undulatum* (Hewd.) P. Beauv. against *Aspergillus fumigatus* (MTCC 343)

Fig. 1 Activity of moss *Atrichum undulatum* (Hewd.) P. Beauv. against undertaken micro-organismsPlate No. 1a: Activity of ethanolic extract of *Atrichum undulatum* (Hewd.) P. Beauv. against *E. coli* (MTCC 40), Plate No. 1b: Activity of ethanolic extract of *Atrichum undulatum* (Hewd.) P. Beauv. against *Proteus mirabilis* (MTCC 425), Plate No. 1c: Activity of ethanolic extract of *Atrichum undulatum* (Hewd.) P. Beauv. against *Staphylococcus aureus* (MTCC 3160), Plate No. 1d: Activity of ethanolic extract of *Atrichum undulatum* (Hewd.) P. Beauv. against *Salmonella typhii* (MTCC 730), Plate No. 1e: Activity of ethanolic extract of *Atrichum undulatum* (Hewd.) P. Beauv. against *Bacillus mycoides* (MTCC 645), Plate No. 1f: Activity of ethanolic extract of *Atrichum undulatum* (Hewd.) P. Beauv. against *Fusarium oxysporum* (MTCC 284), Plate No. 1g: Activity of ethanolic extract of *Atrichum undulatum* (Hewd.) P. Beauv. against *Aspergillus fumigatus* (MTCC 343)

Table 1 Antibacterial activity of moss *Atrichum undulatum* (Hewd.) P. Beauv. against different microbial strains procured

Microbial Strains	[b]Sources/ Strains	Medium	Concentrations (μg/disc)								
			0	25	50	100	250	500	1000	+ve control	
			[a]Zone of inhibition							Tetracycline	Streptomycin
Escherichia coli	MTCC 40	Aqueous	–	–	5.6	5.8	5.9	6.3	6.5	18	19
		Alcoholic	–	5.9	7.2	8.7	12.2	15.4	18.1		
Proteus mirabilis	MTCC 425	Aqueous	–	–	–	–	–	–	–	20	25
		Alcoholic	–	–	–	–	–	–	–		
Staphylococcus aureus	MTCC 3160	Aqueous	–	5.1	5.2	5.6	5.7	7.2	8	25	28
		Alcoholic	–	6.2	9.1	12.4	15.5	18.8	21.1		
Salmonella typhii	MTCC 730	Aqueous	–	6.6	6.7	6.9	7.1	7.5	7.7	20	24
		Alcoholic	–	11.1	15.2	18.2	20.3	23.2	25.4		
Bacillus mycoides	MTCC 645	Aqueous	–	5.6	6.1	6.3	6.4	6.4	7	23	21
		Alcoholic	–	7	8.9	10.8	11.3	13.1	16.7		

[a]values are about the zone of inhibition in mm

[b]Purchased from IMTECH, Chandigarh

The extracts were tested in triplicate and the results are shown as mean value of all experiments

Disc potency of +ve control Tetracycline was 100 μg/disc and of Streptomycin 100 μg/disc

Table 2 Response in moss extract of *Atrichum undulatum* (Hewd.) P. Beauv. against the fungal strains

Microbial strains	Sources/ strains	Medium	Concentrations (μg/disc)							
			0	25	50	100	250	500	1000	+ve control Fluconazole
			[a]Zone of inhibition							
Fusarium oxysporum	MTCC 284	Aqueous	–	–	–	3.4	4.2	5	5.7	20
		Alcoholic	–	–	5.6	6.8	7.6	8.6	10	
Aspergillus fumigatus	MTCC 343	Aqueous	–	–	7.2	8.1	9.1	10.5	10.9	24
		Alcoholic	–	6.2	7.9	9.8	10.6	12.4	14.8	

Applied disc potency of +ve control was 100 μg/disc

[a]values are about the zone of inhibition in mm

Table 3 Minimum and maximum inhibition zone of moss tested *Atrichum undulatum* (Hedw.) P. Beauv. against bacteria

Test pathogens	Alcoholic extract				Aqueous extract				
	SD	Max SD	Min SD	MIC	SD	Max SD	Min SD	MIC	ST (IZ)
S. typhi	19.53 ± 0.50	20	11	3.74		12	7	2.12	25
E. coli	14.66 ± 0.57	15	7	2.40	6.83 0.28	7	5	1.57	27
S. aureus	13.66 ± 0.57	14	7	2.40	8.86 0.23	9	6	0.78	24
P. mirabilis	–	–	–	–	–	–	–	–	25
B. mycoides	9.56 ± 0.51	10	6	0.65	4.26 0.25	4.5	3	2.45	20

Data are average (± SD) diameter (in mm) of inhibition zone from three independent observations; [–], no activity; *MIC* minimum inhibitory concentration (μg/mL); *ST* standard. Standard for bacteria, Ampicillin, *SD* standard deviation

standards (Ampicillin and Fluconazole for fungus). Minimum inhibition zone for *A. fumigatus* was 14.8 mm at the higher concentration 1000 μg/disc while it was 6.2 mm at the lower concentration 25 μg/disc. The MIC of alcoholic extract against fungal pathogen i.e. *F. oxysporum*, was reported 0.851 μg/ml (Table 4). The inhibition zone of the

Table 4 Zone of minimum and maximum inhibition of moss *Atrichum undulatum* (Hedw.) P. Beauv. tested against fungus

Test pathogens	Alcoholic extract				Aqueous extract				
	SD	Max SD	Min SD	MIC	SD	Max SD	Min SD	MIC	ST (IZ)
A. fumigatus	–	–	–	–	–	–	–	–	25
F. oxysporum	10.76 ± 0.40	11	4.5	0.851	–	–	–	–	27

Data are average (± SD) diameter (in mm) of inhibition zone from three independent observations; [–], no activity; *MIC* minimum inhibitory concentration (μg/mL); *ST* standard. Standard for fungus, Fluconazole, *SD* standard deviation

alcoholic extract varied between 2.40 to 4.28 mm for bacteria and 3.74 mm for fungus. However, with aqueous extract, the range of inhibition zone varied between 5.7 to 25.4 mm. The results of this testing are shown in Tables 1 and 2 (Fig. 1a–g).

Discussion

The study revealed that the ethanolic extract of *A. undulatum* had moderate bactericidal and fungicidal action in response to the four bacteria out of five and in one fungal pathogen undertaken. In the bacterial set of test, moss exhibited maximum and minimum values while in the fungal set, the moss responded in terms of highest inhibition zone to alcoholic medium and negligible or no inhibition to aqueous medium.

The results for the first time confirmed that *A. undulatum* has the property of antimicrobial activity. The functional extract of the moss indicated various levels of potency in response to the tested organisms and provided a broad range of antimicrobial efficacy. The maximum susceptibility was shown by *S. typhi* in alcoholic as well as aqueous medium at all concentration gradients i.e. 25, 50, 100, 250, 500 and 1000 μg/disc, however the minimum inhibition was observed in *E. coli.* in alcoholic medium at all above said concentrations.

In aqueous extract, bacterial strain *S. aureus* was reported least affected at all observed concentrations. Any bactericidal effect of alcoholic and aqueous extract could not be observed in *P. mirabilis* at any practiced concentration. This could be attributed to the less number of potent molecules present in the extract.

The enhanced antifungal efficacy of alcoholic extract of *A. undulatum* was noticed in *A. fumigatus* at different degree of concentration. It showed novel activity against *F. oxysporum* at all concentrations of ethanol extract except at 25 μg/disc.

On increasing the concentration, the four out of five bacterial strains responded as increased zone of inhibition specifically on alcoholic medium rather than aqueous medium.

Among all the tested strains, *S. typhi* was proved to be most sensitive followed by *B. mycoides*, *E. coli*, *A. fumigatus* and *F.* oxysporum respectively. However, *P. mirabilis* manifested nil sign of extracts, might be due to the reduced accessibility of active compounds of extract to the cell wall (Basile et al. 1998).

Aqueous as well as ethanol extract of *A. undulatum* exhibited a broad spectrum of antimicrobial potency (Nikolajeva et al. 2012; Banerjee and Sen 1979).This study indicates that *A. undulatum* might have some phyto-compounds which could possess the antimicrobial characteristics (Vats and Alam 2013).The secondary metabolites and antimicrobial chemical constituents have different degree of solubility in the polar solvents, which further isolate the low weight molecular antioxidants (Vats et al. 2012) consecutively increasing the antimicrobial efficacy. Therefore, *A. undulatum* could be used as functional material in antibacterial and antifungal drug development.

This could be owing to some potent antioxidants for wound healing by restricting lipid peroxidation resulting in the increase in superoxide dismutase (SOD) and catalase activity (Singh et al. 2006).

The practice of using traditional medicines has been very prevalent in different parts of India and world in the form of Ayurveda but for the one or other reason, bryophytes have remained under recognized and under explored since a long time. The specific information about appropriate mechanism against microbial cells could be obtained through the extracting active chemical constituents and extending the work further.

Conclusion

It is summarized that the Bryophytes have very low exposure in India, hence *A. undulatum* can be potentially used and incorporated in the list of ethno medicine for its functional activity against bacterial strains. Inclusion of new varieties of plant species would help to share the burden of traditional species as the plant wealth is declining at a faster pace. It can provide a broad spectrum in the therapeutic applications.

Therefore, *A. undulatum* can serve as a potent remedy for certain microbial populations. Further study is required for mapping the key mechanism to combat against bacteria and exact formulation of specific compound.

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