In vitro Screening of Bryophytes for Antimicrobial Activity

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Abstract

Background: Bryophytes, the previous land plants posses medicinally important bioactive compounds but with little information. Traditionally the bryophytes plants posses some bioactive components and therefore used throughout the world as drugs and remedies to cure the various diseases.
Objectives: Evaluation of antimicrobial effect of mentioned bryophytes on some pathogenic microorganisms.
Methods: Different Solvent fractions of selected bryophytes were obtained and dried in vacuum. Antimicrobial effect of these fractions was determined by agar diffusion method on different pathogenic microorganisms. The result was then compared with the standard antibiotics ampicillin and nystatin (10 ug/ml).
Results: Results indicated that the bryophyte extracts were found to be active against at least one of the test organisms except *Racomitrium crispulum*. The extracts that displayed antibacterial activity were neither always effective against the same organisms, nor consistent in magnitude of inhibition. Ethanolic, acetone and chloroform extracts were found to be more effective on *Escherichia coli* and *Staphylococcus aureus.* Among the fungi *Aspergillus niger* was most sensitive to the ethanolic extract of *Plagiochasma appendiculatum* and *Bryum argentium.*
Conclusion: Ethanolic, acetone and chloroform extract of nearly all bryophytes were found to be effective, therefore the study focuses on medicinal significance of bryophytes.

Keywords: Bryophytes, Antimicrobial effect, Agar diffusion method

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Introduction

Bryophytes belong to the group of the oldest known land plants, which includes liverworts, hornworts and mosses. More than 22,000 members of the mosses are exist (Bryophyte) in the world [1]. One of the features that helped bryophytes to survive and maintain their place in today's flora is their content of biologically active compounds. Although Bryophytes are very familiar, their medicinal importance is not exploited completely. They are used in pharmaceutical products, horticulture, household purposes and are also ecologically important as good indicators of environmental conditions [2, 3]. Bryophytes are traditionally used in Chinese, Europe, North American and Indian medicine, to treat illness of cardiovascular system, tonsillitis, bronchitis, tympanitis, in skin diseases and burns. They also possess anticancer and antimicrobial activity due to their unique chemical constituents [4, 5]. A few moss genera *Atrichum*, *Dicranum, Minium, Polytricum*, *Sphagnum, Porella*, and *Reboulia* prevent the soil erosion due to their trample – resistant structure and regenerative ability [6, 7, 8]. Compounds like polygodial from *Porella,* Norpiguisone from *Conocephalum conicum* and Lunularin from *Lunularia cruciata*, 4-hydro-3-methoxybibenzyl and a- and b- pinine-alloromadendrine from *Plagiochila stevensoniana* are useful as antimicrobial compounds [9, 10]. *Plagiochila fasciculata* shows inhibitory effect on P388 cells (Leucemia), *Herpes Simplex type 1*, *Polio type 1*, *Bacillus subtilis*, *Escherichia coli*, *Candida albicans, Trichophyton mentagrophytes* and *Cladosporium resinae* [11]. The antifungal activity of *Herberta aduncus* against *Botrytis cinerea, Rhizoctonia soloni, Pythium debaryanum* is well illustrated [12]. Members of *Fissidens* and *Polytrichum* were used as diuretic and hair growth stimulating drugs [13].

In the present work, different bryophytes liverwort and mosses were screened for determining their antimicrobial activity against the selected bacterial and fungal pathogenic species.

Materials and methods

Source of plants:

Liverwort- *Plagiochasma appendiculatum* and mosses- *Thuidium delicatulum, Thuidium cymbifolium, Bryum cellulare, Bryum argentium* and *Racomitrium crispulum* were collected in the month of November 2007 from Mukteswar (Kumaon Hills, India). The plant material was carefully cleaned from attached litter and dead material under running tap water and finally with sterile distilled water. For experimental work, only green or green brown shoots were used.

Test organisms

Pathogenic bacterial cultures *Escherichia coli, Salmonella typhi, Shigella flexneri, Shigella sonnei, Enterobacter aerogenes, Klebsiella pneumoniae, Staphylococcus aureus, Proteus mirabilis, Proteus vulgaris, Psedomonas aeruginosa* and the fungal strains *Aspergillus niger, Fusarium moniliforme, Rhizoctonia bataticola* were procured from Institute of Microbial Technology, Chandigarh (Table 1). 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^{0}C temperature respectively. Media and growth conditions like temperature (37 ^{0}C for bacteria; 30 ^{0}C for fungi), and incubation period (24-48 hrs for bacteria; 48-72 hrs for fungi) used for culturing these strains were as

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prescribed by Institute of Microbial Technology, Chandigarh respectively. Nutrient and Czapek's Dox medium were also used for bacterial and fungal broth culture preparation. Broth cultures of strains were prepared using the standard method to obtain final population of each organism to 2 x 10^6 cfu/ml for bacterial and fungal (spores) strains.

Solvent Extract preparation

The plant materials (200g) of each were dried, powdered and exhaustively extracted with 95% ethanol separately. These extracts were concentrated and successively fractionated in different solvents (Acetone, Chloroform, Benzene, Petroleum ether and distilled water) and dried in vacuum for 24 hr. The fractions obtained were filtered centrifuged at 3,000 rpm for 10 min and were used for studying antimicrobial activity.

Antimicrobial Assay

Antimicrobial activity of the bryophytes fractions were determined by agar diffusion method [14, 15]. Petri plates containing sterilized Nutrient and Czapek's Dox agar (3% agar) were used as base layer. After solidification of medium each plate was seeded with 0.1 ml of broth culture strains (mixed gently) in respective agar medium (1.5% agar) and poured on the base layer and allowed to solidify. The wells of 6 mm diameter were cut with sterile cork borer. Each well was filled with 0.1 ml solvent extract of each bryophyte. Different solvents alone were used as control, for each test organism. For comparison, the standard drugs 0.1 ml of ampicilln (10 µg/ml) in case of bacteria and 0.1 ml of Nystatine (10 µg/ml) in case of fungi were used. The Petri dishes used for antibacterial activity were incubated at 37 ±0.5 ^{0}C for 24 hrs, whereas the Petri dishes used for antifungal activity were incubated at 28^0 C for 48 hrs. The antimicrobial activity was measured in terms of the zone of inhibition (mm).

Results

Antimicrobial activity of selected bryophyte extracts in different solvents on test microorganisms are represented in Table 1 and 2. Extract of *Thuidium cymbifolium, Thuidium delicatulum, Bryum cellulare, Bryum argentium* and *Plagiochasma appendiculatum* showed antimicrobial activity against test microorganisms. whereas *Racomitrium crispulum* did not revealed any antimicrobial activity.

Table 1: Antimicrobial activity of Bryophyte extracts in different solvents (Inhibition is zone in mm)

Nature of extracts of Bryophytes		Bacteria										Fungi		
		EC	ST	SF	SS	EA	KP	PM	PV	PA	SA	AN	FM	RB
Thuidium cymbifolium	Acetone	-	-	-	-	-	-	-	-	6	8	14	12	16
	Ethanolic	11	-	±	±	-	7	-	11	8	9	13	20	19
	Petroleum ether	-	-	-	-	-	-	-	-	-	-	-	-	-
	Benzene	-	-	-	-	-	-	-	-	-	-	-	-	-
	Chloroform	12	14	-	-	-	9	-	-	-	12	7	4	3
	Distilled water	12	-	±	-	-	-	-	-	-	-	10	2	9
Thuidium delicatulum	Acetone	-	-	-	-	-	-	-	-	7	5	15	10	12
	Ethanolic	10	-	2	4	-	4	-	12	8	6	10	16	14
	Petroleum ether	-	-	-	-	-	-	-	-	-	-	-	-	-
	Benzene	-	-	-	-	-	-	-	-	-	-	-	-	-
	Chloroform	11	14	-	-	-	8	-	-	-	11	3	3	6
	Distilled water	11	8	-	±	-	-	-	-	-	-	6	2	9

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Continuance Table 1. Antimicrobial activity of Bryophyte extracts in different solvents (Inhibition is zone in mm)

Nature of extracts of Bryophytes		Bacteria										Fungi		
		EC	ST	SF	SS	EA	KP	PM	PV	PA	SA	AN	AN	AN
Plagiochasma appendiculatum	Acetone	±	-	-	-	-	-	-	-	10	9	17	16	20
	Ethanolic	12	±	-	±	7	8	±	12	11	14	24	22	20
	Petroleum ether	±	-	-	-	-	-	-	-	-	-	-	-	-
	Benzene	-	-	-	-	-	-	-	-	-	-	-	-	-
	Chloroform	11	13	-	-	-	14	-	-	-	14	10	8	6
	Distilled water	13	10	-	±	±	-	-	±	±	-	12	14	20

Table 2. Antimicrobial activity of Bryophyte extracts in different solvents (Inhibition is zone in mm)

Nature of extracts of Bryophytes		Bacteria										Fungi		
		EC	ST	SF	SS	EA	KP	PM	PV	PA	SA	AN	FM	RB
Bryum cellulare	Acetone	-	-	-	-	-	-	-	-	9	9	12	14	14
	Ethanolic	8	±	-	-	-	9	-	8	9	9	16	10	14
	Petroleum ether	-	-	-	-	-	-	-	-	-	-	-	-	-
	Benzene	-	-	-	-	-	-	-	-	-	-	-	-	-
	Chloroform	10	10	-	-	-	10	-	-	-	9	6	2	2
	Distilled water	10	8	-	±	-	-	±	-	-	-	7	4	2
Bryum argentium	Acetone	-	-	-	-	-	-	-	-	9	10	10	14	14
	Ethanolic	9	-	-	-	±	9	-	9	10	11	23	21	20
	Petroleum ether	-	-	-	-	-	-	-	-	-	-	-	-	-
	Benzene	-	-	-	-	-	-	-	-	-	-	-	-	-
	Chloroform	10.5	10	-	-	-	9	-	-	-	9	8	8	8
	Distilled water	11	6	-	-	-	-	-	-	-	-	10	6	6
Antibiotics	Ampicillin	23	23	21	20	19	21	18	15	22	20	-	-	-
	Nystatin	-	-	-	-	-	-	-	-	-	-	20	19	17

All the solvent extracts of *Racomitrum cripulum* showed no antimicrobial activity.
Data represented is mean of 3 replicates.
EC - *E.coli*; **ST** - *Salmonella typhi*; **SF** - *Shigella flexneri*; **SS** - *Shigella sonnei*; **EA** - *Enterobacter aerogenes*; **KP** - *Klebsiella pneumoniae*; **PM** - *Proteus mirabilis*; **PV** - *Proteus vulgaris*; **PA** - *Pseudomonas aeruginosa*; **SA** - *Staphylococcus aureus*; **RB** - *Rhizoctonia bataticola*; **FM** - *Fusarium moniliforme*; **AN** - *Aspergillus niger*
Not detected - ; ±, Trace activity.

Discussion

The study first time reports the antimicrobial activity of these Indian mosses. The active fractions were prepared in different solvents like ethanol, acetone, benzene, chloroform, petroleum ether and distilled water. Although all the extracts showed varying levels of activity against all the test fungi and bacteria, the ethanolic extract was found to be more active than other fractionated extracts. This also showed similarity with the results of S. Snbisha. Distilled water,

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chloroform and acetone extract of Bryophytes revealed activity against 2 %, 4 % and 2 % bacteria respectively and 100% activity against test fungi. While benzene and petroleum ether extracts of Bryophytes did not revealed any significant activity against test bacteria and fungi. The results obtained are in contrast to Gupta and Singh (1971) who reported that petroleum ether extracts of *Barbula* and *Timmiella* species were active against both gram positive and gram negative bacteria [7]. This may be due to the variations in chemical composition of particular species of plants, which can also vary according to the geographical origin and harvesting seasons [16].

Among the Bryophytes Liverwort *Plagiochasma appendiculatum* was most active against bacteria and fungi and *Bryum argentium* showed maximum antifungal activity. The antimicrobial activity might be due to presence of flavonoids, steroids, terpenoids and other polyphenolic compounds [17, 18].

The results showed that *E. coli* and *S. aureus* were found to be very sensitive test microorganisms followed by *P. aeruginosa* and *K. pneumoniae*. This indicates that gram negative microorganisms are more sensitive than gram positive microorganisms. Conventionally antibiotics are generally more active against gram positive than gram negative bacteria. However antibacterial activity of mosses was found to be active against gram negative bacteria. This makes the advantages of selected bryophytes as antimicrobial agent. Most research studies revealed no antifungal activity of Indian bryophytes. While, other countries study indicates a broad range of antifungal activity including *Porella, Makinoa , Lunularin cruciata , Botryris cinera , Septoria nodorum , Dumortiera hirsute , Sphagnum portoricense , Orthotrichum rupestre* and *Uromyces fabae* [19]. Our study showed that all the selected fungi are more or less sensitive to all the bryophyte fractions. Among these *A. niger* was most sensitive to ethanolic extract *of Plagiochasma appendiculatum* and *Bryum argentium.*

Further research is being carried out for isolation of bioactive chemical constituents from the active fractions and their mode of action on microbial cells.

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