Antimicrobial activity of some bryophytes (liverworts and a hornwort) from Kolhapur district

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ABSTRACT

The *in vitro* antibacterial activity of the extracts of the bryophytes was assessed using agar-well diffusion method against three bacterial strains. The bryophyte extracts were prepared in methanol and dichloromethane. Inhibition of bacterial growth was compared with that of ampicillin and tetracycline as positive control and solvents as negative control. The methanol and dichloromethane extracts of *Plagiochasma intermedium, Asterella wallichiana* and *Targionia hypophylla* had potential activity against all the microorganisms tested. The dichloromethane extract of *P. intermedium,* methanol extracts of *A. wallichiana* and *T. hypophylla* also showed good results. Among all the bryophytes investigated for *in vitro* antimicrobial activity the extracts of *T. hypophylla* showed most promising results than that of all others while the extracts of *Cyathodium cavernarum* was rather inactive.

Key words: antibacterial, *Anthoceros, Asterella, Plagiochasma, Targionia*

INTRODUCTION

In contrast to the extensive utilization of substances from higher plant sources, bryophytes have rarely been considered as a source of substances useful for human beings because they are very small and difficult to collect in large amounts as pure samples. However, they have been used as medicinal plants to cure cut, external wounds, bacteriosis, neurasthenia, pulmonary tuberculosis, etc. Extracts of many bryophytes have been shown to possess varying levels of antibacterial and anticancer activities *in vitro*.[1-4] The classes, liverworts and hornworts of bryophytes represent an interesting groups. These are very small and ubiquitous plants. An interesting feature of bryophytes is that they are neither attacked by either bacteria or fungi nor damaged by insects or snails.[5] Considering such observations bryophytes might have in use as medicinal plants for more than 400 yrs. in China, Europe and North America to cure various types of diseases. The Chinese and the native Americans have used various moss species in the form of paste and applied as poultice. Chinese traditional medicine names 40 kinds of bryophytes that have been used to treat illness of cardiovascular system, tonsillitis, bronchitis, tympanitis, cystitis as well as skin diseases, cuts, burn and wounds. In India the burnt ash of mosses mixed with fat and honey is used as an external application for cuts, burns and wounds in the Himalayan regions. The liverworts *Marchantia polymorpha* is also used as medicine for boils and abscesses. The rosette forming *Riccia* spp. is used as an external application to cure ringworm. When bryophytes screened for antitumor activity it was found that number of mosses and liverworts were active.[6] Antimicrobial and antifungal activities have been reported for wide range of liverworts.[7-9] These activities are due to biologically active compounds present in them particularly lipophylic extracts of several liverworts namely *Bazzania, Frullania, Marchantia, Plagiochilla, Porella and Reitdulla spp.* show antibacterial and antifungal activities. According to some scientists non-ionized organic acids and polyphenolic compounds might contribute the antibiotic properties of bryophytes and it is found that mosses strongly inhibit one or both positive and negative bacteria.[10-11] Gupta and Singh[12] have reported a high occurrence of antibacterial activity in extract of *Barbula* spp. reaching as high as 36.6 % whereas it is only half that in *Timmiella spp* (18.8%). Asakawa and Heidelberger[13] isolated 3 prenyl bitibbenzyl from *Redula spp* and showed that it could inhibit growth of *S. aureus* at the concentration of 20.3 μgml^{-1}. A wide range of antibacterial activity has been observed a in nearly all bryophytes tested.[14-15] Antimicrobial activity has been reported in the extracts of many liverworts.[16]

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The existence of high antibacterial activity in case of liverworts may be due to the biologically active compounds in them. The methanol extract after chromatographic separation gave cyclic bis-bibenzyles, marchantin A and analogues, one of which showed antimicrobial activity against *B. cereus* (MIC 12.5 μgml^{-1}), *B. megaterium* (25 μgml^{-1}), *B. subtilis* (25 μgml^{-1}) and *S. aureus* (3.13-25 μgml^{-1}).[17] Some of the active ingredients that have been isolated and identified are Polygodial from *Porella*, Norpiguisone from *Conocephalum conicum* and Lanularin from *Lanularia cruciata*.[18] 4-hydroxy-3'methoxybibenzyl and α- and ß-pinine-alloromadendrin in *Plagiochila stevensoniana* proved to inhibit *Candida albicans*, and *B. subtilis*.[19] A literature search revealed very little studies on the antimicrobial activity of Indian bryophytes especially those from bryophytes from this region. Therefore the present investigation was undertaken.

MATERIAL AND METHODS

Plant Material

Five different bryophytes viz. *P. intermedium, A. wallichiana, T. hypophylla, C. cavernarum* and *A. subtilis* with the rhizoids were collected from the Panhala region of Kolhapur district, Maharashtra state in the months of August and September. The care was taken to harvest the pure and uniform patch of particular species of bryophyte to be tested.

Fresh gametophytic samples of said bryophytes were extensively washed with tap water and distilled water and surface dried on blotting paper. Any dirt and senescent parts of plants were removed during the washing process.

5 g air dried plant material along with rhizoids was powdered in mortar with pastle and soxhlet extracted in 150-160 ml of two different solvents i.e. dichloromethane and methanol at 40°C. Methanol extract was evaporated to 30 ml under vacuum pressure at 40°C. While dichloromethane extract was dried and redissolved in 30 ml of acetone.These extracts were used for further studies.

Test microorganisms

Three bacterial strains were procured from NCIM, NCL, Pune. *In vitro* antibacterial activity was tested against 2 Gram – positive bacteria *Staphylococcus aureus* (NCIM 5021), *Bacillus subtilis* (NCIM 2010) and a Gram-negative bacterium *Escherichia coli* (NCIM 2089).

Determination of antimicrobial Activity

Plant extracts were tested for antimicrobial activity through agar-plate diffusion method[20] using 100 µl of suspension of the test microorganisms. Bacterial strains were grown on nutrient agar plates. Sterile nutrient agar plates were prepared, 48 hrs cultured suspensions were made and inoculated on sterile agar medium in the respective culture plates. The 10 mm sterile cork borer was used for making wells. The extracts, 0.1 and 0.2 ml were added in assay well carefully. 50 μgml^{-1} tetracycline and ampicillin (Hi-Media) which served as +ve control and methanol and acetone (Hi-Media) used as –ve control were added in the wells. All the plates were kept at low temperature for 1-1½ hrs for sample diffusion and incubated at 35°C for 48 hrs. After incubation, the zone of inhibition was measured.

Statistical analysis

Statistical analysis was done using one-way analysis of variance (ANOVA). $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Adult thalli of said bryophytes (liverworts and hornwort) collected from Panhala region underwent extraction with dichloromethane and methanol. Inhibition of bacterial growth was compared with those of tetracycline and ampicillin. The antibacterial activity results are shown in [Table 1]. Our findings indicated that all the extracts of bryophytes studied showed varying levels of *in vitro* antibacterial activity against all the test bacteria. Of the bryophytes investigated both extracts of three liverworts, *T. hypophylla, P. intermedium* and *A, wallichiana* showed promising results while that of *T. hypophylla* showed very good antibacterial activity. *C. cavernarum* and the hornwort, *A. subtilis* extracts had more or less the same levels of antibacterial effects, whereas, *C. cavernarum* extracts were rather inactive against all the microorganisms under investigation. It may be due to the very thin, papery structure of thallus of *C. cavernarum* which contains very low concentrations of active compounds against microorganisms.

The results revealed that methanol extract of bryophytes had *in vitro* greater potential for antibacterial activity than that of dichloromethane extract against *E. coli* and *B. subtilis*. The dichloromethane extract had greater antibacterial activity against *S. aureus*. It was observed that *B. subtilis* was most susceptible than rest of the bacteria tested with maximum inhibition zone of 14 and 11mm being against methanol extract of *T. hypophylla* and *A. wallichiana* respectively and 12 and 11mm against dichloromethane extracts of *P. intermedium* and *T. hypophylla* respectively. But *S. aureus*, a Gram-positive bacterium gave weak response.

In case of *E. coli* no response was observed to the *C. cavernarum* extracts and same was true with that of dichloromethane extract for *B. subtilis*. In case of both the *A. subtilis* extracts and the lower concentrations of *C. cavernarum* extracts same results were observed i.e. no antibacterial activity was observed against *S. aureus*. Our findings showed that extracts of liverwort had interesting activity against Gram-positive and Gram-negative bacteria. It is common that the sensitivity of

Table 1: Antibacterial activity of methanol and dichloromethane extracts of some bryophytes (expressed as inhibition zones in mm)

Plant /substance	Test organism	Inhibition zone (mm)			
		Concentration of extract (µl)			
		Methanol Extract		Dichloromethane Extract	
		100	200	100	200
P. intermedium					
	E. coli	6.0 ± 2.0*	9.67 ± 0.5*	4.67 ± 0.5	10.67 ± 0.5*
	B. substilis	4.67 ± 2.0	7.67 ± 2.08	8.67 ± 5.50	12.33 ± 4.04
	S. aureus	5.0 ± 1.0	7.67 ± 1.52*	5.0 ± 1.0	9.0 ± 1.0
A. wallichiana					
	E. coli	6.67 ± 1.53	12.33 ± 0.58*	0.0	0.67 ± 1.15
	B. substilis	7.0 ± 0.0	11.00 ± 1.73	0.0	3.67 ± 0.58
	S. aureus	5. 67 ± 1.15	7.67 ± 2.08	2.67 ± 1.52	4.67 ± 2.31
T. hypophylla					
	E. coli	8.0 ± 1.0*	12.3 ± 2.89*	4.67 ± 0.58	8.0 ± 1.73*
	B. substilis	9.0 ± 1.0*	14.33 ± 0.58*	6.33 ± 1.54	11.33 ± 2.5*
	S. aureus	3.67 ± 1.53	9.67 ± 0.58*	5.67 ± 2.08	12.0 ± 1.0*
*C. cavern*arum					
	E. coli	0.0	0.6 ± 0.57	0.0	0.0
	B. substilis	0.33 ± 0.57	1.67 ± 1.57	0.0	0.0
	S. aureus	0.0	0.33 ± 0.58	0.0	1.33 ± 2.31
A. subtilis					
	E. coli	0.0	3.33 ± 0.58	2.33 ± 4.04	6.67 ± 6.42
	B. substilis	0.67 ± 1.15	1.67 ± 1.55	0.33 ± 0.58	3.0 ± 1.73
	S. aureus	0.0	0.0	0.0	0.33 ± 0.58
Methanol					
	E. coli	0.0			
	B. substilis	0.0			
	S. aureus	0.0			
Acetone					
	E. coli	0.0			
	B. substilis	0.0			
	S. aureus	1.0 ± 0.0			
Tetracyclin					
	E. coli	22.33 ± 0.58			
	B. substilis	11.67 ± 1.25			
	S. aureus	15.0 ± 1.0			
Ampicillin					
	E. coli	17.77 ± 0.58			
	B. substilis	16.66 ± 0.58			
	S. aureus	1.00 ± 0			

Values are mean ± SD. $n = 3$ in each group. *$P < 0.001$ compared to negative control (Tukey test). The activity was assayed by disc diffusion method.

Gram-negative bacteria is generally higher than that of Gram-positive ones. This is important from medicinal standpoint because antibacterial substances which are normally employed in therapy are active chiefly against Gram-positive.

The present study reported that liverworts showed varying levels of activity against all the three test bacteria. This suggests that the extracts of three liverworts, *T. hypophylla, P. intermedium, and A. wallichiana* in both the solvents have a broad spectrum of activity although the degree of susceptibility could differ between different organisms. Thus the above extracts are worthy of further investigation for their use as antibacterial agents. There is a need of purification and characterization of active principle(s) which can help in new drug development.

ACKNOWLEDGEMENT

The first author is thankful to the Co-ordinator, Department of Agrochemicals and pest management, Shivaji University, Kolhapur for providing necessary laboratory facilities.

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