

Bactericidal Activity of Bioactive Compounds Extracted from Bryophyte *Barbula vinilis*

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Abstract: Natural product and their components are become increasingly popular as a bactericidal agent. Bryophytes are a group of simple land plants, well-adapted to moist habitats but are also found in grassland and deserts, where they endure prolonged dry periods. In the present investigation fresh samples of bryophyte *Barbula vinilis* were collected from locality of district Chirang B.T.A.D. of state Assam, India. The present study aimed at evaluating *in vitro* antimicrobial activity of Hexane extract of *Barbula vinilis* against *Escherichia coli* (NCIM 2501), *Pseudomonas aeruginosa* (NCIM 5029), *Staphylococcus griseus* (NCIM 2621), *Bacillus murcesens* (NCIM5037), *Staphylococcus marcesens* (NCIM 2079), *Lactobacillus casei* (NCIM 2651), *Saccharomyces cerevaceae* (ARIFCC 1248), *Rhizobium indigofera* (ARIFCC 2818), *Bacillus subtilis* (NCIM 2921) and *Salmonella typhimurium* (NCIM 2501). The Hexane extract of *Barbula vinilis* presented its highest bactericidal activity against *Staphylococcus griseus* (NCIM 2621), *Bacillus subtilis* (NCIM 2921) and *Salmonella typhimurium* (NCIM 2501).

Keywords: *Barbula vinilis*, Bacterial cultures, Hexane.

1. Introduction

Bryophytes belong to the group of the oldest known land plants. They are a group of simple land plants, well-adapted to moist habitats but are also found in grassland and deserts, where they endure prolonged dry periods. Bryophytes do not have true stems, leaves, roots, or a vascular system. Like the rest of the land plants, they evolved from green algal ancestors, closely related to the Charophytes. They are attached to the substrate (ground, rock, or bark) by *rhizoids*, which are one or a few-celled, root-like threads that serve only for anchoring and are not capable of absorbing water and nutrients from the substrate. The bryophytes are generally considered as “Key” group (Mitchell and Rook, 1979). Bryophytes have successfully exploited many environments, perhaps partly because they are rarely in direct competition with higher plants (Anderson, 1980). They are vital plants and have indirect use in the formation of soil and rocks in vegetation covers and soil conservation and act as a pioneer plant in plant succession. Although Bryophytes are very familiar, their medicinal importance is not exploited complete. Bryophytes are having several applications such as, Anti-Microbial activity, Anti-Tumor Activity, Production of biopharmaceuticals in bryophytes, Anti-microbial activity of lipid etc. (Mahesh and Satish, 2008 and Bishnu et al, 2009).

2. Materials and Methods

Collection & Processing of Samples

Fresh samples of bryophyte *Barbula* Sp. were collected from locality of district Chirang B.T.A.D. of state Assam, India. For the present study, only developed gametophyte plants were collected. Brownish or pale plants and dried plants were rejected. The gametophytes entering into reproductive stage may start to produce different compounds than simple

vegetative ones and therefore they were avoided. The intact moss carpets were sampled and transported to the laboratory where these were carefully cleaned from all dead material and attached litter within two weeks after sampling. The soil moiety was removed by proper washing. Only green and green-brown shoots were included. Mosses were sun dried until dryness and stored in polythene bag at 4°C for further analysis (Jager A. K, 2003).

Chemicals

The reagents used in this study were chloroform, methanol, petroleum ether, diethyl acetate, glacial acetic acid, n-hexane, H₂SO₄ 50% (v/v), peptone (Qualigens), yeast extract (Loba chemie), campicillin 500 mg (CADILA Pharmaceuticals), agar (Qualigens), potato dextrose broth (Himedia M403). All the chemicals used are of analytical grade.

1) Chloroform: Methanol extraction:

A 5gm of moss sample was taken in 20 ml of chloroform: methanol (2:1 ratio) and crushed in motor and pestle under 4°C. Supernatant was taken in separate vial after centrifugation at 5000 rpm for 10min. Component was dried up to small quantity in water bath set at 50°C. Extract were used to check their antimicrobial activity under different test organisms.

2) Soxhlate extraction:

5 gm of moss powders were loaded in Soxhlate assembly with 50ml n-hexane and then distilled (at 55°C) to concentrate the extract separately. These are stored at 4°C and used as sample for further analysis.

Anti-microbial sensitivity test

Test organisms- Following micro-organisms used in the present investigation. *Escherichia coli* (NCIM 2501), *Pseudomonas aeruginosa* (NCIM 5029), *Staphylococcus griseous* (NCIM 2621), *Bacillus murcesens* (NCIM5037),

Staphylococcus marcesens (NCIM 2079), *Lactobacillus casei* (NCIM 2651), *Saccharomyces cerevaceae* (ARIFCC 1248), *Rhizobium indigofera* (ARIFCC 2818), *Bacillus subtilis* (NCIM 2921) and *Salmonella typhimurium* (NCIM 2501).

These were obtained from National Collection of Industrial Micro-organisms (NCIM). The collected cultures of bacteria were sub cultured on nutrient agar (HiMedia) slants respectively and stored at 4°C until required for study.

Anti-microbial sensitivity test:

1) Activation of culture: Inoculums preparation

a) Bacterial culture: - Antibacterial activity of the crude extracts in different solvents was tested by disc diffusion assay. Mueller Hinton agar no. 2 (HiMedia, India) was used as the bacteriological medium. Medium was prepared and poured 20 ml each in sterilized Petri plates of 9 cm diameter and allowed to solidify. Bacterial cultures grown in nutrient broth and on agar slants were used. All bacterial suspensions were prepared aseptically in 10 ml of saline (0.085 g NaCl in 10 ml Distilled water) using laminar air flow (LAF). The plates, cultured with microbial suspension (100-150 µl) by spread plate technique. The bacterial cultures were maintained on Nutrient agar (pH 7.0± 0.2) at 4°C temperature respectively. Media and growth conditions like temperature (37°C), and incubation period (24-48 hrs for bacteria) used for culturing these strains were as prescribed by Agarkar Research Institute (ARI), Pune and National Collection of Industrial Microorganisms (NCIM); NCL, Pune. Nutrient medium were also used for bacterial broth culture preparation. Broth cultures of strains were prepared using the standard method to obtain final population of each organism to 2×10^6 cfu/ml for bacterial strains (Elizabeth K.M, 2005 and Mahesh and Satish, 2008).

2) Disc Diffusion Method

Anti bacterial tests were carried out using the disk diffusion method in sterile condition. A 0.1ml of bacterial activated suspension was inoculated on solid nutrient agar Petri plate. The bacterial inoculums were spreaded by glass spreader until totally absorbed in agar layer for the development of uniform bacterial growth. Sterile filter paper disc of 3mm diameter were prepared by drying with 50 µl (50mg/ml) of hexane extract in three different discs of 25 % (12.5mg/ml), 50 % (25mg/ml) and 100 % (50mg/ml) of above concentration. A 50µl solvent for blank and 10 µl anti bacterial drug (Campicillin 500mg) were also loaded as negative and positive control. The plates were incubated for 24 hours at 37°C. For each bacterial strain controls were maintained where extract free pure solvents were used. The control zones were subtracted from the test zones and the resulting zone diameter is shown in the Table 2. The experiment was performed thrice and the mean values were presented with standard error (Ekundayo E.O and Ezeogu, 2006).

3) Measurement of zone:

The anti bacterial activity was evaluated by measuring the diameter of the inhibition zone formed around the disc. The experiments were performed in triplicate and the mean diameter of the zone of inhibition was calculated (Bodade et al., 2008 and Bishnu et al, 2009).

3. Result and Discussion

For thousands of years, there has been target interest in biologically active compounds, isolated from plant species for the elimination of pathogenic micro-organisms, because of the resistance that micro-organisms have built against antibiotics (Bodade et al., 2008) or because they are ecologically safe compounds (chaugale et al., 2009).



Figure 1: Moss *Barbula vinilis* collected from Assam (collected by MSA & AK)

Result obtained in antibacterial activity of bioactive compounds isolated from *Barbula vinilis* is summarized in table 1 and 2. In the present study in an all total 10 microbial culture including gram positive and negative bacteria have

been tested against isolated compounds. For antimicrobial assay it was slotted into two parts; first is chloroform: methanol (2:1) extracts and seconds the n-hexane extract by soxhlet. Amongst these two only n-hexane extract exhibited

the presence of bioactive compound and showed antimicrobial activity against microorganism *Escherichia coli* (NCIM 2501), *Pseudomonas aeruginosa* (NCIM 5029), *Staphylococcus griseus* (NCIM 2621), *Bacillus murcesens* (NCIM5037), *Bacillus subtilis* (NCIM 2921) and *Salmonella typhimurium* (NCIM 2501).

Table 1: Screening of antimicrobial activity for different solvent extract against experimental micro organisms.

Test organism	Chloroform: Methanol (2:1) extract	Hexane extract (soxhlet)
<i>Escherichia coli</i> (NCIM 2501)	Absent	Present
<i>Pseudomonas aeruginosa</i> (NCIM 5029)	Absent	Present
<i>Staphylococcus griseus</i> (NCIM 2621)	Absent	Present
<i>Bacillus murcesens</i> (NCIM5037)	Absent	Present
<i>Staphylococcus marcesens</i> (NCIM 2079)	Absent	Absent
<i>Lactobacillus casei</i> (NCIM 2651)	Absent	Absent
<i>Saccharomyces cerevaceae</i> (ARIFCC 1248)	Absent	Absent
<i>Rhizobium indigofera</i> (ARIFCC 2818)	Absent	Absent
<i>Bacillus subtilis</i> (NCIM 2921)	Absent	Present
<i>Salmonella typhimurium</i> (NCIM 2501)	Absent	Present

The obtained zone of inhibition (ZOI) for hexane extract has been mentioned in table 2. The data was compared with standard antibiotic campicillin. It was found that *S. griseus* showed 16mm ZOI while *S. typhimurium* exhibited 12mm, *B. subtilis* (Fig. 1 A) had 11mm ZOI value under 100% (50mg/ml) of n-hexane extract. That was found higher than the standard drug i.e. 12, 5 and 10 mm respectively. The *Barbula vinilis* bioactive components showed antimicrobial activity ranging from 12.5-50 mg/ml concentration.

The antibacterial activity of mosses against microorganisms has been shown earlier in different investigations; for instance , *Rhyncostegium riparioides* and *Pleurochaete squarrosa* extracts were more active against gram negative bacteria than gram positive bacteria (Basile et al., 1998a, 1998b). In another study, Ilhan et al., (2006) found that acetone extract of *Palustriella commutate* was effective against all gram negative bacteria tested and some gram positive bacteria such as *Bacillus mycoides*, *B. cereus*, *B. subtilis* and *Micrococcus luteus*. However acetonic and methanolic extract of *P. ommutate* were inactive against yeast and mould strains. Moreover, Dulger et al., (2005) showed that while 8 different species of mosses were active against both Gram positive and Gram negative bacteria, these mosses displayed weak antiyeast activity. All these studies suggest that mosses have potential antimicrobial activity. The determination of the antimicrobial activities of mosses and their utilization in preparation of the new antimicrobials is crucial. As around 22.7% of world bryophytes are present in India (Banerjee, 1978).

Table 2: Evaluation of Zone of inhibition (ZOI) of hexane extract against micro organisms. Zone of inhibitions are after subtracting 3mm disc diameter from the total diameter ZOI

Micro organism tested	Hexane extract (MIC Value)			
	25% ,12.5mg/ml (50µl)	50%, 25mg/ml (50µl)	100%, 50mg/ml (500µl)	Camphicillin (500µl)
<i>Escherichia coli</i>	1 mm	2 mm	5 mm	15 mm

(NCIM 2501)				
<i>Pseudomonas aeruginosa</i> (NCIM 5029)	4 mm	-	2 mm	17 mm
<i>Staphylococcus griseus</i> (NCIM 2621)	7 mm	9 mm	16 mm	12 mm
<i>Bacillus murcesens</i> (NCIM5037)	-	-	4 mm	10 mm
<i>Staphylococcus marcesens</i> (NCIM 2079)	-	-	-	14 mm
<i>Lactobacillus casei</i> (NCIM 2651)	-	-	-	12 mm
<i>Saccharomyces cerevaceae</i> (ARIFCC 1248)	-	-	-	7 mm
<i>Rhizobium indigofera</i> (ARIFCC 2818)	-	-	-	11 mm
<i>Bacillus subtilis</i> (NCIM 2921)	2 mm	6 mm	11 mm	10 mm
<i>Salmonella typhimurium</i> (NCIM 2501)	3 mm	8 mm	12 mm	5 mm

In present investigation, it was found that bioactive compound on n-hexane extract from *Barbula Vinilis* has significant antimicrobial activity. It concludes the study indicate that *Barbula vinilis* may be used for protection against pathogenic microorganisms having economically cheaper than synthetic drug.

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