International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 29-08-2021, Accepted: 14-09-2021, Published: 29-09-2021
Volume 6, Issue 5, 2021, Page No. 909-912

Investigation on the biological activities of *Bryum argenteum* Hedw.-a moss plant (Bryophyte) from Kolli Hills, Eastern Ghats, Tamil Nadu, India

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Abstract
The present studies reported that the antimicrobial analysis on *Bryum argenteum* Hedw. a moss plant. The whole plant was screened for antimicrobial evaluation. The antimicrobial activities were tested using various extracts viz. ethyl acetate, chloroform, ethanol and aqueous extracts against nine bacterial and seven fungal strains. The antibacterial studies discovered that the most susceptible microorganisms were *Escherichia coli* and *Pseudomonas aeruginosa.* The ethanolic extracts showed good antifungal activities against the tested fungi. *Bryum argenteum* could be exploited in the infectious management of various bacterial and fungal diseases.

Keywords: *Bryum argenteum*, antimicrobial activity, ethanol extract

Introduction
Bryophytes play an important place in the plant kingdom, as they have an ecological value. In various ways the bryophytes are beneficial to human beings, other organisms and also for the environment in different ways. It plays an important key role in forming communities in environment (Pant and Tewari, 1981). In worldwide the attention for the antimicrobial activity of plants has gained huge preference in recent years, because of the frightening increase in the rate of infection by drug-resistant pathogens (Salvat *et al*., 2004) [16]. Many of the experiments have been investigated with variety of extracts from different plants, to assess the antimicrobial activity that lead to the discovery of novel compounds (Cowan 1999, Abu-Shanab *et al*., 2004) [5, 1].
In spite of the richness of moss flora in the country, there is a dearth of researchers in bryology and in the amount of work done on their taxonomy owing primarily, lack of knowledge of their economic value and reluctance on the part of scholars to expose themselves to exhaustive field study (Sahaya Sathish *et al.*, 2013) [15]. Keeping all these in mind the present study was carried out to evaluate the antimicrobial potencies of a moss *Bryum argenteum*.

Material and Methods
The plant material were collected from Kolli hills and the plant was identified and authenticated by Dr. S. Sahaya Sathish, Centre for Cryptogamic studies, St. Joseph's College,Tiruchirappalli-620 002.

Plant description
Plants glossy, silvery green, leaves closely imbricate, costa faint, ending far below apex and Plants small to robust, densely tufted, stem tomentose, mostly with subapical innovations, simple or branched rhizoids numerous, brown, tubers present in some; leaves large, bordered or non-bordered, ovate or ovate- lanceolate, acuminate, lower leaves smaller, distant, upper larger; cells sub-rectangular at base, narrow-rhomboidal above but not linear, smooth, seta long, erect, mostly red, arcuate at tip; capsule clavate, pyriform, with a distinct neck, sometimes broadly ovate, apophysis tapering, peristome usually double; spores rounded, 10-15 µm diagonally.

Methodology
Preparation of Plant Extracts
100g of sample was soaked in 150ml of various solvents such as ethyl acetate, chloroform, ethanol, aqueous and stored in brown bottles. It was stirred occasionally and maintained in room temperature for 3 days. After 3 days the extract was filtered through Whatman no 1 filter paper and stored for further use.

Collection of Micro Organisms
The bacterial and fungal pathogens were collected from the department of microbiology, K.A.P. Viswanatham Government Medical College, Tiruchirappalli – 620001. Collected micro organisms are as follows:

Bacterial pathogens: *Bacillus cereus*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Enterobacter aerogenes*, *Salmonella typhi*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*.

Fungal pathogens: *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus fumigates*, *Candida albicans*, *Geotricum candidum*, *Microsporum gypseum*.

Antimicrobial activities
Disc-diffusion test (Maruzzella and Henry, 1958)
The disc diffusion method provides a simple and reliable test in routine clinical microbiology in order to find out the effect of a particular substance on specific bacterium. This

method consists of impregnating small circular discs of standard filter paper with given amount of chosen concentration of substance. The discs are placed on the plates of culture medium previously spread with bacterial inoculums to be tested. After incubation the degree of sensitivity is determined by measuring the incubation zone produced by diffusion of the antibiotic substances from the discs of the surrounding medium.

Preparation of discs

Discs usually consisted of absorbent paper impregnated with the compound (plant extract). It is most convenient to use Whatman № 1 filter paper for preparing the discs. Dry discs of 6 mm diameter were prepared from Whatman № 1 filter paper and sterilized in an autoclave. These dry discs were used for the assay.

Circular discs of 6 mm diameter were prepared from Whatman № 1 filter paper and sterilized in an autoclave. These paper discs were impregnated with test compounds (plant extract) in the respective solvents for overnight and placed on nutrient agar plates seeded with the test bacterium. The plates were incubated at 37° C for 24 hr. After 24 hr the zone of inhibition around each disc was measured and the diameter was recorded. Gentamycin (10 mcg/disc) was used as the reference. A negative control was prepared using only the solvent used for extraction and kept for comparison. The tests were repeated 4 times to ensure reliability of the result.

Agar Well Diffusion Method (Perez *et al*., 1990)

Agar well diffusion method is also known as Hole Plate Diffusion Method (Brantner *et al*., 1993) or Cup Diffusion Method (Vikas Dhingra *et al*., 1999). Nutrient agar was used as the culture medium for this assay. The molten nutrient agar was dispensed in pre-sterilized petridishes (25 ml each) and allowed to cool. These agar plates were homogenously inoculated with the test bacterium previously suspended in tryptose broth (10^6 cells/ml). The plates were allowed to solidify. After solidification holes/ wells (cups) of 6 mm diameter were punched into the agar with the help of flamed cork borer. Five wells were prepared for each plate. Of these five, three holes were filled with 0.2 ml of the plant extract and the fourth hole was filled with 0.2 ml of standard antibiotic solution (Gentamycin, 500 µg/ml) and the fifth hole was filled with blank (extracting solvent alone). The petridishes were incubated at 37° C for 24 hr. After this incubation period the diameter of the inhibition zone formed around each hole (well/cup) was measured and the values were recorded. The antimicrobial activity was expressed as the ratio of the inhibition zone produced by the plant extract and the inhibition zone caused by the standard. Two sets of control were used. One control was the organism control where standard antibiotic solution was used and the other control was the blank where only the extracting solvent was used. This was just to ensure the validity of the test. Testing was carried out for each bacterium in quadruplicates.

Results and Discussion

Disc Diffusion Method

a. *Bryum argenteum* Hedw.

The antibacterial activity of various extracts of *Bryum argenteum* Hedw. against the test bacteria by disc diffusion method has shown in the Table. It is observed from the result that the chloroform extract was found to have inhibitory effect against *Escherichia coli* and *Pseudomonas aeruginosa* and the zone of inhibition was from 1.5 and 2.0 mm respectively. However ethyl acetate extract showed inhibitory effect against *Bacillus cereus* in addition to the above said two bacteria, with almost same diameter of inhibition zone.

Table 1: Antimicrobial activity of *Bryum argenteum* Hedw. Against various bacteria (Disc diffusion method)

Organism	Diameter of inhibition zone in mm (Mean*)				
	Ethyl acetate extract (30 µg/ disc)	Chloroform extract (30 µg/ disc)	Ethanolic extract (30 µg/ disc)	Aqueous extract (30 µg/ disc)	Standard# antibiotic
Gram-positive bacteria:					
Bacillus cereus	–	–	1.7 ± 0.4	–	4.4
Streptococcus faecalis	–	–	1.5 ± 0.2	–	5.2
Staphylococcus aureus	–	–	–	–	4.1
Gram-negative bacteria:					
Escherichia coli	–	1.7 ± 0.2	2.3±0.2	–	4.4
Proteus vulgaris	–	–	–	–	4.4
Enterobacter aerogenes	–	–	2.4 ± 0.4	–	4.1
Salmonella typhi	–	1.4 ± 0.3	–	–	2.1
Klebsiella pneumonia	–	–	–	–	4.8
Pseudomonas aeruginosa	–	2.2 ± 0.18	2.8 ± 0.3	–	4.1

*: Mean of triplicate

±: Standard Deviation

#: Gentamycin (Hi-media)

–: Absence of measurable inhibitory action

Agar Well Diffusion Method

The various solvent extracts of this moss did not show any significance result against most of the tested bacteria. However the ethyl acetate extract produced little effect on *Escherichia coli* and *Pseudomonas aeruginosa* and the diameter of inhibition zones were very less.

Table 2: Antimicrobial activity of *Bryum argenteum Hedw.* Against various bacteria (Agar Well diffusion method)

Organism	Diameter of inhibition zone in mm (Mean*)				
	Ethyl acetate extract (30 µg/ ml)	Chloroform extract (30 µg/ ml)	Ethanolic extract (30 µg/ ml)	Aqueous extract (30µg/ml)	Standard# antibiotic
Gram-positive bacteria:					
Bacillus cereus	–	–	2.4 ± 0.5	–	4.8
Streptococcus faecalis	–	–	–	–	4.2
Staphylococcus aureus	–	–	–	–	4.9
Gram-negative bacteria:					
Escherichia coli	–	–	2.2± 0.3	–	4.2
Proteus vulgaris	–	–	–	–	4.5
Enterobacter aerogenes	–	–	–	–	4.2
Salmonella typhi	–	1.4 ± 0.2	1.8 ± 0.4	–	4.6
Klebsiella pneumoniae	–	–	–	–	5.5
Pseudomonas aeruginosa	–	–	1.8 ± 0.1	–	5.0

*: Mean of triplicate
±: Standard Deviation
#: Gentamycin (Hi-media)
–: Absence of measurable inhibitory action

Antifungal activities

In antifungal activity *Bryum argenteum* Hedw, did not show any inhibitory effect on the fungal organisms tested. However the diameter of the inhibition zone is only lesser than the standard antibiotic.

Table 3: Antifungal activity of various extracts of *Bryum argenteum Hedw* against various fungi (Disc diffusion method)

Organism	Diameter of inhibition zone in mm (Mean*)				
	Ethyl acetate extract (30 µg/ disc)	Chloroform extract (30 µg/ disc)	Ethanolic extract (30 µg/ disc)	Aqueous extract (30 µg/ disc)	Standard# antibiotic (30 µg/ disc)
Aspergillus niger	–	–	1.5 ± 0.2	–	7.5
Aspergillus flavus	–	–	–	–	5.3
Aspergillus parasiticus	–	1.8 ± 0.3	–	–	5.2
Aspergillus fumigatus	–	–	–	–	6.7
Candida albicans	–	–	1.2 ± 0.4	–	7.8
Geotricum candidum	–	–	2.2 ± 0.3	–	7.9
Microsporum gypseum	–	–	–	–	6.8

*: Mean of triplicate
#: Nystatin
±: Standard Deviation
–: Absence of measurable inhibitory action

Agar Well Diffusion Method

Antifungal activity of various solvent extracts of test mosses against various fungi by agar well diffusion method has revealed that only the ethanolic extract of all the mosses showed some notable effect, on *Aspergillus niger*, *Aspergillus flavus* and *Geotricum candidum.*

Table 4: Antifungal activity of various extracts of *Bryum argenteum* Hedw. Against various fungi (Agar well diffusion method)

Organism	Diameter of inhibition zone in mm (Mean*)				
	Ethyl acetate extract 10 mg/ml	Chloroform extract 10 mg/ml	Ethanolic extract 10 mg/ml	Aqueous extract 10 mg/ml	Standard# antibiotic 10 mg/ml
Aspergillus niger	–	–	3.8 ± 1.7	–	8.2
Aspergillus flavus	–	–	2.1 ± 1.0	–	5.8
Aspergillus parasiticus	–	–	–	–	5.7
Aspergillus fumigatus	–	–	1.4 ± 0.3	–	5.0
Candida albicans	–	1.7 ± 0.2	–	–	4.8
Geotricum candidum	–	–	1.7 ± 0.5	–	7.6
Microsporium gypseum	–	–	–	–	6.5

*: Mean of triplicate
#: Nystatin
±: Standard Deviation
–: Absence of measurable inhibitory action

Similar antimicrobial studies have been done in same plant *Bryum argenteum* by Sabovljevic *et al*., (2006) [14], in which Minimal inhibitory concentration (MIC) was evaluated. They have analyzed the ethanolic extract of moss plant against four bacterial organisms such as, *Escherichia coli*, *Bacillus subtilis*, *Micrococcus luteus*, *Staphilococcus aureus* and four fungal organisms such as *Aspergillus niger*, *Penicillium ochrochloron*, *Candida albicans*, *Trichophyton mentagrophyes*. They have obtained significant activity (MIC) that coincides with our results.

Oyesiku and Caleb (2015) [12] tested three different mosses with 3 various solvents. They reveled that the ethanolic extract showed the high zone of inhibition when compared to acetone and methanolic extract. Savaroglu *et al*., (2011) [17] determined the antimicrobial activities of four different solvents. Among them ethyl acetate showed the promising activity which is in contrast to our results were ethyl acetate extract doesn't shows any activity.

Conclusion

The Bryophytes are abundant in the Indian sub-continent, there has been a neglect in research among these groups. There is a prime need to prepare a Bryophyte Flora of India. The antimicrobial studies of the selected moss species were done using various techniques such as disc diffusion method and agar well diffusion method, which clearly revealed that the disc diffusion method showed significant antimicrobial property. To conclude the *Bryum argenteum* have shown a high degree of inhibition especially in ethanolic extract. Hence these moss could be used for therapeutic purposes and the active compounds can be isolated from these mosses plants and used potentially for controlling the pathogenic microbes. Thus it is proved that this plant is potent enough to produce various therapeutically used phytocompounds.

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