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Evaluationn of Antibacterial Activity of *Macromitrium sulcatum* (Hook.) Brid. an *In vitro* Study

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The present study revealed with an antimicrobial screening of *Macromitrium sulcatum* (Hook.) Brid. collected from Bhimashankar, Pune district of Maharashtra. The plants were extracted in acetone, benzene, methanol and ethanol evaluated against two microorganisms *viz. Bacillus cereus* Frankland & Frankland and *Enterobacter aerogenes* Hormaeche & Edwards by Agar well diffusion method. Among the extracts tested both bacterial strains were found most sensitive.

Key words: Antibacterial activity, *Macromitrium sulcatum* (Hook.) Brid., *In vitro.*

Bryophytes, which are phylogenetically placed between vascular plants and algae, form a unique division in the plant kingdom[1]. There exist more than 22,000 members of the Mosses (Bryophyta) in the world. This figure represents 5.5% of the 400,000 plant types spread throughout the world[2].

An interesting character of bryophytes is that they as relatively free from parasitism. Herbarium specimens of these plants need no special treatment like those of the higher plants[3].

Antibiotic activity of bryophytes has drawn the attention of botanists and microbiologists in past few years. The search for plants with antimicrobial activity has gained increasing importance in recent years, due to a growing worldwide concern about the alarming increase in the rate of infection by antibiotic-resistant microorganisms[4].

Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds[5,6,7].

Macromitrium sulcatum (Hook.) Brid. an epiphytic moss member of family Orthotrichaceae. Plants grow in dens tufts; leaves spirally twisted when dry; capsule spherical to oblong-ovoid, sulcate and peristome absent. It is commonly found in various part of Maharashtra.

To the best of our knowledge, there have been no published reports concerning the antibacterial activity of *Macromitrium sulcatum* (Hook.) Brid. extracts. We have, therefore, focused on its biological activity in our study. This was achieved with the Agar well diffusion method.

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MATERIAL AND METHODS

Plant material

Macromitrium sulcatum (Hook.) Brid. was collected from Bhimashankar area of Pune district of Maharashtra at an altitude 1100 m. in October 2007. The specimens were identified in the Bryology Laboratory, Department of Botany, University College of Science, Mohanlal Sukhadia University, Udaipur. A voucher specimen was deposited at the Herbarium of the Department.

Preparation of Extract

The plants were washed thoroughly with running tap water, shed dried and grinded to powder using a table model grinder and stored in air tight bottles. Powdered materials of the fresh plants were extracted with 100 ml acetone; benzene; methanol; ethanol for 96 hours at room temperature. Filtering it with Whatman filter paper No.1 and the crude extract were obtained by evaporating the solvent in open air.

Test microorganisms

Two test microorganisms were obtained from the Microbial Type Culture Collection and Gene Bank (IMTECH, Chandigarh, India) were employed. They were the gram positive bacteria, *Bacillus cereus* Frankland & Frankland (MTCC-1305) and the gram negative bacteria, *Enterobacter aerogenes* Hormaeche & Edwards (MTCC-2822).

Determination of antibacterial activity

The plant extracts were tested for antibacterial through the Agar well diffusion method (8). Both bacterial strains were plated out on nutrient Agar plates and incubated for 24 hours at 37°C and colonies from this fresh culture were used for making suspension. Bacterial suspension of approximately 0.6 optical densities and 100 µl of it was uniformly spread on nutrient Agar medium when the temperature reached 35-40 °C in sterile glass Petri plates. After complete solidification, holes were made aseptically with a 6 mm sterile cork borer. 1000 µg/ml concentrated crude extracts dissolved in Di-methyl formamide and 100 µl of extract were poured in the wells. The plates were incubated at 37 °C for 24 hrs and antibacterial activity of the extracts was determined by measuring the diameter zone of inhibition in mm including 6 mm wells. All the tests were performed in triplicates and average results were recorded.

RESULTS AND DISCUSSION

The antibacterial activity tested against two bacterial strains such as *Bacillus cereus and Enterobacter aerogenes* by Agar well diffusion method (Plate-1). The bioactivity measured in terms of zone of inhibition exhibited by the different extracts against the respective bacterial strains are summarized mentioned in Table 1.

All the plant extracts (Acetone, Benzene, Methanolic and Ethanolic) showed activity against the tested bacteria. The methanolic and ethanolic extract of plant showed maximum zone of inhibition against *Bacillus cereus* and *Enterobacter aerogenes*. The minimum inhibition zone observed in Benzene extract of this plant against *Enterobacter aerogenes*.

Table 1. Antibacterial activity of different crude extracts of *Macromitrium sulcatum* (Hook.) Brid. by Agar well diffusion method.

Microorganism	Zone of Inhibition in (mm)*			Control		
	Acet. extract (1000µg/ml)	Benz. extract (1000µg/ml)	Meth. extract (1000µg/ml)	Etha. extract (1000µg/ml)	DMF (Negative) (1000µg/ml)	Str. (Positive) (1000µg/ml)
Bacillus cereus	10	11	14	12	NI	21.5
Enterobacter aerogenes	9	7	13	15	NI	20.0

*= Values include cup borer diameter (6.00) and are mean of three replicates; NI= No Inhibition zone; Acet. = Acetone; Benz. =Benzene; Meth. = Methanolic; Etha. = Ethanolic; Str. = Streptomycin; DMF = Di-methyl formamide.

CONCLUSION

To the best of our knowledge, the antibacterial activity of *Macromitrium sulcatum* (Hook.) Brid. extract has not been previously reported; thus, our results can be evaluated as the first report about the antibacterial properties with respect to the four different extracts. The results of this study suggest that *Macromitrium sulcatum* (Hook.) Brid. might possess a novel antibacterial molecule, which has an effect against gram-positive and gram-negative bacteria. Many reports of antibacterial activity of plants extracts against human pathogens and their pharmaceutical application are available[9,10], but not much has been reported on the antibacterial activity of plants extract against plant pathogens[11]. Further research is needed in order to obtain information about the chemical composition of moss species, as well as to reveal their mode of action on bacterial cells.

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