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ANTIFUNGAL ACTIVITY OF *PLAGIOCHASMA APPENDICULATUM* AND *PELLIA ENDIVIIIFOLIA*

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ABSTRACT: Bryophytes are the closest modern relatives of the predecessors to the earliest terrestrial plants and have shown to be brilliant chemists since they have long been a favorite treatment among tribal peoples all over the world, who use its pharmacological characteristics to treat a wide range of ailments. As bryophytes' Phytochemistry contains a diverse range of biologically active compounds such as lipids, proteins, steroids, organic acids, alcohols, aliphatic and aromatic compounds, polyphenols, terpenoids, acetogenins and phenylquinones, it's no surprise that substances derived from these ancestral plants are widely used as antitumor, antipyretic, insecticidal and antimicrobial agents. The present investigation has been an attempt to evaluate the antifungal potential of two ethnic botanically important liverworts, *Plagiochasma appendiculatum*, and *Pellia endiviifolia*. The methanolic extract was tested against two fungal species, *Candida albicans* MTCC 1637 and *Aspergillus niger* MTCC 1235. *In-vitro* antifungal activity was evaluated by disc diffusion method, which revealed inhibition of *Candida albicans* MTCC 1637 and *Aspergillus niger* MTCC 1235 growth. *Plagiochasma appendiculatum* proved to be more effective against both tested fungi; especially *Candida albicans*. These results confirm the antifungal activities in liverwort extracts.

INTRODUCTION: Fungal infections signify a constant and severe risk to human health and they are related to at least 1.5 million deaths globally every year. In immune-compromised patients, fungal infections are common due to aggressive treatments (*e.g.* chemotherapy, long-term corticosteroid treatment, organ transplant, AIDS). Fungal species belonging to genera Candida, Aspergillus, Cryptococcus, Pneumocystis, Mucor and Rhizopus are reported to cause 90% of these deaths [1, 2.]

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Fungi also produce superficial infections, which include the skin and mucosal surfaces and have more occurrence than the invasive infections fading the quality of life of affected individuals [3, 4, 5]. The increased use of antifungal agents has given rise to drug resistance development. The spread of multidrug-resistant strains of fungus and the reduced number of drugs available make it necessary to discover new classes of antifungals from natural sources [6].

Bryophytes are stated to synthesize various secondary metabolites to deal with several biotic and biotic stresses. Compounds like Bisbenzyl derivatives isolated from various bryophytes are reported to possess antifungal potential. The innovative biomolecules from exclusive natural sources like bryophytes can resolve the problem of drug resistance development in pathogenic fungi [7].

Almost all species of bryophytes are not spoiled by fungi, bacteria, and insect larvae [8], Because biological compounds like phenylquinone, aromatic and phenolic substances, oligosaccharides, polysaccharides, sugar alcohols, amino acids, fatty acids and aliphatic compounds in bryophytes protect these organisms, bryophytes have the potential for medical use [9, 10, 11].

In this study, we investigated the liverwort *Plagiochasma rupestre* and *Pellia endiviifolia,* both well-known species from all the bryo-geographical regions of the world. This work aimed to determine the antifungal effects of liverworts and contribute to the pharmaceutical industry to deal with the problem of drug resistance.

MATERIALS AND METHODS:

Plant Material: Liverworts samples, grown as carpet on walls in shade and humidity environments, were collected in September from D.S.B. Campus Nainital, hill region of western Himalayas lies between 29°22' 49.0944" North latitude and 79°27’48.8520 East longitude. The collected liverworts were transferred in air-tight zip-lock bags and brought to The Botanical Survey of India (BSI), Dehradun for identification. Dr. S.K. Singh, Director, Botanical Survey of India, Dehradun, identified the sampled as *P. appendiculatum* Lehm. & Lindenb (family Aytoniaceae) and *P. endiviifolia* (Diks.) Dumort (family Pelliaceae) kept them preserved with accession numbers 578 and 579 respectively in BSI herbarium.

Preparation of Extract: Extracts were prepared by soaking 100 gm. of powdered samples in Methanol (250 ml) for 96 h. The mixture was stirred every 24 h using a sterile glass rod. At the end of extraction, the extract was passed through Whatman filter paper no. 1 (Whatman, UK). The filtrates were concentrated on a rotary evaporator under a vacuum at 40ºC.

Source of Fungal Strain: Two fungal strains were tested for antifungal properties of methanolic extract of *Plagiochasma appendiculatum* and *Pellia endiviifolia* the *Candida albicans* MTCC 1637 and *Aspergillus niger* MTCC 1235. These fungal strains were obtained from the Department of Biotechnology, Graphic Era Hill University, Dehradun and they were maintained at 4°C on potato dextrose agar slants for further use.

Media Preparation and Sterilization: The potato dextrose agar media was used in antifungal activity investigations. 65 gm. of Potato dextrose agar was suspended in 1000 ml. of distilled water and boiled to dissolve the medium completely. The pH was adjusted to 5.4 at 25℃. 15-20 ml of Potato dextrose agar were transferred to test tubes and sealed with non-absorbent cotton. They were then autoclaved at a pressure of 15 psi (121^0C) for not less than 15 minutes.

Inoculum Preparation: The inoculum for the experiment was prepared fresh in potato dextrose broth from preserved frozen slant culture. It was incubated at 25℃ for 24-48 h.

Disc Diffusion Method: The organisms (*Candida albicans* MTCC 1637 and *Aspergillus niger* MTCC 1235) were inoculated in the plates by dipping a sterile swab in the inoculum, removing the excess inoculum by pressing and rotating the swab firmly against the sides of the culture tube above the level of the liquid and finally streaking a swab all over the surface of the medium three times, rotating the plates through the angle of 60℃ after each application. Finally, the swab was pressed around the edges of the agar surface. The inoculated medium was allowed to dry at room temperature with the lid closed. The sterile disc containing methanol extract of both plants, standard (Fluconazole 25µg/disc) and blank, were placed on the previously inoculated surface of the Potato dextrose agar plates and they were kept in the refrigerator for 1 h to facilitate uniform diffusion of the drug. Plates were prepared in triplicate, and the petri-dishes were incubated for 24-48 h at 25℃. The diameter of the zone of inhibition around the discs was measured and compared with that for Fluconazole [12, 13].

RESULTS AND DISCUSSION: In the present study, the antifungal activity of the methanol extract of *Plagiochasma appendiculatum* and *Pellia endiviifolia* was tested against two pathogenic fungal species. For comparison of antifungal activity, a synthetic fungicide Fluconazole was used. The methanolic extract showed a higher zone of inhibition in *Candida*

albicans fungal strain, which ranges between 12.45±0.4mm to 15.55±0.3mm, than *Aspergillus niger* fungal strain, which is between 9.45±0.3mm to 12.11±0.4mm. Here the zone of inhibition showed a direct relationship with concentration. The standard drug showed a higher degree of inhibition against the fungi *Candida albicans.*

TABLE 1: ANTIFUNGAL ACTIVITY OF *PLAGIOCHASMA APPENDICULATUM* ASSESSED BY DISC DIFFUSION METHOD

PAME concentration(µg/ml)	Zone of inhibition (mm)	
	Candida albicans	***Aspergillus niger***
100	12.45±0.4	9.45±0.3
200	13.6±0.6	10.59±0.4
400	13.89±0.10	11.25±0.4
800	14.9±0.4	11.90±0.3

Values are expressed as Mean±SD of three parallel measurements.

PAME: *Plagiochasma appendiculatum* methanolic extract.

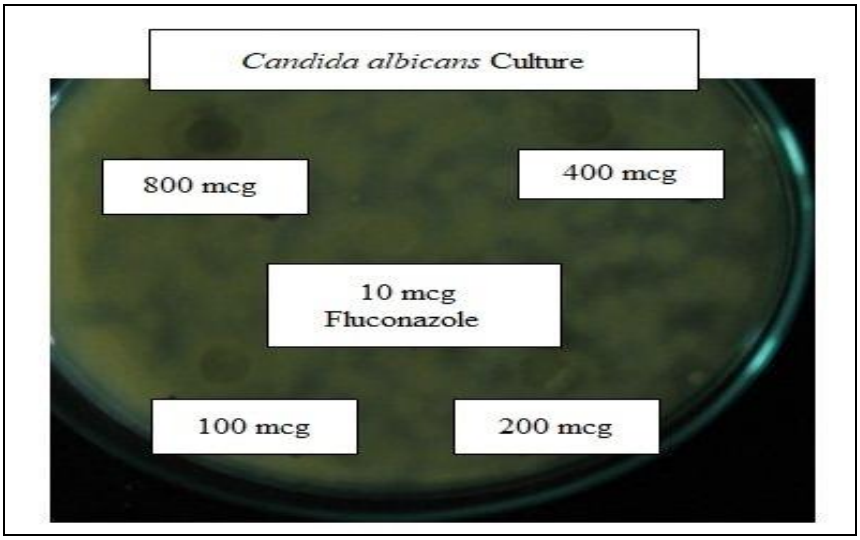

FIG. 1: ZONE OF INHIBITION BY PAME ON *CANDIDA ALBICANS* CULTURE

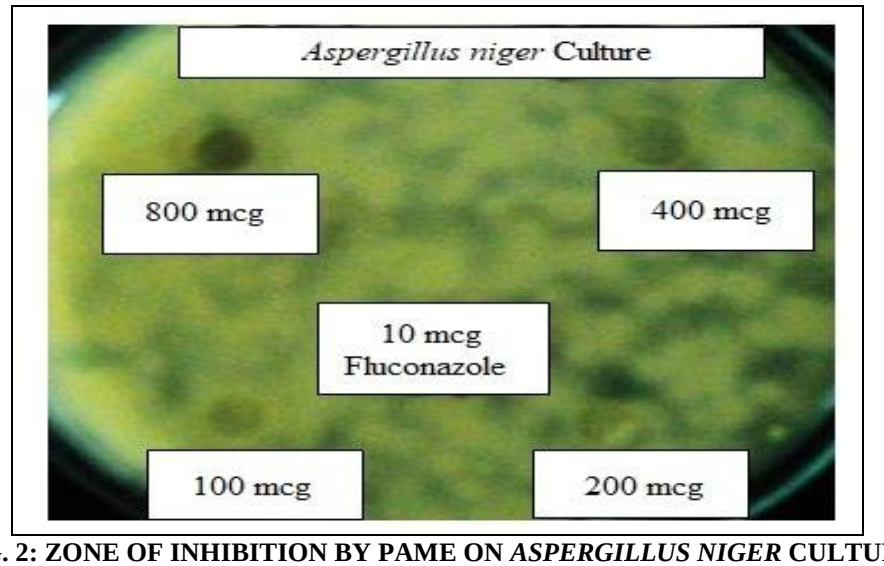

FIG. 2: ZONE OF INHIBITION BY PAME ON *ASPERGILLUS NIGER* CULTURE

The methanolic extract of *Pellia endiviifolia* showed zone of inhibition is directly proportional to extract concentration. The methanolic extract showed a higher zone of inhibition in *Candida albicans* fungal strain, which ranges between 10.10±0.1mm to 15.55±0.3mm, than *Aspergillus niger* fungal strain, which is between 8.4±0.3mm to 12.11±0.4mm.

TABLE 2: ANTIFUNGAL ACTIVITY OF *PELLIA ENDIVIIFOLIA* ASSESSED BY DISC DIFFUSION METHOD

PEME concentration(µg/ml)	Zone of inhibition (mm)	
	Candida albicans	*Aspergillus niger*
100	10.10±0.1	8.4±0.3
200	11.77±0.4	9.69±0.6
400	12.31±0.6	10.15±0.4
800	13.25±0.5	10.89±0.4
Standard drug (Fluconazole) 10 µg/ml	15.55±0.4	12.11±0.5

Values are expressed as mean±SD of three parallel measurements.

PEME: *Pellia endiviifolia* methanolic extract.

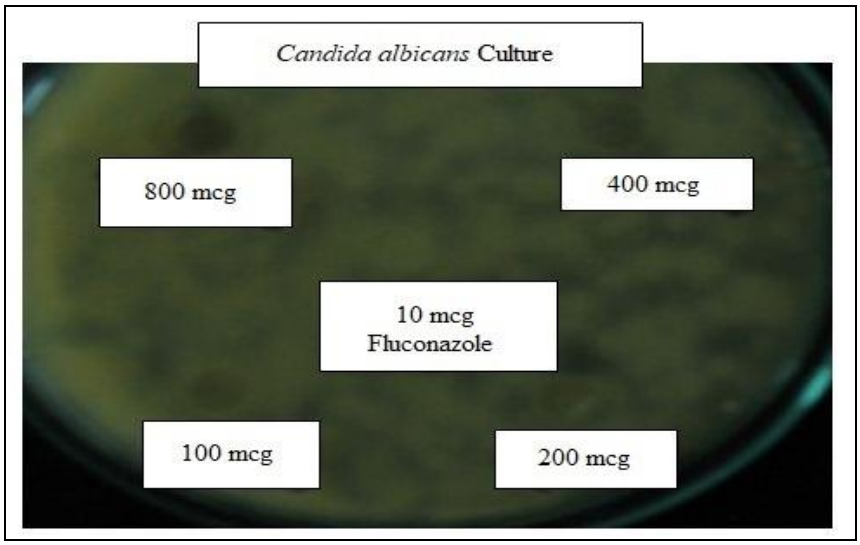

FIG. 3: ZONE OF INHIBITION BY PEME ON *CANDIDA ALBICANS* CULTURE

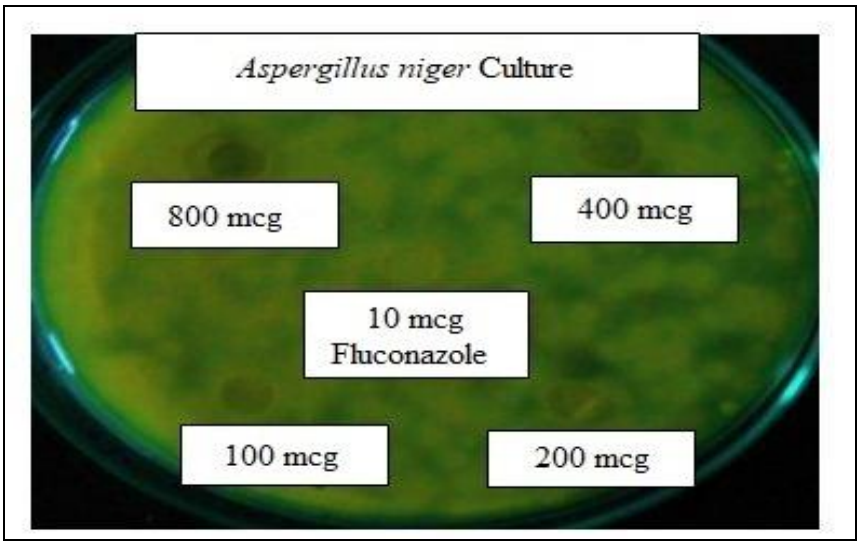

FIG. 4: ZONE OF INHIBITION BY PEME ON *ASPERGILLUS NIGER* CULTURE

Antifungal activity of methanolic extract of both liverworts may be due to the presence of a rich variety of appropriate secondary metabolites that can interfere with the growth of fungi.

On the basis of the present study, it can be concluded that the extracts of *Plagiochasma appendiculatum* and *Pellia endiviifolia* show a considerable effect as an antifungal agent. Moreover, fungi like *Candida albicans* and *Aspergillus niger*are sensitive to this extract. The results obtained are similar to the results reported by Sharma *et al.*, 2014 [14]. This study will help in the establishment of novel antifungal molecules from widely available liverwort species.

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CONFLICTS OF INTEREST: There is no conflict of interest.

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