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ANTIFUNGAL ACTIVITY OF *HYBANTHUS ENNEASPERMUS* ON WET CLOTHES

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ABSTRACT
During rainy season, when clothes are not properly dried they develop spots. In clothes the spots appear as black or greenish black in color and these spots or mildews were cultured and microscopically examined. It was identified as fungi, viz. *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus*. Antifungal activities of different extracts of *Hybanthus enneaspermus* were screened. The antifungal activity was graded, based on the zone of inhibition. Among the three extracts used for the present studies, methanolic extract exhibited the maximum growth inhibition, followed by chloroform and petroleum ether extract.
KEYWORDS: antifungal, mildews, *Hybanthus enneaspermus*, methanolic extract,

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INTRODUCTION

Hybanthus enneaspermus F. Muell (Syn. *Ionidium enneaspermum* DC and I. suffruticosum Ging; Family: Violaceae) is native of high altitude Himalaya region and warmer parts of India from Delhi to Bengal and throughout Deccan Peninsula. It is reported to be used as a tonic, diuretic[1], anti-gonorrhoetic and demulcent[2]. Root is used in the treatment of urinary infections and for bowel complaints of children[3].

Many plants are gaining importance due to fungitoxicity. Until recently very little antifungal[4] research has been conducted. An upsurge of fungal infections has resulted in demand for new antifungal compounds. In the present investigation, efforts are made to find out the antifungal activity of different extracts of H.enneaspermus on the wet clothes with black and greenish black spots.

Fungi are ubiquitous organisms found on earth, in water, in moist soil, in compost and in decomposing organic matter. Fungi prefer to grow in darkness and they inhabit usually in moist places, so they easily attack on wet clothes[5]. During rainy season, when clothes are not properly dried and when it is dumped, it develops spots. In clothes, spots appear as black or greenish black in color, called mildew. It is caused by fungi.

In the present study, antifungal activities of different extracts of *Hybanthus enneaspermus* were screened. The antifungal activity was graded, based on the zone of inhibition. Among the three extracts used for the present studies, methanolic extract exhibited the maximum growth inhibition, followed by chloroform and petroleum ether extract.

MATERIALS AND METHODS

The fungal attacked clothes were cut into small pieces and then placed in the petridish containing Potato Dextrose Agar (PDA) medium (Hi-Media Pvt. Ltd., Mumbai, India). The cultures were maintained in the PDA slants for further studies. The petriplates were placed in the inoculation chamber at 37°C for 7-14days to identify the fungi. Smear from culture was mounted on a slide in 10% glycerine and stained with methylene blue (1%), for 1 minute and it was observed under microscope[6]. The cultures were maintained in slants and agar plates for further studies. The sensitivity disc was placed in the pure culture to know the sensitivity of the plant extracts. The fungi identified were Aspergillus niger, Aspergillus flavus and Aspergillus fumigatus.

Disc Diffusion Method

Sterile disc of 5mm diameter were cut from a standard Whatmann No.1 filter paper. Petroleum ether, chloroform and methanol extracts of the plant were taken for antimicrobial studies[7]. The plates were heavily inoculated with standard inoculums of fungi to the respective plants by means of sterile cotton swab. 500mg/mL concentrations of various extracts were prepared. Each plate is divided into 5 sectors by drawing lines on the bottom of each plate. Sterile disc of 6mm

diameter soaked in 3 different concentrations viz. 25mg/mL, 40mg/mL and 60mg/mL concentrations of the petroleum ether extract using micropipette were taken and allowed it to dry and placed at specific locations on the surface flooded plates of fungi[8].

The same technique was repeated in the case of the remaining test organisms for chloroform extract and methanol extract. One disc with fungicide (Bavistin 20 mg/disc) was used. The plates with disc were incubated at 27°C for 7 days for fungal culture. After that the colonies were counted using the colony counter and the diameter of zone of inhibition produced by the microorganisms were measured in mm and compared accordingly.

RESULTS AND DISCUSSION

Profuse growth of Aspergillus niger, Aspergillus flavus and Aspergillus fumigatus were seen in control as it contained extract free medium. It was confirmed from the experimental data that varied rates of inhibition were shown by extracts of 25mg/mL, 40mg/mL 60mg/mL concentrations. Aspergillus niger recorded a growth inhibition zone of 16mm in methanol extract (60mg/mL) and 14mm in both petroleum ether and chloroform extract (60mg/mL). Aspergillus flavus recorded a growth inhibition zone of 16 mm in both chloroform and methanol extract (60mg/mL) and 15mm in petroleum ether extract (60mg/mL). Aspergillus fumigatus recorded a growth inhibition zone of 15mm in both petroleum ether and methanol extract (60mg/mL) and 14mm in chloroform extract (60mg/mL).

In the present investigation, the methanol extract showed maximum inhibitory effect on the three fungal strains, viz. Aspergillus niger, Aspergillus flavus and Aspergillus fumigatus than petroleum ether extract and chloroform extract. The antifungal activity of these extracts could be due to the presence of naturally occurring antimicrobial compounds like phenols, catechols, marmalosin, furocoumarin, alkaloids, tannins and secondary metabolites[9].

The results indicated that as the concentration of the plant extract increased, the zone of inhibition produced also found to be increased. All the extracts showed high to moderate zone of inhibition against three fungal strains tested. Among them, methanolic extract exhibited the maximum growth inhibition followed by chloroform extract and petroleum ether extract.

The results obtained in this study justify the efficacy of the extracts of petroleum ether, chloroform and methanolic on the fungi-infected clothes. Therefore this research work offers a scientific basis for the traditional use of plant extracts in removing the stains or spots (mildew) from clothes.

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Table 1: Antifungal activity of the plant extracts of *Hybanthus enneaspermus* Linn.

S.No	Extracts	Concentration (mg/ml)	*Aspergillus niger*	*Aspergillus flavus*	*Aspergillus fumigatus*
			Diameter of zone of inhibition (mm)		
1	Drug (Bavistin)	20	18	19	17
2	Petroleum ether	20	8	10	13
		40	10	13	14
		60	14	15	**15**
3	Chloroform	20	10	10	11
		40	12	14	13
		60	14	**16**	14
4	Methanol	20	10	12	12
		40	12	14	14
		60	**16**	**16**	**15**

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