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ANTIBACTERIAL ACTIVITY OF MOSS *ENTODON MYURUS* (HOOK) HAMP. AGAINST SOME PATHOGENIC BACTERIA

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ABSTRACT
The crude extract with solvents such as ethanol, petroleum ether, acetone and benzene of *Entodon myurus* were tested for antibacterial activity against some pathogenic bacteria by agar well diffusion method. All the plant extracts showed activity against the tested microorganisms. Maximum antibacterial activity showed by acetone (29 mm) and methanol (29 mm) extract against *Enterobacter aerogenes* while minimum antibacterial activity showed by acetone extract(20 mm) against *klebsiella pneumoniae.*

INTRODUCTION

Bryophytes belong to the group of the oldest known land plants, which includes liverworts, hornworts and mosses. More than 22,000 species of the mosses (Bryophyte) exist in the world (Frahm, 2004). One of the features that helped bryophytes to survive and maintain their place in today's flora is their content of biologically active compounds (Glime and Saxena, 1991). Although Bryophytes are very familiar, their medicinal importance is yet to be exploited completely. They are used in pharmaceutical products, horticulture, household purposes and are also ecologically important as they are good indicators of environmental conditions (Kumar *et al.,* 1999).

It is common knowledge that bryophytes are not infected by bacteria and fungi, even though most live in close connection to the forest floor or organic decomposing substrates. All living plants must protect themselves against microbial infection through cuticle or bark. Bryophytes lack such shields, but use chemical weapons that are part of their alternative poikilohydric life strategy. If a fungal spore falls on a bryophyte thallus or leaf, phenolic compounds are released when the surface is wet, thus inhibiting spore germination. This is at least one important factor for the evolutionary success of bryophytes and the fact that they survived for more than 350 million years (Frahm, 2004).

Since the sixties of the last centuary, these effects were tested in laboratories. An evaluation of literature (Asakawa, 1981,1984,1988 and 1990; Asakawa *et al.,* 1976,1980; Banerjee and Sen, 1979; Basile *et al.,* 1999; Belcik and Weigner, 1980; Castaldo-Cobianchi *et al.,* 1988; Dikshit *et al.,* 1982; Gupta and Singh, 1971; Hayes, 1947; Hoof *et al.,* 1981; Kamory *et al.,* 1995; Lorimer and Perry, 1993,1994; Madson and pates, 1952; Matsuo *et al.,* 1982; McCleary *et al.,* 1960, 1966; Pavletic and Stilinovic, 1963; Wolters, 1964) indicates that alcoholic and aquaceous extracts or isolated compounds of about 150 species of hepatics and mosses were used for agar plate tests. They showed antimicrobial effects against various groups of fungi and yeasts, as well as against gram positive and negative bacteria. Compounds like polygodial from *Porella*, Norpiguisone from *Conocephalum conicum* and lunularin from *Lunularia cruciata*, 4 – hydro -3-methoxy bibenzyl and a – and b – pinine-alloromadendrine from *Plagiochila stevensoniana* are useful as antimicrobial compounds (Kamory et al., 1995, Lorimer and Perry, 1993). Besides the said biological implications, the potential microbicidal properties of bryophytes may be harnessed for therapeutic purposes against the respective susceptible pathogenic organism, provided the active principle(s) is effectively isolated and purified, its chemical nature is worked out and the yields is adequate (Banerjee and Sen,1979). For investigation of the antibacterial potential of *Entodon myurus* plants were collected from different localities of Maharastra (India). *Entodon myurus* an epiphytic moss belonging to family Entodontaceae (Bryophyte),is fairly robust, golden green, glossy, plants grow in dense tufts, leaves terete often complanate; capsule erect, longly ovate cylindrical and peristome present.

MATERIALS AND METHODS

Plant material

Entodon myurus was collected from different localities of

Maharastra such as Purandhar fort, Bhimashankar (Pune District), Mahableshwar, Panchgani (Satara District). The Specimens were identified in the Bryology laboratory Department of Botany, University College of Science, Mohanlal Sukhadia University, Udaipur 313001 India. The specimen was deposited at the Herbarium, Department of Botany University college of Science Udaipur.

Extract Preparation

The plants were thoroughly washed with sterile distilled water. Plants were shade dried at room temperature and pulveriged into fine powder using an electric blender. Powdered plants were extracted with 100 mL acetone, benzene, petroleum ether, methanol and ethanol for 96 hr at room temperature. Filtering it with whatman filter paper No.1 and the crude extract was obtained by evaporating the solvent in open air.

Test Microorganisms

Test microorganisms were procured from the Microbial Type Culture Collection (MTCC) and Gene Bank IMTECH, Chandigarh, India.

Antibacterial assay of crude extract

The plant extracts were tested for antibacterial assay through the Agar well diffusion method (Perez *et al*.,1990). All bacterial strains were plated out on nutrient agar plates and incubated for 24 hr at 37ºC and colonies from this fresh culture were used for making suspension. 100 μL of bacterial suspension was uniformly spread on nutrient agar medium in sterile petri plates. After solidification of nutrient agar, wells were made with a 6 mm sterile cork borer. Different concentrations of extracts were made with DMSO (Dimethyle sulfoxide) and 100 mL of it were poured in the wells. The plates were incubated at 37ºC for 24 hr and antibacterial activity of plant extract was observed by measuring the diameter zone of Inhibition and average result was recorded.

RESULTS AND DISCUSSION

The different crude extract viz. petroleum ether, benzene, acetone, ethanol and methanol of *Entodon myurus* were tested against various gram +ve and gram –ve bacteria. The results illustrated in Table1 (Fig. 1) revealed that except aqueous extract, all crude extracts showed satisfactory inhibitory activity against all type of bacterial strains. Maximum antibacterial activity showed by acetone and methanol extract against *Enterobacter aerogenes* while acetone extract showed minimum activity against *Klebsiella pneumonie*.

It is interesting that moss and liverworts species are important source of tetraterpenoids carotenoids.These activities may be due to the presence of carotenoids like α- and β-carotene, lutein, neo-, viola-, crypto-andexanthin (Sabovljevic and Sabovljevic, 2008). Many antibiotics have been isolated from bryophytes, but few have been developed for medical use, despite their demonstrated effectiveness. In Germany, *Ceratodon purpureus* and *Bryum argenteum* are used to cure fungal and bacterial infections of horses. Several medical uses to promising, such as antileukemic properties and anticancer agents. The most promising uses of bryophytes in medicine seem to lie in genetic engineering. Bryophytes are being used already to produce human blood-clotting proteins, while others are known to reduce thrombin activity (Frahm, 2004).

Use of synthetic chemicals against microorganisms contributed to management of such losses but these chemicals attributed the emergence of resistant microorganisms (Shah *et al.,* 1992; Akimpelu, 2001) and led to many ecological and medical problems due to residual toxicity, carcinogenicity and

Table 1: Results of antibacterial activity of *Entodon myurus* by agar well diffusion method against some pathogenic bacteria

Microorganisms	Extracts	Different Concentration of the Plant extracts (μg/mL)				
		1000	750	500	250	175
Bacillus cereus	Petroleum ether	25	24	22	20	17
	Benzene	25	22	21	19	17
	Acetone	27	26	23	20	17
	Methanol	24	21	20	18	17
	Ethanol	22	19	18	16	15
Staphylococcus aureus	Petroleum ether	26	25	24	21	18
	Benzene	21	20	16	15	14
	Acetone	28	25	22	21	18
	Methanol	23	21	18	16	13
	Ethanol	24	23	21	20	15
Escherichia coli	Petroleum ether	28	25	24	22	18
	Benzene	27	26	24	21	19
	Acetone	28	25	22	20	18
	Methanol	22	20	19	17	16
	Ethanol	29	28	25	21	19
Enterobacter aerogenes	Petroleum ether	23	22	20	19	16
	Benzene	28	25	23	21	18
	Acetone	29	26	24	21	20
	Methanol	29	26	24	21	19
	Ethanol	25	22	19	17	16
Klebsiella pneumoniae	Petroleum ether	26	25	22	21	19
	Benzene	25	23	22	20	18
	Acetone	20	19	18	16	14
	Methanol	25	22	21	19	18
	Ethanol	27	24	22	21	20

Values of zone of Inhibition included cup borer diameter (6.00) in mm and are mean of three replicates

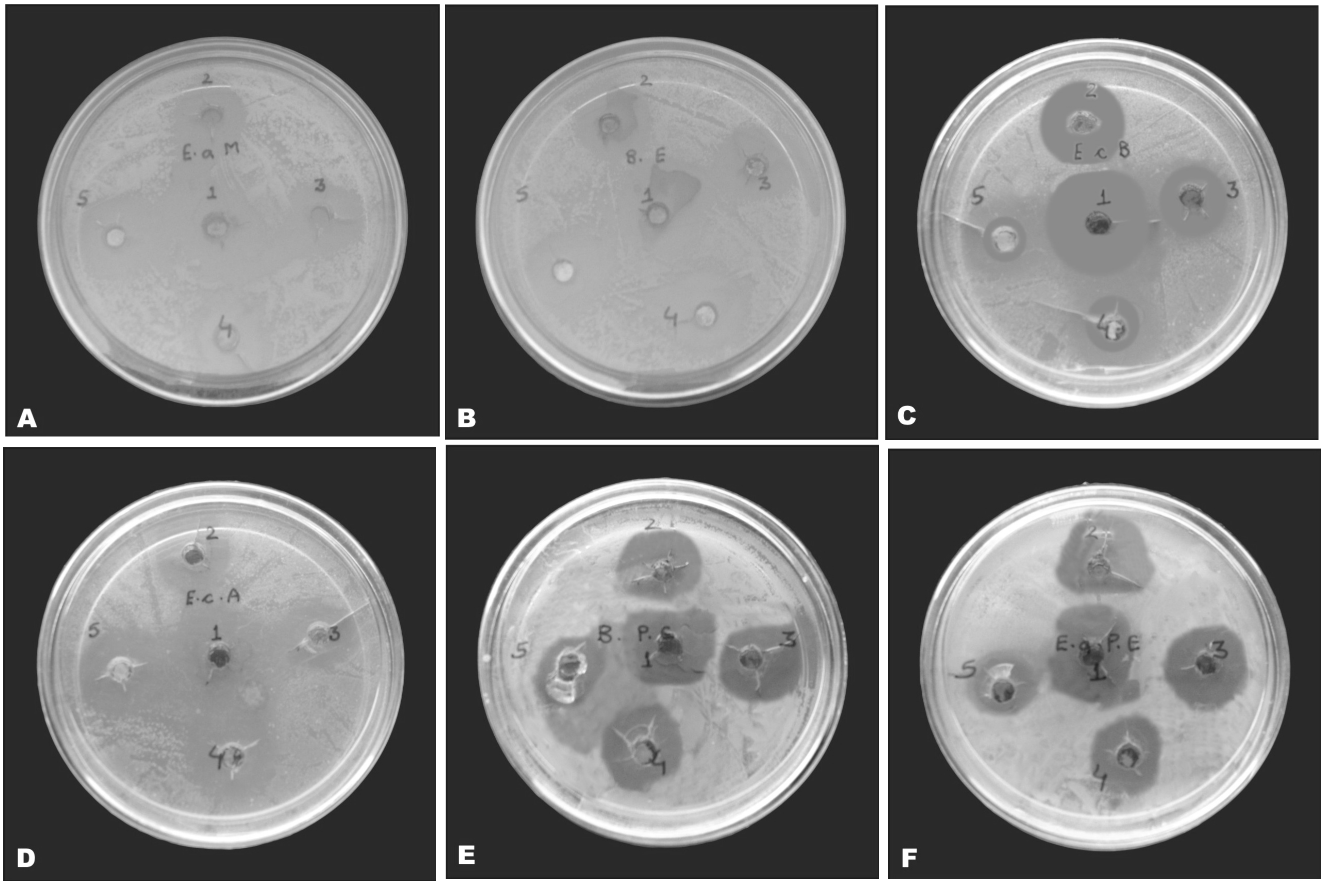

Figure 1 A to F: Antibacterial activity of crude extract of *Entodon myurus* (Hook) Hamp. (A) Methanol extract against *E. aerogenes* (B) Ethanol extract against *Bacillus cereus* (C) Benzene extract against *E. coli* (D) Acetone extract against *E. coli* (E) Petroleum ether extract against *B. cereus* (F) Petroleum ether extract against *E. aerogenes*

hormonal imbalance (Omura *et al.,* 1997; Pandey, 2003). To reduce this problem there is a need of alternative biological method including use of plants. Further studies involving the isolation, characterization and purification of the chemical compounds of the plant and screening for antibacterial may result in the development of a potent entity which will be of lower toxicity and a high therapeutic value to mankind.

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