Antibacterial activity of the alcoholic extracts of *Entodon nepalensis* Mizush. against some pathogenic bacteria

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Abstract: The antibacterial activity of the ethanolic and methanolic extracts from the moss- *Entodon nepalensis* Mizush. was evaluated against three bacterial species. The antibacterial activity was assessed by well diffusion method. The ethanolic extract of plant showed a stronger effect than the methanolic extract. The maximum antagonistic effect showed against tested *Escherichia coli* followed by *Salmonella typhimurium* and least against *Bacillus subtilis*.

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Keywords: *Entodon nepalensis,* ethanol extract, antibacterial activity, well diffusion method.

1. Introduction

Since time immortal plants have been a valued source of natural products for maintaining human health, particularly in the last decade, with more intensive studies for natural therapies like Ayurveda etc. They are used for treatment of various skin problems and wounds (Flowers, 1957). Various kinds of biological activities are reported so far from bryophytes (Asakawa, 2001; 2004). The use of plant compounds for pharmaceutical purposes has increasingly amplified in the world, especially in developing countries, where there is dependence on traditional medicine for a variety of diseases.

According to World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use conventional medicine, which has compounds derived from medicinal plants. Consequently, certain little known plants should be investigated to better understand their properties, safety and efficiency (Basile et al., 1998a). Secondary metabolites which are derived from plants have long been use as drugs and there is an increasing demand for these natural products (Frahm and Kirchhof, 2002).

Bryophytes belong to the group of the oldest known land plants, which includes liverworts, hornworts and mosses. More than 22,000 members of the mosses (Bryophyte) are present in the world. One of the characteristics that made bryophytes to endure and keep their place in today's flora is the presence of biologically active compounds in them.

Although Bryophytes are very common, their medicinal importance is not exploited wholly. They are used in pharmaceutical products, horticulture, household purposes and are also ecologically important as good indicators of environmental conditions (Kumar et al., 1993; Glime and Saxena, 1991). Conventional medical use of bryophytes in China initiated more than 400 years ago. For example, *Polytrichum* and *Fissidens* species were used as diuretic and hair growth stimulating drugs in China (Asakawa, 1990). Moreover, North American Indians used *Polytrichum juniperinum*, *Bryum, Mnium* and *Philonotis* mosses to heal burns, bruises and wounds (Ilhan et al., 2001). Many other bryophytes also exhibit antimicrobial effects against fungi and bacteria (Basile et al., 1998b; 1999; Banerjee, 2001; Scher et al., 2004; Subhisha and Subramoniam, 2005; Sabovljevic et al., 2006; Vats et al., 2012; Dulgar et al, 2009; Alam et al., 2011; 2012; Alam, 2012; Vats, 2012; Mathur et al, 2007). Liverwort like *Marchantia tosana* exhibited antifungal, antibacterial and antitumor activity (Lahlou et al., 2000). It has also been shown that *Ptilidium pulcherrimum* have antibacterial and antifungal activity (Veljic et al., 2010).

Almost all species of bryophytes are not damaged by fungi, bacteria, insect larvae (Asakawa, 1984) because, biological compounds like phenylquinone, aromatic and phenolic substances, oligosaccharides, polysaccharides, sugar alcohols, amino acids, fatty acids, aliphatic compounds in bryophytes provide protection against these organisms, therefore, bryophytes have the potential for medical use (Asakawa, 1981; Asakawa et al., 2000).

At present, several environmental problems are caused by the rigorous use of synthetic antibiotics. Natural plant-derived products as antibacterial agents have less shock on the environment. Usually, the secondary metabolites produced by bryophytes are known for antibacterial properties. However, various studies have confirmed that extracts and bryophytes

play various biological roles and appear to function as allelopathic agents in nature.

In this study, we investigated the liverwort *Entodon nepalensis* which is a terricolous as well as corticolous taxa, it grows on tree trunks, exposed rocks and soil surfaces. This species is well known from all the bryo-geographical regions of the Indian continent. In Rajasthan, this moss- *Entodon nepalensis* is distributed in Mount Abu and Ranthambore Tiger Reserve (Alam et al., 2011). The aim of this work was to determine the antibacterial effects of this moss against some selected pathogenic bacteria and to make a contribution to the pharmaceutical botany studies to be done in the future in India.

2. Material and Methods

2.1 Plant material:

Plant materials of this study were collected from the Mount Abu, western Rajasthan, at an altitude of 1600m, 24°31' to 24°43'N and 72°38' to 72°53' E, in October, 2011. Specimens: 7860015-BVH 7860019/2011; Legit. A. Alam and S. C. Sharma; Det.: A. Alam) are deposited in the Bryophyte Herbarium of Banasthali Vidyapith (BVH), Rajasthan (India).

2.2 Test Microorganism:

The pathogenic bacteria were obtained from the Type culture collection (MTCC), Institute of Microbial Technology, Chandigarh those were *Bacillus subtilis* (MTCC 619) is a Gram-positive, bacterium, *Escherichia coli* (ATCC 118) is a Gram negative, rod-shaped bacterium, *Salmonella typhimurium* (MTCC 98), rod shaped, Gram negative, non-spore-forming, mostly motile enterobacteria, through the Department of Bioscience and Biotechnology, Banasthali University.

2.3 Solvent Extract preparation

The plant materials (200g) of each were dried, powdered and exhaustively extracted with 95% ethanol separately. These extracts were concentrated and successively fractionated in different solvents and dried in vacuum for 24 hr. The fractions obtained were filtered centrifuged at 3,000 rpm for 10 min and were used for studying antimicrobial activity. The filtrate was evaporated until dry with a rotary evaporator.

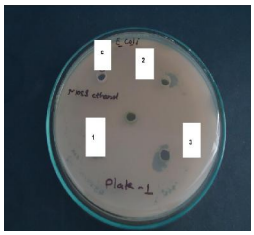
2.4 Test for antibacterial assay

The following bacterial species were used: *Bacillus subtilis* (MTCC 619), *Escherichia coli* (MTCC 118*), Salmonella typhimurium* (MTCC 98). The bacterial species were cultured overnight at 37ºC in LB medium. Inoculum suspensions containing ~10 cells/ml were used for the experiments. Antimicrobial activity of the moss fractions were determined by agar diffusion method(Gould and Bowie, 1952; Anna King and Brown, 2001). Petri plates containing sterilized Nutrient agar (3% agar) were used as base layer. After solidification of medium each plate was seeded with 0.1 ml of broth culture strains (mixed gently) in respective agar medium (1.5% agar) and poured on the base layer and allowed to solidify. The wells of 6 mm diameter were cut with sterile cork borer. Each well was filled with 0.1 ml solvent extract (ethanol and methanol) of each moss. Different solvents alone were used as control, for each test organism. For comparison, the standard drugs 0.1 ml of tetracycline (10 µg/ml) was used. The Petri dishes used for antibacterial activity were incubated at 37 ±0.5 °C for 24 hrs. The antibacterial activity was measured in terms of the zone of inhibition (ZOI) in mm (Plate 1; Table1).

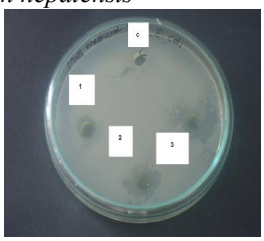
3. Results

Antibacterial activity of *Entodon nepalensis* extracts in distilled water, methanol and ethanol on three bacterial strains are shown in the Table 1. The ethanolic extract is more effective in all the cases. However, *Escherichia coli* is more affected by the three extracts than the other two bacteria-*Bacillus subtilis* and *Salmonella typhimurium* (Figure 1).

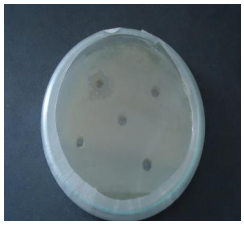
Plate 1: Antibacterial activity of Alcoholic extracts of moss *Entodon nepalensis*



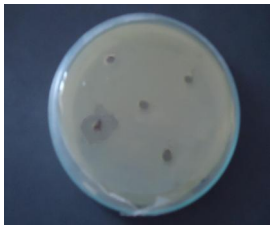
Antibacterial activity *Entodon nepalensis* ethanolic extract against *E. coli*



Antibacterial activity of *Entodon nepalensis* methanolic extract against *E. coli*

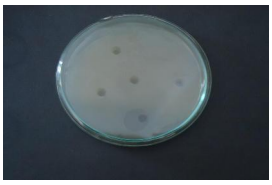


Antibacterial activity ethanolic *Entodon nepalensis* extract against *Salmonella typhimurium*



Antibacterial activity methanolic *Entodon nepalensis* extract against *Salmonella typhimurium*

Antibacterial activity ethanolic *Entodon nepalensis* extract against *Bacillus subtilis*



Antibacterial activity methanolic *Entodon nepalensis* extract against *Bacillus subtilis*

Table 1: Antibacterial activity of *Entodon nepalensis* extracts (Inhibition Zone in mm) against selected bacterial strains

	E. coli (MTCC 118)		***S. typhimurium*** (MTCC 98)		***B. subtilis*** (MTCC 619)	
	ZOI (mm)	MIC (g/ml)	ZOI (mm)	MIC (g/ml)	ZOI (mm)	MIC (g/ml)
Aqueous	7	0.04	6	0.05	0	0.06
Methanol	10	0.04	8	0.05	4	0.06
Ethanol	14	0.04	12	0.05	4	0.06

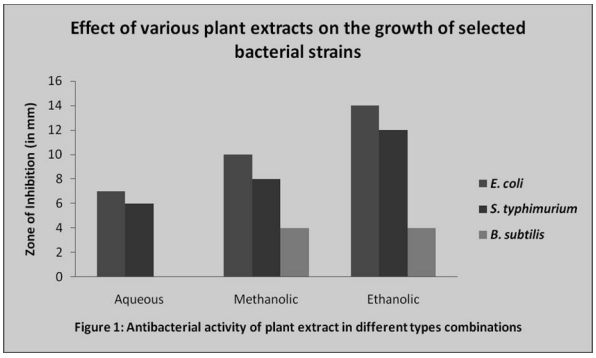


Figure 1: Antibacterial activity of plant extract in different types combinations

3. Results and Discussion

In the present study, the antibacterial activity of the aqueous, methanol and ethanolic extracts of *Entodon nepalensis* were tested against three bacterial species. For comparison of antibacterial activity a tetracycline was used. According to obtained results it is evident that the extract of *Entodon nepalensis* at a concentration of 0.04-0.06g/ml has significant antibacterial activity. In case of *E. coli* and *Salmonella typhimurium* this concentration showed better inhibition than *Bacillus subtilis*. Moreover, the ethanolic extracts are more efficient in terms of bactericidal capacity than the methanolic and aqueous extracts of moss.

4. Conclusion

On the basis of the present study it can be concluded that the extracts of *Entodon nepalensis* show considerable effect as antibacterial agent. The alcoholic extract is more capable in this activity than the aqueous extract as antibacterial agent against some well known as resistant species which are also sensitive to this ethanolic extract. The results obtained are similar to some earlier reports that extracts from mosses and liverworts exhibit antibacterial and antifungal activities (Castaldo et al., 1998). This study helps in the establishment of bryophytes as bio-control agents against infectious diseases caused by these bacterial species which can be eradicated in an eco-friendly way.

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