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Phytochemical screening and *in vitro* evaluation of crude extracts of moss *Funaria hygrometrica* (Funariaceae) for potential antibacterial activity

Shabna Khanam* and BL Chaudhary
Bryology Laboratory, Department of Botany, University College of Science, Mohanlal Sukhadia University, Udaipur-313001, India

Abstract
Traditionally bryophytes are known to possess some bioactive components and, therefore, used throughout the world as drugs and remedies to cure various diseases. In the present study, bioassay for antibacterial activities was carried out using whole plant of *Funaria hygrometrica* Hedw. Different solvent fractions and aqueous extracts of this moss were obtained and dried in vacuum. Antibacterial effect of these fractions was determined by disc diffusion technique on different human pathogenic gram positive bacteria i.e. *Bacillus subtilis* and *Staphylococcus aureus*, gram negative bacteria i.e. *Escherichia coli* and *Pseudomonas aeruginosa*. The result was then compared with the standard antibiotic streptomycin. Both the aqueous and organic crude extracts showed considerable activity against all the bacteria but maximum antibacterial activity was observed in ethanol extract against *Staphylococcus aureus*. The phytochemical analysis of the extract indicated the presence of steroid, flavonoids, alkaloids starch and oil.
KEYWORDS. .

Keywords: *Funaria hygrometrica*, moss, crude extracts, agar well diffusion, microorganism, antibacterial activity, phytochemical constituents

Bryophytes are the second largest group of land plants after the flowering plants with about 20,000 to 28,000 species. Three main groups of bryophytes are hornworts (Anthocerotopsida), liverworts (Marchantiopsida) and mosses (Bryopsida).They live in all zonobioms from the desert to the polar, but not in the seas (Sabovljevic 2006). Bryophytes are closely linked with civilization, culture, beliefs, and ethics of humankind (Pant 1990). Bryophytes are used by different cultural groups for cuts, wounds and skin diseases suggesting that they protect the skin and open wounds from microbial pathogen (Subramoniam 2005). Extracts of many bryophytes have been shown to possess varying levels of antibacterial and anticancer activities in vitro (Ando 1984; Adio 2004 ; Subramoniam 2003) and many chemical constituents were isolated from bryophytes (Asakawa 1982)

Chemical components of these plants can be used as biologically active agents since many compounds isolated from bryophytes have shown interesting biological activity with particular reference to their application in medicine and agriculture for all round benefit of living beings (Pant 1998). In Asia, already 500 bryophytes have been studied with respect to their chemistry, pharmacology and application as cosmetics and medicinal drugs. (Asakawa, 2001 a). Compounds like polygodial from *Porella*, Norpiguisone from *Conocephalum conicum* and Lunularian from *Lunularia cruciata*, 4-hydro-3-methoxybibenzylel 1 and a-and b-pinine-alloromadendrine from *Plagiochila stevensoniana* are useful as antimicrobial compounds (Kamory 1995; Lorimeres 1993). *Plagiochila fasciculate* shows inhibitory effect on P388 cells (Leukemia), Herpes simplex type 1, Polio type1, *Bacillus subtilis*, *Escherichia coli*, *Candida albicans*, *Trichophyton mentagrophytes* and *Cladosporium resinae* (Lorimeres 1994). The antifungal activity of *Herberia aduncus* against *Botrytis cinerea*, *Rhizoctonia solani*, *Pythium debaryanum* is also well illustrated (Matsuo 1983). Members of *Fissidens* and *Polytrichum* were used as diuretic and hair growth stimulating drugs (Basile 1998).

Funaria hygrometrica Hedw. belong to family Funariaceae and the plants grow in loose to compact tufts, in large patches, green to yellowish green, simple or branched. Stem slender, erect, 11 to 12.5 mm height. Lower leaves small sparse; Costa poorly developed, upper leaves large crowded at apex; leaves (dry) curved and folded, (moist) spreading up to 3mm long and 1mm broad, concave, obovate to oblong lanceolate, acute,entire; costa strong, ending below the apex. Plants grow on moist soil or on rocks in large patches; or on slopes near water resources.

In this context, antibacterial potential of *Funaria hygrometrica* against some pathogenic bacteria was studied. The study includes effect of some organic and aqueous extracts of *Funaria hygrometrica* against four

*Corresponding author:
E-mail: pkhan07@yahoo.com

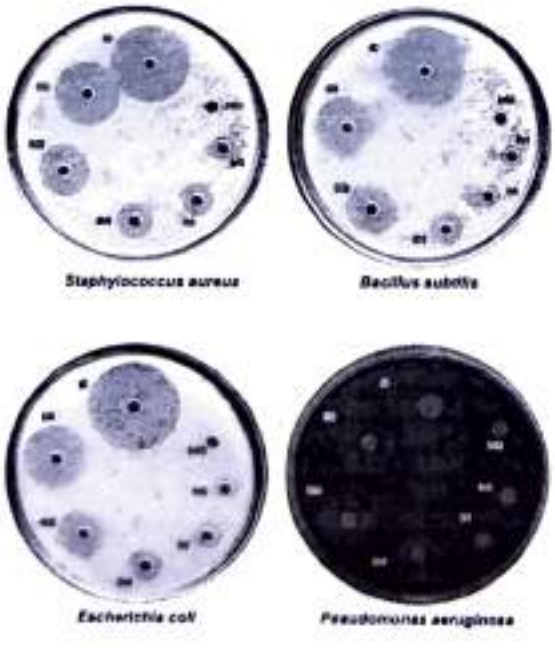
bacterial strains. Preliminary phytochemical screenings were also conducted.

Materials and Methods

Collection of plant material. *Funaria hygrometrica* was collected from Nakki Lake (Alt.1160) and Sunset point (Alt.1195) of Mount Abu district of Sirohi, Rajasthan, in the month of September 2006. The plants were identified and voucher specimens have been deposited in Bryology Laboratory, Dept. of Botany, Univ. College of science, Udaipur for future reference.

Extraction procedure and phytochemical screening: The plant material was carefully cleaned from attached litter and dead material under running tap water and finally with sterile distilled water. Air-dried and powdered approximately 20 g plant material of *Funaria hygrometrica* was extracted by cold percolation in either petroleum ether, benzene, acetone, methanol, ethanol or about 200 ml autoclaved water. The extracts were decanted, filtered with whatman No.1 filter paper and concentrated at reduced pressure below 40°c through rota vapour and lyophilized (Buchi, Labconco, US) to obtain dry extract. 0.1mg crude extracts were taken up for biological screening and also to observe the presence and absence of different phytochemical constituents.viz. alkaloids (Dragendorff's test), saponins (foam formation), flavonoids (using magnesium (Mn) and dil.HCl), terpenes (Liebermann-Burchard's test) according to standard methods (Sofowora, 1982, Trease and Evans, 1987).

Test microorganisms. Four test microorganisms were used in antibacterial sensitivity test were procured from Microbial Type Culture Collection And Gene Bank (IMTECH, Chandigarh,India), ie., the Gram positive bacteria *Bacillus subtilis* (MTCC-441) and *Staphylococcus aureus* (MTCC-740) and Gram negative bacteria *Escherichia coli* (MTCC-41) and *Pseudomonas aeruginosa* (MTCC-424). All the bacterial strains were maintained at 4°C on nutrient agar slants and sub cultured as required.



(i) 1000µg/ml (ii) 800µg/ml (iii) 500µg/ml (iv) 250µg/ml (v) 125µg/ml (vi) 65µg/ml (vii) DMSO

Fig1: Control plates of various bacteria against antibiotic Streptomycin as +ve and DMSO as –ve control

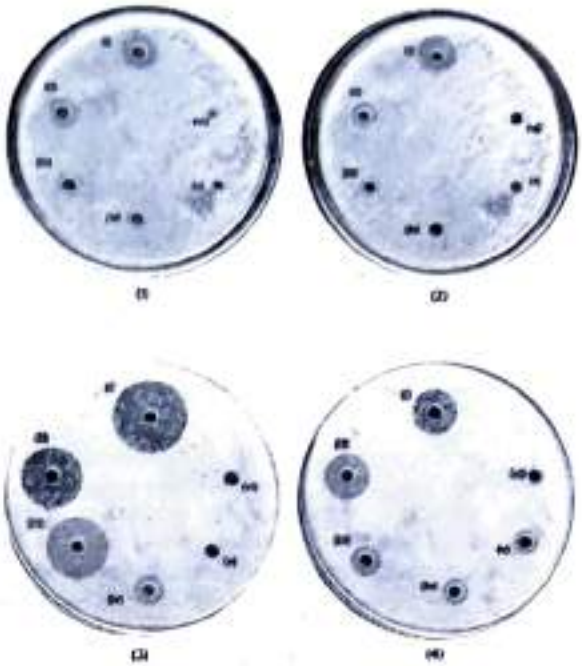


Fig 2. Antibacterial screening of benzene and acetone extract of *F. hydgrometrica* against *B. subtilis* (1,2) activity of ethanol and methanol extract against *Staphylococcus aureus* (3,4)

Antibacterial activity. The agar well diffusion method (Murray et al., 1995) evaluates the antibacterial activity. Bacteria were cultured overnight at 37°C in nutrient broth (Hi-media, Bombay) and were used as inoculum. 20 ml nutrient agar medium was poured in sterilized petri plates and allowed to solidify at room temperature. 24 h broth culture of test bacteria was used as inoculum under sterile condition. The freshly activated 100µl of organisms was set to 0.5 optical density and spread with a sterile L shaped bent glass rod. Using cork borer several wells of 6mm diameter were punched. To each well 100 µl crude extracts of various concentration (1000, 800, 500, 250, 125, 65µg/ml) were added. DMSO (Dimethyl sulfoxide) were used in making of extracts concentration and neutralized with 0.1N NaOH and 0.1N HCl. The plates were incubated at 37°C. Streptomycin and DMSO were used as positive and negative control respectively. The experiment was performed in triplicate and average results were recorded. Finally the diameter of zone of inhibition including laterally around the well was measured with antibiotic zone scale in mm

Results and Discussion

Preliminary phytochemical screening of the plant showed the presence of steroids, alkaloids and flavonoids in *Funaria hygrometrica* while saponins were absent (Table-1). The results of testing the

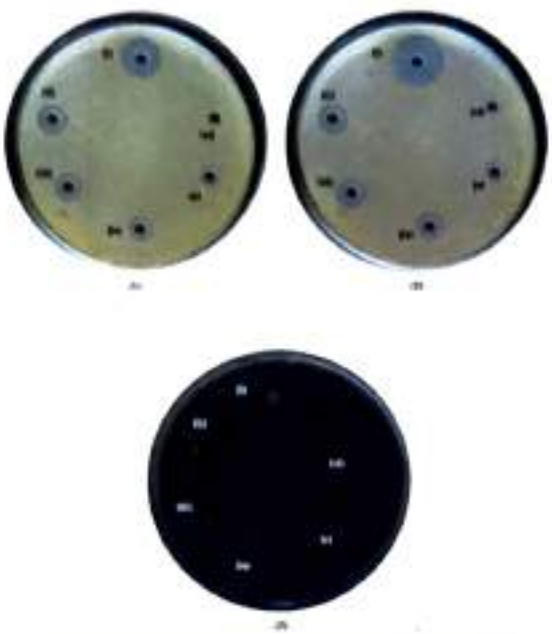


Fig 3 Antibacterial screening of benzene and acetone extract of *F. hygrometrica* against *E. coli* (1,2) activity of ethanol and methanol extract against *Pseudomonas aeruginosa* (3)

antibacterial activity of crude extracts of moss *F.hygrometrica* are presented. These were obtained by the disc diffusion method against various bacterial strains. Streptomycin and DMSO were used as positive and negative control respectively. Plant was extracted using both aqueous and organic solvents. The maximum antibacterial activity (approximately 24mm zone of inhibition) was observed in ethanol extract of the plant against the *Staphylococcus aureus* and minimum zone of inhibition observed in petroleum ether extract against *P.aeruginosa* (Figs.1-3)

Data obtained demonstrates that the antibacterial activity of plant depends largely upon the types of solvent used for extraction. Data indicate that almost all organic extracts of the plant showed antibacterial activity against the tested bacterial isolates (Table-2).

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References

Ado, A.M., Paul, C., Kloth, P., Koning, W.A. Sesquiterpenes of the liverwort, Scapania undulate. Phytochemistry 2004; 65: 199-206

Ando, H. Matsua, A. Applied Bryology. In: Schultze-Motel W, editor. Advances in Bryology Vol.2. J Cramer. Germany Vaduz, 1984.

Asakawa, Y and Heidelberger, M. Chemical constituents of the Hepaticae. Progress in the Chemistry of Organic Natural Products Bd.42.Wien-New York: 1082.

Asakawa, Y.Pharmacologically active substances from oriental bryophytes and inedible mushrooms and Fijian kava, In : World Conference on Medicinal and Aromatic plants, Book of Abstracts, Budapest, Hungary, pp 86: 2001a.

Basile, A. Vuotto, M.L. Ielpo, M.T. 1998 a. Antibacterial activity in *Rhynchostegium riparioides* (Hedw.) Card Extract (Bryophyta). *Phytotherapy Research*. 12: 146-148.

Kamory, E. et. al. 1995. Isolation and antibacterial activity of Marchgantiin A, a cyclic bis (bi phenyl) Constituent of Hungarian *Marchantia polymorpha*. *Planta Medica* 61:387-388.

Khobragade, C.N. et. al. 2008. In vitro screening of Bryophytes for Antimicrobial Activity. Journal of Medicinal Plants 7(4): 23-28.

Lorimeres, S. D. Perry, N. B. and Tangney, R.S. 1993. An antifungal bi benzyl from the New Zealand liverwort, *Plagiochila stevensoniana*. Bioactivity directed isolation, synthesis and analysis. *J. of Natural products* (Lloydia); 56: 1444-1450.

Lorimeres, S. D. Perry, N.B. 1994. Antifungal Hydroxy acetophenones from the New Zealand liverwort, *Plagiochila fasciculata*. *Planta Medica*. 60:386-387.

Maria de, Fda S. P.Regina, C. L. L, Ruth, V. B. 1989. Contribuicao ao estudo de briofitas como fontres de antibioticos. Acta Amazonica 19:139-145.

Matsuo, A. Yuki, S. and Nakayama, M. 1983. - (-) - herbertenediol and (-) - Herbertenolide, two new sesquiterpenoids of the ent-herbertane class from the liverwort *Herberta adunca*. , *Chemical Letters*. 1041-1042.

Murray, P.R. Baron, E. J. Pfallar, M.A. Tenover, F.C. Yolke, R.H. 1995. Manual of Clinical Microbiology, Sixth ed. ASM, Washington, DC, National Institute of Health, 1985. Guide for the Care and Use of Laboratory Animals, second ed. DHEW Publication, Bethesda, USA.

Pant, G.Medicinal uses of bryophytes.In: Chopra, R.N., ed., Topics in Bryology. New Delhi, Mumbai, Calcutta, Lucknow, Chennai, Nagpur, Bangalore, Hyderabad, Ahmedabad.Pp.112-124:1998.

Pant, G. and Tiwari, S. D. Bryophytes and mankind. Ethnobotany 1990; 2:97-103.

Sabovljevic A., Sokovic[b],M., Sabovljevic,M[a, d] and Grubisic,D.2006. Antimicrobial activity of *Bryumargenteum* Fitoterapia2006:77:2:144-145.

Sofowora, E.A. 1982. Medicinal plants and Traditional Medicine in Africa. Wiley, Chichester, 256.

Subramoniam, A., Subhisha, S., Ajikumaran Nair, S., Shylesh, B.S., Nair, G.M.Bryophytes of Kerala: A potential source of anti-cancer agents.Amla Res Bull 2003:23:78-83.er, M.

Subramoniam, An and Subhisha, S. Bryophytes of India: A potential source of anti-microbial agents. In: Khan IA, Khanum A, editors. Role of biotechnology in medical and aromatic plants.Vol.11. Hyderabad, India: Ukaaz Publictions.2005.

Trease, G.E. Evans, W.C. 1987. A Text Book of Pharmacognosy. ELSB Baillere Tindal, Oxford. 1055.