



Antibacterial Effect of Some Indian Liverworts

Kanchna Devi*, Sunita Kapila

Department of Botany, Punjab University, Chandigarh, India.

*Corresponding author's E-mail: s_kapila0802@yahoo.co.in

Accepted on: 02-04-2013; Finalized on: 31-05-2013.

ABSTRACT

The antibacterial activity of aqueous extracts of six liverworts (*Marchantia nepalensis*, *Marchantia palmata*, *Plagiochasma appendiculatum*, *Asterella pathankotensis*, *Cyathodium cavernarum* and *Reboulia hemisphaerica*) was evaluated against five bacterial species (*Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Micrococcus luteus* and *Bacillus subtilis*). All the studied liverworts showed antibacterial activity against all the strains of microorganisms as evident from the diameter of zones of inhibition in each case. *Ocimum sanctum* and *Azadirachta indica* were used to find synergistic or antagonistic effect with the extracts of the studied liverworts. Maximum synergistic activity was shown by *Marchantia palmata* with *Ocimum sanctum* as indicated by zone of inhibition 1.4cm against *Micrococcus luteus*, 1.3 cm against *E. coli*, 1.1 cm against *S. pyogenes*, 0.9 cm against the *B. subtilis* and 0.8 cm against *S. aureus*. *Reboulia hemisphaerica* showed synergistic effect with *Ocimum sanctum* against *Bacillus subtilis* as indicated by 0.8 cm zone of inhibition. All the liverworts showed antagonism with *Azadirachta indica* against the studied bacterial species.

Keywords: Liverworts, antibacterial effect, synergism, antagonism.

INTRODUCTION

Liverworts or hepatics under Division Bryophyta, with about 6000 species in the world grow as flattened leafless thalli or leafy forms. Oil bodies in the liverworts contain a number of lipophilic terpenoids with a variety of aromatic compounds, several of which show biological activities such as allergenic contact dermatitis, insecticidal, insect antifeedant, cytotoxic, piscicidal, muscle relaxing, plant growth regulatory, anti-HIV, DNA polymerase beta inhibitory, anti-obesity, neurotrophic, antimicrobial and antifungal activities¹⁻³.

Many liverworts exhibit antimicrobial effects against fungi and bacteria. Bodade *et al.*⁴ (2008) observed a wide range of activity of extracts of the liverwort *Plagiochasma appendiculatum* in different solvents like ethanol, acetone, benzene, chloroform, petroleum ether and distilled water against fungi and bacteria, out of which ethanolic extract showed the highest activity. The methanol extract of liverwort *Ptilidium pulcherrimum* was found to exhibit antibacterial and antifungal activity against five bacterial and six fungal species⁵. The methyl alcohol extract of acrocarpous mosses showed highest antimicrobial effect, whereas the chloroform extract showed lowest effect against bacterial and fungal strains⁶.

Ocimum sanctum commonly known as tulsi is a valuable medicinal plant used for the treatment of diseases and for the promotion of health. The leaves of plants have been used as anticancer, antiseptic, expectorant diaphoretic and tonic rejuvenator. Antibacterial activity of *Ocimum sanctum* was analyzed by Baskaran⁷ (2008) against four bacterial strains (*Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia*) in acetone, benzene and chloroform.

Azadirachta indica commonly known as neem is a native of India and distributed all over the world. It is of great medicinal value. Leaves of this plant are used to treat ringworm, various skin infections, eczema, chronic wounds etc. The antibacterial, antimalarial, antiulcer and contraceptive activities have been found in various preparations of neem⁸⁻¹⁰. Bark of neem showed antimicrobial effect against *Streptococcus sobrinus* in aqueous as well as acetone extract¹¹.

Ocimum sanctum and *Azadirachta indica* were used to find synergistic or antagonistic effect with the extracts of the studied liverworts.

MATERIALS AND METHODS

Collection of plant material

Six species of liverworts, namely *Marchantia palmata*, *Marchantia nepalensis*, *Plagiochasma appendiculatum*, *Asterella pathankotensis*, *Cyathodium cavernarum* and *Reboulia hemisphaerica* were collected from Western Himalaya at an altitude of 750m and 1927m from Mandi and Kasauli (Himachal Pradesh).

Preparations of plant extract

Plants were washed with distilled water to remove soil particles, dried at room temperature, powdered and prepared the aqueous extract gm/10ml and centrifuged at 2000 rpm for 15 minutes. The supernatant was used to check the antibacterial activity.

Preparation of Muller-Hinton Agar

Muller-Hinton agar was prepared, autoclaved and allowed it to cool. Wells were made with sterile beads. After pouring the freshly prepared and cooled medium into the petri-plates, the agar medium was allowed to

cool at room temperature and the beads were removed to make the intact wells.

Preparation of inocula

Five strains of bacteria i.e. *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Micrococcus luteus* and *Streptococcus pyogenes* were selected to check the antibacterial activity. The top of each colony was touched with a loop to transfer the growth into a tube containing 4 to 5 ml of a Luria broth. The broth culture was incubated at 35 °C until it achieved turbidity.

Inoculation of test plates

The dried surface of a Muller- Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface. Then the plates were incubated in the incubator at 37°C.

Transfer of plant extracts to inoculated agar plates

Dispensed 20 µl of the extract into the wells. Each bryophyte extract was mixed in equal concentration with

that of *Azadirachta indica* and that of *Ocimum sanctum* and loaded in the wells to check antagonism and synergism activity. Incubated the plates at 37°C overnight. After incubation, the results were recorded by measuring the zone of inhibition around the wells. Then synergistic or antagonistic activity was determined. In case of synergism, the diameter of zone of inhibition of the mixture was more than that of the individual components and antagonism was recorded if the diameter of zone of inhibition of mixture was less than that of the individual components.

RESULTS AND DISCUSSION

Different strains of gram negative and gram positive microorganisms namely *E. coli*, *S. aureus*, *S. pyogenes*, *M. luteus* and *B. subtilis* were taken to test the antimicrobial activity of the six species of liverworts.

Table 1 shows antibacterial activity of the liverwort taxa expressed as diameter of zone of inhibition in cm.

Table 1: Antibacterial activity of the liverwort taxa

	<i>Escherichia coli</i> (Gm -ve)	<i>Bacillus subtilis</i> (Gm+ ve)	<i>Staphylococcus aureus</i> (Gm+ ve)	<i>Micrococcus luteus</i> (Gm+ ve)	<i>Streptococcus pyogenes</i> (Gm+ ve)
Plant samples					
<i>Plagiochasma appendiculatum</i>	0.3 cm	0.2 cm	0.1 cm	0.3 cm	0.2 cm
<i>Asterella pathankotensis</i>	0.6 cm	0.6 cm	0.3 cm	0.3 cm	0.4 cm
<i>Marchantia palmata</i>	0.1 cm	0.3 cm	0.2 cm	0.6 cm	0.2 cm
<i>Marchantia nepalensis</i>	0.4 cm	0.5 cm	0.3 cm	0.7 cm	0.5 cm
<i>Reboulia hemisphaerica</i>	0.4cm	0.5cm	0.1cm	0.3 cm	0.6 cm
<i>Cyathodium cavernarum</i>	0.2 cm	0.4 cm	0.7 cm	0.6 cm	0.5 cm
Reference plants					
<i>Ocimum sanctum</i>	–	–	–	–	–
<i>Azadirachta indica</i>	0.9 cm	0.5 cm	0.7 cm	1.0 cm	0.8 cm

As observed by the diameter of zones of inhibition, all the studied liverworts showed resistance against all the strains and showed antibacterial activity against different strains of microorganisms *Cyathodium cavernarum* showed maximum zone of inhibition against *S. aureus* and *M. luteus* i.e. 0.7 cm, 0.6 cm respectively and against *S. pyogenes*, *B. subtilis* and *E. coli* it showed a diameter of 0.5 cm, 0.4 cm and 0.2 cm respectively. *Asterella pathankotensis* showed maximum zone of inhibition i.e. 0.6 cm against each of *E. coli* and *B. subtilis* and microorganisms, the zone of inhibition lies in the range of 0.4 cm with *S. pyogenes* and 0.3 with each of *S. aureus* and *M. luteus*.

The reference plant *Ocimum sanctum* individually showed no result against any of the micro organisms taken. *Azadirachta indica* showed a range of diameter between 0.5-1.0 cm against different gram –ve and gram +ve bacteria.

The antibacterial activity of each liverwort in combination with extracts of *Ocimum sanctum* and *Azadirachta indica* used to check antagonistic and synergistic activity of plants. Only *Marchantia palmata* with a combination of *Ocimum sanctum* showed enhanced antimicrobial activity with an increased diameter of inhibition zone is 1.4 cm against *M. luteus*, 1.3 cm against *E. coli*, and 1.1 cm against *S. pyogenes*, 0.9 cm against *B. subtilis* and 0.8cm against *S. aureus*. *Reboulia hemisphaerica* with the

combination of *Ocimum sanctum* showed enhanced antimicrobial activity against *B. subtilis* with diameter of zone of inhibition 0.8 cm, and showed no result against all other strains. The combination of *Ocimum sanctum* with *Plagiochasma appendiculatum*, *Asterella pathankotensis*, *Marchantia nepalensis* and *Cyathodium cavernarum* respectively showed no inhibition zone against *E. coli* and *M. luteus* and showed negligible zones against *B. subtilis*, *S. aureus* and *S. pyogenes*, thereby indicating antagonism.

All the studied liverworts taxa in combination with *Azadirachta indica* showed antagonism. The present experimental studies reveal that studied liverwort taxa alone show better antibacterial activity against the bacterial strains under study.

REFERENCES

- Asakawa Y, Terpenoids and aromatic compounds with pharmacological activity from bryophytes, *In* Zinsmeister HD, Mues R (eds.), *Bryophytes: Their Chemistry and Chemical Taxonomy*, Oxford University Press, UK, 1990, 369-410.
- Asakawa Y, Phytochemistry of Bryophytes: Biologically active terpenoids and aromatic compounds from liverworts, *In* Romeo JT (ed.), *Phytochemicals in human health protection, nutrition, and plant defense*. Kluwer Academic/Plenum, New York, 1999, 319-342.
- Asakawa Y, Chemosystematics of the Hepaticae, *Phytochemistry*, 65, 2004, 623-669.
- Bodade RG, Borkar PS, Arfeen MS, Khobradgade CN, *In vitro* Screening of Bryophytes for Antimicrobial Activity, *Journal of Medicinal Plants*, 7, 2008, 23-28.
- Veljic M, Ciric A, Sakovic M, Janackovic P, Marin PD, Antibacterial and antifungal activity of the liverwort (*Ptilidium pulcherrimum*) methanol extract, *Archives of Biological Science Belgrade*, 62, 2010, 381-395.
- Elibol B, Ezer T, Kara R, Celik GY, Colak E, Antifungal and antibacterial effects of some acrocarpic mosses, *African Journal of Biotechnology*, 10, 2011, 986-989.
- Baskaran X, Preliminary Phytochemical Studies and Antibacterial Activity of *Ocimum sanctum* L., *Ethanobotanical Leaflets*, 12, 2008, 1236-1239.
- Siddiqui S, Faizi S, Siddiqui BS, Ghiasuddin. Constituents of *Azadirachta indica*: isolation and structure elucidation of a new antibacterial tetranortriterpenoid, mahmoodin, and a new protolimonoid, naheedn, *Journal of Natural Products*, 55, 1992, 303-310.
- Jones IW, Denholm AA, Ley SV, Lovell H, Wood A, Sinden RE, Sexual development of malaria parasites is inhibited *in vitro* by the neem extract Azadirachtin, and its semi-synthetic analogues, *FEMS Microbiology Letters*, 120, 1994, 267-273.
- Garg GP, Nigam SK, Ogle CW, The gastric antiulcer effects of the leaves of the Neem tree, *Planta medica*, 59, 1993, 215-217.
- Bhuyan MM, Nishimura M, Matsumura S, Shimono T, Antibacterial effects of the crude *Azadirachta indica* Neem bark extract on *Streptococcus sobrinus*, *Pediatric Dental Journal*, 7, 1997, 61-64.

Source of Support: Nil, Conflict of Interest: None.

