ISSN-2249-5746

International Journal Of Ayurvedic And Herbal Medicine 2:2 (2012)229-233

Journal Homepage http://interscience.org.uk/index.php/ijahm

In Vitro Antifungal Efficacies Of Aqueous Extract Of *Targionia Hypophylla* L. Against Growth Of Some Pathogenic Fungi

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Methanol extracts of the thalloid liverwort Targionia hypophylla L., a thalloid liverwort (bryophyte), was found to be effective against five plant pathogenic fungi: Aspergillus niger, Botrytis cinerea, Trichoderma viridae, Penicillium chrysogenum, and Penicillium expansum. In vitro antifungal activity was assessed by micro dilution method, which exhibit total or strong inhibition on Trichoderma viridae and Botrytis cinerea growth. These results confirm the antifungal activities in liverwort extracts.

Key words: *Targionia hypophylla* L., Antifungal, Extract, Phytopathogens

Introduction

Phytochemicals, being safe and biodegradable, form better option as an antimicrobial agent. Various natural plant products are reported by various workers which are gaining importance due to their abundant antifungal activity postharvest pathogens (Sharma and Verma, 1991; Sharma, 1992; Bishop and Thornton, 1997; Tripathi and Dubey, 2004; Sharma *et al.*, 2006; Sharma and Tripathi, 2006; Sharma and Tripathi, 2008a; 2008b; Sharma *et al*., 2008).

Since ancient time bryophytes has been used as medicinal plants in customary medicines. 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; 2003). 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*., 2006).

Many other bryophytes also show antimicrobial effects against fungi and bacteria [Basile *et al*., 1998a; 1998b; 1999; Banerjee, 2001; Frahm and Kirchhoff, 2002; Scher *et al*., 2004; Subhisha and Subramonium, 2005; sabovljevic *et al*, 2006; Bodade *et al*., 2008; Lahlou *et al*, 2000; Dülger *et al*., 2009). Liverwort like *Marchantia tosana* exhibited antifungal, antibacterial and antitumour activity (Veljic *et al*., 2010). It has also been shown that *Ptilidium pulcherrimum* have antibacterial and antifungal activity (Asakawa, 1981).

Almost all species of bryophytes are not damaged by fungi, bacteria, insect larvae 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; 1984; 2000).

At present, several environmental problems are caused by the rigorous use of synthetic fungicides in agriculture. Natural plant-derived products for agriculture have less shock on the environment. Usually, the secondary metabolites produced by bryophytes are known for antifungal and 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 *Targionia hypophylla* L. (Figure 1) which is a terricolous taxa, it grows on exposed rocks and soil surfaces. This species is well known from all the bryo-geographical regions of

the world. The aim of this work was to determine the antifungal effects of this liverwort and to make a contribution to the pharmaceutical botany studies to be done in the future in India.

Materials and Methods

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 August 2011. Specimens (BHBV 78610- BHBV 78622/2011; Legit.: A. Alam and S. C. Sharma; Det.: A. Alam) are deposited in the Bryophyte Herbarium of Banasthali Vidyapith (BHBV), Rajasthan (India).

Pytopathogenic Fungi

The phytopathogenic fungi were obtained from the culture collection of Plant Pathology Laboratory, Department of Bioscience and Biotechnology, Banasthali University. The fungal species used in the experiment were *Aspergillus niger* BVPPL 17 (An), *Botrytis cinerea* BVPPL 30 (Bc), *Trichoderma viridae* BVPPL 44 (Tv), *Penicillium chrysogenum* BVPPL 38 (Pc), and *Penicillium expansum* BVPPL 58 (Pe). The culture of the phytopathogenic organisms was maintained on the PDA at 4^oC.

Preparation of the extracts

A Sample (10 g) was treated with 0.8% Tween 80 aqueous solution to remove epiphytic hosts found on the plant surface. Then, the samples were washed in tap and distilled water, and dried on filter paper. The samples were extracted with methanol (100 x 2ml) for 24 h at 40°C. the extract was filtered with a cellulose-acetate membrane. The filterate was evaporated until dry with a rotatory evaporator and 100 mg of dry extract was then dissolved with 1 ml dimethyl sulfoxide (Ilhan *et al*., 2006).

Determination of antimicrobial activity

Micro dilution method: The modified micro dilution method was also used to obtain quantitative data for the compounds under study (Hanel and Raether, 1988). The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v) and adjusted with sterile saline to a concentration of 1.0×10^5 in a final volume of 100 µl/ml. the inocula were stored at 4^oC for further use. Dilutions of the inocula were cultured on solid MA to verify the absence of contamination and to check the validity of the inoculum.

Minimum inhibitory concentration (MIC) determination was performed by serial dilution technique. The investigated compounds were dissolved in broth medium with inoculum to obtain the required concentration (0.05-20 mg/ml). Micro plates were incubated at 28^oC for 72 h. The minimum fungicidal concentration (MFCs) was determined by serial sub cultivation of 2 µl into microtitre plates containing 100 µl of broth per well and further incubation at 28^oC for 72 h. The lowest concentration with no visible was defined as MFC, indicating 99% killing of original inoculum. DMSO was used as control and Bifonazol was used as positive control.

Results and Discussion

In the present study, the antifungal activity of the methanol extract of *Targionia hypophylla* L. was tested against five fungal species. For comparison of antifungal activity a synthetic fungicide Bifonazol was used. According to obtained results it is evident that the extract of *Targionia hypophylla* L. at a concentration of 20mg/ml has significant antifungal activity. In case of *Trichoderma viridae*, *Penicillium expansum* and *Botrytis cinerea* this concentration showed better inhibition than the Bifonazol. The concentration of 2.5 mg/ml or higher exhibit a more significant antifungal activity against the susceptible fungal species (Table 1).

Table 1. **Antifungal activity of *Targionia hypophylla* L. methanol extract assessed by micro dilution method**

Concentration of plant extract and Bifonazol (mg/ml)				
	Targionia hypophylla L.		Bifonazol	
Fungal Species	MIC	MFC	MIC	MFC
Bc	0.5	2.5	1.0	1.0
An	2.5	5.0	0.1	0.1

Pc	0.5	2.5	1.0	1.0
Tv	0.5	2.5	1.0	1.0
Pe	0.5	2.5	1.0	1.0

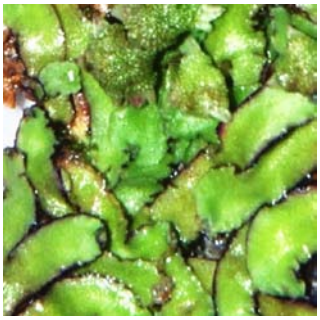

Figure 1: A patch of *Targionia hypophylla L.*(BHBV 78620/2011)

Conclusion

On the basis of the present study it can be concluded that the extracts of *Targionia hypophylla* L. show considerable effect as antifungal agent. Moreover, fungus like *Trichoderma viridae* and *Botrytis cinerea*, which are known as resistant species were also sensitive to this extract. The present study also showed that *Targionia hypophylla* L. has significant antifungal activity against four out of five selected fungi. The results obtained are similar to some researchers' report that extracts from mosses and liverworts exhibit antifungal activities (Castaldo *et al*., 1988; Alam *et al*., 2011). The present study clearly shows the mode of action of extract against the test pathogen *A. niger*. The potential antifungal compound can be isolated in mass volume by various techniques like tissue culture so that the antifungal compound can be of economic value in controlling the phytopathogens in postharvest commodities in eco-friendly way. This study helps in the establishment of bryophytes as bio-control agents against infectious diseases caused by these fugal species.

Acknowledgement

The author is grateful to Prof. Aditya Shastri, Vice Chancellor, Banasthali Vidyapith, Rajasthan (India) for providing necessary support during the study.

References

1. Alam, A., Tripathi, A., Vats, S., Behera, K. K. and Sharma, V. 2011. *In vitro* antifungal efficacies of aqueous extract of *Dumortiera hirsuta* (Swaegr.) Nees against sporulation and growth of postharvest phytopathogenic fungi. *Archive for Bryology*.*103*: 1-9.
2. Asakawa, Y., Toyota, M., Tori, M and Hashimoto, T. 2000. Chemical structures of macrocyclic bis (bibenzyls) isolated from liverworts (Hepaticae). Spectroscopy.*14*: 149-175.
3. Asakawa, Y. 1990. *Biologically active substances from bryophytes*. In: Chopra RN, Bhatla SC (Eds.). Bryophytes Development: Physiology and Biochemistry. CRC Press, Boca Raton. pp. 259-287.
4. Asakawa, Y. 1981. Biologically active substances obtained from bryophytes. *The Journal of the Hattori Botanical Laboratory*. *50*: 123-142.
5. Asakawa, Y. 2004. Chemosystematics of the hepaticae. *Phytochemistry. 65*: 623-669.
6. Asakawa, Y. 2001. Recent advances in phytochemistry of bryophytesacetogenins, terpenoids and bis (bibenzyl)s from selected Japanese, Taiwanese, New Zealand, Argentinean and European liverworts. *Phytochemistry*.*56*: 297-312.
7. Asakawa, Y. 1984. Some biologically active substances isolated from hepaticae: terpenoids and lipophilic aromatic compounds. *The Journal of the Hattori Botanical Laboratory. 56*: 215-219.

8. Banerjee, R. D. 2001. *Antimicrobial activities of bryophytes: A Review*. In: Nath V, Asthana AK. (eds.), Perspectives in Indian Bryology. Bishen Singh Mahendra Pal Singh Publisher, Dehradun, India. pp. 55-74.
9. Basile, A., Giordano, S., Lopez-Saez, J. A. and Castaldo Cobianchi, R. 1999. Antibacterial Activity of pure flavonoids isolated from mosses. *Phytochemistry.52*: 1479-1482.
10. Basile, A., Sorbo, S., Giordano, S., Lavitola, A. and Castaldo Cobianchi, R. 1998a. Antibacterial activity in *Pleurochaete squarrosa* extract (Bryophyta). *International Journalof Antimicrobial Agents. 10*: 169-172.
11. Basile, A., Vuotto, M. L., Ielpo, M.T.L., Moscatiello, V., Ricciardi, L., Giordano, S. and Castaldo Cobianchi, R. 1998b. Antibacterial Activity in *Rhynchostegium riparioides* (Hedw.) Card. Extract (Bryophyta). *Phytother. Res. 12*: 146-148.
12. Bishop, C. D. and Thornton, I. B. 1997. Evaluation of the antifungal activity of the essential oils of *Monarda citriodora* var. *citriodora* and *Melaleuca alternifolia* on the post-harvest pathogens. *Journal of Essential Oil Research. 9*(1): 77–82.
13. Bodade, R. G., Borkar, P. S., Saiful Arfeen, M. D. and Khobragade, C. N. 2008. *In vitro* Screening of bryophytes for antimicrobial activity. *Journal of Medicinal Plants. 7*(4): 23-28.
14. Castaldo, C. R., Giordano, S., Basile, A. and Violante, U. 1988. Occurrence of antibiotic activity in *Conocephalum conicum, Mnium umdulatum* and *Leptodictyum riparium* (Bryophyta). *Giardino Botanico Ital.122*: 303-311.
15. Daouk, D., Dagher, S. and Sattout, E. 1995. Antifungal activity of essential oil *Origanum syriacum* L. *Journal of Food Protect. 58*: 1147-1149.
16. Dülger, B., Hacıo,_lu N. and Uyar, G. 2009. Evaluation of Antimicrobial Activity of Some Mosses From Turkey. *Asian Journal of Chemistry. 21*(5): 4093-4096.
17. Flowers, P. 1957. Ethnobryology of the Gosuite Indians of Utah. *Bryologist. 60*: 11-14.
18. Frahm, J. P. and Kirchhoff, K. 2002. Antifeeding effects of bryophyte extracts from *Neckera crispa* and *Porella obtusata* against slug *Arion lusitanicus*. *Cryptogamie Bryologie.23*: 271-275.
19. Hanel, H. and Raether, W. 1988. A more sophisticated method of determining the fungicidal effect of water insoluble preparation with a cell harvester, using miconazoleans an example. *Mycoses. 31*:148-154.
20. Ilhan, S., Savaroglu, F., Colak, F., Iscen, C. and Erdemgil, F. 2006. Antimicrobial activity of *Palustriella commutata* (Hedw.) Ochyra extract (Bryophyta). *Turkish Journal of Biology. 30*: 149-152.
21. Lahlou, E. H., Hashimoto, T. and Asakawa, Y. 2000. Chemical constituent of the liverworts *Plagiochasma japonica* and *Marchantia tosana. The Journal of the Hattori Botanical Laboratory. 88*: 271-275.
22. Sabovljevic, A., Sokovic, M., Sabovljevic, M. and Grubisic, D. 2006. Antimicrobial activity of *Bryum argenteum. Fitoterapia. 77*: 144-145.
23. Scher, J. M., Speakman, J. B., Zapp, J. and Becker, H. 2004. Bioactivity guided isolation of antifungal compounds from the liverwort *Bazzania trilobata* (L.) Gray SF. *Phytochemistry. 65*: 2583-2588.
24. Sharma, N. 1992. Control of *Alternaria alternata* by *Clerodendron aculeatum*. *National Academy of Science Letters. 15*(4): 107-109.
25. Sharma, N. and Tripathi, A. 2006. Fungitoxicity of the essential oil of *Citrus sinensis* on post- harvest pathogens. *World Journal of Microbiology and Biotechnology. 22*(6): 587-593.
26. Sharma, N. and Tripathi, A. 2008a. Effects of *Citrus sinensis* (L.) Osbeck epicarp essential oil on growth and morphogenesis of *Aspergillus niger* (L.)Van Tieghem. *Microbiological Research. 163*: 337-344.
27. Sharma, N. and Tripathi, A. 2008b. Integrated management of postharvest *Fusarium* rot of gladiolus corms using hot water, UV-C and *Hyptis suaveolens* (L.) Poit. Essential oil. *Postharvest Biology and Technology*. *47*: 246-254.

28. Sharma, N. and Verma, H. N. 1991. Effect of leaf extracts of *Clerodendron* spp. on fruit rotting fungi. *Fitoterapia. 62* (6): 517-518.
29. Sharma, N., Tripathi, A. and Verma R. R. 2006. Synergistic bio-efficacy of three plant extracts on sporulation of fruit rotting fungi. *Journal of Mycopathology Research*. *44* (1): 55-60.
30. Subhisha, S. and Subramoniam, A. 2005. Antifungal activities of a steroid from *Pallavicinia lyellii,* a liverwort. *Indian Journal of Pharmacology*. *37*(5): 304-308.
31. Tripathi, P. and Dubey, N. K. 2004. Exploitation of natural products as alternative strategy to control post-harvest fungal rotting of fruits and vegetables. *Postharvest Biology and Technology*. *32*: 235–245.
32. Veljic, M., Ciric, A., Sokovic, M., Janackovic, P. and Marin, P. D. 2010. Antibacterial and antifungal activity of the liverwort (*Ptilidium pulcherrimum*) methanol extract. *Archive of Biological Science*. *62* (2): 381-395.