

ANTIFUNGAL ACTIVITY EVALUATION OF *BERGENIA CILIATA*

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ABSTRACT

Phytopharmaceutical drug of medicinal importance *Bergenia ciliata* was subjected to bioactivity analysis. A battery of assays were performed on different extracts of *Bergenia ciliata* for antifungal effects of ethanol, hexane, ethyl acetate, chloroform, butanol and aqueous extracts of the roots and leaves extract of *Bergenia ciliata* exhibited various degree of inhibition activity. It was observed that root and leaves extract were promising as antifungal agent. The root and leaves extract were effective against *Microsporum canis*, *Pleurotus oustreatus* and *Candida albicans*. This study showed that *Bergenia ciliata* could inhibit selectively fungus.

INTRODUCTION

Bergenia ciliata (How. Sternb. belongs to family Saxifragaceae. This family comprises of 30 genera and 580 species, mostly distributed in the cold and temperate regions. The genus *Bergenia* comprises of 6 species distributed in the temperate Himalays and Central and East Asia, represented in Pakistan by 2 species namely *Bergenia ciliata* and *Bergenia stracheyi*. It is perennial herb upto 50 cm tall. *Bergenia ciliata* is used in the traditional medicine of Asian countries. In Nepal, one tea spoonful of the juice of dried rhizome of *B. ciliata* along with an equal amount of honey has been taken orally 2-3 times a day by post-partum-women against the digestive disorders as carminative and tonic as well. This preparation, however, was prescribed at least a week or longer for bearing healthy build up (Bhattarai N.K. *et al.*, 1994). It is also used orally for anthelmintic (Manandhar N.P., 1995).

In the North West and trans-Himalayan region in Jammu and Kashmir state the roots of *B. ciliata* are boiled in water and table salt is added in the decoction. Traditionally this decoction is taken orally by human adults of this region for the treatment of asthma (Singh

V., 1995). The hot water extract of whole dried plant of *Bergenia ciliata* has been employed orally by human adults for renal or urinary calculi (Mukerjee T. *et al.*, 1984). All species of *Bergenia* are reported to dissolve gravel and stones in the kidney. Root is used as a tonic, in fever, diarrhoea and pulmonary affections as an anti-scorbutic and in ophthalmia (Mehra P.N. *et al.*, 1971).

Antifungal Activity

The root and leaves ethanol, hexane, ethyl acetate, chloroform, butanol and aqueous extracts were dissolved in distilled water (5 mg/ ml) and the aliquots were used to test the antifungal activity. All the extracts were tested for their activity against following fungi.

Fungi

Aspergillus niger (ATCC 16019), *Alternaria solani* (ATCC 11078), *Penicillium funiculosium* (ATCC 9846), *Fusarium solani* (ATCC 26533), *Microsporum canis*, *Nigrospora oryzae*, *Curvularia lunata*, *Pleurotus oustreatus* and *Candida albicans*.

Penicillin 100 I.U/ml and *Streptomycin* (1mg/ml) were used as control for comparison. Test microorganisms were

maintained on stock culture saboraud dextrose agar (SDA). From stock culture 24 hours fresh culture was prepared. Inocula were prepared by diluting 24 hours old culture in saline. A dilution of 1:100 was used in all the tests.

MATERIALS AND METHODS

Saboraud dextrose agar (Merck), Culture Plates, Sterile cork borer, Wire loop, Fungi test culture, Autoclave, Incubator, Penicillin (Merck), Streptomycin (Merck).

Antifungal Assay

The tests were run in triplicate. Petri plates (10x10) were prepared with saboraud dextrose agar (SDA). The diluted culture 0.1 ml was poured on each plate and plates were dried for 30 minutes at 37°C. Wells of 6 mm (approximate) diameter were cut with sterile cork borer in the inoculated agar. The wells were filled with the plant extracts and control solutions for comparison. Plates were incubated at 25°C±1°C for 5 days. At the end of incubation period the clear zone of inhibition around the wells were measured in millimeter (mm). The results are given in table land table 2.

Table 1
Antifungal activity of roots extract of *Bergenia ciliata*

Organisms	Zone of inhibition (in mm.)							
	Control		Ethanol	Hexane	Ethylacetate	Chloroform	Butanol	Aqueous
	Streptomycin	Ampicillin						
<i>Aspergillus tiger</i> (ATCC 16019)	12	15	6	N/A	N/A	N/A	N/A	N/A
<i>Alternaria solani</i> (ATCC 11078)	10	15	12	N/A	N/A	N/A	N/A	N/A
<i>Penicillium fitniculosium</i> (ATCC9846)	12	18	12	N/A	N/A	N/A	N/A	N/A
<i>Fusarium solani</i> (ATCC 26533)	18	10	N/A	N/A	N/A	N/A	N/A	N/A
<i>Microsporum canis</i>	20	20	8	6	6	8	8	10
<i>Nigrospora oryza</i>	15	12	N/A	N/A	N/A	N/A	N/A	N/A
<i>Curvularia lunta</i>	12	15	N/A	N/A	N/A	N/A	N/A	N/A
<i>Pleurotus oustreatus.</i>	18	12	8	6	8	8	10	8
<i>Candida albicans</i>	18	10	10	14	8	6	8	10

N/A= No Activity (No zone of inhibition observed)

Table 2
Antifungal activity of leaves extract of *Bergenia ciliata*

Organisms	Zone of inhibition (in mm.)							
	Controls		Ethanol	Hexane	Ethyl acetate	Chloro form	Buta not	Aqueous
	Strepto mycin	Peni cillin						
<i>Aspergillus niger</i> (ATCC 16019)	10	12	N/A	N/A	N/A	N/A	N/A	N/A
<i>Alternaria solani</i> (ATCC 11078)	10	12	N/A	N/A	N/A	N/A	N/A	N/A
<i>Penicillium funiculosium</i> (ATCC9846)	15	10	10	N/A	N/A	16	10	20
<i>Fusarium solani</i> (A TCC 26533)	18	10	N/A	N/A	N/A	N/A	N/A	N/A
<i>Microsporum canis</i>	15	18	12	N/A	16	N/A	N/A	N/A
<i>Nigrospora oryza</i>	16	18	N/A	N/A	N/A	N/A	N/A	N/A
<i>Curvularia lunta</i>	12	15	20	N/A	N/A	10	N/A	N/A
<i>Pleurotus oustreatus.</i>	15	10	22	N/A	N/A	N/A	20	10
<i>Candida albicans</i>	20	12	N/A	N/A	N/A	N/A	20	10

N/A= No Activity (No zone of inhibition observed)

RESULTS AND DISCUSSION

Plants have been a source of medicinal compounds since pre-historic time. It is well established that all parts of plants were used in *Unani* systems of medicine for centuries. However, the discovery and use of synthetic drugs led to a dramatic decline in the popularity of herbal products used in the therapy. Nevertheless the realization of harmful toxic effect of a large number of synthetic drug led to alternative sources which would be safe and effective in various ailments. A resurgence of interest in the study and use of medicinal plant taking place during the last three decades. A considerable growth has occurred in official and commercial

interest in the use of natural products (Akerele O., 1992). In recent years there has been a growing trend to evaluate the bioactivity of the medicinal plants, so that a systematic approach could be adopted for their therapeutic utilization. The present study is an attempt to investigate and evaluate the bioactivity of *Bergenia ciliata*, which is of considerable medicinal importance.

The biological screening of medicinal plant extracts has most frequently been carried out to determine the antifungal profile. These evaluations are usually done through different techniques to ascertain the inhibition effect on pathogenic and non-pathogenic bacteria. In this study anti-fungal screening

of the different extracts of roots and leaves of *Bergenia ciliata* have been separately attempted by agar-well diffusion method. In the experimental, nine fungal strains were selected for the screening activity. These fungal strains used are *Aspergillus niger*, *Alternaria solani*, *Penicillium funiculosium*, *Fusarium solani*, *Microsporum canis*, *Nigrospora oryza*, *Curvularia lunta*, *Pleurotus ostreatus* and *Candida albicans*

Anti Fungal Activity of Root Extracts

Antifungal activities were conducted by employing *Aspergillus niger*, *Alternaria solani*, *funiculosium*, *Microsporum canis*, *Nigrospora oryza*, *Curvularia lunta*, *pleurotus ostreatus* and *Candida albicans* with different extracts of the root of *Bergenia ciliata*.

The results obtained on antifungal studies are given in table 1, which depicts that ethanol, hexane, ethyl acetate, chloroform, butanol and aqueous root extracts of *Bergenia ciliata* exhibited antifungal activity against *Pleurotus ostreatus*, *Microsporum canis* and *Candida albicans* (zone of inhibition range 6mm-14mm). Whereas only ethanol extract exhibited activity against *Aspergillus*

niger, *Penicillium funiculosium* and *Alternaria solani* (zone of inhibition range 6mm-12mm). The hexane, ethyl acetate, butanol and aqueous extracts found inactive against the subjected nine fungi. The results indicated that all the extracts found inactive against *Fusarium solani*, *Nigrospora oryza* and *Curvularia lunta*.

The graphic presentation of antifungal activity of roots extracts of *Bergenia ciliata* is given in Fig. 1. On the whole antifungal screening of *Bergenia ciliata* extracts is not very remarkable, that this plant can be utilized for antifungal lesions.

As such the different chemical constituents elaborated by different spp. of *Bergenia* displayed are coumarin benzenoids, steroids and tannins and the antifungal activity of *Bergenia ciliata* may emancipate due to either of these constituents.

Antifungal Activity of Leave Extracts

The screening results of *Bergenia ciliata* leaves extract are as shown in Fig.2 that exhibited no response against *Aspergillus niger*, *Alternaria solani*, and *Fusarium solani* and *Nigrospora oryza*. Whereas ethanol

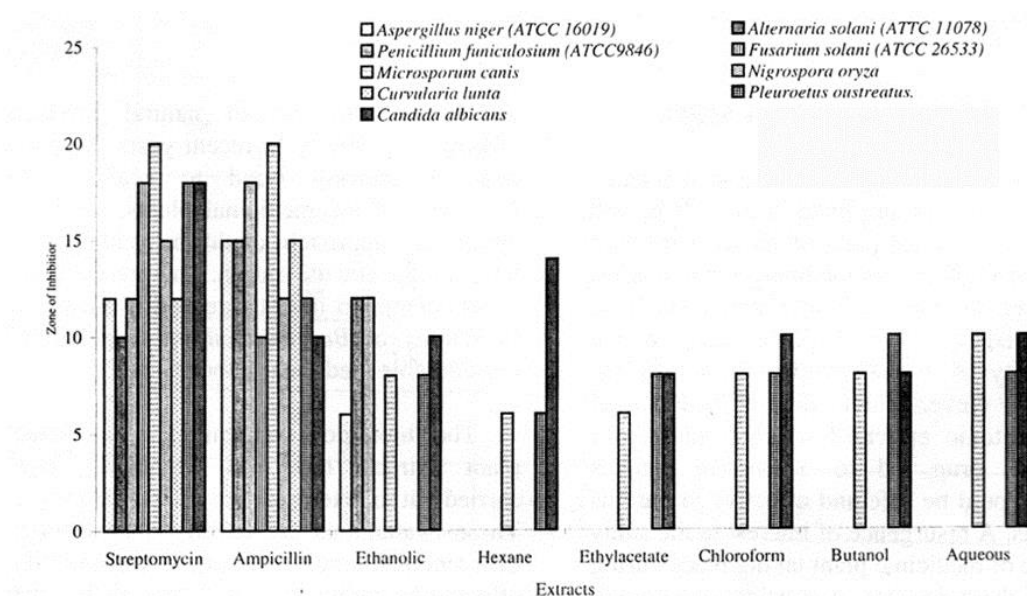


Fig. 1: Graphical representation of antifungal activity of root extracts of *Bergenia ciliata*

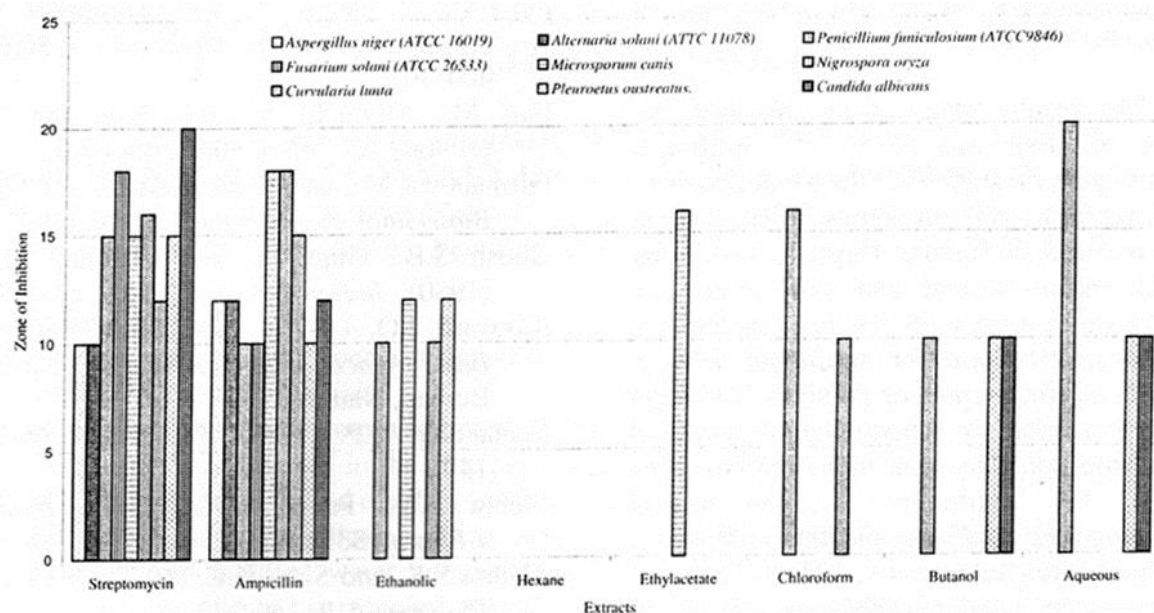


Fig. 2: Graphical representation of antifungal activity of leaves extracts of *Bergenia ciliata*

extract has exhibited antifungal activity against *Penicillium funiculosium*, *Microsporunz canis*, *Curvularia lunta*, and *Pleuroetus oustreatus* (zone of inhibition 10mm-22mm). Hexane extract did not exhibit positive activity against any of the subjected fungi. Ethyl acetate extract has shown activity against *Microsporium canis* (zone of inhibition 16mm). Chloroform extract has shown activity against *Penicillium funiculosium* and *Curvularia lunta* (10mm-16mm). Butanol extract has shown activity against *Penicillium funiculosium*, *Pleuroetus oustreatus* and *Candida albicans* (zone of inhibition range 10mm-20mm). Aqueous extract exhibited activity against *Penicillium funiculosium*, *Pleuroetus oustreatus* and *candida albicans* (zone of inhibition range 10mm-20mm). The literature search showed the different studies carried out on the antifungal activity of different medicinal plants. As such ether and chloroform extracts of *Mucuna pruriens* seeds and *Curcuma longa* stems were found to be fungistatic, while the ether extract of *Shorea robusta* resin, chloroform extract of *Azadirachta indica* and *Pongamia glabra* were fungicidal (Mishra S.K. & Sahu K.C., 1977). Essential oils of *Curcuma aromatica* (Rao J.T., 1976),

Curcuma caesia (Benerjee A. & Nizam S.S., 1976) and *Curcuma angustifolia* (Benerjee A. & Nizam S.S., 1978) and rhizomes of *Curcuma amada* (Ghosh S.B. et al., 1980) showed potent antifungal activity *in vitro*. Antifungal activity of *Alpinia officinarum* (Ray P.J. & Majumdar S.K., 1976a), *Cassia alata* (Radhakrishnan et al., 1976) and *Saussurea lappa* (Ray P.J. & Majumdar S.K., 1976b) showed potent antifungal activity. *Arnebia* spp. also exhibited a potent antifungal activity against experimental fungal infection in guinea pig (Whab S. et al., 1982).

The chemical compounds particularly polyphenols such as arbutin, hydroquinone, methylarbutin and hydroquinone, methyl ether which are generally present in the different species of *Bergenia* (Fuji M. et al., 1996a; 1996b; Furmanowa M & Rapczewska L., 1993; Ostrowska B. & Gorecki P., 1988) can be suspected for the antibacterial activity. The test results of ethanolic extracts (crude) of both leaves and roots proved to be strongly effective against a variety of disease producing microorganisms. Further evidences can be cited where *Bergenia* spp. have shown to possess weak bacteriophage activity which

is rather good choice for inhibition of antibacterial gram positive strain.

The results obtained on the root and leaves of *Bergenia ciliata* for antifungal activity provide support to the folkloric use of skin wound healing properties in Pakistan i.e. it is referred as Zakhme Hayat in vernacular which means wound and skin protection. Although no prior study has been undertaken on *Bergenia ciliata* for antifungal activity. This is the first report of its kind. Although there is a reference where ethanol extract of dried root of *Bergenia schmidtii* has been tested for antifungal activity against *Aphanomyces euteiches* and this exhibited a weak activity (Kakwaro O., 1976). Therefore it can be concluded that skin infections can be checked by applying the extract of *Bergenia ciliata* but it is toxic in nature, therefore authentication of claim for folklore use is rather still an unresolved issue for its application.

In conclusion, the summary of the antifungal activity evaluation of the leaves and root extracts of *Bergenia ciliata* can be delineated as follows. The antifungal activity of roots and leaves extract were effective against *Microsporum canis*, *Pleurotus oustreatus*, and *Candida albicans*.

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REFERENCES

- Akerele, O. *Fitoterapia* (1992). **LX111** (2): 99.
- Bhattarai N. K. (1994). *int. J. Pharmacog.* **32**(1): 13.
- Benerjee A. and Nigam S.S. (1976). *Indian J. Med. Res.* **64**: 1318-1321.
- Benerjee A. and Nigam S.S. (1978). *Indian J. Med. Res.* **68**: 864-866.
- Fuji M., Miyaichi Y. and Tomimori T. (1996a). *Natural Medicines*. **50**(6): 404-407.
- Fuji M., Miyaichi Y. and Tomimori T. (1996b). *Nat. Med.* **50**(6): 404-407.
- Furmanowa M., and Rapczewska L. (1993). *Biotechnol. Agr. Fores.* **2**: 18-33 (1993).
- Ghosh S.B., Gupta S. and Chandra A.K. (1980). *Indian J. Exp. Biol.* **18**: 174-176.
- Kakwaro J.O. (1976). *Medicinal Plants of East Africa*. East Africa Literature Bureau, Nairobi.
- Manandhar N.P. (1995). *Fitoterapia*. **66**(2): 149.
- Mehra P.N., Paina M.K. (1971). *Indian J. Pharm.* **33**: 126.
- Mishra S.K. and Sahu K.C. (1977). *Indian J. Pharmacol.* **9**: 269-272.
- Mukerjee T., Bhalla N., Singh G., Aulakh H.C. (1984). *Jain, Indian Drugs*. **21**(6): 224.
- Ostrowska B. and Gorecki P. (1988). *Herba Pol.* **34**(1/2): 21-25.
- Radhakrishnan N., Namsveni R., Uma R. and Thyagarajan R. (1976) *J. Res. Indian Med. Yoga Homoeo.* **11**: 70-74.
- Rao J.T. (1976). *Indian J. Pharmacol.* **38**: 5354.
- Ray P.J. and Majumdar S.K. (1976a). *Indian J. Exp. Biol.* **14**: 712.
- Ray P.J. and Majumdar S.K. (1976b). *Indian J. Exp. Biol.* **15**: 334.
- Whab S., Tandon R.N., Jacob Z., Chandra B. and Srivastava O.P. (1982). *Indian J. Med. Res.* **76**(suppl.): 77-82.