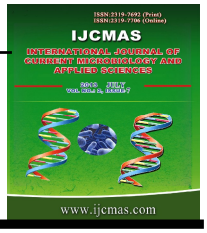
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 7 (2013) pp. 69-78**
http://www.ijcmas.com



Original Research Article

Antifungal activity evaluation of different extracts of *Bergenia stracheyi*

Vinesh Kumar* and Devendra Tyagi

Department of Chemistry, D.A.V. (PG) College, Dehradun, Pincode-248001,Uttarakhand, India
**Corresponding author e-mail: vkresearch47@gmail.com*

A B S T R A C T

The herb *Bergenia stracheyi* belongs to genus bergenia and family Saxifragaceae. The *Bergenia stracheyi* has been reported from Afganistan to Uttarakhand (India) between 3300 to 4500 m in Alpine slopes. On the basis of literature review it was observed that no previous work done on antifungal activity of *Bergenia stracheyi.* Therefore, present research work was undertaken to determine the antifungal activity of different extracts of *B. stracheyi.* The antifungal activity was tested against six fungal species viz. *Alternaria alternate, Aspergillus niger, Colletotrichum gloeosporioides, Fusarium oxysporium, Ganoderma lucidum* and *Rhizoctonia solani.* Antifungal study was carried out by poisoned food technique using different concentrations of extracts against all the six test fungi. The results of antifungal activities were reported in terms of Inhibition Concentration (IC_{50}), Minimum Inhibitory Concentration (MIC) and zone of inhibition. The results of antifungal screening showed that different extracts exhibit different extent of antifungal activity against all test fungi.

Keywords

Bergenia stracheyi; saxifragaceae; antifungal and poisoned food technique.

Introduction

The genus *Bergenia* belongs to family Saxifragaceae. The plant of genus commonly known as Paashanbheda (Paashan = rockstone, bheda = piercing) in Hindi, which itself indicates that the plant grows between rocks and appears to break them or that it possesses lithotriptic property.

The *Bergenia stracheyi* has been reported from Afganistan to Uttarakhand (India) between 3300 to 4500 m altitude in the form of vast patches in the sub-alpine and alpine areas. Literature review suggested that the Bergenia species have been used in folklore and Indigenous system of *Bergenia species* has been reported possess astringent, tonic, antiscorbutic, and laxative properties. the species of genus bergenia has been given for the treatment of pulmonary affection, dysentery, ulcers, dysuria, spleen enlargement, cough and fever.

There is a large demand for new fungicides for use in food protection, agriculture and medicine. In recent years there has been a growing trend to evaluate the antimicrobial activity of the extracts and isolates of medicinal plants, because of resistance developed by pathogens, gross side effects of synthetic drugs due to

indiscriminate use and their expensive treatment regimen (Nychas, 1995; Tauxe, 1997; Cowan, 1999; Smid and Gorris, 1999; Sharif, 2001; and Tomoko et al., 2002).

In recent times, the plant extracts and isolates have been subjected to rigorous bio-chemical, chemical, clinical, pharmacological, toxicological investigations and many new therapeutic applications have been emerged out (Pinheiro, 1987, and Takahashi, 2001). The present study was an attempt to investigate and evaluate the antifungal activity of different extracts prepared from *B. stracheyi.*

In our previous study, it was observed that no previous work done on antifungal activity of *Bergenia stracheyi* (Vinesh Kumar and Devendra Tyagi, 2013). Therefore, present research work was undertaken to determine the antifungal activity of different extracts of *B. stracheyi.*

Materials and Methods

Plant collection and Extraction

The plants of *Bergenia stracheyi* were collected from the Alpine slope of Kumaun Himalaya of Uttarakhand State, India. The identification of *Bergenia stracheyi* was done with the help of Department of Botany, D.A.V. (P.G.) College, Dehradun, Uttarakhand. Before extraction plant tissue were dried under controlled conditions in laboratory. The dried roots and rhizomes of *B. stracheyi* were subjected to reduction to coarse powder using hammer grinding mill.The coarse powder was extracted with organic solvents of different polarity (i.e. petroleum ether, chloroform, ethyl acetate and ethanol) in a Soxhlet extractor. The crude extracts were evaporated to dryness in rotator evaporator under low temperature and reduced pressure.

Antifungal activity

The antifungal activity had been tested against six fungal species viz. *Alternaria alternate, Aspergillus niger, Colletotrichum gloeosporioides, Fusarium oxysporium, Ganoderma lucidum* and *Rhizoctonia solani*.different fungi. The antifungal study was carried out in two parts.Inhibition Concentration (IC_{50}) and Minimum Inhibitory oncentration (MIC).

Inhibition Concentration (IC_{50})

The IC_{50} is the concentration at which 50 percent inhibition of mycelia growth of the test fungus occurs. Study was carried out by poisoned food technique using different concentrations of extracts against all the six test fungi.

Minimum Inhibitory Concentrations (MIC)

The MIC was determined as that concentration above which the fungal growth was totally suppressed and below which the fungus resumed growth. The MIC at which no mycelial growth of the test fungus was seen or 100 percent inhibition of the fungus growth was determined. Study was carried out by poisoned food technique using different concentrations of extracts against all the six test fungi.

Procedure of IC_{50} and MIC

A culture of the test fungi was grown on Potato Dextrose Agar (PDA) medium for certain period (generally 7 days) at the

optimum temperature (25±1°C) for growth. The solvent used for dissolving extract was taken on the basis of polarity. PDA supplemented with different plant extracts at four concentrations (0.5, 1.0, 1.5, and 2.0 %) was poured in the Petri plates under aseptic conditions. After solidification, small dish (0.5 cm dia.) of the fungus culture was cut with a sterile cork borer and transferred aseptically upside down at the centre of petri dish. Suitable checks were maintained, where the culture discs were grown under same conditions on PDA without extract. Solvent checks were maintained to check out the inhibitory effect of solvent on fungi in which PDA was mixed with solvent (a solvent, which is used for dissolving extracts). Petri plates were incubated at 25 ±1°C. The radial six fungi were selected for bioassay viz. *Alternaria alternate, Aspergillus niger, Colletotrichum gloeosporioides, Fusarium oxysporium, Ganoderma lucidum* and *Rhizoctonia solani.* Growth of fungus colony was measured after every 24h till the fungus in the control plates completely occupied it. Three replications were maintained for each treatment. The antifungal activity was evaluated by measuring the relative growth of fungus in treatment vis-à-vis control. To maintain aseptic condition (the word aseptic means "without microorganisms") throughout the experiment is very important. The percent growth inhibition over control was worked out using the formula.

$$I = C\text{-}T/C \times 100$$

Where, I is inhibition percent, C is colony diameter in control (cm) and T is colony diameter in treatment (cm) (Janssen et *al*., 1986; and Barrata *et al*., 1998).

Results and Discussion

Antifungal activity of all extracts of *Bergenia stracheyi* was carried out in two steps and accordingly results are discussed as follows:

Inhibition Concentration (IC_{50})

The ethyl acetate, chloroform, ethanol and petroleum ether extracts of *B. stracheyi* was screened by poisoned food technique to select out their concentration on the criterion of more than 50 percent inhibition of radial growth of various fungi.

Antifungal screening of ethanol extract

The results of antifungal screening of ethanol extract of *Bergenia stracheyi* are given in table no.1.1 and comparative effectiveness is shown in fig. no. 1.1. When interaction between fungus and concentration was studied, it was observed that no fungi inhibited more than 50 percent at lowest concentrations 0.5 percent. The results indicate that at 0.5 percent concentration ethanolic extract had maximum 40 percent growth inhibition against *R. solani* and lowest 10 percent against *Fusarium oxysporium.* Two test fungi, *F. oxysporium* and *G. lucidium* had lower than 50 percent growth inhibition at 1.5 percent concentration, while the remaining four fungi were inhibited more than 50 percent. At 2.5 percent concentration extract is most effective against *A.niger.* At 3.5 percent concentration, complete growth reduction observed against *A. alternate, A. niger, G. lucidium* and *R. solani.* The antifungal activity of ethanolic extract of *B. stracheyi* was not very remarkable effective against two test fungi i.e. *C. gloeosporiodes* and *F. oxysporium.*

Table.1.1 Effect of different concentrations of ethanol extract of *Bergenia stracheyi* on the radial growth of fungi.

Fungus species	Concentration/Growth inhibition (%)					Mean
	Control	0.5	1.5	2.5	3.5	
Alternaria alternate	0	35	55	79.5	100	67.375
Aspergillus niger	0	40	58.5	88.7	100	71.8
Colletotrichum gloeosporiodes	0	25	45	66	87.4	55.85
Fusarium oxysporium	0	10	35	58.3	79.2	45.625
Ganoderma lucidum	0	38	61	83.2	100	70.55
Rhizoctonia solani	0	45	65.3	87	100	74.325

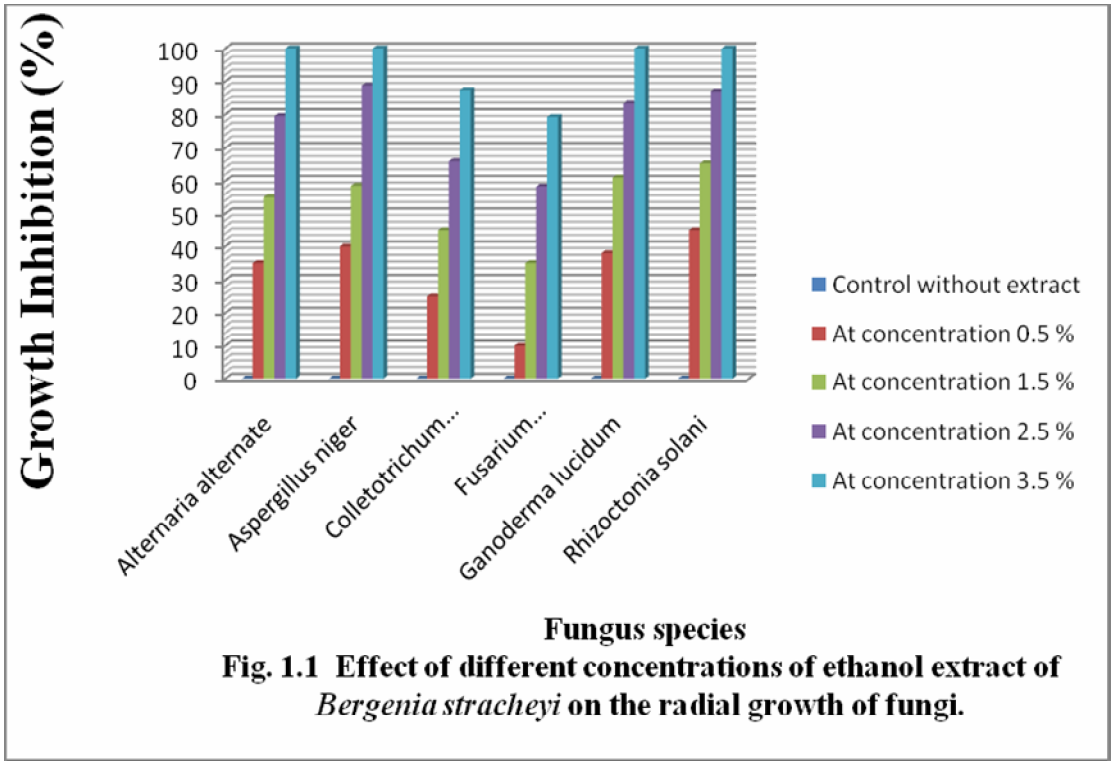


Fig. 1.1 Effect of different concentrations of ethanol extract of *Bergenia stracheyi* on the radial growth of fungi.

Antifungal screening of chloroform extract

The results of antifungal screening of chloroform extract of *Bergenia stracheyi* are given in table no.1.2. and comparative effectiveness is shown graphically in fig. no.1.2. The results indicated that at 0.5 percent concentration chloroform extract had some growth reduction against *C. gloeosporiodes, R. solani,* and *G. lucidium* but no activity was observed for *A. alternate, A. niger* and *F. oxysporium*. The 37.7 percent growth inhibition at 1.5 percent was observed against *G. lucidium,* 36.8 percent against *R. solani,* 27.8 percent against C. *gloeosporiodes,* 12.3 percent against *A. alternate,* 10 percent against *F. oxysporium,* and 7.5 percent against *A. niger.* At 2.5 percent concentration the maximum growth inhibition reported 57.3 percent against *G. lucidium.* At 3.5 percent concentration the growth inhibitions were 45 percent, 25 percent, 79.2 percent, 49.7 percent, 83.5 percent and 72.2 percent for *A. alternate, A. niger, C. gloeosporiodes, F. oxysporium, G. lucidium* and *R. solani* respectively. Thus it can be concluded that chloroform extract of *B. stracheyi* was not very effective for antifungal activity.

Antifungal screening of ethyl acetate

The results of antifungal screening of ethyl acetate extract of *Bergenia stracheyi* are given in table no.1.3, and comparative effectiveness is shown graphically in fig. no.1.3. The results showed that 0.5 percent concentration ethyl acetate has some antifungal activity. The growth inhibition at 1.5 percent was observed 27.8 percent against C. *gloeosporiodes,* 26.2 percent against *A. niger,* 25 percent against *A. alternate,* 22.2 percent against *R. solani,*13 percent against *G. lucidium,* and 9 percent against *F.oxysporium.* At 2.5 percent concentration the maximum growth inhibition observed 38.4 percent against *C. gloeosporiodes.* At 3.5 percent concentration the growth inhibitions were 45 percent, 42.3 percent, 43.5 percent, 24 percent, 26.5 percent and 38 percent for *A. alternate, A. niger, C. gloeosporiodes, F. oxysporium, G. lucidium* and *R. solani* respectively.

When interaction between fungus and concentration was studied, it was observed that up to 3.5 percent all test fungi had less than 50 percent growth reduction. Thus it can be concluded that ethyl acetate extract of *B. stracheyi* was not very effective for antifungal activity.

Antifungal screening of ether extract

The results of ether extract of *B. stracheyi* are given in table no.1.4, and graphical representation of antifungal activity of ether extract is shown in fig. no.1.4. The results showed that 0.5 percent concentration ether extract has some antifungal activity. The growth inhibition at 1.5 percent concentration observed 27.9 percent against *C. gloeosporiodes,* 24.4 percent against A. alternate, 23 percent against *A. niger,* 19.6 percent against *R. solani,* 16.4 percent against *F.oxysporium* and 8 percent against *G. lucidium*. At 2.5 percent concentration the maximum growth inhibition observed 38.3 percent against *C. gloeosporiodes*. At 3.5 percent concentration the growth inhibitions were 50 percent, 49.5 percent, 55.5 percent, 35.6 percent, 23.8 percent and 43 percent for *A. alternata, A. niger, C. gloeosporiodes, F. oxysporium, G. lucidium* and *R. solani* respectively. Thus, it can be concluded that ether extract of *B. stracheyi* is not very effective for antifungal activity.

Table.1.2 Effect of different concentrations of chloroform extract of *Bergenia stracheyi* on the radial growth of fungi

Fungus species	Concentration/Growth inhibition (%)					Mean
	0	**0.5**	**1.5**	**2.5**	**3.5**	
Alternaria alternata	0	0	12.3	25	45	20.575
Aspergillus niger	0	0	7.5	15	25	11.875
Colletotrichum gloeosporiodes	0	15	28.7	50.4	79.2	43.325
Fusarium oxysporium	0	0	10	26.4	49.7	21.525
Ganoderma lucidum	0	18	37.7	57.3	83.5	49.125
Rhizoctonia solani	0	21	36.8	53.3	72.2	45.825

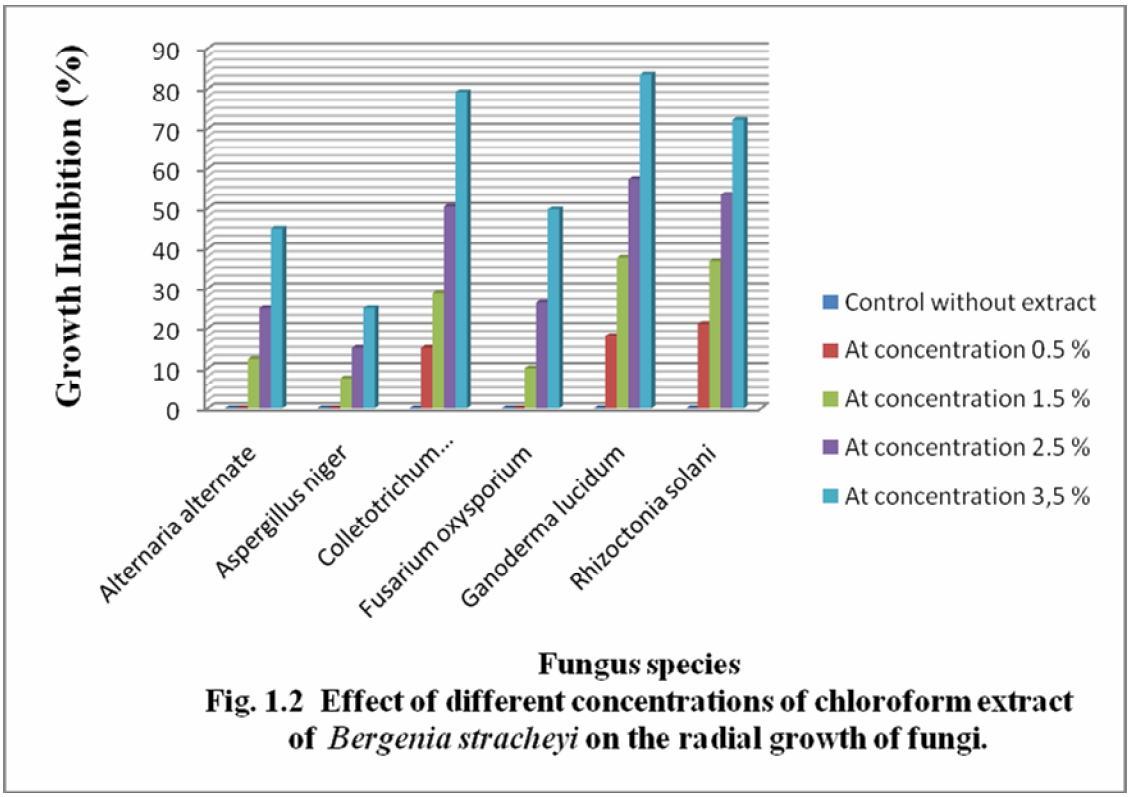


Fig. 1.2 Effect of different concentrations of chloroform extract of *Bergenia stracheyi* on the radial growth of fungi.

Table.1.3 Effect of different concentrations of ethyl acetate extract of *Bergenia stracheyi on* the radial growth of fungi.

Fungus species	Concentration/Growth inhibition (%)					Mean
	0	**0.5**	**1.5**	**2.5**	**3.5**	
Alternaria alternata	0	10	25	36	45	29
Aspergillus niger	0	17	26.2	32.5	42.3	29.5
Colletotrichum gloeosporiodes	0	18.5	27.8	38.4	43.5	32.05
Fusarium oxysporium	0	5	9	18	24	14
Ganoderma lucidum	0	9	13	19	26.5	16.875
Rhizoctonia solani	0	12	16.4	22.2	38	22.15

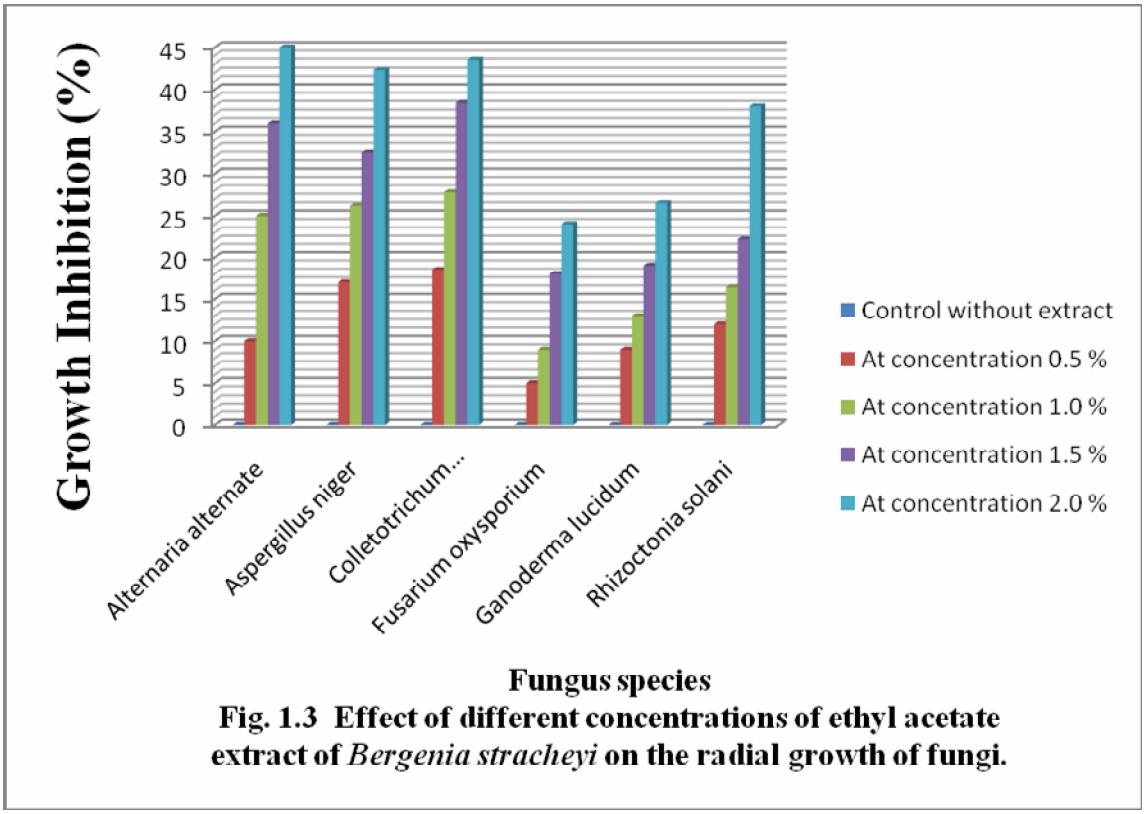


Fig. 1.3 Effect of different concentrations of ethyl acetate extract of *Bergenia stracheyi* on the radial growth of fungi.

Table.1.4 Effect of different concentrations of ether extract of *Bergenia stracheyi* on the radial growth of fungi.

Fungus species	Concentration/Growth inhibition (%)					Mean
	0	0.5	1.5	2.5	3.5	
Alternaria alternata	0	14	24.4	35.34	50.6	31.085
Aspergillus niger	0	14.2	23	38.3	49.5	31.25
Colletotrichum gloeosporiodes	0	17.4	27.9	38.3	55.5	34.775
Fusarium oxysporium	0	10	16.4	25.7	35.6	21.925
Ganoderma lucidum	0	0	8	14.6	23.8	11.6
Rhizoctonia solani	0	12	19.6	27.5	43.4	25.625

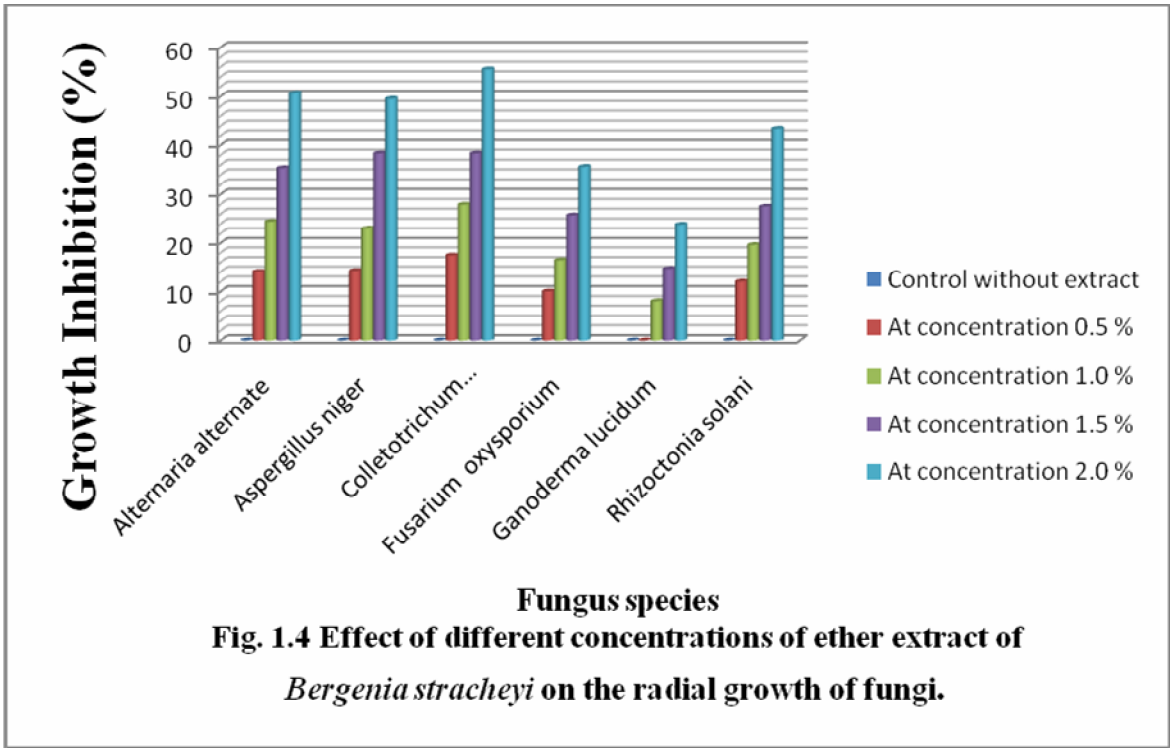


Fig. 1.4 Effect of different concentrations of ether extract of *Bergenia stracheyi* on the radial growth of fungi.

Minimum Inhibotory Concentration (MIC)

The MIC was determined as that concentration above which the fungal growth was totally suppressed and below which the fungus resumed growth. Experiments were carried out by poisoned food technique using different concentrations of ethanol. The MIC at which no mycelial growth of the test fungus was seen or 100 percent inhibition of the fungus growth was determined.

The results of MIC of ethanol extract of *B. stracheyi* showed that at lower concentrations i.e. 0.5 percent, 1.5 percent and 2.5 percent no fungi had 100 percent growth inhibition. Only four fungi i.e., *Alternaria alternate, Aspergillus niger, Ganoderma lucidum* and *Rhizoctonia solani* had total inhibition of growth at 3.5 percent concentration of ethanol extract. The remaining two fungi, *Fusarium oxysporium* and had complete growth reduction at concentration of 5.5 percent. Due to the weak antifungal activity of chloroform, ethyl acetate and ether extract of *B. stracheyi,* the MIC was not studied. The test results of ethanolic extracts to be strongly effective against a variety of disease producing microorganisms.

The chemical compounds particularly polyphenols such as bergenin, arbutin, hydroquinone, β-sitosterol, methyl arbutin, are reported in different species of *Bergenia* can be responsible to be for antifungal activity (Jamal *et al*. 2009, and Holland *et al.,* 1978).

The results obtained on the *B. stracheyi* for antifungal activity support to the folkloric use of skin wound healing properties in Kashmir i.e. it is referred as Zakhme Hayat in vernacular which means wound and skin protection. Although no prior study have been reported for antifungal activity of *Bergenia stracheyi*. This is the first report of its kind. Although, there are two references, where ethanol extract of dried root of *Bergenia schemidtii* and *Bergenia ciliata* have been tested for antifungal activity (Kakwaro, 1976; Mazhar-Ul-Islam *et al*., 2002). Therefore it can be concluded that skin infections and plant infections can be checked by applying the extract of *Bergenia stracheyi* but it was toxic in nature, therefore authentication of claim for folklore use in rather still an unresolved issue for its application.

The present work exclusively deals with a greater scope of analysis using four more solvents: ethyl acetate, chloroform, ethanol and petroleum ether which was not being undertaken by earlier workers for *Bergenia stracheyi* herb. The results of antifungal screening showed that ethanol extract of *Bergenia stracheyi* is more effective against all test fungi. The chloroform, ethyl acetate and ether extract have shown antifungal activity but not sufficient to inhibit the total growth of fungus at lower concentrations. In future, further research should be carried out on antifungal activity of the isolated compounds from this herb.

References

Barrata, T.M., Dorman, H.J.D. et al., 1998. Antimicrobial and antioxidant properties of some commercial essential oils. Flavour Fragr. J. 13: 335-244.

Cowan, M.M., 1999. Plants products as antimicrobial agents. Clin. Microbial. Rev. 12: 564-582.

Holland, H. L.,P.R.P. Diakow and Taylor, G. J. 1978. Can. J. Chem. 56: 3121-3127.

Jamal, A., W.A. Nasser, Yaacob, B. Laily, Din, M. Bohari, Yamin and Jalifah Latip. 2009. ARPN. J.Engineer. App. Sci.4: 92-95.

Janssen, A.M., Scheffer, J.J.C. *et al*., 1986. Antimicrobial activity of essential oil: 1976-86 literature review aspect of test methods. Planta Med.53: 395-398.

Kakwaro J.O., 1976. Medicinal Plants of East Africa. East Africa Literature Bureau, Nairobi.

Mazhar-Ul-Islam, Iqbal Azhar, Khan Usmanghani, Mohammad Aslam Gill, Shahab-Ud-Din and Afzal Ahmad. 2002. Antifungal activity evaluation of *Bergenia ciliata.* Pak. J. Pharm. 19(2):1-6.

Nychas G.J., 1995. Natural antimicrobials from plants. in new methods of food preservation; Gould, G. W. Ed; Blackie Academic and Professional: London, United Kingdom, pp.58-59.

Pinheiro, C.E., 1987. Effect of guarana and *Stevia rebaudiana* bertoni (leaves) extracts, and stevioside, on the fermentation and synthesis of extracellular insoluble polysaccharides of dental plaque. Rev. Odont. Usp. 1: 4:9-13.

Sheriff, Z.U., 2001. Modern herbal therapy for common ailments. Nature Pharmacy Series (Volume 1), Spectrum Book Limitid, Ibadan, Nigeria in Association with Safari Books (Export) Limited, United Kingdom, pp. 9-84.

Smid E.J., and Gorris L.G.M. 1999. Natural antimicrobials for food preservation. in handbook.

Takaki, M., 1985. Antimicrobial activity in leaves extracts of Stevia rebaudiana. Antiviral Res. 49: 1: 15-24.

Tauxe R.V., 1997. Emerging food borne diseases: an evolving public challenge. Diary Food Environ. Sanit. 17:788-795.

Tomoko, N., Takashi, A. *et al*., 2002. Antimicrobial activity of extracts prepared from tropical and subtropical plants on methicillin-resistant *Staphylococcus aureus*. *J*. Health Sci. 48: 273-276.

Vinesh Kumar and Devendra Tyagi., 2013. Review on phytochemiocal, ethanomedical and biological studies of medically useful genus *Begenia*. Int. J. Curr. Microbiol. App. Sci. 2 (5): 328-334.