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EVALUATION OF ANTIFUNGAL ACTIVITIES OF *BERGENIA CILIATA* PLANT

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ABSTRACT

There are many synthetic and natural product-based drugs available for treating fungal infections, but they are not consistently effective. The increased use of antifungal agents has resulted in the development of resistance to these drugs. The spread of multidrug-resistant strains of fungus and the reduced number of drugs available make it necessary to discover new classes of antifungal from natural products including medicinal plants. Screening of plants for biologically active compounds against fungi is a renewed interesting research field. In this study Bergenia ciliate plant is screened against six fungal strains viz Aspergillus niger ,Aspergillus flavus, Rhizopus stolonifer , Trichoderma viride,Saccharomyces cerevisiae and Pleurotus ostreatus. The antifungal activity was determined in ethanol,methanol ,acetone and aqueous extracts of this plant. MIC was determined by agar well method between concentrations 800-200mg/ml. The results of antifungal screening showed that ethanol extract of B.ciliata is effective against all test strains and can be used to treat infections with pathogenic fungi.The activity of ethanolic extract might be due to the maximum solubility of chemical compounds in this solvent.

***Key Words*:** *Antifungal, discover, multidrug-resistance, synthetic and strain*

1. INTRODUCTION

Fungal kingdom comprises of a tremendous diversity of taxa with different ecology,life-cycle, strategy and morphology. But, little is known about the true biodiversity of this kingdom. There are millions of fungal species on the Earth, but only about 300 species are known to cause diseases in humans **[1]**. Fungal diseases are ofsten caused by fungi that are common in the environment. Fungi are present in soil, on plants and trees as well as on many surfaces and on human skin. Most fungi are not dangerous, but some may be harmful to health. Fungal infections are causing serious problems in aged persons and immunocompromised patients worldwide. Generally, healthy individuals have a strong immunity against fungal infections. However, individuals who have a weak immune system such as children, the elderly, those with HIV, and patients who have received a transplant surgery, chemotherapy, or have been taking immunosuppressant's for long periods are the most vulnerable to fungal infections **[2].** There is little understanding of the true impact of fungal infections on patients and healthcare systems since often superficial infections are diagnosed at a general practice level, there is little routine fungal specific surveillance and diagnosing invasive fungal infections is notoriously difficult.

Knowledge about the global incidence of fungal diseases has been impaired by lack of regular national surveillance systems, no obligatory reporting of fungal diseases, poor clinician suspicion outside specialised units, poor diagnostic test performance (especially for culture) and few well-designed published studies. Some fungal diseases are only recently recognised **[3,4** and **5].**

There are many synthetic and natural product-based drugs available for treating fungal infections, but they are not consistently effective **[6,7**and **8]**. Furthermore, the development of resistance in fungi against most of the drugs has now been reported for several years **[9]**. The use of amphotericin B, known as the "gold standard", is limited because of its infusion-related problems and nephrotoxicity **[10** and **11]**. In addition, the low efficacy, and side-effects and resistance associated with the existing drugs, highlight the advent of safe, novel, and effective antifungal drugs. Plants produce a great deal of secondary metabolites, many of them with antifungal activity.Based on the knowledge that plants develop their own defence against fungal pathogens **[12]**, they appear as an interesting source for antifungal compounds. Thus, aim of present study is to evaluate the antifungal activity of *Bergenia ciliata* plant which is of great medicinal importance. It is used in traditional Ayurvedic medicine for the treatment of several diseases in Nepal, India, Pakistan, Bhutan and some other countries. *Bergenia ciliata* plant was screened against six fungal species and was found active against all species.

Medicinal plants have also been reported in traditional systems of medicine for the treatment of both human and animal mycoses, and are considered to be a valuable source for the discovery of new antifungal drugs **[13]**. In my previous study, it was observed that little work is done on antifungal activity of *Bergenia ciliata* . Therefore, present research work was undertaken to determine the antifungal activity of different extracts of *B.ciliata* plant.

2. MATERIALS AND METHODS

2.1 Plant Collection

Fresh plants were collected from hilly areas of Kashmir valley from Apharvat nearby Gulmarg and authenticated at the Botany department of Kashmir University. The root and stem of the authenticated herbal plant were then collected and dried under sun for six weeks. The dried sample was cut into smaller pieces and ground into fine particles with a grinder. Lowering particle size increases surface contact between sample and extraction solvents. The powdered sample was bagged in transparent poly bags and stored in air tight container for further use.

2.2 Preparation of extracts

Extraction is an important step in the screening of bio-active compounds from plant materials. The purpose of all extraction is to separate the soluble plant metabolites, leaving behind the insoluble cellular marc (residue) that is not required, with the help of solvents. Different extracts were prepared in different solvents viz, methanol, ethanol, acetone and aqueous.

For aqueous extraction, 8g of dried plant powder was added to 100ml distilled water and boiled on slow heat for 15-20min. It was then filtered through 7 layers of muslin cloth and centrifuged at 5000rpm for 15 min. The supernatant was then collected and concentrated (evaporated) to make the final volume one-tenth of its original

volume ie.,80% extract slikewise 60%,40% and 20% extracts were prepared **[14]**.It was stored at 4^{o}C in air tight bottles for rurther work.

For solvent extraction, 8g of dried plant powder was taken in 100 ml of organic solvent (methanol,ethanol and acetone) in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 24 hrs. After 24 hours the supernatant was collected and the solvent was evaporated to make the final volume one-tenth of its original volume **[14]** thereafter, stored at 4^{o}C in airtight bottles for further work.

2.3 Microorganisms

Six fungal cultures, *Aspergillus niger*, *Aspergillus flavus, Rhizopus stolonifer*, *Trichoderma viride*, *Saccharomyces cerevisiae* and *Pleurotus ostreatus* were used in antifungal assay. Cultures were obtained from Codon biotech. All the strains were maintained on agar slants at 4℃ for antimicrobial tests.

2.4 Screening for antifungal activity

A culture of the test fungi was grown on Potato Dextrose Agar (PDA) medium for certain period (generally 3 days) at the optimum temperature (25±1°C) for growth. The required amount of each fungal strain was introduced in 2 ml Potato Dextrose broth. This suspension was homogeneously spread on Petri plates containing Potato Dextrose agar media using sterile swabs. Wells of 8 mm diameter were punched into the agar medium and sealed with molten agar to prevent leaching of the compound. The wells are filled with 150µl of plant extract of known concentration and solvent blank separately. The plates are incubated at 25°C, for 72 hrs and average diameter of the inhibition zones surrounding the wells were measured for the result **[15** and **16]**.

2.5 Determination of Minimum Inhibition Concentration

Minimum inhibition concentration was determined using the agar well method **[17].** The MIC was evaluated on plant extract that showed antifungal activity in the Agar well diffusion assay on any organism**. [18]** for this method two-fold serial dilutions of *Bergenia ciliata* extrat were prepared in the solvent.8mm diameter wells were cut in PDA plates,bases of wells were sealed using sterile molten PDA. Thereafter, plates were inoculated with 72hr test culture of fungi using swabs. After inoculation, plates were allowed to dry for 15 min. at room temperature in laminar air flow before dispensing 150 μL of diluted extract and pure solvent. The plates were incubated at 37°C for 72hrs.The lowest concentration of the drug that completely inhibits the growth was determined after 3day incubation at 37 °C. All tests were conducted in triplicate.

3. RESULT AND DISCUSSION

Plant products have been part of phytomedicines since time immemorial. These can be derived from any part of the plant like bark, leaves, flowers, seeds, etc **[19]** i.e., any part of the plant may contain active components. The biological activities of a plant extract depend on quantity of its bioactive markers or metabolites, which is affected by various factors such as the plant species, the time and season of harvest, climate, altitude, latitude, longitude, place of collection, age and size of a plant/plant part and phenology **[20].**

Knowledge of the chemical constituents of plants is desirable because such information will be valuable for the synthesis of complex chemical substances. Such phytochemical screening of various plants is reported by many workers **[21,22** and **23]**.One of the species Bergenia ciliata under present study is good source of secondary metabolites in controlling many diseases. *Bergenia* species have a number of secondary

metabolites. These are Bergenin, Tannic acid, Gallic acid, Stigmesterol, β-Sitosterol, Catechin, (+)-Afzelechin, 1,8-cineole, Isovalaric acid, (+)-(6S)-parasorbic acid, Arbutin, Phytol, Caryophyllene, Damascenone, β-eudesmol, 3-methyl-2-buten-1-ol, (Z)-asarone, Terpinen-4-ol, Paashaanolactone **[24].**

Bergenia ciliata plant was screened against six fungal strains *viz Aspergillus niger , Aspergillus flavus, Rhizopus stolonifer , Trichoderma viride, Saccharomyces cerevisiae* and *Pleurotus ostreatus.* The antifungal activity was determined in ethanol, methanol ,acetone and aqueous extracts of this plant. Ethanol extract of leaf and root was effective against all strains but ethanol extract of stem was effective against *Pleurotus ostreatus* and *Saccharomyces cerevisiae* only, methanol extract of both root and stem were effective against *Saccharomyces cerevisiae* only ,acetone extract of root showed activity against *Rhizopus stolonifer* and *Saccharomyces cerevisiae* while stem extract of acetone showed activity against *Pleurotus ostreatus* and *Saccharomyces cerevisiae.*Aqueous extract showed no activity at all. The chemical compounds particularly asarone and afzelechin can be responsible for antifungal activity of this plant as visualized from the chromatograms of LC-MS.

Table (3.1) Antifungal Activity Of Different Extracts Of *Bergenia ciliata* Leaves

Fungal strains	Zone of inhibition in mm							
	Ethanol		Methanol		Acetone		Aqueous	
	80%	40%	80%	40%	80%	40%	80%	40%
A.niger	22	20	NA	NA	18	NA	NA	NA
A.flavus	18	10	NA	NA	28	NA	-	-
T.viride	18	17	NA	NA	24	NA	-	-
R.stolonifer	24	NA	15	NA	NA	NA	-	-
S.cerviasiae	34	12	17	13	25	13	-	-
P.ostreatus	NA	NA	NA	NA	NA	NA		

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Table (3.2) Antifungal Activity Of Different Extracts Of *Bergenia Ciliata* Root

Fungal strains	Zone of inhibition in mm							
	Ethanol		Methanol		Acetone		Aqueous	
	80%	40%	80%	40%	80%	40%	80%	40%
A.niger	25	NA	NA	NA	NA	NA	NA	NA
A.flavus	26	25	NA	NA	NA	NA	-	-
T.viride	25	15	NA	NA	NA	NA	-	-
R.stolonifer	47	23	NA	NA	25	NA	-	-
S.cerviasiae	22	11	32	10	22	13		

P.ostreatus	NA	NA	NA	NA	NA	NA	-	-

NA=no activity

Table (3.3) Minimum Inhibitory Concentration MIC of *Bergenia Ciliata* Against The Tested Fungi;

Fungal cultures	MIC mg/ml Root extract	MIC mg/ml Leaf extract
Aspergillus niger	200	200
Aspergillus flavus	400	200
Tricoderma viride	400	200
Rhizopus stolonifer	400	400
Saccharomyces cerviasiae	400	200

From the above results it can be stated that in case of root extract *Rhizopus stolonifer* has the maximum zone of inhibition with 47 mm value in ethanol extract while *Saccharomyces cerevisiae* has the lowest value with 13 mm zone in acetone extract and in case of leaf extract again ethanol possesses the maximum zone of inhibition with 34mm value in *Saccharomyces cerevisiae* and acetone possess the lowest with 13mm value showing that ethanol root extract of *Bergenia ciliata* has higher antifungal activity as compared to leaf extract perhaps due to the maximum solubility of secondary metabolites in it. Determination of MIC value further showed antifungal activity of *Bergenia ciliata* . The MIC was defined as the lowest concentration of antimicrobial that inhibited the visible growth of a microorganism after 72hr incubation. MIC values did not exhibit substantial variations when compared to the trend of inhibition shown with the plate diffusion method.Generally, larger inhibition zone values correlated with lower MIC. The study showed that *Bergenia ciliata* can inhibit all tested fungi with very low concentration.This result further strengthens the strong antifungal activity of this plant and also concludes that ethanolic extract of this plant can be used to treat infections with pathogenic fungi.

4. CONCLUSION

It can be concluded from the above results that all extracts except aqueous extract possess antifungal activity but, ethanol extract of *Bergenia ciliata* has significant activity against all test fungi perhaps due to the maximum solubility of compounds which have significant fungal activity like, asarone and afzelechin present in this plant. In future,further research should be carried out on antifungal activity of the isolated compounds from this herb.

REFERENCES

1. Garcia- solace, and Ma,Casadevall, A.(2010).Global warming will bring new fungal diseases for animals. *m Bio*1.

2. Jain, A., Jain, S., and Rawat, S.(2010). Emerging fungal infections among children: a review on its clinical manifestations, diagnosis, and prevention. *J Pharm Bioallied Sci 2:314–320.*

3. Denning, D.W. (2016).Minimizing fungal disease deaths will allow the UNAIDS target of reducing annual AIDS deaths below 500,000 by 2020 to be realized. *Philos. Trans. R. Soc*. B *371.*

4. Denning, D.W.(2015). The ambitious "95-95 by 2025" roadmap for the diagnosis and management of fungal diseases.*Thorax 70:613–614.*

5. Pegorie, M., Denning, D.W., and Welfare, W.(2017). Estimating the burden of invasive and serious fungal disease in the United Kingdom. *J. Infect 74(1):60–71.*

6. Lazar, J.D., and Wilner, K.D .(1990). Drug interactions with fluconazole. *Rev Infect Dis 12:S327–S333.*

7. Gearhart, M.O.(1994). Worsening of lung function with fluconazole and review of azole antifungal hepatotoxicity. *Ann Pharmacother 28:1177–1181.*

8. Goa, K.I., and Barradell, L.B.(1995).Fluconazole: an update of its pharmacodynamic and pharmacokinetic properties and therapeutic use in major superficial and systemic mycoses in immunocompromised patients. *Drugs 50:658–690.*

9.Cuenca-Estrella, M., Mellado, E., Diaz-Guerra, T.M., Monzon, A., and Rodriguez-Tudela, J.L. (2000).Susceptibility of fluconazole-resistant clinical isolates of Candida spp. to echinocandin LY303366, itraconazole and amphotericin B. *J Antimicrob Chemother 46:475–477.*

10. Grasela, T.H., Goodwin, S.D., and Walawander, M.K .(1990). Prospective surveillance of intravenous amphotericin B use patterns. *Pharmacother 10:341–348.*

11. Fanos, V., and Cataldi, L.(2000).Amphotericin B-induced nephrotoxicity: a review. *J Chemother 12:463–470.*

12. Gurgel, L.A., Sidrim, J.J.C., Martins, D.T., Filho,C.V., and Rao, V.S .(2005). In vitro antifungal activity of dragon's blood from Croton urucurana against dermatophytes. *J Ethnopharmacol 97:409–412.*

13. Mathias-Mundy, E., and McCorkle, C.(1995). Ethnoveterinary medicine and development: a review of the literature. In: Warren DM, Surrerwer L, Broshenka D (eds) The cultural dimension of indigenous knowledge systems. *Intermediate Technology Publications, London* 488–498.

14. Parekh, J., Nair, R., and Chanda, S.(2005). Preliminary screening of some folklore medicinal plants from western India for potential antimicrobial activity. *Indian J.Pharmacol* 37: 408-409.

15. Srinivasan, D., Sangeetha, N., Suresh, T., and Lakshmanaperumalsamy, P.(2001). Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *J. Ethnopharmaco*l *74: 217-220.*

16. Sen, A., and Batra, A.(2012).Evaluation of antimicrobial activity of different solvent extracts of Medicinal plant:Melia Azedarach L.*International journal of current and pharmaceutical research 4(2).*

17. Myer, and Koshi,. (1982). Manual of diagnostic procedures in medical microbiology and immunology and serology, Christian Medical College and Hospital. *Vellore 72:109.*

18. Bhoj, R.S. (2013).Evaluation of Antibacterial Activity of Sage (Salvia officinalis) Oil on Veterinary Clinical Isolates of Bacteria.*Noto-are Medicine.*

19. Cragg, G.M., and David, J.N.(2001). Natural product drug discovery in the next millennium. *J. Pharm. Biol 39: 8-17.*

20. Kunle, O.F., Egharevba, H.O., and Ahmadu, P.O. (2012).Standardization of herbal medicines: a review. *Int J Biodivers Conserv 4:101–112.*

21. Siddiqui, S., Verma, A., Rather, A.A., Jabeen, F., and Meghvansi, M.K.(2009). Preliminary phytochemicals analysis of some important medicinal and aromatic plants. *Advan. Biol. Res 3(5-6): 188-195.*

22. Kumar, A., Rajkumar, P., and Kanimozhi, M.(2010). Phytochemical screening and antimicobial activity from five Indian medicinal plants against human pathogens. *Middle-East J. Sci. Res 5(3): 157-162.*

23. Chitravadivu, C., Manian, S., and Kalaichelvi, K.(2009). Qualitative analysis of selected medicinal plants, Tamilnadu, India. *Middle-East J. Sci. Res 4(3*): *144-146.*

24. Chauhan, R., Ruby, K.M., and Dwivedi, J .(2012). *Bergenia ciliata* mine of medicinal properties: a review *Int. J. Pharm. Sci 15(2)*:*20-23*.