ANTIFUNGAL ACTIVITY OF AQUEOUS LEAF EXTRACT OF *OCIMUM SANCTUM* ON DOMINANT FUNGAL SPECIES OF MONUMENTS

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Four common species of fungi isolated *A. niger, Rizopous, Cladpsporium and Curvularia lunata* from archaeological site were subjected to laboratory experiment involving in vitro control of the fungal species using plant extracts. Aqueous leaves extract at 10 %, 20 %, 30 % and 40 % with the control (basal medium) concentrations tested on potato dextrose agar (PDA) for activity against mycelium growth were determined at 26±1 ^{0}C with three replicated plates. Fungal growth values recorded were generally low compared with the control (without extract petri plate). Inhibitory action of the extract on fungal growth increased with increases in concentration of extract. A study was carried out to evaluate the antifungal properties of *Ocimum Sanctum Linn*. Aqueous extract on common fungal species, isolated from Bhand Deol temple at Arang of Chhattisgarh state using the well in PDA media. The in vitro studies have been performed by using leaf aqueous extract of *Ocimum Sanctum Linn.* (Tulsi plant). Extract showed antifungal activity. Different concentration viz. 10 %, 20 %, 30 % and 40 % of solution prepared for the study. It was found that the plant extract at 40 % concentration were effective in reducing the mycelial growth of *A. niger, Rizopous, Cladpsporium sp*. whereas 30 % of plant extract effective for *Curvularia lunata.* Concentrations of extract which inhibit 75 % and above having known as effective in this study. Plant extracts readily available and affordable and environmentally friendly in the control of fungal disease.

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INTRODUCTION

The application of synthetic chemical on the monuments as a biocides are toxic and hazardous to the environment and to public health other than the stone itself. The biocide application can be harmful for conservators and the environment[1] and little is known about the consequences of repeated applications.[2] **The EC regulations (BPD 98/8/EC n 20 June 2004)** have had as consequence the elimination from the market of the most active (and toxic) compounds applied to this aim and new approaches are made in several sectors in order to overcome this problematic.

Alternative new approach based on natural antifouling substances, are recently experimented in the marine sector for the prevention of bio settlement on ships submerged structures. Natural sources or synthetic analogues must be found to ensure supplying at a reasonable cost.[3] Medicinal plants have been used for different ailments of human beings all over the world just from the beginning of civilization. Indian traditional medicinal system includes hundreds of medicinal plants related to multiple effects.[4] Throughout the old world and especially in the tropics *Ocimum sanctum* otherwise known as Tulsi or Holy Basil is cultivated abundantly for religious and medicinal purposes. It is commonly used in Ayurveda and across the wide region of south-east Asia is widely known as a medicinal plant as well as an herbal tea.[5] Exploitation of plant metabolites in monuments protection and preservation against bio-deterioration of stone caused by fungi appear to be promising. In view of these, the authors screened some extracts one of them Tulsi (*Ocimum Sanctum Linn*.) against bio-deterioration causes by fungal species isolated and identified from the sample of Bhand Deol temple at Arang of Chhattisgarh.

MATERIALS AND METHODS

Sampling and isolation of fungi

Sample of monument was collected from Bhand Deol temple, Arang of Chhattisgarh for isolation and identification of fungal species. During the investigation period PDA media was used for the isolation of microorganisms. Sample was collected from the surface of the monument. Few drops of sample pour in the petridish and kept this petridish at 28±1 ^{0}C for 7 days for incubation.[6] At the end of incubation period fungal colonies were counted, isolated and identified with the help of available literature and finally send this culture to authentic authority: National Center of Fungal Taxonomy Delhi for identification.

Preparation of plant leaf powder

The fully grown leaf of *Ocimum Tenuiflorum* (also known as *Ocimum sanctum*) was collected from campus of various temples at Bhilai (Chhattisgarh). The collected plant material thoroughly washed with tap water and then rinsed with sterile distilled water. The leaf of Tulsi was shed dried and grind in electric mixer. The powder material was kept in air tight glass bottles. This stock powder was used for further extraction.

Preparation of aqueous leaf extract

5.00±0.05 g of dried and ground leaves powder of Tulsi was placed in a thimble of soxlate apparatus. Sample was extracted in a Soxhlet extraction system using 150 ml of distilled water. The heating power was set to two cycles/h so that six cycles of extraction were achieved within 3 h. Distilled water used in this extraction process. The crude extract solutions obtained was then concentrated using a water bath at very low temperature to remove the solvent and completely dried in an atmospheric oven. High temperature treatment was avoided to minimize the component degradation[7]. Extract was then stored at room temperature before weighing gravimetrically to determine the yields after that prepared various dilution viz 10 %, 20 %, 30 %, 40 % and 50 % concentrations of extracts for inhibition of growth of fungal species. Control treatment was done without any plant extract in petriplate.

Percentage inhibition of fungi growth by the leaf extracts was calculated using the formula.[8]

$$FG = 100\,\frac{D_c - D_r}{D_c}$$

where:

FG = inhibition of fungi growth in %

D_c=diameter of control (mm)

D_r = diameter of test (mm)

Well in agar method

A loopful of the inoculums suspension of pure 04 cultured identified fungal organism were spread uniformly on the solidified sterile culture media (PDA) in the petriplate for uniform distribution of the organism. Using a sterile cork borer a well of 0.5 cm was made in the media and in each well, plant extract was filled so as to allow the diffusion of plant extract in the media. The petriplate were incubated at for 24 hours at 30±1°C temperature and the observations were recorded as diameter of inhibitory zone in mm. Well in agar plate filled with sterile distilled water was used as control in all the experiments[9]. All the experiments were in triplicate and mean has been considered in observation Table 1.

Table 1. Effect of *Ocimum tenuiflorum* aqueous extract on fungi growth (in %)

Fungal species↓	**Concentration of extract**				
	10 %	**20%**	**30%**	**40%**	**50%**
A. niger,	10	27	67	75	88
Rizopous,	16	27	70	82	90
Cladpsporium	17	28	71	77	87
Curvularia Lunata	19	31	77	88	91

RESULT AND DISCUSSION

At a concentration of 40 %, the leaf aqueous extract of Tulsi recorded effective for *A. niger, Rizopous* and *Cladpsporium* and their percentage of inhibition are 75 %, 82 % and 77 % for that concentration respectively while concentration of 30% leaf aqueous extract is effective for *Curvularia Lunata.* The minimum inhibition by leaf extract was recorded 10 % of *A. niger* sp. by 10 % of extract while 19 % of inhibition recorded for *Curvularia Lunata* by same concentration of the extract. Percentage of inhibition increases with the concentration of plant extract. Among the extracts assayed, the leaf aqueous extract of Tulsi having antifungal properties was observed. Results showed that radial growth in all the test organisms was impaired by the addition of the extracts in the culture medium used. The test organisms differed in their reaction to the different extracts but on the whole growth inhibition increased with the concentration of plant extract. The antifungal activity of the plant for the organisms was found is in increasing order with the concentration of extract.

This study showed that the leaf aqueous extract of *Ocimum Sanctum Linn*.(Tulsi) has fungicidal activity. Few previous studies have comprehensively investigated the activity of medicinal plant leaves, bark and other parts of plant against dermatophytes and other filamentous fungi.[10]

Many researchers already reported that, plant metabolites and plant based pesticides or biocides appear to be one of the better alternatives as they are known to have minimal environmental impact and eco-friendly to conservators/ scientist involved in this fields as well as stone components in contrast to synthetic chemicals used as pesticides/biocides.[11-13] Studies on antifungal activity of different extracts of Cassia fistula and bioactivity guided isolation and identification of antifungal agent has been performed by Shilpakala et al.[14] Thus, there is a need to search for alternative eco-friendly approaches for conservation and preservation for our heritage.

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REFERENCES

[1]Price, C. A., In: *Stone conservation: an overview of current research*, Keys, A. (ed). The Getty Conservation Institute, **1996.**

[2]Fortune, I. S., Alakomi, H. L., Young, M. E., Gorbushina, A. A., Krumbein, W. E., Maxwell, I., McCullagh, C., Robertson, P., Saarela, M., Valero, J. and Vendrell, M., *Assessing the suitability of novel biocides for use on historic surfaces in Heritage Microbiology and Science – Microbes, monuments and maritime materials*, Springer Verlag, Great Britain, **2008.**

[3]Yebra, D. M., Kiil, S. and Dam-Johansen, K., *Progr. Org. Coat.*, **2003,** *50*, 75-104.

[4]Rahal, A., Singh, V., Mehra, D., Rajesh, S., and Ahmad, A. H., *J. Nat. Prod.*, **2009,** *2*, 110-115.

[5]Kumar, A., Rahal, A., Chakraborty, S., Tiwari, R., Latheef S. K. and Dhama, K., *Int. J. Agron. Plant Product.*, **2013,** *4(7)*, 1580-1589.

[6]Sharma, K. and Lanjewar, S., *J. Phytol.*, **2010,** *2(11)*, 47-49.

[7]Kumoro, A. C., Hasan, M. and Singh, H., *Sci. Asia*, **2009,** *35*, 306–309.

[8]Mondall, N. K., Mojumdar, A., Chatterje, S. K., Banerjee, A., Datta, J. K., and Gupta, S., *J. Appl. Sci. Environ. Manage*, **2009,** *13(1)*, 49-53.

[9]Shinde, V. and Dhale, D. A., *J. Phytol.*, **2011,** *3(12)*, 41-44.

[10]Rajan, S., Jeevagangai, T. J., *J. Basic Appl. Biol.*, **2009,** *3(1-2)*,76-81.

[11]Varma, J., and Dubey, N. K., *Curr. Sci.*, **1999,** *76(2)*, 172–179.

[12]Harborne, J. B., *Phytochemical methods: A guide tomodern techniques of plant analysis*, 3rd ed. Chapman & Hall Pub., London, U. K, **1998,** 7–8.

[13]Gottlieb, O. R., Borin, M. R. and Brito, N. R., *Phytochemistry*, **2002,** *60(2)*, 145–152.

[14]Shilpakala, S. R., Prathiba, J. and Malathi, R., *Eur. Rev. Med. Pharmacol. Sci.*, **2009,** *13*, 371-374.

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