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ECO-FRIENDLY CONSERVATION OF ARCHAEOLOGICAL MONUMENT WITH DHATURA LEAF EXTRACT AGAINST DOMINANT FUNGAL SPECIES

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Abstract

Four common and dominant species of fungi viz *A. niger*, *Rizopous*, *Cladpsporium and Curvularia lunata* isolated from archaeological site were subjected to laboratory experiment involving in vitro control of the fungal species using medicinal plant extracts. Aqueous leaves extract at 10%, 20%, 30%, 40% and 50% with the control (basal medium) concentrations tested on potato dextrose agar (PDA) for activity against mycelium growth were determined at 26 ± 1^0C 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 aqueous extract of *Datura stramonium* (Dhatura) dominant 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 aqueous leaf extract of *Datura stramonium* (Dhatura) plant. Extract showed antifungal activity. Different concentration of extract solutions prepared and standardized for the study. It was found that the 5 ml of 26% extract was effective in reducing the mycelial growth of *Curvularia lunata* which inhibit 74.9 % and not effective for *A. niger, Rizopous and Cladpsporium* in this study. Plant extracts readily available and affordable and environmentally friendly in the control of fungal disease.

Keywords: Mycelial growth; fungal species; *Dhatura* plant extract; antifungal activity; concentration and culture media.

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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 and little is known about the consequences of repeated applications [1,2]. **The EC regulations (BPD 98/8/ECn 20 June 2004)** 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. Exploitation of plant metabolites in heritage conservation against biodeterioration of stone caused by fungi appear to be promising. In view of these, the author screened some extracts one of them Dhatura against bio-deterioration causes by fungal species isolated and identified from the sample of Bhand Deol temple at Arang of Chhattisgarh.Bhand Deol temple **(FigA-B)** is situated in Tehsil place called Arang of District Raipur of Chhattisgarh state. Approaches for the monument are by air from Raipur airport and by rail from Raipur railway station. Monument is located with walking distance from Arang bus stand. Ge- coordinates of Bhand Deol temple is **Lat.** $21^0$11'43" N and **Long.** 81^0 58'10"E. declared as Government protected on 26-10-1922 by Central province PWD, B&R Branch with notification number, Nagpur No. 1219-D.A.B.Bhand Deol temple is locally known as Bhand Deol having a stellate Garbhagriha, enshrining images of three Tirthankaras namely Ajitanatha, Neminatha and Sreyansanatha. On plan it has once consisted of a sanctum, mandapa and a porch of which the later two are lost now. The Garbhagriha of temple and its Rekha Sikhara are richly carved and adorned with beautiful sculptures including some erotic figures on the surface of wall of the monument. Stylistically the temple is assignable to the early Haihaya period i.e. circa 9^{th} century AD [3].

Materials and Methods

Sampling and isolation of fungi

Samples of monument were 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. Samples were collected from the surface of the monument. Few drops of sample pour in the petridis and kept this petridis at 28 ± 1^0C for 7 days for incubation [4]. At the end of incubation period, fungal colonies were counted, isolated of

pure culture and identified with the help of available literature and finally send this pure culture to authentic authority: National Centre of Fungal Taxonomy Delhi for identification.

A **B**

Fig (A&B): Showing front and lateral view of Bhand Deol temple, Arang, Distt.-Raipur

Preparation of plant leaf powder

The fully grown leaf of Dhatura was collected from Bhilai (Chhattisgarh). The collected plant leaf thoroughly washed with tap water and then rinsed with sterile distilled water. The leaf of dhatura was shed dried and grind in electric mixer. The powder material was kept in airtight glass bottles. This stock powder was used for further extraction [5].

Preparation of aqueous leaf extract

5.00±0.05 g of dried and ground leaves powder of dhatura 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 [6]. 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 following formula [7].

FG = (100 x Dc-Dr)/Dc,

FGI = 100-FG

Where: FG= Fungi growth in %, FGI = Inhibition of fungi growth in %,

*D*c=diameter of control (mm), *D*r = diameter of test (mm)

Well in agar method

A lapful 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 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 [8]. All the experiments were in Triplicate and mean has been considered in observation table [1-5].

TABLE-1: Standardization of plant extract for the study of inhibition of mycelium growth of fungal species with *Dhatura*

Fungal Sp.	**Volume of drop (in ml)**	**Effect of extract on fungal gro**	**Volume of drop (in ml)**	**Effect of extract on fungal gro**	**Volume of drop(in ml)**	**Effect of extract on fungal growth**	**Volume of drop (in ml)**	**Effect of extract on fungal growth**	**Volume of drop (in ml)**	**Effect of extract on fungal gro**	**Volume of drop (in ml)**	**Effect of extract on fungal growth**

		wth		wth						wth		
Concentration →	Control (0%)		10 %		20 %		30 %		40%		50 %	
Aspergillus niger	-	-	1	X	1	X	1	X	1	X	1	X
	-	-	3	X	3	X	3	X	3	X	3	X
	-	-	5	X	5	X	5	X	5	X	5	X
	-	-	7	X	7	X	7	X	7	X	7	X

Extract of *dhatura* was not effective for *A. niger*

X = Shown no any effect of extract of dhatura on *A. niger*

TABLE-2: Standardization of plant extract for the study of inhibition of mycelium growth of fungal species with *dhatura*

Fungal Sp.	**Volume of drop (in ml)**	**Effect of extract on fungal growth**	**Volume of drop (in ml)**	**Effect of extract on fungal growth**	**Volume of drop (in ml)**	**Effect of extract on fungal growth**	**Volume of drop (in ml)**	**Effect of extract on fungal growth**	**Volume of drop (in ml)**	**Effect of extract on fungal growth**	**Volume of drop (in ml)**	**Effect of extract on fungal growth**
Concentration →	Control (0%)		10 %		20 %		30 %		40%		50 %	
Rizopous	-	-	1	X	1	X	1	X	1	X	1	X
	-	-	3	X	3	X	3	X	3	X	3	X
	-	-	5	X	5	X	5	X	5	X	5	X
	-	-	7	X	7	X	7	X	7	X	7	X

Extract of *dhatura* was not effective for *Rizopous*

X = Shown no any effect of extract on *Rizopous*

TABLE-3: Standardization of plant extract for the study of inhibition of mycelium growth of fungal species with *dhatura*

Fungal Sp.	**Volume of drop (in ml)**	**Effect of extract on fungal growth**	**Volume of drop (in ml)**	**Effect of extract on fungal growth**	**Volume of drop (in ml)**	**Effect of extract on fungal growth**	**Volume of drop (in ml)**	**Effect of extract on fungal growth**	**Volume of drop (in ml)**	**Effect of extract on fungal growth**	**Volume of drop (in ml)**	**Effect of extract on fungal growth**
Concentration →	Control (0%)		10 %		20 %		30 %		40%		50 %	
Cladosporium	-	-	1	X	1	X	1	X	1	X	1	X
	-	-	3	X	3	X	3	X	3	X	3	X
	-	-	5	X	5	X	5	X	5	X	5	X
	-	-	7	X	7	X	7	X	7	X	7	X

Extract of *dhatura* was not effective for *Cladosporiu*

X = Shown no any effect of extract on *Cladosporium*

TABLE-4: Standardization of plant extract for the study of inhibition of mycelium growth of fungal species with *Dhatura*

Fungal Sp.	**Volume of**	**Effect of extr**	**Volume of**	**Effect of extr**	**Volume of**	**Effect of extr**	**Volume of**	**Effect of extr**	**Volume of**	**Effect of extr**	**Volume of**	**Effect of extra**

	drop (in ml)	**action fungal growth**	**drop (in ml)**	**action fungal growth**	**drop (in ml)**	**action fungal growth**	**drop (in ml)**	**action fungal growth**	**drop (in ml)**	**action fungal growth**	**drop (in ml)**	**ct on fungal growth**
Concentration →	Control (0%)		10 %		20 %		30 %		40%		50 %	
Curvularia Lunata	-	-	1	X	1	X	1	x	1	X	1	x
	-	-	3	X	3	X	3	x	3	✓	3	✓
	-	-	5	X	5*	✓	5*	✓	5*	✓	5*	✓
	-	-	7	X	7	✓	7	✓	7	✓	7	✓

*5 ml of aqueous *dhatura* extract was standard for study on *Curvularia Lunata*

√= shown effect of extract on *Curvularia Lunata.* X = shown no any effect of extract on *Curvularia Lunata*

Table -5 : Measurement of % inhibition of fungal sp. growth by standardized plant extract after 03 days

Fungal Sp.	**Conc. of extract in %**	**%FGI**
Curvularia Lunata	0 (Control)	0
	10	60
	20	70
	30	78
	40	86
	50	90
	21	70.3
	22	71.7
	23	72.6
	24	73.9

	25	74.2
	26*	74.9*
	27	75.1
	28	76.1
	29	76.3

* 5 ml of 26% aqueous extract of *dhatura* was shown effective for inhibition of fungal growth.

RESULTS AND DISCUSSION

At a concentration of 26 % and their 5 ml leaf aqueous extract of Dhatura in this study recorded effective for *Curvularia lunata* and their percentage of inhibition for fungal growth was 74.9% whereas the minimum inhibition by leaf extract was recorded 60% of *Curvularia lunata* sp. by 5 ml of 10 % of the plant extract[9] **[TABLE 4-5]** and no any effect recorded of plant extract on other dominant fungal pathogens viz. A.niger, Rizopous and *Cladosporium.*

Percentage of inhibition increased with the concentration of plant extract. Among the extracts assayed, the leaf aqueous extract of Dhatura was recorded to have antifungal properties. 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 *Curvularia lunata* growth inhibition increased with the concentration of plant extract. The antifungal activity of the Dhatura plant for the organism was recorded increasing with the concentration of extract. This study showed that the leaf aqueous extract of Dhatura tree had fungicidal activity [9].

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.,2009 9[14]. Thus, there is a need to search for alternative eco-friendly approaches for conservation and preservation for our heritage [5,9].

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