WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 6, Issue 1, 1022-1031. Research Article ISSN 2277– 7105

ANTIFUNGAL ACTIVITIES OF CALOTROPIS GIGANTEA AGAINST DOMINANT FUNGAL PATHOGENS OF MONUMENT WITH REFERENCE TO BHAND DEOL TEMPLE OF CHHATTISGARH

***Sanjay Prasad Gupta**

Archaeological Survey of India, Raipur Circle, Raipur, Chhattisgarh, India.

Article Received on
08 Nov. 2016,

Revised on 28 Nov. 2016,
Accepted on 18 Dec. 2016

DOI: 10.20959/wjpr20171-7632

***Corresponding Author**
Dr. Sanjay Prasad Gupta
Archaeological Survey of India, Raipur Circle, Raipur, Chhattisgarh, India.

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^{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 aqueous extract of *Calotropis gigantea* (Aak plant) on common and 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 *Calotropis gigantea* (Aak plant). Extract showed antifungal activity. Different concentration of extract solutions prepared and standardized for the study. It was found that the 3 ml of 37% and 7 ml of 47% extracts were effective in reducing the mycelial growth of *A. niger* and *Curvularia lunata* respectively whereas not effective of Aak extract for *Cladosporium and Rizopous.* 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.

KEYWORDS: Mycelial growth; fungal species; *Calotropis gigantea* (Aak plant). plant extract; antifungal activity; concentration and culture media.

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 aak 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 9th century AD.[3]

A **B**

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

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^{0}C 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.

Preparation of plant leaf powder

The fully grown leaf of aak was collected from Bhilai (Chhattisgarh). The collected plant leaf thoroughly washed with tap water and then rinsed with sterile distilled water. The leaf of aak 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 aak 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[1-6].

RESULTS AND DISCUSSION

At a concentration of 37% and their 3 ml leaf aqueous extract of Aak was taken in this study and found effective **(TABLE-1-2)** for *A. niger* and their percentage of inhibition for fungal growth was 75.2%. The minimum inhibition by leaf extract was recorded 10% of *A. Niger* sp. by 3 ml of 10% of the plant extract **(TABLE-2)**. At any concentration and any volume of leaf aqueous extract of Aak was taken and not found effective for *Rhizopus nigricans* and did not show their percentage of inhibition for fungal growth. The minimum inhibition by leaf extract was not found for any concentration of *Rhizopus nigricans* sp**(TABLE-3)**. At any concentration and any volume of leaf aqueous extract of Aak was taken and not found effective for *Cladosporium cladosporioides* and did not show their percentage of inhibition for fungal growth. The minimum inhibition by leaf extract was not found for any concentration of *Cladosporium cladosporioides* sp. of the plant extract **(TABLE-4)**. At a concentration of 47% and their 7 ml leaf, aqueous extract of Aak was taken and found effective **(TABLE-5-6)** for *Curvularia lunata* and their percentage of inhibition for fungal growth was 74.8%. The minimum inhibition by leaf extract was found 19% of *Curvularia lunata* sp. by 7 ml of 10% of the plant extract **(TABLE-5)**. Percentage of inhibition increased with the concentration of plant extract for *A. niger* and *Curvularia lunata* sp. Among the extracts assayed, the leaf aqueous extract of Aak was found 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 overall, growth inhibition increased with the concentration of plant extract except *Cladosporium cladosporioides* and *Rhizopus nigricans*. The antifungal activity of the Aak plant for the organisms was found increasing with the concentration of extract. In this study showed that the leaf aqueous extract of *Aak* 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]

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

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 %	
Aspergillus niger	-	-	1	X	1	X	1	X	1	✓	1	✓
	-	-	3	X	3*	X	3*	✓	3*	✓	3*	✓
	-	-	5	X	5	✓	5	✓	5	✓	5	✓
	-	-	7	X	7	✓	7	✓	7	✓	7	✓

3 ml of aqueous *Aak* extract was standard for study on *A. niger*

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

TABLE -2: Measurement of % inhibition of fungal sp. growth by standard plant extract after 03 days

Fungal Sp.	Conc. of extract in %	%FGI
Aspergillus niger	**0 (Control)**	**0**
	10	10
	20	27
	30	67
	40	77
	50	88
	31	67.2
	32	67.6
	33	67.8
	34	69.8
	35	71.2
	36	73.1
	37*	75.2*
	38	75.9
	39	76.9

*3 ml of 37% aqueous extract of *Calotropis* was shown effective for inhibition of fungal growth.

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

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 *Aak* was not effective for *Rizopous.*

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

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

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 *Aak* was not effective for *Cladosporium.*

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

TABLE-5: Standardization of plant extract for the study of inhibition of mycelium growth of fungal species with *Calotropis gigantea*

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 %	
Curvularia Lunata	-	-	1	X	1	X	1	X	1	X	1	X
	-	-	3	X	3	X	3	X	3	√	3	√
	-	-	5	X	5	X	5	X	5	√	5	√
	-	-	7	X	7	X	7	√	7	√*	7	√*

Extract of *Aak* was not effective for *Curvularia Lunata.*

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

TABLE -6: Measurement of % inhibition of fungal sp. growth by standard plant extract after 03 days

Fungal Sp.	Conc. of extract in %	%FGI
Curvularia Lunata	0 (Control)	0
	10	19
	20	31
	30	66
	40	72
	50	77
	41	73.3
	42	73.7
	43	73.7
	44	73.9
	45	73.9
	46	74.3
	47*	74.8*
	48	75.6
	49	75.9

*7 ml of 47% aqueous extract of *Calotropis* was effective for inhibition of fungal growth.

ACKNOWLEDGMENT

Authors are gratitude thanks to Dr. Dilip Kumar Khamari Superintending Archaeologist, Archaeological Survey of India, Raipur Circle for their encouragement and guidance**.** We are also thankful to Dr. P.N. Chowdhry for Identification of fungi, Principal, Govt. Arts and commerce Girls College and Vice Chancellor, MATS University, Raipur for providing necessary laboratory facilities for isolation and identification of fungi and extraction of plant extract.

REFERENCE

1. Price, C.A., "In: Stone conservation: an overview of current research, Keys, A. (end)", *The Getty Conservation Institute*, 1996.
2. Fortune, I. S., Aliakmon, H. L., Young, M. E., Gorbushina, A. A., Krumbein, W. E., Maxwell, I., McCullough, C., Robertson, P.,Saarela, M., Valero, J. and Ventral, M., "Assessing the suitability of novel biocides for use on historic surfaces in Heritage Microbiology and Science – Microbes, monuments and maritime materials", *Springer Vela*, Great Britain, 2008.
3. Mishra, P.K., Shriwastava, K.C., Kamahi, D.K. and Yadav, S.N., "Samsmriti (Protected monuments of Raipur circle at a glance)", *Archaeological Survey of India, Raipur Circle, Chhattisgarh, India*. 2012; 36-37.
4. Sharma, K. and Laneway, S. J., "Photo", 2010; 2(11): 47-49.
5. Gupta, S.P., Rana, K.S., Sharma, K. and Chhabra, B.S., "Antifungal activity of aqueous leaf extract of osmium sanctum on Dominant fungal species of Monuments", *European Chemical Bulletin*, United state of America, 2014; 2(6): 609-611.
6. Kumoro, A.C., Hasan, M. and Singh, H., "Sci." *Asia*, 2009; 35: 306–309.
7. 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.
8. Shinde, V. and Dhale, D. A., "J. Phytol.", 2011; 3(12): 41-44.
9. Gupta, S. P., "Novel Chemical Approaches for conservation of out door cultural heritage of Chhattisgarh", *Ph.D. Thesis*, MATS University, Raipur, C.G., 2014; 1-121.
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.