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Antimicrobial Activity of Plant Extract against Fungi Associated with Monument Deterioration of Gwalior Fort in India

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Abstract:

Heritage sites are damaged by various means. A number of magnificent palaces like Gwalior fort, Datia Fort in Madhya Pradesh have survived from medieval period. The composition & structure of artifact can be modified by Microorganisms and other deteriorating agent. Amongst microorganisms Fungi are of prime interest because of their simple ecological and nutritional requirement can develop easily on outdoor objects and monuments. Once established, fungi degrade stone chemically as well as mechanically. For conservation reasons and maintain the original status and integrity of the heritage site it is preferable to eliminate biological growth. Relatively little research has been conducted on antifungal treatments for stone so there is a need of finding antifungal treatments with sufficient persistence. Thus present study was taken to determine the efficacy of natural products against monument associated fungi so as to determine their antifungal potential to prevent colonization of these fungi.

Fungi were isolated from stone samples collected from Gwalior fort, Gwalior, India. During this study Antimicrobial activity of leaf extracts of 36 plants extracts were examined against Alternaria sp, A.

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nidulans, Curvularia sp, Penicillium sp, Fusarium sp. The plants extracts were prepared by soxhlet extraction.

Amongst tested extract Azadirachta indica was effective only against Alternaria sp. While most of other plants were either showing very moderate/ least activity against test fungi or was resistant to it. However, Rosa centrifolia and Punica granatum plant extract were found to have very good activity against all tested organisms. The data revealed that the highest activity in the Rosa centrifolia and Punica granatum plant extracts. It is evident from present study that the some of the examined plant possess good antifungal potential. These plants along with their antimicrobial property can be used as effective measure to control deteriogenic fungi. The active principle can be used in the development of effective and new formulations that can support conservator.

Key words: Biodeterioration, Fungi, Plant Extract

Introduction

Stone monuments are damaged by physical, chemical, or physicochemical deterioration processes. Conservative interventions have been applied in order to stop or slow-down the biodeterioration process. Several preventive and remedial methods have been used in tropical environments for control and eradication of microorganisms on stone monuments (Richardson 1988; May *et al* 1993). Such treatments must meet several conditions, and this can prove difficult in the outdoor environment, where there is a continual supply of moisture to promote re-growth. They must not only kill the growth in the first place, but they must also be resistant to new strains. They must not have any harmful effect on the stone itself, nor must they change its appearance. They must be safe both to the person applying them and to the wider environment. The development of pest resistance and problems of environmental pollution have accompanied excessive reliance on pesticides.

These problems can be avoided or minimized by using the natural material products such as plant extracts.

Caneva *et al*. (1991) mentioned the possibility of preventive conservation by the deliberate introduction of suitable vegetation in the vicinity. Relatively little research has been conducted on antifungal treatments for stone. In view of the extensive work on the role of fungi in decay, but it may reflect the difficulty of finding antifungal treatments with sufficient persistence. Preventive measures aimed at forestalling the possibilities of destruction & control measures. It is preferable to eliminate biological growth for conservation reasons and maintain the original status and integrity of the heritage site. Thus present study was taken to determine the efficacy of natural products against monument associated fungi so as to determine their antifungal potential to prevent colonization of these fungi.

Nature has evolved numerous biochemical solutions to many different problems, and has a superior ability to fabricate stereospecific compounds with very specific bioactivities (Knutsen, 1997; Metting and Pyne, 1986). India has about 45000 plant species and among them, several thousands have been claimed to possess antimicrobial properties. Present study was undertaken to investigate the antimicrobial activity of some of the traditionally used medicinal plants and some ornamental plants. Plants are thus an enormous reservoir of new bioactive molecules (Hostettman *et al* 1998)**.** Therefore, it is advantageous to derive new bio-chemical substances from this source, because these are more likely to be "well tested", biologically functional and degradable.

Many organic and inorganic compounds have been used, as biocidal agents, to eliminate the biodeteriogens from cultural objects. Caneva, Nugari, and Salvadori (1991) provided a valuable account of the many available biocides, which are normally applied to the surface of the stone by brush or spray. Plant extracts as well as essential oils and other compounds are

of considerable interest because of their antifungal activity (Pepeljnjak, *et al* 2003). It has been found that the efficacy of biocides in killing target organisms is usually not the same on different stone substrates, as surface conditions and stone mineralogy often affect biocidal activity (Grant and Bravery, 1981). Plants are one of the bedrocks for modern society to attain new principles (Evans *et al* 2002). These molecules can be used to prevent monument deterioration by significantly reducing the microbial load at a damaged site.

Material & Methods

Test organisms:

For isolation of the fungi samples were collected from Gwalior fort, India. Fungal species isolated from the monument site were maintained and studied in the Department of Microbiology, CHRI and Gwalior, India, by growing them on potato dextrose agar. Five Fungal species i e *Alternaria sp, A. nidulans, Curvularia sp, Penicillium sp, Fusarium sp* were used in the study.

Plant Material:

Antimicrobial activity of leaf extracts from 36 plants was tested against five fungi isolated from the deteriorating monument. Fresh leaves were collected from plants growing in Gwalior District in Madhya Pradesh, India, and listed in Table 1.1

Methanolic (Qualigens Fine Chemicals, GSK, Mumbai) extracts were prepared by soxhlet extraction taken to dryness by evaporating the methanol.

Processing of leaf extracts before activity testing

Of the solid residue from each leaf extract, 500 mg were dissolved in 1000 µl of methanol and mixed by vortexing to get homogenize preparation. Forty µl of each of the methanol solutions was added to a separate well on an agar plate, four

wells per plate. The methanol was allowed to evaporate from the wells overnight and diffusion of extracts.

Anti-microbial assay

Anti-microbial assays were performed in wells on plates containing Mueller-Hinton agar supplemented with 2% glucose and 0.5 µg methylene blue per ml (MHA) (NCCLS M44-P).] An inoculum of conidial or sporangiospore suspension was prepared from each test mold and its concentration adjusted spectrophotometrically to contain $0.4x10^4$ to $5x10^4$ CFU/ml by OD determination at 530 nm. For *Aspergillus* sp. the OD was adjusted to 0.09-0.11; for *Fusarium* sp. the OD was adjusted to 0.15-0.17; and for Alternaria sp. the OD was adjusted to 0.2. Tween 80 (0.05%) was added as wetting agent in the preparation of each inoculum.

Antifungal activity determination

Each well on a test plate received 40 µl of leaf extract solids that had been redissolved in methanol. To allow proper diffusion of the extract and evaporation of the methanol, each plate was incubated for 24 hrs at room temperature. Antifungal activity was tested on the following day. The well plates were then seeded with one of the fungal test strains by even streaking of swab. All plates were then incubated at 28 ± 2^0C for 48 hrs. A set of controls was also run in same way for each test organism using Amphotericin B.

The diameter of the zone of inhibition around each test fungus was measured and (in mm) & an average of three independent determination was recorded (Table1.2).

Results

The results of the tests of antifungal activity with methanolic extracts from the leaves of 36 plants are listed in Table 1.2. A

total of 25 out of the 36 leaf extracts were active against at least one of the five test fungi.

It is well known that higher plants produce a number of agents capable of fungal inhibition. The highest activities were exhibited by leaf extracts from *Rosa centrifolia* and *Punica granarum* against test fungi.

Extracts obtained from *Artocarpus heterophyllus, Vitis vinifera, Withania somnifera, Solenostemon* sp, *Pongamia pinnata, Boswellia serrata, Annona squamosa, Delonix regia, Bougainvillea glabra, Psidium guajava, Araucaria heterophylla* were found ineffective against any of the test fungi. Extract obtained *from Azadirachta indica* was effective only against *Alternaria sp*., whereas most of the other leaf extracts showed either very moderate/minimal activity or no activity against any of the test fungi. On the other hand, leaf extract from *Rosea centrifolia* and from *Punica granarum* exhibited significant activity against all test fungi.

Discussion

Plants have shown ability to synthesize antimicrobial substances, most of which are secondary metabolites. In many cases they serve as a plant defense mechanism against predation by microorganisms, insects, herbivores (Cowan, 1999). In the present study, we found antifungal activity in many leaf extracts from different plants (Table 1.2). Among tested plant species we found that the methanolic extracts from *Rosa centrifolia* and *Punica granatum* shows highest activity. While most of other plants were either showing very moderate/ least activity against test fungi or were found ineffective against fungi. Methanolic extracts from *Azadirachta indica ,Bougainvillea glabra, Psidium guajava, Annona squamosa Delonix regia, Araucaria heterophylla, Coleus ,kathal, vitis ,Ashwagandha, Boswelia, Karanj* were not found to be effective against fungi(Gangwal *et al* 1995). *O*ils of *P. pinnata* were

reported to show strong inhibition against *S. aureus, P. aeruginosa, A. niger* and *A. fumigatus* (Wagh *et al* 2007). In vitro studies of water extracts of plants such as *Terminalia chebula, Punica granatum, Delonix regia* and *Emblica officinalis* were found to be inhibitory to dermatophytes (Dutta *et al* 1998).Jackfruit (*Artocarpus heterophyllus*), , has lectins present in the fruit and seeds which may be antibacterial, antifungal, and antiviral (www.naturalstandard.com). However in present study the plant extract obtained from leaves of A*rtocarpus heterophyllus* were not found effective. Coleus was found ineffective against our test fungi, which is in agreement with previous findings (Perumal et al. 2004). Ginger has been reported to contain antifungal (Kapoor, A.1997), however, we found it to have only a moderate effect on the growth of Fusarium and Curvularia and no effect on the growth of Alternaria sp., A. nidulans, and Penicillium sp." Ashwagandha was reported to have potent antifungal activity against *Aspergillus flavus, Fusarium oxysporum, F. verticilloides* and antibacterial activity against *Clvibacter michiganensis subsp. Michiganensis* (Girish *et al* 2006) but we found it to be ineffective against our test fungi.

Rose extracts were found to have very good activity against most of the tested sp. Traditionally, rose extracts have been used in some medicinal preparations. Rose petals were used by Ayuvedic physicians. Most of the medicinal properties are related to the floral part of plants. The Rose plant contains citronellol, eugenol, linalool, cyanin and many other active compounds (Herbalpedia, 2000).Antibacterial activity of rose petals has been reported several times. The distribution of such compounds appears to vary in different varieties.

Our study evaluated the effect of extracts from leaves of the rose plant instead of rose petals against environmental fungi. We found the leaf extracts to be a very good antifungal agent. Previous studies also found that it has antifungal potential (Rattanapitigorn P, *et al* 2006).

Our second most active antifungal leaf extract was that from Punica *granatum*. This plant was previously reported to produce a potent antimicrobial (Kurokawa et al. 1993). Duraipandiyan (2006) reported antifungal activity of Punica *granatum* against Candida albicans. *Punica granatum also* possesses various medicinal properties including antibacterial, antiviral and astringent (Yeung, 1985; Polunin &Huxley, 1987) Neem leaf and its constituents have been demonstrated to exhibit, antifungal, properties. (Verma, *et al* 1998, Subapriya and Nagini ,2005**)** But in present study neem doesn't inhibit the growth of test fungi though slight inhibition of growth of *Alternaria sp* was observed.

From our investigation of screening different plant species it is evident that some of the leaf extracts from different plants were active against our test organisms. They possessed good antifungal potential. The antimicrobials of these plants can be used as effective measure to control deteriogenic fungi. In addition, these results form a good basis for selection of candidate plant species for further phytochemical investigation. The active compounds from these plants can be used to develop new, effective formulations to aid conservators in the preservation of monuments without detrimental effect to the environment. Further phytochemical studies are required to determine the type of compounds responsible for the antimicrobial effect of these plants. In vitro assessment of activity should be done before field trial.

Vegetation may also be used as a preventive and protective measure in archaeological sites or outdoor environments. Vegetation for landscaping around monuments and other sites may help modify the microclimate and thereby alter biological colonization of masonry structures. A careful choice of plants is critical to optimize results and minimize risks related to destructive effects of their root systems. Suitably chosen vegetation may lower the water table,

minimize evaporation, reduce air salinity and pollution, and reduce erosion (Fosberg 1980; De Marco *et al* 1990)

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Table1.2 Antimicrobial activity of plant extracts against fungi

S No	Plant Extract	zone of inhibition against test fungi(in mm*)				
		A.nidulans	*Fusarium sp*	*Penicillium sp*	*Curvularia sp*	*Alternaria sp*
1	*Azadirachta indica*	R	R	R	R	10mm
2	*Bougainvillea glabra*	R	R	R	R	R
3	*Eugenia jambolana*	R	10	15	18	11
4	*Psidium guajava*	R	R	R	R	R
5	*Michelia champaca*	10	R	10	13	10
6	*Gymnema sylvestris*	10	15	R	20	R
7	*Annona squamosa*	R	R	R	R	R
8	*Mangifera indica*	R	21	R	16	13
9	*Rosa centrifolia*	17	20	19	27	28
10	*Thuja orientalis*	R	R	R	12	11
11	*Saraca Indica*	11	R	R	14	10
12	*Ocimum sanctum*	R	R	R	R	12
13	*Calendula officinalis L*	10	R	R	R	11
14	*Citrus limon*	R	20	R	11	13
15	*Chrysanthemum coronarium*	R	R	11	R	11
16	*Colocasia esculenta*	R	R	10	R	11
17	*Albizia lebbek*	R	R	R	R	11
18	*Calotropis procera*	R	R	R	10	10
19	*Dhatura stramonium*	R	R	12	19	14
20	*Aegle marmelos*	R	13	R	13	10
21	*Hibiscus rosa sinensis*	10	R	R	10	13
22	*Delonix regia*	R	R	R	R	R
23	*Araucaria heterophylla*	R	R	R	R	R
24	*Cerbera thevetia*	R	10	R	R	R
25	*Vinca rosea*	R	10	R	16	20
26	*Punica granatum*	11	21	16	19	25
27	*Jasminum grandiflorum*	R	12	R	10	11
28	*Solenostemon* sp	R	R	R	R	R
29	*Pongamia pinnata*	R	R	R	R	R
30	*Boswellia serrata*	R	R	R	R	R
31	*Sapindus trifoliatus*	10	25	R	37	R
32	*Zingiber officinalis*	R	13	R	10	R
33	*Withania somnifera*	R	R	R	R	R
34	*Murraya koenigii*	11	11	R	10	R
35	*Artocarpus heterophyllus*	R	R	R	R	R
36	*Vitis vinifera*	R	R	R	R	R
37	Control	19	R	21	31	27

R= resistant, *= mean of three replicates