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Original Research Article

Evaluation of Antifungal Activity of Crude Leaf Extracts of Indian Sacred Trees

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ABSTRACT

Sacred trees are plants with a socio-economic, medicinal value which associates them with the Gods. Herbal medicines have been the basis of treatment and cure for various diseases and physiological conditions in traditional methods practiced in India such as Ayurveda, Unani and Siddha. Medicinal plants have been reported to have antimicrobial properties against many microbial organisms. Fungi are secondary invaders of an already weakened human body. Mycoses are still a critical cause of mortality second only next to bacterial diseases. Though significant advances have been made in antibacterial chemotherapy, there is a lack of serious strides in the area of antifungal drug discovery. In the present study, an attempt has been made to study and compare the antifungal efficacy of five leaf crude extracts of Indian sacred trees viz., *Aegle marmelous* Linn. Correa., *Feronia elephantum* Linn., *Ficus benghalensis* Linn., *Ficus religiosa* Linn., and *Mimusops elengi* Linn. The extracts which showed the highest activity were analysed and the minimum inhibitory concentration was determined. Leaf extracts of *Aegle marmelos* and *Mimusops elengi* showed good anticandidal and antifungal activity against most of the strains tested. Therefore, these medicinal trees should be conserved as sacred grooves and exploited commercially for drug development properties.

Keyword: Sacred trees; mycoses; antifungal activity

INTRODUCTION

In India, any form of nature such as trees, rivers, mountains, animals are considered sacred. Several villages had their own sacred groves which were mini biosphere reserves containing indigenous plants and wild life. The Pancha Bhootas such as air, land, water, fire (energy) and space were revered as sacred and essential for life. Planting and nurturing trees

was a routine practice in ancient India. Sacred groves are forest fragments of various sizes which are communally protected and have a religious connection with the protecting community. Sacred groves are usually associated with temples, monasteries, shrines or burial grounds. Logging and hunting are prohibited strictly within these patches of trees; therefore they acted as a huge repository for various Ayurvedic medicines [1].

Opportunistic systemic mycoses are those infections, which are found in patients with underlying predisposing conditions whose immunological defense mechanisms are weakened by endogenous causes like cancer, leukemia or exogenous causes like immunosuppressive therapy and AIDS. Causative agents of opportunistic mycoses include *Candida albicans* and different species of *Aspergillus*. There is an emergence of drug resistance among certain fungi causing opportunistic mycoses [2]. Medicinal plants represent a rich source of antimicrobial agents. These plants are used in the form of extracts as raw drugs but they are not evaluated properly [3].

Today, though the number of sacred groves in different parts of India has declined, few are still protected as they serve as a biodiversity hotspot for the conservation of endangered flora and fauna. The present study is an attempt to re-establish the identity of sacred groves in India by instilling the ethno medicinal importance of the trees in a sacred grove in today's fast paced changing scenario.

MATERIALS AND METHODS

Collection and preparation of plant powder [4]

The medicinal trees included in the study were *Aegle marmelous* (vilvam), *Feronia elephantum* (wood apple), *Ficus benghalensis* (banyan), *Ficus religiosa* (peepal) and *Mimusops elengi* (Magilam).The leaves of the sacred trees were collected from different temples in and around Chennai. The leaves were separated from the twigs and washed twice with double distilled water and then surface sterilised using 70% ethanol. The leaves were shade dried for 1-2 weeks. The leaves were then ground into a coarse powder form using a mixer.

Preparation of crude extract [5]

The crude extracts were prepared by hot and cold method of extraction. In the hot method of extraction, about 1 gram of the powdered plant material was mixed with 10 ml of the solvent, incubated in a shaker at 37ºC for 4 hours at 250 rpm after which it was placed in a water bath at 60°C for 2 hours. The supernatant was filtered and dried in air at room temperature. For the cold method of extraction, about 1 gram of the of the powdered plant material was mixed with 10 ml of the solvent, incubated in a shaker at 37°C for 4 hours at 250 rpm.The supernatant filtered and then dried in air at room temperature. The solvents used were water,ether, ethanol, methanol, chloroform, acetone and dichloromethane. The residue obtained after drying was dissolved in the appropriate solvent and used for anticandidal and antifungal screening.

Microbial cultures used

The test organisms used for screening were *Candida albicans,Aspergillus niger, Aspergillus fumigatus* and *Aspergillus flavus*.All the strains were laboratory isolates of the Department of Microbiology, J.B.A.S college for Women, Chennai.

Preliminary Screening using Disc Diffusion Assay [6,7]

Discs(6mm) prepared from Whatmann No.1 filter paper was sterilised and impregnated with 20µl of various crude solvent extracts (concentration:100mg/ml). Saline cultures of the microorganisms were prepared. The culture was checked for turbidity by comparing with the McFarland Standard (4).A lawn culture of the organisms to be tested was made on the

Saboraud's Dextrose agar media. The prepared discs were placed on the plate in a way such that each disc was at least 20mm from one another. The plates were then incubated at 37°C for 24 hours for Candida albicans and 48 hours for fungal molds. The inhibition zone around each disc both in the experiment and the control were measured. Standard antibiotics as per the organisms tested were included as positive control and respective solvents without the plant extracts were used as the negative control.

Determination of Minimum Inhibition Concentration/ Minimum Bactericidal Concentration [8]

The Microbroth dilution was performed on a microtitre plate. Doubling dilutions of the crude extract were prepared in Saboraud's dextrose broth. Fungal and candidal cultures of 10^6 cfu/ml dilution were prepared with McFarland standard (4) and 10µl were added to each well of the microtitre plate and mixed well. The microtitre plates were incubated at 37ºC overnight for Candida albicans and 48 hours at room temperature for molds and a loopful of the culture was streaked on to Saborauds' dextrose agar plates. The plates were incubated at the same temperature and time as before. The growth/no growth pattern of the organisms corresponded to the MIC /MBC of the crude extract.

RESULTS

In the preliminary screening of plant extracts for anticandidal activity (table 1), the ethanol and ether extracts of the medicinal trees showed maximum activity, followed by acetone, methanol and chloroform. Water extracts were least effective. In the preliminary screening of plant extracts for anti-fungal activity (table 2), the ethanol extracts of the medicinal trees showed maximum activity, followed by ether, acetone, and methanol. Chloroform and water extracts were least effective. For *Candida albicans*, most of the extracts showed an MIC value of 3.125 and 1.56 mg/ml, for *Aspergillus flavus* and *Aspergillus fumigatus*, all the extracts failed to grow at the least concentration of 1.56 mg/ml, for *Aspergillus niger*, most of the strains showed an MIC value of 6.25 and 12.5 mg/ml (table 3).

DISCUSSION

Indian Medicinal plants are considered a vast source of several pharmacologically active principles and compounds, which are commonly used in home remedies against multiple ailments [9, 10]. In the present study, for the determination of anticandidal activity, the ethanol leaf extracts of *Aegle marmelos* and *Mimusops elengi* had shown maximum activity followed by *Ficus religiosa* , *Ficus benghalensis* and *Feronia elephantum.*

In the determination of antifungal activity, for *Aspergillus flavus* and *Aspergillus fumigatus*, the ethanol extracts of *Aegle marmelous* and *Feronia elephantum* showed maximum activity followed by *Ficus benghalensis*, *Ficus religiosa* and *Mimusops elengi*. For *Aspergillus niger*, the ether extracts of the medicinal trees showed maximum activity.

In MIC assay to determine the MIC of the crude extract, for *Aspergillus niger*, *Mimusops elengi, Feronia elephantum* showed an MIC value of 12.5mg/ml whereas *Aegle marmelous* and *Ficus benghalensis* showed an MIC value of 25mg/ml and *Ficus religiosa* showed an MIC of 3.25mg/ml. For *Aspergillus flavus* and *Aspergillus fumigatus*, the MIC value could not be determined, so further dilution of plant extract should be taken to determine the MIC value.

Among the solvents used in the extraction procedures, ethanol and ether was found to be highly effective in extracting the antifungal components, followed by acetone, methanol, and chloroform. Water was ineffective in extracting the components of the medicinal plants.

Table 1: Preliminary screening for anticandidal efficacy
Method employed: Agar disc diffusion assay
Concentration of disc: 100mg/ml
Temperature of incubation: 37°C

Organisms tested	*Aegle marmelous*		*Feronia elephantum*		*Ficus benghalensis*		*Ficus religiosa*		*Mimusops elengi*	
	H (mm)	C (mm)	H (mm)	C (mm)	H (mm)	C (mm)	H (mm)	C (mm)	H (mm)	C (mm)
Solvent: ethanol										
Candida albicans -1	24	24	6	21	20	21	22	22	24	24
Candida albicans MTCC	24	24	20	22	22	22	18	24	21	24
Solvent :methanol										
Candida albicans -1	14	13	15	12	9	9	16	11	14	12
Candida albicans MTCC	15	15	13	13	11	16	13	18	15	13
Solvent: acetone										
Candida albicans -1	17	9	15	19	14	18	15	19	15	17
Candida albicans MTCC	15	20	14	21	11	21	9	21	20	22
Solvent: ether										
Candida albicans -1	20	20	20	22	20	22	20	20	20	21
Candida albicans MTCC	19	22	14	22	19	21	22	20	22	24
Solvent: chloroform										
Candida albicans -1	15	16	16	17	13	15	13	16	16	17
Candida albicans MTCC	11	11	13	9	14	12	11	11	13	9
Solvent: aqueous										
Candida albicans -1	-	11	-	-	9	-	11	-	-	-
Candida albicans MTCC	9	-	-	-	9	-	-	-	9	-

Table 2: Preliminary screening for antifungal efficacy
Method employed: Agar disc diffusion assay
Concentration of disc: 100mg/ml
Temperature of incubation: 25°C

Organisms tested	Aegle marmelous		Feronia elephantum		Ficus benghalensis		Ficus religiosa		Mimusops elengi	
	H (mm)	C (mm)	H (mm)	C (mm)	H (mm)	C (mm)	H (mm)	C (mm)	H (mm)	C (mm)
Solvent:ethanol										
Aspergillus niger	27	25	22	24	25	23	22	21	29	29
Aspergillus flavus	13	14	14	13	12	11	5	7	8	12
Aspergillus fumigates	15	13	13	11	9	0	13	15	12	13
Solvent :methanol										
Aspergillus niger	11	11	-	-	10	9	14	12	12	12
Aspergillus flavus	10	-	-	-	9	6	6	8	-	14
Aspergillus fumigatus	12	-	-	-	9	6	6	6	-	12
Solvent:acetone										
Aspergillus niger	12	11	13	10	8	8	8	9	9	9
Aspergillus flavus	13	13	12	9	12	13	14	13	13	14
Aspergillus fumigates	-	-	-	-	11	11	9	7	9	9
Solvent:ether										
Aspergillus niger	13	12	14	13	9	9	12	12	12	13
Aspergillus flavus	18	21	25	26	18	26	22	22	26	27
Aspergillus fumigatus	20	20	20	22	22	18	18	19	21	21
Solvent:chloroform										
Aspergillus niger	-	-	-	-	-	-	-	-	7	9
Aspergillus flavus	-	-	-	-	-	-	-	-	8	8
Aspergillus fumigatus	-	-	-	-	-	-	-	-	5	7
Solvent:aqueous										
Aspergillus niger	-	-	-	-	-	-	-	-	-	-
Aspergillus flavus	-	-	-	-	-	-	-	-	-	-
Aspergillus fumigatus	-	-	-	-	-	-	-	-	-	-

Table 3: Determination of MIC/MBC for fungi

Extracts tested*	CONCENTRATION OF THE EXTRACTS IN MG/ML								
Candida Albicans-1	100	50	25	12.5	6.25	3.125	1.56	BROTH CULTURE	BROTH ONLY
HVEt,HWEt,HMA,CBEt,CMET,CPEt	-	-	-	-	-	-	+	+	-
HBEt,CVE	-	-	-	-	-	-	-	+	-
Candida albicans MTCC									
HVEt,HMEt,CBEt,CWEt	-	-	-	-	-	+	+	+	-
HPE,CVE,CME,CBE	-	-	-	-	-	-	+	+	-
Aspergillus flavus									
HVE,HBE,HPE,HME,HWE, CVE,CBE,CPE,CME,CWE	-	-	-	-	-	-	-	+	-
Aspergillus fumigates									
HVE,HBE,HPE,HME,HWE, CVE,CBE,CPE,CME,CWE	-	-	-	-	-	-	-	+	-
Aspergillus niger									
HVEt,HBEt,CVEt	-	-	-	-	+	+	+	+	-
HMEt,HWEt,CBEt,CPEt,CMEt,CWEt	-	-	-	-	-	+	+	+	-
HPEt	-	-	-	+	+	+	+	+	-

*only those extracts which showed a inhibition zone of more than 15mm were included in determination of MIC,

+-presence of bacterial growth; - absence of bacterial growth,

H-denotes hot extracts,C-cold extracts,Et-solvent ethanol,E-solvent ether,V- Aegle marmelous, B-Ficus benghalensis, P-Ficus religiosa, M-Mimusops elengi, W-Feronia elephantum

The present study confirms the previous reports, indicating that the aqueous extracts were the least active extracts [11]. Several methods for preparing an initial extract of the plant material have been reported and ethanol appears to be the most useful solvents [12]. Similar observation was found in our study where good antifungal efficacy was demonstrated with ethanol. The hot method of extraction was more effective in eluting out the antifungal components than the cold method of extraction.

CONCLUSION

Fungal diseases have become very common as secondary infections to various predisposing factors as well as agents of primary diseases. Although many antifungal agents have been developed so far only a few are clinically effective and safe to use. Some of the fungi do not respond to the antifungal treatment in many diseases due to development of drug resistance. In this situation, it is necessary for exploring new possibilities of an antifungal like component from medicinal plants that are conserved and endemic to a particular region. The ether and ethanol extracts of the medicinal trees should be further investigated to characterize and isolate the active components which serve as potential antifungals for the treatment of drug resistant fungal pathogens.

CONFLICT OF INTEREST STATEMENT

None Declared

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