Original Article

Antifungal activity of some wild plant extracts against fungal pathogens

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ABSTRACT

The antifungal activity and minimum inhibitory concentration (MIC) of plant extracts in methanolic solvents such as soxhlet extract of plants traditionally used asmedicines were as Leaf extracts of five wild plants viz:, Lantana camara Linn., Oscimum basilicum Linn., Tribulus terrestris Linn., Withania somnifera Dunal., were evaluated against the Clinical pathogens Alternaria alternata, Aspergillus niger, Curvularia lunata and Candida albicans organisms were found to have maximum antifungal activity in soxhlet extracts, MIC value of 0.5 and 0.3mg/ml respectively. Soxhlet extracts of Lantana camara Linn., and Oscimum basilicum Linn., showed highest MIC value of 0.7 mg/ml against Aspergillus niger.

KEYWORDS: Soxhlet extracts, methanolic solvent, clinical pathogens, antifungal activity.

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INTRODUCTION

Plants have formed the basis for traditional systems of medicine that have been in existence for thousands of years and continue to provide remedies to mankind (Gurib- Fakim, 2006). In developing countries and particularly in India low income people such as farmers, people of small isolated villages and native communities use folk medicine for the treatment of common infection [1]. Traditional healers claim that their medicine is cheaper and more effective than modem medicine. Patients of these communities have a reduced risk to get infectious diseases from resistant pathogens than people from urban areas treated with traditional antibiotics. One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents. Traditional healers claim that some medicinal plants are more efficient to treat infectious diseases than synthetic antibiotics. It is necessary to evaluate, in a scientific base, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens. Medicinal plants might represent an alternative treatment in non-severe cases of infectious diseases [2]. They can also be possible source for new potent antibiotics to which pathogen strains are not resistant. We chose a wild plants *Lantana camara Linn., Oscimum basilicum Linn., Tribulus terrestris Linn., Withania somnifera Dunal.,* used in folk medicine to determine their antifungal activity against clinical pathogens i.e., *Alternaria alternata, Aspergillus niger, Candida albicans Curvularia lunata.*

MATERIALS AND METHODS

Experimental Section: All the chemicals and reagents used were from Hi-Media Pvt. Limited, Bombay, India. Glass wares used were from Borosil.

Plant material: The selected plants growing in different localities in Ahmednagar district were used to understand the antifungal activity. The identification of this plant was confirmed with the help of Cooke (1958) flora [4]. The voucher specimens are labeled and preserved in the herbarium in the research centre. Information about regionally important plant used in medicine was collected by consulting and interviewing local traditional healers.

The shade dried leaves were ground into fine powder and used for soxhlet extraction for further use. 50gms of plant material was extracted in 250ml of methanol in soxhlet apparatus for 48h. For better extraction of secondary metabolites ammonia drops were added into the plant powder. Then the extract was filtered and concentrated under reduced pressure on the water bath. The residue thus obtained was collected and stored in amber bottles at 4° C for further experiment.

The authenticated sample was collected from different regions of Maharashtra, India and was

Further confirmed in Botanical Taxonomist from P.V.P Arts science and commerce college P.G

Center, Loni. Maharashtra, India.

Preparation of plant extracts: Plant leaves were collected, washed with tap water, dried in shade, packed in brown paper bags for bioassay and phytochemical analyses. Precaution was taken to dry the leaves in shade to prevent the degradation of bioactive components in plants due to sunlight [5]. The shade dried leaves were ground into fine powder and used for hot extraction for further use.

Hot Extraction: 50gms of plant material was extracted in 250ml of methanol in soxhlet apparatus for 48h. For better extraction of secondary metabolites ammonia drops were added into the plant powder. Then the extract was filtered and concentrated under reduced pressure on the water bath. The residue thus obtained was collected and stored in amber bottles at 4° C for further experiment.

Inoculum: The fungal strains were inoculated separately in Potato dextrose broth for 6 hrs and the suspensions were checked to provide approximately 10^5 CFU/ml.

Fungal strains used: The clinical fungal test organisms used for study are ***Alternaria alternata*** MCIM 718 **,** ***Aspergillus niger*** *MTCC 2202***,** ***Candida albicans*** *ATCC 10231*, ***Curvularia lunata*** *NCIM 716* **.** The following test microorganisms used in the present study were procured from Department of Microbiology, Rural Medical College of Pravara Medical Trust, Loni, Dist: Ahmednagar, Maharashtra state in India.

Determination of antifungal activity: The agar well diffusion method C. Perez was modified. This technique avoids volatilization of active plant extract compounds. Potato dextrose agar (PDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in Potato dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with plant extracts and solvent blanks (DMSO). Standard antibiotic (Fucanazole, concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 37°C for 72 h. The diameters of zone of inhibition observed were measured.

Determination of MIC and MLC: The antifungal plant extracts were then after evaluated to determine MIC and MLC values. The broth dilution method was adopted by using DMSO for diluting the plant extract and was incubated for 48 h. The minimum dilution of the plant extract that kills the fungal growth was taken as MLC (Minimum lethal count) while the minimum dilution of plant extract that inhibits the growth of the organism was taken as MIC.

RESULTS AND DISCUSSION

The zone of inhibition was determined by measuring the record in mm for antifungal activity the methanolic plant extracts. All the plants were found to have maximum antifungal activity in comparison to antifungal drug Fucanazole. The Methanolic extract of *Lantana camara Linn.,and Oscimum basilicum Linn.* Possessed potent antifungal activity amongst all the methanolic extracts of other plants against

Table 1: Zone of Inhibition of Methanolic and Aqueous extract in comparison to antifungal drug Fucanazole.

S.No	Plant Extract /Fucanazole (1mg/ml)	Diameter zone of inhibition (mm) METHANOLIC EXTRACT				Diameter zone of inhibition (mm) AQUEOUS EXTRACT			
		A.a	A.n	C.a	C.l	A.a	A.n	C.a	C.l
1	*Lantana Camara (leaf)*	18	20	20	16	NA	NA	NA	NA
2	*Oscimum basilicum (leaf)*	16	18	19	15	NA	NA	NA	NA
3	*Tribulus terrestris (stem)*	14	11	13	15	NA	NA	NA	NA
4	*Withania somnifera (stem)*	14	12	13	16	NA	NA	NA	NA
5	Fucanazole	11	10	11	10	11	10	11	10

A.a= *Alternaria alternata,* A.n=*Aspergillus niger, C.a=Candida albicans, C.l=,Curvularia lunata*

S.No	Plant Extract /Fucanazole (1mg/ml)	MIC(mg/ml)				MLC(mg/ml)			
		A.a	A.n	C.a	C.l	A.a	A.n	C.a	C.l
1	*Lantana Camara (leaf)*	0.9	0.4	0.3	0.8	0.7	0.9	0.5	NA
2	*Oscimum basilicum (leaf)*	0.7	0.3	0.4	0.7	0.5	0.8	0.3	NA
3	*Tribulus terrestris (stem)*	0.5	0.5	0.5	0.3	NA	0.5	0.3	NA
4	*Withania somnifera (stem)*	0. 4	0.6	0.7	0.4	1	0.9	NA	NA

Table 2: Minimum Inhibitory Concentration and Minimum Lethal Concentration.

A.a= *Alternaria alternata,* A.n=*Aspergillus niger, C.a=Candida albicans, C.l=,Curvularia lunata*

Aspergillus niger and Candida albicans showing diameter of zone of inhibition viz. 20 mm while other plant extracts of *Tribulus terrestris Linn., Withania somnifera Dunal* showed similar antifungal activity against *Alternaria alternata, Curvularia lunata* showing zone of inhibition viz. 14-16 mm. The plant extracts extracts of *Tribulus terrestris Linn., Withania somnifera Dunal* showed antifungal activity against *Aspergillus niger and Candida albicans* (diameter of zone of inhibition viz. 11-13 mm).Aqueous extracts of Lantana *camara Linn.,Tribulus terrestris Linn., Withania somnifera Dunal* showed no antifungal activity against all four pathogens.

The MIC values of the plant extracts of *Lantana camara Linn.*, against Alternaria alternate showed (0.9 mg/ml), Aspergillus niger (0.4 mg/ml) Candida albicans (0.3 mg/ml) , Curvularia lunata(0.8 mg/ml), showing more MIC and MLC for Aspergillus niger & Candida albicans.

Whereas *Oscimum basilicum Linn* extract has showed against Alternaria alternate showed (0.7 mg/ml), Aspergillus niger (0.3 mg/ml) Candida albicans (0.4 mg/ml), Curvularia lunata(0.7 mg/ml), showing more MIC potency to Aspergillus niger & Candida albicans fungal strains.

Tribulus terrestris Linn extract showed Alternaria alternate showed (0.5 mg/ml), Aspergillus niger (0.5 mg/ml) Candida albicans (0.5 mg/ml), Curvularia lunata(0.3 mg/ml), with MIC more potency towards Curvularia lunata. While the same plant extract of *Tribulus terrestris Linn.,* showed MIC value of 0.5 mg/ml against *Candida albicans* and 0.5 mg/ml against *Aspergillus niger*.

Finally *Withania somnifera* showed Alternaria alternate showed (0.4 mg/ml), Aspergillus niger (0.6 mg/ml) Candida albicans (0.7 mg/ml), Curvularia lunata(0.4 mg/ml), with MIC more potent for Alternaria alternate & Curvularia lunata found to be 0.4 mg/ml, While *Withania somnifera Dunal* extracts showed showed MIC value of 0.7 mg/ml against *Candida albicans* and 0.6 mg/ml against *Aspergillus niger.* Antifungal activity of aqueous extracts of all these plants was not observed against *Alternaria alternate, Aspergillus niger, Candida albicans, Curvularia lunata,*.But Methanolic extracts of Lantana *camara Linn.,Tribulus terrestris Linn., Withania somnifera Dunal* showed antifungal activity against all the pathogens.The results are illustrated in **Table 1** and **Table 2.** The present study thus states that all the plants are effective against fungal infections caused by *Aspergillus niger & Candida albicans*.

CONCLUSION

The extracts of the plant (s) part used showed prominent antifungal activity against *Aspergillus niger* and *Candida albicans* which are severe pathogens. Thus the use of these plants in the treatment of pathogenic diseases associated with the infection of these pathogens is validated, scientifically supported by the results obtained in this work.

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