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Antifungal activity of some plant extracts against Clinical Pathogens

Abhishek Mathur*[1], Ravinder Singh[1], Sajad Yousuf[1], Aprajita Bhardwaj[1], Satish K. Verma[1], Pradeep Babu[2], Vinay Gupta[2], GBKS Prasad[3] and V.K. Dua[4]

Sai Institute of Paramedical & Allied Sciences, Dehradun (U.K), India
Uttarakhand Technical University, Dehradun (U.K), India
Jiwaji University, Gwalior (M.P), India
National Institute of Malaria Research, Hardwar (U.K), India

ABSTRACT

The antifungal activity and minimum inhibitory concentration (MIC) of various plant extracts in different solvents such as hydro-alcohol (50 % v/v) and hexane of plants traditionally used as medicines as Valeriana jatamansi (Sugandhbala), Coleus barbatus (Pathar choor), Berberis aristata (Kingore), Asparagus racemosus (Satrawal), Andrographis paniculata (Kalmegha), Achyranthes aspera (Latjiri), Tinospora cordifolia (Giloei), Plantago depressa (Isabgol) were evaluated against Aspergillus niger and Candida albicans. Hydro-alcoholic extracts of all the plants were found to have maximum antifungal activity in comparison to hexane extracts. Hydro-alcoholic extracts of Andrographis paniculata and Achyranthes aspera showed maximum potency against Aspergillus niger and Candida albicans at highest MIC value of 0.5 and 0.3 mg/ml respectively. Hexane extracts of Andrographis paniculata showed highest MIC value of 0.7 mg/ml against Aspergillus niger.

Key words: Hydroalcoholic extracts, hexane extracts, clinical pathogens, antifungal activity.

INTRODUCTION

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 infections [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]. Antimicrobial, antioxidant and anti-inflammatory properties of some Indian plants were reported [3-9]. They can also be a possible source for new potent antibiotics to which pathogen strains are not resistant. We chose eight species used in folk medicine to determine their antifungal activity against clinical pathogens i.e. *Andrographis paniculata* (Acanthaceae), *Valeriana jatamansi* (Valerianaceae), *Asparagus racemosus* (Liliaceae), *Tinospora cordifolia* (Menispermaceae), *Coleus barbatus* (Lamiaceae), *Berberis aristata* (Berberidaceae), *Achyranthes aspera* (Amaranthaceae), *Plantago depressa* (Plantaginaceae).

MATERIALS AND METHODS

Experimental Section

All the chemicals and reagents used were from C.D.H and Ranchem. Glass wares used were from Borosil. The media and broth used for microbial culture were from Hi-Media Pvt. Limited, Bombay, India.

Plant material

The authenticated sample was collected from different regions of Uttarakhand, India and was further confirmed in Botanical Survey of India (BSI), Dehradun. Voucher specimens have been deposited in BSI, Dehradun, India.

Preparation of plant extracts

The method [10] was adopted for preparation of plant extracts with little modifications. Briefly four 20 g portions of the powdered plant material were soaked separately in 100 ml of hydro alcohol (50% v/v) and hexane for 72 h. Each mixture was stirred after every 24 h using a sterile glass rod. At the end of extraction, each extract was passed through Whatmann filter paper no1 (Whatmann, England). The filtrate obtained were concentrated in vacuo using rotary evaporator at 30^{o}C.

Culture Media

The media used for antifungal test was Sabouraud's dextrose agar/broth of Hi media Pvt. Bombay, India.

Inoculum

The fungal strains were inoculated separately in Sabouraud's dextrose broth for 6 h and the suspensions were checked to provide approximately 10^5 CFU/ml.

Fungal strains used

The clinical fungal test organisms used for study are *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404 were procured from National Chemical Laboratory (NCL), Pune, Maharashtra, India.

Determination of antifungal activity

The agar well diffusion method [11] was modified. Sabouraud's dextrose agar (SDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in Sabourauds dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with plant extracts and solvent blanks (hydro alcohol, and hexane). Standard antibiotic (Fucanazole, concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 37^{o}C for 72 h. The diameters of zone of inhibition observed were measured.

Determination of MIC and MBC

The antifungal plant extracts were then after evaluated to determine MIC and MLC values. The broth dilution method was adopted by using N-saline 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 antifungal activity was determined by measuring the diameter of zone of inhibition recorded. Hydro-alcoholic extracts of all the plants were found to have maximum antifungal activity in comparison to hexane extracts. Hydro-alcoholic extract of *Andrographis paniculata* possessed potent antifungal activity amongst all the hydroalcoholic extracts of other plants against *Aspergillus niger* showing diameter of zone of inhibition viz. 20 mm while hydroalcoholic extracts of *Achyranthes aspera* showed similar antifungal activity against *Candida albicans* and *Aspergillus niger* showing diameter of zone of inhibition viz. 20 mm. Hexane extracts of *Andrographis paniculata* showed antifungal activity against *Aspergillus niger* (diameter of zone of inhibition viz. 10 mm) but no antifungal activity against *Candida albicans*. Hexane extracts of *Achyranthes aspera* showed no antifungal activity against both the pathogens. Hydroalcoholic extract of *Anrographis paniculata* showed no antifungal activity against *Candida albicans*. The MIC values of the hydroalcoholic extract of *Andrographis paniculata* against *Aspergillus niger* was found to be 0.5 mg/ml while hydroalcoholic extract of *Achyranthes aspera* showed MIC value of 0.3 mg/ml against *Candida albicans* and 0.5 mg/ml against *Aspergillus niger*. Hydroalcoholic extracts of *Valeriana jatamansi, Berberis aristata, Asparagus racemosus, Tinospora cordifolia* and *Plantago depressa* showed almost similar antifungal activity against *Aspergillus niger* but minimum in comparison to hydroalcoholic extract of *Coleus barbatus* (diameter of zone of inhibition viz. 18 mm) against the same pathogen but no antifungal activity of hydroalcoholic extracts of all these plants was observed against *Candida albicans*. Hexane extracts of *Valeriana jatamansi, Coleus barbatus, Achyranthes aspera, Tinospora cordifolia* and *Plantago depressa* showed no antifungal activity against any of the pathogens. Hexane extracts of *Berberis aristata* and *Asparagus racemosus* showed similar antifungal activity against *Aspergillus niger* (MIC: 0.7 mg/ml) but no activity against *Candida albicans*. The results are illustrated in **Table 1** and **Figure 1**. The present study thus stated that all the plants are effective against fungal infections caused by *Aspergillus niger* in comparison to *Candida albicans*.

Table 1: Antifungal activity of the plant(s) extracts

S.No	Plants/ Fucanazole (1 mg/ml)	Solvent Extract	Diameterof zone -inhibition (mm)		MIC(mg/ml)		MLC(mg/ml)	
			AN	CA	AN	CA	AN	CA
1.	*Valeriana jatamansi* (Sugandhbala)	HA	12	NA	0.5	NA	0.7	NA
		HX	NA	NA	NA	NA	NA	NA
2.	*Coleus barbatus* (Pathar choor)	HA	18	NA	0.7	NA	0.8	NA
		HX	NA	NA	NA	NA	NA	NA
3.	*Berberis aristata* (Kingore)	HA	10	NA	0.8	NA	0.9	NA
		HX	08	NA	0.7	NA	0.9	NA
4.	*Asparagus racemosus* (Satrawal)	HA	10	NA	0.5	NA	0.7	NA
		HX	06	NA	0.7	NA	0.9	NA
5.	*Andrographis paniculata* (Kalmegha)	HA	20	NA	0.5	NA	0.7	NA
		HX	10	NA	0.7	NA	0.9	NA
6.	*Achyranthes aspera* (Latjiri)	HA	20	20	0.5	0.3	0.7	0.5
		HX	NA	NA	NA	NA	NA	NA
7.	*Tinospora cordifolia* (Giloei)	HA	10	NA	0.5	NA	0.7	NA
		HX	NA	NA	NA	NA	NA	NA
8.	*Plantago depressa* (Isabgol)	HA	11	NA	0.9	NA	1.0	NA
		HX	NA	NA	NA	NA	NA	NA

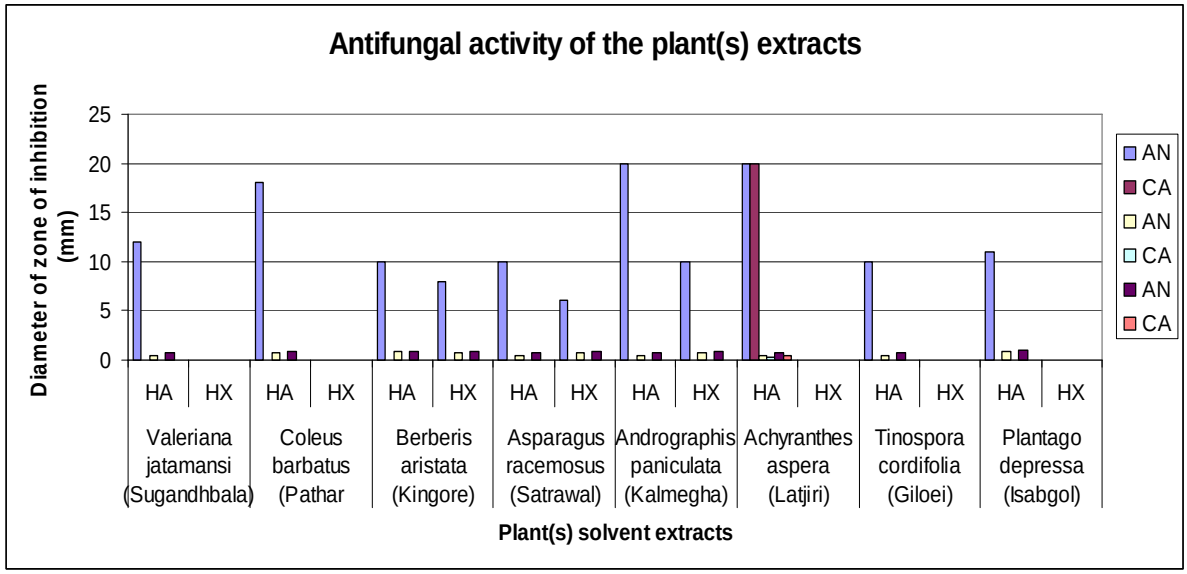

Fig. 1: Antifungal activity of the plant(s) extracts

CONCLUSION

The extracts of the plant (s) part used showed prominent antifungal activity against *Aspergillus niger* and less activity against *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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