Antifungal Activity of Alkaliphiles

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Abstract- *The aim of this study is to produce antifungal metabolites from alkaliphilic isolates and evaluate antifungal activity of these metabolites against Aspergillus niger and Candida albicans. Cell free extracts were used to study antifungal activity of the alkaliphilic isolates. Antifungal activity exhibited by metabolites of most of the alkaliphilic isolates, showed significant antifungal activity against Aspergillus niger, but these metabolites exhibited low antimycotic activity against Candida albicans.*

Keywords- Antifungal metabolites, Cell free extracts, Alkaliphilic isolates.

I. INTRODUCTION

Nowadays invasive aspergillosis is an increasing fungal infection in intensive care, transplant and burn units (1). Otomycosis is a superficial mycotic infection of the outer ear canal. The infection may be either acute or sub-acute and is characterized by inflammation, pruritis, scaling, feeling of fullness and severe discomfort (2). There are alarming reports of opportunistic fungal infections. The infections caused by opportunistic fungi are increasing awareness amongst clinicians and microbiologists. *Aspergillus fumigatus* and *Aspergillus niger* were also isolated from patients suffering from tuberculosis (3).

Candida albicans is part of the indigenous microbial flora in humans and can be found in oral cavity, digestive and vaginal tract. However an increased prevalence of candidiasis is well documented (6). Hence it is essential to develop the remedy to fight against these issues.

II. MATERIAL AND METHODS

Production of antimicrobial compound:

The alkaliphilic microorganisms were isolated on R2A and Horikoshi-II agar medium (pH-11) on the basis of colony characteristics. These isolates were inoculated into Tryptic soy broth (pH-11) and Horikoshi-II broth (pH-11) medium, which were incubated for 10-14 days at 32^0C.

Extraction of antimicrobial compound:

Cell free extracts from alkaliphilic isolates were prepared by centrifugation at 13500 rpm for 30 minutes. Supernatant was collected and was further used for antifungal assay.

Preparation of inoculum:

Candida albicans culture used in this study was inoculated into broth and incubated at 25^0C for 24hrs and adjusted to obtain turbidity comparable to 0.5 McFarland standards (1×10^8 cfu/ml). *Aspergilllus niger* culture was standardized (1×10^5 cfu/ml).

Antimicrobial assay:

Antimicrobial assay was carried out by agar well diffusion method (Kirby Bauer). The 24hr old cultures of *Candida albicans* was evenly streaked on sterile Sabouraud dextrose agar plates with the sterile cotton swab. Spore suspension of *Aspergillus niger* was evenly streaked on sterile Sabouraud dextrose agar plates with the sterile cotton swab. These plates were kept for few minutes to set the culture on Sabouraud dextrose agar plates. The wells were made on Sabouraud dextrose agar plates. The wells were loaded with cell free extracts. These plates were incubated at 25^0C for 48hrs. This was followed by recording diameter of zone of inhibition.

III. RESULTS AND DISCUSSION

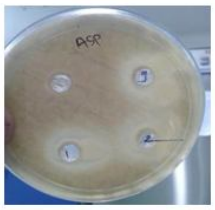
Table No.1

Alkaliphilic isolates From H-II medium	Zone of inhibition (mm)	
	Aspergillus niger ATCC 16404	*Candida albicans ATCC 10231*
1	23	0
2	25	0
5	15	0
6	13	0
7	23	0
8	18	0
9	22	0
15	20.5	0
16	14.5	0
17	17	0

Table No.2

Alkaliphilic isolates from R2A medium	Zone of inhibition (mm)	
	Aspergillus niger ATCC 16404	*Candida albicans ATCC 10231*
8	15	0
9	20	0
10	18	0
11	15.5	0
16	23.5	0
17	12.5	16
22	0	15
23	13.5	11
24	14	11

Photograph No.1

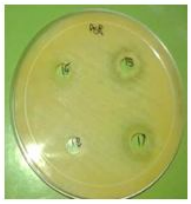


Antifungal activity of cell free extract of alkaliphilic isolates (1, 2 and 9) from H-II medium against *Aspergillus niger*

Photograph No.2

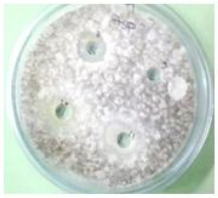
Antifungal activity of cell free extract of alkaliphilic isolates (5, 6, 7 and 8) from H-II medium against *Aspergillus niger*

Photograph No.3



Antifungal activity of cell free extract of alkaliphilic isolates (15 and 17) from H-II medium against *Aspergillus niger*

Photograph No.4



Antifungal activity of cell free extract of alkaliphilic isolates 8, 9,10 and 11 from R2A medium against *Aspergillus niger*

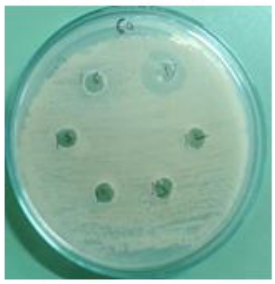
Photograph No.5

Antifungal activity of cell free extract of alkaliphilic isolates 12, 13, 14, 15, 16 and 17 from R2A medium against *Aspergillus niger*

Photograph No.6

Antifungal activity of cell free extract of alkaliphilic isolates 22, 23 and 24 from R2A medium against *Aspergillus niger*

Photograph No.7

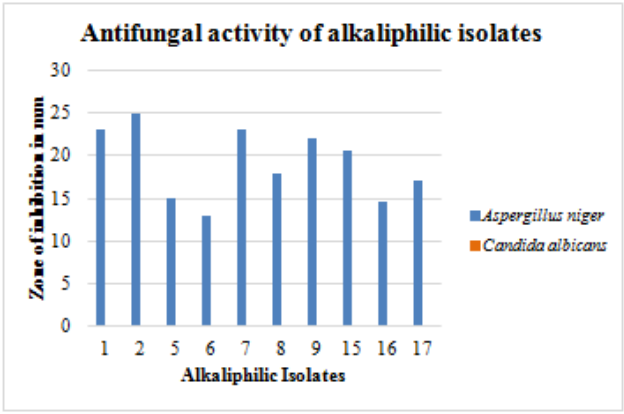


Antifungal activity of alkaliphilic isolates 12, 13, 14, 15, 16 and 17 from R2A medium against *Candida albicans*

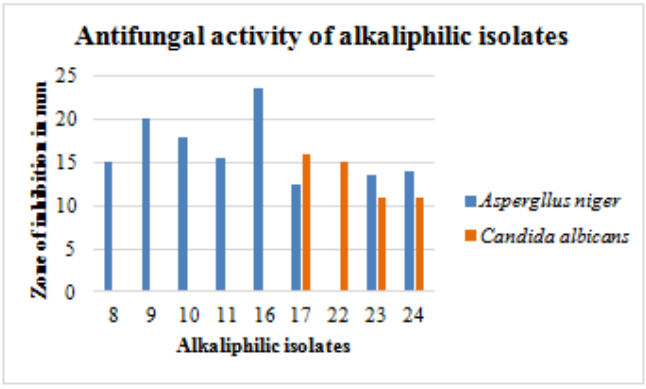
Photograph No.8

Antifungal activity of alkaliphilic isolates 22, 23 and 24 from R2A medium against *Candida albicans*

Graph No.1



Graph No.2



In this study metabolites of alkaliphlic isolates from H-II medium exhibited antifungal activity against *Aspergillus niger* but there were no antifungal activity against *Candida albicans.* Metabolites of alkaliphilic isolates from R2A medium showed antifungal activity against *Aspergillus niger* and *Candida albicans.* Most of the metabolites of alkaliphilic

isolates from R2A medium showed antifungal activity against *Aspergillus niger*. Metabolites of few isolates were found to show antifungal activity against *Candida albicans.* Metabolites of alkaliphilic isolates from H-II medium demonstrated higher antifungal activity against *Aspergillus niger* as compared to metabolites of alkaliphilic isolates from R2A medium.

Bansod Sunita and Rai Mahendra (3) isolated *Aspergillus fumigatus* and *Aspergillus niger* from sputum samples of pulmonary tuberculosis patients from civil hospital of Washim. They have carried out antimycotic assay with essential oils from Indian medicinal plants against these isolates. They got maximum antimycotic activity by *C. martini* followed by *C. citratus, Eucalyptus globosus* and *Cinnamomum zylenicum.*

S. Satish and D.C. Mohana et al (4) tested some plant extracts against seed borne pathogens of *Aspergillus sp*. They recorded high susceptibility for *A. flavus* as compared to other *Aspergillus* species. Among various solvents tested they found methanol was effective for extraction of plant metabolites.
Moiz A. Ansari and Das Shrayanee et al (11) tested antifungal activity of resorcinol against *Candida albicans*. They found that antifungal activity exhibited by resorcinol is independent of the drug efflux pump transporter, which is major cause of the drug resistance in *Candida albicans.* They found that resorcinol is potent inhibitor of yeast to hyphal transition that could be used for treatment *Candida* infections.

IV. CONCLUSION

Antimicrobial activity of alkaliphilic isolates with cell free extracts were evaluated against *Aspergillus niger* and *Candida albicans.* The study revealed that cell free extracts of alkaliphilic isolates from H-II medium exhibited promising activity against *Aspergillus niger*. Antifungal activity can be further enhanced by optimization of conditions for production of bioactive metabolites. The active constituents responsible for antifungal activity can be explored.

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