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ASSESMENT OF ANTIFUNGAL ACTIVITY OF A CYANOBACTERIUM *CALOTHRIX JAVANICA* DE WILDE

J.N. Nehul
Dada Patil Rajale College, Adinathnagar Tal-Pathardi Dist-Ahmednagar- 414505(MS) India
*Corresponding Author: jnnehul@gmail.com

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ABSTRACT:

A cyanobacterium *Calothrix javanica* was isolated from the collected soil samples from different locations. Identification was carried out using morphological variation and taxonomical approaches according to Desi kachary (1959) and Prescott (1962) .The axenic culture of *Calothrix javanica* was obtained by using the method recommended by Bolch and Blackburn (1996). The isolated species of *Calothrix javanica* was grown autotropically in BG-11 medium as described by Rippka *et al.*, (1979) and incubated at 30±2°c. After 25 days, biomass was harvested by filtration through double layered muslin cloth and dried using air blower. The biomass of this *Calothrix javanica* species was used for the assessment of antifungal activity against *Aspergillus niger*, *Trichophyton capitanum*, *Aspergillus flavus*, *Fusarium Monelliforme*, *Helminthosphorium* sp., *Alternaria* sp. *and Candida albicans*. In the light of the experimental result concerning the antifungal activity against the tested fungal organisms, the results recorded clearly showed that the methanol extracts of *Calothrix javanica* towards the tested fungi gave positive results.

Key words: - *Calothrix javanica,* Antifungal Activity, BG-11, *Candida albicans, Alternaria sp.*

INTRODUCTION:

Fungal infections remain a significant cause of morbidity and mortality despite the advances in medicines and the emergence of new antifungal agents (McNeil *et al.*, 2001). *Candida albicans*, the agent of candidiasis, is an increasingly important disease that has a worldwide distribution due to the fact that it is a frequent opportunistic pathogen in AIDS patients (De Pavia *et al.*, 2003). It is commonly found in the gastrointestinal and urinogenital tracts of human (Black, 1996). The available antifungal drugs produce many adverse effects, show reappearance or lead to the development of resistance. Therefore, the new antifungal agents without these disadvantages are strongly needed (Selitrennikoff, 2001).

Screening of cyanobacteria for antibiotics and other pharmacologically active compounds has received ever-increasing interest as a potential source for new drugs (Borowitzka 1995, Fish and Codd, 1994, stensvik *et al.*, 1998, Schlegel *et al.*, 1999). Several species of cyanobacteria produce substances with antibiotic activity. Antifungal activity was observed in a large percentage of the cyanobacterial extracts tested (Ishibashi *et al.*, 1986). The cryptophycins comprise the largest class of cyanobacterial depsipeptides (Trimurtulu *et al.*, 1994) in which cryptophycin-1, was isolated from *Nostoc* sp. ATCC 53787 as an antifungal agent (Hirsch *et al.*, 1990). The scytophycins are also found to be potent antifungal agents (Patterson and Carmeli, 1992). Scytophycins are highly cytotoxic and fungicidal macrolides. In spite of the studies carried out so far, many cyanobacterial compounds are still largely unexplored, thus giving a great opportunity for the discovery of a new bioactive compound.

The expected rate of discovery is far lower than that of other better-studied group of organisms (Olaizola, 2003).

Therefore the aim of the current research was to investigate the antifungal activity of *Calothrix javanica* isolated from terrestrial habitat of Ahmednagar district of Maharashtra state, India.

MATERIALS AND METHODS:

Collection, Isolation and Identification of Westiellopsis prolifica

Calothrix javanica was isolated from the collected soil samples from different locations. The axenic culture of *Calothrix javanica* was obtained by using the method recommended by Bolch and Blackburn (1996). The isolated *Calothrix javanica* was grown auto tropically in BG-11 medium as described by Rippka *et al.*, (1979) and incubated at 30±2°c . Identification was carried out using morphological variation and taxonomical approaches according to Desikachary (1959) and Prescott (1962).

Extraction procedure

Five g of finely powdered biomass was successively extracted in 50 ml of chloroform, methanol, hexane and water by using soxhlet apparatus at 40°C for 24 h. The filtered extract was concentrated in vacuo at 40°C. Final volume of the extract was made 1ml with respective solvents.

Test organisms

Pure cultures of *Aspergillus niger*, *Trichophyton capitanum*, *Aspergillus flavus*, *Fusarium Monelliforme*, *Helminthosphorium sp.*, *Alternaria sp. and Candida albicans* were procured from NCIM, NCL, Pune and used for antifungal assay. Test cultures were grown on 25 ml potato dextrose agar of composition (in g L^{-1}) potato 200g, dextrose 20, and agar 20 at pH 5.6 for sporulation in 500 ml Erlenmeyer flasks. Only *Candida albicans* was grown on MGYP medium. Spore suspension was prepared by pouring 5 ml sterile 0.01% triton X-100 solution, vortexing for 1 min and sieving through cheesecloth. Final inoculum density of 10^6 spores ml^{-1} was calibrated using a hemocytometer.

Screening of isolates for antifungal activity

Agar diffusion assay

Agar wells were prepared with borer and poured with 400µl extracts of *Calothrix javanica*, dried, and placed on potato dextrose agar plates, prior inoculated with 0.1 ml fungal spore suspension (1 X10^6 spores ml^{-1}). Antifungal activity was assessed by measuring the diameter of growth inhibition zone after incubation at 30°C for 48 h. Itraconazol (Hi Media, India) was used as the positive control at 10 µg ml^{-1}concentrations.

RESULTS AND DISCUSSION:

In the light of the experimental result concerning the antifungal activity against the tested fungal organisms, the results recorded in Table -1 clearly showed that the antifungal activity of the methanol extract of *Calothrix javanica* towards the tested fungi gave positive results but with varying degree. Methanol extract of *Calothrix javanica* gave the largest inhibition zones against *Aspergillus flavus*. Methanol extract exhibited activity against all the tested fungi and the zone of inhibition ranged from 06 mm to 17 mm.

Activity of chloroform extract shown by *Calothrix javanica* was only against *Aspergillus niger* and *Aspergillus flavus*.No activity of chloroform extract was shown by *Calothrix javanica* against *Trichophyton capitanum, Fusarium monelliform Candida aibicans,* and *Helminthosporium* sp. Hexane and aqueous extracts did not show any activity against any tested fungal organism. Results are the mean of diameter values ± standard deviation. Effective zone of inhibition

= (Total zone of inhibition minus diameter of disk). A few studies have been carried out to screen *Calothrix javanica* species for production of antimicrobial substances from paddy-fields. Possibly the synthesis of highly active toxin is a defense option of cyanobacteria in these environments against other organisms like bacteria, fungi, viruses and eukaryotic microalgae (Mundt *et al.*, 2001).Maximum antifungal activities in case of methanol extraction as observed in the present study are in accordance with earlier reports (Østensvik *et al.*, 1998; Soltani *et al.*, 2005). The effect of antimicrobial activity of cyanobacteria has been reported in other studies such as Patterson *et al.*, (1994), Falch *et al.*, (1995), and Smitka *et al.*, (1992).

It seems *Calothrix javanica* is being reported for the first time as producer of antifungal substances. The results of this work indicate that this species displays a potential that warrants further investigations. No antifungal activity was detected in the hexane and aqueous extracts. This is probably because of polar nature of the active components. It shows that the chance of finding antifungal activity is higher in methanol extracts. The variation in antifungal activities could be due to different permeability of bioactive substances into the test organisms. The production of bioactive compounds and expression of antimicrobial activity depends on physiological factors such as stage of growth and culture conditions (Schlegel *et.al.*1999).

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Table no.–1: Antifungal activity of methanol and chloroform extract of *Calothrix javanica* at 400µg/ml concentration against some species of fungi.

	Diameter of effective zone of inhibition (mm)						
Extract	*Aspergillus niger*	*Trichophyton capitanum*	*Fusarium monelliforme*	*Candida aibicans*	*Helminthosporium* sp.	*Alternaria* sp.	*Aspergillus flavus*
Methanol	6±1.3	11±1.8	16±1.5	10±1.7	8±1.8	11±1.3	17±1.5
Chloroform	11±1.3	---	---	---	---	----	09±1.4
Hexane	---	---	---	---	---	---	---
Aqueous extract	---	---	---	---	---	---	---
Itraconazole(10µgml$^{-1)}$	22±2.4	28±1.3	25±1.7	22±1.6	28±1.2	30±1.2	28±1.2