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ANTIFUNGAL ACTIVITY OF SELECTED CYANOBACTERIA AGAINST FUNGAL PATHOGENS

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ABSTRACT

Cyanobacterial secondary metabolites have a diverse antagonistic activity that lead to disintegration of microbial growth. The present study was conducted to evaluate the antifungal property possessed by two cyanobacterial Tolypothrix nodosa and Tolypothrix tenuis strains were isolated from Water and Soil samples of various sites of Warangal district, Telangana State, India. Cyanobacterial strains were extracted in acetone, methanol and petroleum ether and tested for antifungal activity against four pathogenic fungal strains (Aspergillus niger (MTCC-4325), Aspergillus fumigatus (MTCC-4163), Trichophyton rubrum (MTCC-3272) and Macrophomina sp. (MTCC-10576). Crude extracts of each strain show differential antifungal response to test organisms. Based on zone of inhibition formation, it was concluded that the Acetone culture crude extracts of T.Tenuis had maximum antifungal efficacy13.00 ± 0.57 mm against A. Fumigatus and the minimum zone of inhibition 7.00 ± 0.00 mm was observed in the same extract of acetone against Macrophomina sp. Also, the T.Nodosa methanol extract exhibited significant activity (11.33 ± 0.88 mm) against A. fumigatus, and minimum inhibition zone 7.66 ± 0.33 mm exhibited against T. rubrum in the acetone extract respectively. Acetone extract was showed highest antifungal activity among all the solvents tested; methanol and petroleum ether were the next preferred solvents. The Cyanobacteria can be excellent biocontrol sources for human pathogenic fungi as they can be easily cultured and less expensive compared to synthetic fungicides and they are ecofriendly. The results of present investigation, clearly indicates the presence of antimicrobial compounds in the two-cyanobacterial species. Therefore, further studies are necessary to elucidate the components responsible for antifungal activities against microorganisms.

KEY WORDS

Cyanobacteria, Tolypothrix nodosa, Tolypothrix tenuis, Organic solvent extracts, fungal trains, antifungal activity and zone of inhibition

INTRODUCTION:

Cyanobacteria or Blue-green algae are oxygenic photosynthetic microorganisms commonly found in diverse aquatic environments [1, 2]. Cyanobacteria have a capability to grow in diverse habitats, and adaptation of these ecological niches has triggered the production of some of the novel bioactive compounds by the certain mechanisms. The screening of cyanobacteria from different habitats have some out with the discovery of novel secondary metabolites with good bioactivity as well as implications for environmental health [3, 4].

Some of the Cyanobacterial strains are known to produce intracellular and extracellular metabolites with diverse biological activities such as antibacterial, antifungal, antiviral and anticancer activity [5]. Recently there are numerous reports of cyanobacteria derived compounds that have a broad range of biological activities, such as antibacterial, antifungal, antiviral, anti-neoplastic, antifouling, antioxidant, anti-inflammatory, anticoagulant, anti-enzymatic, cytotoxic and antimitotic [4,6,7,8,9,10,11].

A number of research papers have been published about the antimicrobial activities from Cyanobacteria [12,13]. The cyanobacteria such as *Nostoc commune* [14,15], *Anabaena variabilis* [16], *Nostoc spongiaeforme* [17], *Microcystis aeruginosa*, *Anabaena flos-aquae* [18], *Calothrix brevissima* [19], *Nostoc commune* and *Spirulina platensis* [20], *Oscillatoria* sp., *Nostoc* sp., *Nostoc muscorum, Nostoc piscinale, Phormidium* sp., *Anabaena flos-aquae* and *Spirulina platensis* [21], *Anabaena circinalis, Nostoc muscorum, Stigonema ocellatum* and *Hapalosiphon welwitschii* [22] and *Nostoc calcicola* [23] have been popularly reported to produce antimicrobial substances.

Further, cyanobacterial metabolites show interesting and exciting biological activities, including antimicrobial, immuno suppressant, anticancer, anti-HIV (human immunodeficiency virus), antibacterial, anticoagulant, antifungal, antiinflammatory, antimalarial, antiprotozoal, antituberculosis, antiviral, and antitumor activities [24, 25, 26].

The aim of present study is an attempt to evaluate the antifungal activity of *Tolypothrix nodosa* and *Tolypothrix tenuis* against human fungal pathogens.

MATERIALS AND METHODS:

i). Collection of sample and culture condition: Water and Soil samples containing algae/cyanobacteria were collected from various sites of Warangal district, Telangana state and were kept in the plastic vials to transfer to the lab. The cyanobacterial samples were cultured directly in inorganic BG-11 media [27]. A standard plating and streaking techniques were used for isolation and purification of cyanobacterial strains. Unialgal cultures were prepared using sub-culturing methods. Purified Cyanobacteria cultures were transferred into 250 ml conical flasks containing 100 ml of inorganic BG-11 medium and incubated under controlled conditions of continuous fluorescent light of regime of 16 h light / 8 h dark cycles with temperature 26 ± 2 ºC for 28 days. Finally, the uni algal cultures were established for further studies.

ii). Identification of cyanobacterial species:

Cyanobacteria were identified by morphological as well as taxonomical observation methods. The nature of filament, shape, and size of the vegetative cell, heterocysts, and Akinete was viewed at 400x and 1000x using Olympus 21Xi microscope and identification of cyanobacterial species was made based on the morphological observations using standard monographs and protocols [28, 29]. The obtained species were identified as *T.nodosa* and *T. Tenuis.*

Iii). Preparation of cyanobacterial extracts: Cyanobacterial biomass were harvested in the stationary growth phase by centrifugation at 5000 rpm for 15 minutes to obtain the biomass. The obtained biomass was dried in the hot-air oven at 60^{o}c for 24 hrs, resulted biomass powdered was used for the extraction by different organic solvents. The one gram (1.0 grm) of dried powdered of each strain were extracted in 15 ml of solvents, i.e., Acetone, Methanol and Petroleum ether left overnight it gets to saturation and filtered the extract. The filtrate crude of each strain was again evaporated to dryness at 40 ^{o}c and resultant crude extract 1 mg was weighed and dissolved in 1 ml of same organic solvent as stock solution and it was preserved at 4ºC [30] until it uses for bioassay.

iv). Test organisms: Microorganisms were used for the present study, i.e., fungal strains of *Aspergillus fumigatus* (MTCC-4163), *Aspergillus niger* (MTCC-4325), *Mucor* sp. (MTCC-3340) and *Trichophyton mentagrophytes* (MTCC-8476) were obtained from Department of Microbiology, Kakatiya University, Warangal, Telangana State, India. The fungal strains were inoculated onto glucose peptone broth and incubated at 30^{o}C for 5 days.

v). Agar disc diffusion assay method:

Antifungal activity of culture crude extracts of two cyanobacterial species were determined by using the agar disc diffusion assay by using Sabouraud Dextrose Agar (SDA) method [31]. The 20 ml of sterilized Sabouraud Dextrose Agar medium (Hi Media- Mumbai, India) was poured into petri plates, allowed to cool and solidify. 100 µl 5-day glucose peptone broth culture of fungal suspension was poured in each plate and inoculated with L-shaped spreader. Sterile filter paper disc (6mm) impregnated with 50 µl of crude extract were placed on the surface of the agar containing media, similarly disc (6mm) impregnated with 50 µl of respective organic solvent (Acetone, Methanol and Petroleum ether) were placed on the surface of the agar containing media were used as negative control and Nystatin 50 µg/disc was used as standard control. The plates were incubated for 48-72 hrs at 28^{0} C. At the end of incubation period the zone of inhibition was recorded in millimeters (mm) by using Hi Antibiotic Zones Scale-C^{TM} Hi Media (Mumbai, India). Antifungal activity was

evaluated by measuring the zone of inhibitions against the tested microorganisms and their mean and standard errors was calculated.

Data analysis:

The results of the present data obtained were statistically analyzed and expressed as the mean (x-) and standard error (SE) for inhibition zones of three experiments (n=3) by using Graph Pad Prism version 5.03 (Graph Pad Software, Inc.,).

RESULTS AND DISCUSSION:

Assessment of antifungal activity:

The antifungal activity of *Tolypothrix nodosa* in different solvents of Acetone, Methanol and Petroleum ether culture extracts were tested against four pathogenic fungi shown in **(Table-1 & Figure-1: A)**. The Methanol culture extract showed highest zone of inhibition (11.33 ± 0.88 mm) against *A. fumigatus*, (10.00 ± 0.00 mm) against *A. niger*, (9.33 ± 0.33 mm) against *Macrophomina* sp. and (9.00 ± 0.57 mm) against *T. rubrum* respectively. The Petroleum ether culture crude extract was also expressed with zone of inhibition (9.00 ± 0.57 mm) against *A. fumigatus* and *Macrophomina* sp. followed by (8.00 ± 0.57 mm) against *T. rubrum*. The Acetone extract was also inhibited the growth of two fungal pathogens with significant zone of inhibitions (8.00 ± 0.57 mm and 7.66 ± 0.33 mm) against *A. niger*, and *T. rubrum*, respectively. All the culture extracts which were shown with antifungal activity in various solvents were expressed by a prominent zone of inhibitions, but in the Acetone culture extract it was observed with low inhibition zones (7.66 ± 0.33 mm) against *T. rubrum*. The Acetone culture crude extract was not expressed any kind of inhibition zone against *A. fumigatus* and *Macrophomina* sp. Similarly, the petroleum ether culture crude extract did not show any kind of biological activity against *A. Niger*. All the extracts were showed the lower zone of inhibition when compared to the standard control Nystatin (50 µg/disc, **Figure-1:C**).

Table-1: Antifungal activity of *Tolypothrix nodosa*

Test Organisms	**Zone of Inhibition (**ZOI) (diameter in mm) **Organic extracts (1mg/ml)**						
	Acetone		**Methanol**		**Petroleum ether**		**Standard Control**
	Extract	Control	Extract	Control	Extract	Control	Nystatin (50 µg/disc)
Aspergillus niger (MTCC-4325)	8.00 ± 0.57	0.00	10.00 ± 0.00	0.00	--	0.00	23.00 ± 0.57
Aspergillus fumigatus (MTCC-4163)	--	0.00	11.33 ± 0.88	0.00	9.00 ± 0.57	0.00	23.33 ± 0.88
Trichophyton rubrum (MTCC-3272)	7.66 ± 0.33	0.00	9.00 ± 0.57	0.00	8.00 ± 0.57	0.00	21.66 ± 0.88
Macrophomina sp. (MTCC-10576)	--	0.00	9.33 ± 0.33	0.00	9.00 ± 0.57	0.00	20.66 ± 0.33

" --" No inhibition zone

Diameter of the inhibition zone including disc diameter (6 mm).

Values were with mean **±** SE of three separate experiments (n=3).

Table-2: Antifungal activity of *Tolypothrix tenuis*

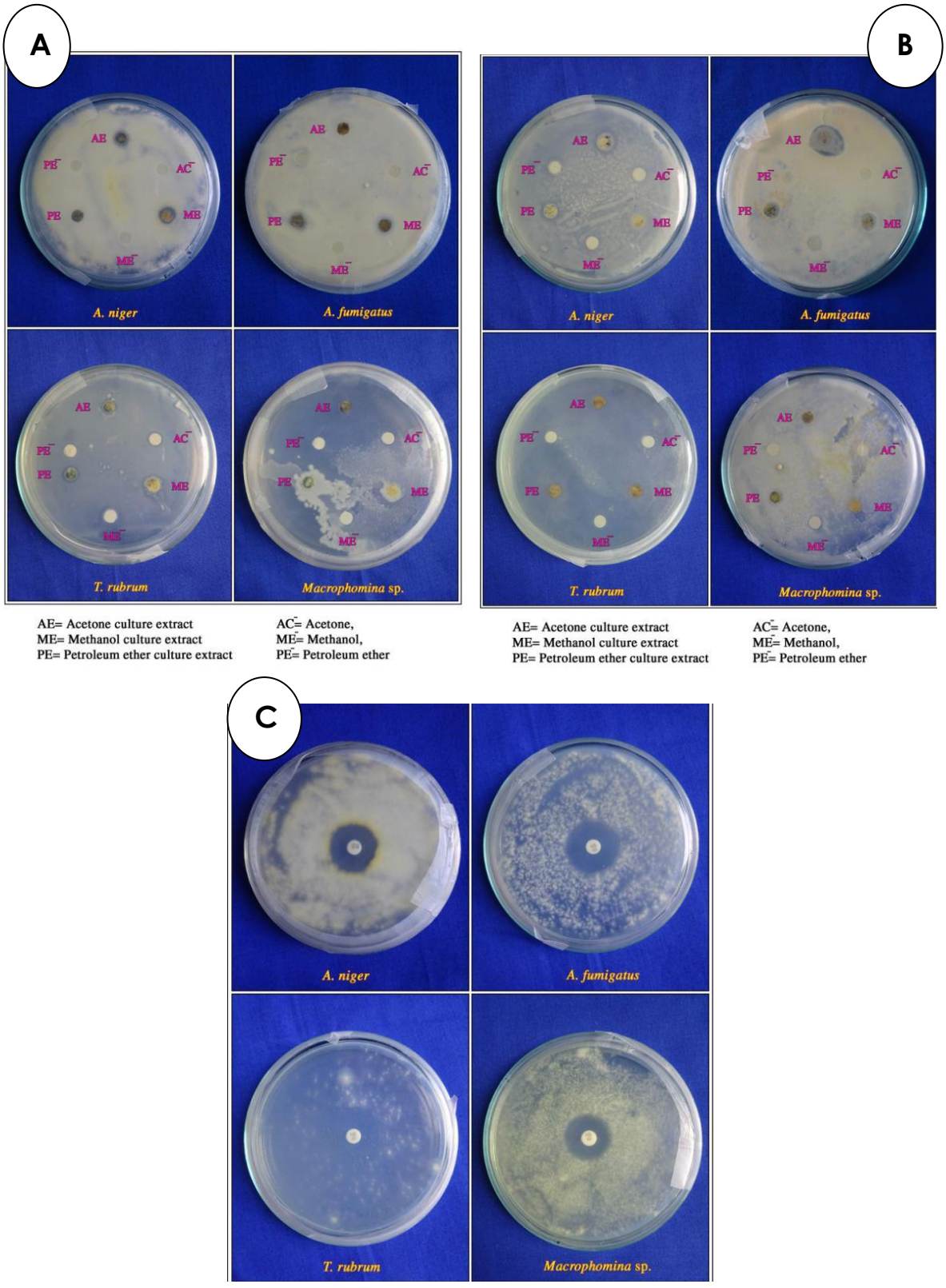
Test Organisms	**Zone of Inhibition (**ZOI) (diameter in mm) **Organic extracts (1mg/ml)**						
	Acetone		**Methanol**		**Petroleum ether**		**Standard Control**
	Extract	Control	Extract	Control	Extract	Control	Nystatin (50 µg/disc)
*Aspergillus niger (*MTCC-4325)	10.00 ± 0.57	0.00	7.66 ± 0.33	0.00	9.00 ± 0.57	0.00	23.00 ± 0.57
Aspergillus fumigatus (MTCC-4163)	13.00 ± 0.57	0.00	8.66 ± 0.33	0.00	8.00 ± 0.57	0.00	23.33 ± 0.88
Trichophyton rubrum (MTCC-3272)	8.00 ± 0.57	0.00	9.33 ± 0.33	0.00	7.66 ± 0.33	0.00	21.66 ± 0.88
Macrophomina sp. (MTCC-10576)	7.00 ± 0.00	0.00	--	0.00	10.66 ± 0.33	0.00	20.66 ± 0.33

"--" No inhibition zone
Diameter of the inhibition zone including disc diameter (6 mm).
Values were with mean **±** SE of three separate experiments (n=3).

The antifungal activity of *Tolypothrix tenuis* culture crude extracts was evident in all the tested pathogenic fungi. Almost all the extracts evaluated showed variable degree of inhibition zones against different pathogenic fungi shown in **(Table-2 & Figure-1: B)**. The results indicate that the Acetone culture crude extract notably inhibited the pathogen with the highest inhibition zone (13.00 ± 0.57 mm) against *A.fumigatus*, followed by (10.00 ± 0.57 mm) against *A. niger*, (8.00 ± 0.57 mm) against *T. rubrum* and (7.00 ± 0.00 mm) against *Macrophomina* sp. The Petroleum ether culture extract was also exhibited antifungal activity against *Macrophomina* sp. (10.66 ± 0.33 mm), *A. niger* (9.00 ± 0.57 mm), *A. fumigatus* (8.00 ± 0.57 mm) and *T. rubrum* (7.66 ± 0.33 mm). While, the Methanol culture extract was exhibited with desirable activity against *T. rubrum* (9.33 ± 0.33 mm), *A. fumigatus* (8.66 ± 0.33 mm) and *A. niger* (7.66 ± 0.33 mm). The *Macrophomina* sp. have shown with lowest inhibition zone (7.00 ± 0.00 mm) in the solvent of Acetone culture extract under observation. Whereas, in the Methanol culture crude extract no antifungal activity was found against *Macrophomina* sp. All the extracts were showed the lower zone of inhibition when compared to the standard control Nystatin (50 µg/disc, **Figure-1:C**).

The results obtained from the present investigations are in agreement with the reports of that the methanol extract of cyanobacterial species *A.variabilis* showed highest anfifungal activity against *A. niger* and *R. Stolonifer* [16]. In one study, the culture media of cyanobacteria belonging to Nostaceae, Microchaetaceae and Scytonematacaea isolated from the Argentinian agricultural fields were found to be active against *S. aureus* and *Candida albicans* [32]. The chloroform, diethyl ether, ethanol and methanol extracts of *Spirulina major, Oscillatoria limosa* and *Nostoc linckia* showed the maximum inhibitory effect against different fungal pathogens [33]. The ethanol was the most appropriate solvent to extract bioactive compounds from two macros and microalgal species with antimicrobial activities against four microorganisms (*E. coli, Staphylococcus aureus, Candida albicans* and *Aspergillus niger*) [34]. Moreover, the cyanobacterial chloroformic extracts displayed antifungal activity against *Aspergillus terreus* F98 [21]. The antifungal activities of different extracts of cyanobacteria tested towards fungi given similar results but with varying degrees and these observations are in line with those of the present study [35,36,37].

Figure-1: Antifungal activity of *Tolypothrix nodosa* and *Tolypothrix tenuis* crude extracts against pathogenic fungi



A) *Tolypothrix nodose;* B). *Tolypothrix tenuis;* C*).* Standard Control, Nystatin (50 µg/disc)

CONCLUSION:

The Cyanobacteria are the rich source of secondary metabolites having diverse antifungal activity. The present investigation revealed that the methanol and acetone extracts of *T.Nodosa* and *T.Tenuis* were proved with the antimicrobial activity against the tested species of fungi. Improved analysis of bioactive compounds with respect to antimicrobial activity would assist in efforts for the pharmaceutical application. Hence, further studies are necessary to elucidate the components from these cyanobacteria against antifungal activity.

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