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# Antimicrobial activity of Blue-Green Algae, Calothrix braunii (A. Br.) Bornet et Flahault.

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## Abstract

Cyanobacteria (Blue-green algae) are rich source of structurally novel and biologically active metabolites with antibacterial, antifungal, antiviral and anticancer activities. The present study was an attempt to assess the antifungal and antibacterial efficacy of the Aqueous, Chloroform, Ethyl acetate, Hexane and Methanol culture crude extract of cyanobacterium *Calothrix braunii* (A. Br.) Bornet et Flahault. *In vitro* antimicrobial activity was evaluated by agar disc diffusion assay method against four pathogenic fungi *Aspergillus fumigatus* (MTCC-4163), *Aspergillus niger* (MTCC-4325), *Mucor* sp. (MTCC- 3340) and *Trichophyton mentagrophytes* (MTCC-8476) and four pathogenic bacteria in which two are Gram-positive, *Bacillus subtilis* (MTCC-1427), *Staphylococcus aureus* (MTCC-1430) and two are Gram-negative, *Escherichia coli* (MTCC-1302), and *Klebsiella pneumoniae* (MTCC-4030). The cyanobacterium, *Calothrix braunii* showed varying degree of inhibitory activity against all the tested fungi and bacteria. The zone of inhibition of five various extracts were varied from solvent to solvent and it was determined and compared with the standard control Nystatin and Ciprofloxacin. *Calothrix braunii* was exhibited with maximum antifungal activity 12.66 mm against *A. fumigatus* in the Hexane extract and antibacterial activity was found high 17.66 mm in Chloroform extract against *S. aureus* under investigation.

**KEYWORDS:** *Calothrix braunii,* Antifungal activity, Antibacterial activity, Nystatin and Ciprofloxacin.

## Introduction

More number of species of cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activities such as antialgal, antibacterial, antifungal and antiviral activity [19, 6] In addition, they are known to produce wide array of bioactive compounds (secondary metabolites) with different biological activities including antibacterial, antifungal, antiviral, antimalarial, antitumoral and anti inflammatory properties, also having

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industrial, therapeutic and agricultural significance [9]. Microalgae represent a large and underexplored resource of antimicrobial compounds [14, 27, 8]. Cyanobacteria produces natural products which have the ability of antimicrobial characters [11, 7, 21]. Micro algae produce the various antimicrobial active substances from the green alga, *Chlorella vulgaris* and the cyanobacterium *Pseudanabaena* sp. [16]. Cyanobacteria from local habitats seem to be a source of potential new bioactive substances that could contribute to reduce of the number of bacteria, fungi, viruses and other microorganisms [12, 18, 13]. Additionally, the bioactive products of cyanobacteria exhibited more active against bacteria, fungi, and virus [1, 24, 5] with reference to their growth control.

## Materials and Methods

**Culturing and maintenance of cyanobacteria:** Water and soil samples of cyanobacterium, *Calothrix braunii* (A. Br.) Bornet et Flahault was collected from various locations of Warangal district, Telangana State. The samples were brought to laboratory in plastic vials and washed with distilled water to prevent potential contaminants. The axenic cyanobacterial strain of *Calothrix braunii* (A. Br.) Bornet et Flahault was grown for 28 days in BG-11 [28] inorganic media under controlled laboratory conditions (Temperature at 26±2 $^0$C and light at 4000 Lux) and a regime of 8 h dark/ 16 h light, before using for the experiment.

**Identification of cyanobacteria:** The identification and taxonomy of cyanobacterium *Calothrix braunii* was done based on the morphological variation like presence of heterocyst and akinetes. The standard monographs like [4, 2, 26 ] were used.

**Preparation of extracts for antimicrobial activity:** The 28 days harvested algal cultures were centrifuged at 5000 rpm and the pellets were collected, weighted and used for the extraction. The dried biomass was extracted with the five solvents. 1 (one) gram of dried biomass powder was extracted in 20 ml of each Aqueous, Chloroform, Ethyl acetate, Hexane and Methanol for 24 hrs for extraction and extracts were filtered and evaporated under reduced pressure. The resultant crude extract was weighed and dissolved in a known volume 1ml of Dimethyl Sulfoxide to obtain a final concentration. The extracts were preserved at 4 $^0$C as a stock solution for further

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bioassay studies. For the bioassay study 50 µg/ml concentration of crude cyanobacterial extract was taken from the stock solution.

**Test organisms**: Pure cultures of microorganisms like four fungal species, *Aspergillus fumigatus* (MTCC-4163), *Aspergillus niger* (MTCC-4325), *Mucor* sp. (MTCC- 3340) and *Trichophyton mentagrophytes* (MTCC-8476) and four bacteria in which two are Gram- positive bacteria *Bacillus subtilis* (MTCC-1427), *Staphylococcus aureus* (MTCC-1430) and two are Gram- negative bacteria *Escherichia coli* (MTCC-1302) and *Klebsiella pneumoniae* (MTCC-4030) were obtained from Department of Microbiology, Kakatiya University. All test organisms were maintained on specific media slants at 4°C and revived prior to use.

**Antimicrobial susceptibility assay**: Antifungal and antibacterial activity was determined by agar disc diffusion assay method. The Sabroud Dextrose Agar (SDA) and Muller Hinton Agar (MHA) media were prepared according to the procedure [22, 23]. The media was sterilized by autoclaving at 121 $^0$C for 15 minutes at 15 lbs pressure and was used for the fungal and bacterial assay. After sterilization, the media was poured on to the sterile petriplates, and then the plates were allowed to cool and solidify. After solidification, 100 µl of fungal and bacterial cultures were inoculated on to the petriplates with a lawn of cultures. Then the filter paper discs (6 mm) saturated with 50 µl of the culture crude extracts, allowed for air dried and placed on Sabouraud Dextrose Agar plates (Fungi) and Muller Hinton Agar plates (Bacteria). The plates were incubated at 28 $^0$C for the period of 48-72 hrs for fungi and at 37 $^0$C for the period of 24 hrs for bacteria. At the end of incubation period the zone of inhibition around the paper disc (6 mm) was calculated and expressed in millimeter (mm) and compared with the standard control Nystatin (Fungi) and Ciprofloxacin (Bacteria). All the experiments were done in aseptic condition under laminar air-flow cabinet in triplicates and their mean and standard errors were presented. The various extracts containing antimicrobial components produced distinct zones of inhibition around the discs were determined and used as an indication of antimicrobial activity.

**Statistical analysis**

The results of the present data were statistically analysed and the values are mean ± standard error (SE) of the three measurements (N=3).

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## Results

The cyanobacterial strain *Calothrix braunii* was extracted and tested against respected pathogenic fungal and bacterial strains for its antimicrobial activity. The results revealed from the present study concerning the biological activity of the antimicrobial agents produced by the cyanobacterium, *Calothrix braunii* against different species of fungi and bacteria were recorded in (**Table-1**). It is clear from the present investigation that the diameter of the zone of inhibition depends mainly on type of the cyanobacterial species, type of the solvent used and the tested fungal and bacterial organisms.

### Antifungal activity

The antifungal activity of cyanobacterium, *Calothrix braunii* was tested against four fungal pathogens *Aspergillus fumigatus, Aspergillus niger*, *Mucor* sp. and *Trichophyton mentagrophytes*. As per the formation of zone, Hexane extract expressed the high visibility of zone of inhibition 12.66 mm against *A. fumigatus* as compared to the remaining culture crude extracts. Followed by the Chloroform extract 11.00 mm against *T. mentagrophytes*. Similarly, the Chloroform, Ethyl acetate and Methanol culture crude extracts were showed the moderate inhibitory effect 10.33 mm against *A. fumigatus*, *Mucor* sp., *T. mentagrophytes* and *A. fumigatus* and also the Chloroform culture crude extract exhibited the zone of inhibition 10.00 mm against *A. niger* and Aqueous extract was expressed with 9.33 mm against *Mucor* sp. under study. Likewise all the culture crude extracts were showed the significant zone of inhibitions against respective fungal pathogens. Whereas, the low inhibition activity 7.33 mm was expressed in the culture crude extract of Hexane against *T. mentagrophytes*. While all other culture crude extracts were showed the growth of inhibition, except Aqueous extract which did not show any clear zone of inhibition on fungi of *A. niger.* All the extracts were showed the lower zone of inhibition when compared to the antifungal standard control Nystatin.

### Antibacterial activity

The antibacterial activity of five different extracts of *Calothrix braunii* against four selected test organisms is shown in (**Table-1**). The highest effective zone of inhibition 17.66 mm was noticed in Chloroform extract against *S. aureus* followed by *E. coli* 16.66 mm. Similarly, 16.00 mm zone of inhibition against *E. coli* in the culture crude extract of Hexane, 15.00 mm against *K. pneumoniae* in Ethyl acetate extract. The Ethyl acetate culture crude extract was found with the

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potential activity 14.66 mm against *E. coli*, likewise the Chloroform extract 13.33 mm against *K. pneumoniae*, Ethyl acetate and Hexane culture crude extracts were showed with same inhibition zones 13.00 mm against *B. subtilis* and *S. aureus*. The moderate inhibitory effect 12.66 mm was noticed in the culture crude extract of Aqueous against *B. subtilis*, followed by 12.33 mm in the solvent of Methanol against *K. pneumoniae*, 11.66 mm against *E. coli* in the culture extract of Methanol and 11.33 mm was observed in the culture crude extract of Hexane against *B. subtilis*. The minimum zone of inhibition was observed in the Aqueous culture crude extract with the zone of inhibition 10.00 mm against *E. coli*. The cyanobacterium, *C. braunii* was not responded with any inhibition zones in the culture crude extracts of Aqueous against *S. aureus* and *K. pneumoniae* and it was followed by Chloroform extract against *B. subtilis*, Hexane extract against *K. pneumoniae* and the Methanol culture extract against *B. subtilis* and *S. aureus*. All the observation of different solvent culture crude extracts were noticed with lower zone of inhibitions when compared to the standard control Ciprofloxacin under investigation.

**Discussion & Conclusion**

According to the previous reports, cyanobacterium *Calothrix braunii* are rich sources of antimicrobial and bioactive compounds. In our study we observed that Hexane and Chloroform extracts of the *C. braunii* exhibited maximum potential antifungal and antibacterial activity. The culture crude extract of *Cylindrospermum majus* was shown with significant antibacterial activity in the solvent of Chloroform against *K. pneumoniae* and in fungal activity the Aqueous extract showed maximum inhibition zone against *A. fumigatus* [15]. The Methanolic extract of *Oscillatoria* sp. expressed with high inhibitory effect against *E. coli*, *P. aeruginosa*, *B. cereus* and *S. aureus* [25]. Antibacterial activity of bloom forming cyanobacteria against clinically isolated human pathogenic microbes [3]. Diethyl ether crude extract of *Rivularia* sp. possessed potential antibacterial activity against Gram-negative bacteria when compared to the standard antibiotics [17]. In antibacterial activity the *Bacillus subtilis* and *Staphylococcus aureus* were more susceptible whereas *Micrococcus luteus* and *Escherichia coli* were comparatively less susceptible against all the four chosen solvent extracts [10]. Therefore the present study confirms that the cyanobacterium, *Calothrix braunii* are potential source of bioactive compounds against various pathogens, which can be used as natural non-toxic preservative and may be more acceptable to society. Concerning the antifungal and antibacterial effects, the present

investigation revealed that the Hexane and Chloroform extracts were proved with the highest biological activity against the tested species of fungi and bacteria. Further work has to be made to screen, indentify and characterization of the active compounds from the cyanobacterium under study.

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**Table-1:** Antimicrobial activity of *Calothrix braunii*

| Zone of inhibition (diameter in mm) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Fungal species used** | | | | **Bacterial species used** | | | |
| Culture crude extract | *A. fumigatus* MTCC-4163 | *A. niger* MTCC-4325 | *Mucor* sp. MTCC-3340 | *T. mentagrophytes* MTCC-8476 | *B.subtilis* MTCC-1427 | *S. aureus* MTCC-1430 | *E. coli* MTCC-1302 | *K. pneumoniae* MTCC-4030 |
| Aqueous | 8.33 ± 0.33 | -- | 9.33 ± 1.45 | 8.00 ± 0.00 | 12.66 ± 0.33 | -- | 10.00 ± 1.15 | -- |
| Chloroform | 10.33 ± 0.33 | 10.00 ± 0.00 | 10.00 ± 1.15 | 11.00 ± 0.57 | -- | 17.66 ± 0.33 | 16.66 ± 0.33 | 13.33 ± 0.66 |
| Ethyl acetate | 8.00 ± 0.57 | 8.33 ± 0.88 | 10.33 ± 0.88 | 10.33 ± 0.88 | 13.00 ± 0.57 | 11.33 ± 0.66 | 14.66 ± 0.66 | 15.00 ± 1.15 |
| Hexane | 12.66 ± 0.66 | 9.33 ± 0.33 | 9.00 ± 0.57 | 7.33 ± 0.33 | 11.33 ± 0.88 | 13.00 ± 1.15 | 16.00 ± 0.57 | -- |
| Methanol | 10.33 ± 0.33 | 7.66 ± 0.66 | 7.66 ± 0.33 | 8.00 ± 0.57 | -- | -- | 11.66 ± 0.88 | 12.33 ± 0.88 |
| **Nystatin (50 µg/disc)** | 23.33 ± 0.33 | 22.00 ± 0.57 | 21.33 ± 0.88 | 21.66 ± 0.66 | | | | |
| **Ciprofloxacin (10µg/disc)** | | | | | 25.33 ± 0.33 | 24.66 ± 0.66 | 28.33 ± 0.33 | 29.66 ± 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).