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ANTIMICROBIAL AND ANTIFUNGAL ACTIVITY OF ISOLATED BETAGLUCAN FROM *CHROOCOCCUS TURGIDUS*

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ABSTRACT

Cyanobacteria (blue-green algae) are rich sources of structurally novel and biologically active metabolites. Recent studies indicate the presence of some bioactive compounds in the blue green algae which are shown to exhibit antimicrobial, antifungal or anti-inflammatory and other pharmacological activities. In the present study, the isolated beta-glucan from *Chroococus turgidus* was assayed for antibiotic activities against the three fungal and three bacterial pathogens.

Key words: Cyanobacteria, Blue green algae.

INTRODUCTION

Cyanobacteria are nature's unique gift to mankind, as they possess several innate properties that make them ideal organisms with potential for multifaceted biotechnological applications. They are large and morphologically diverse group of unique photosynthetic organisms of great importance because of their very long existence for well over 3.5 billion years and cosmopolitan distribution in terrestrial, freshwater and marine habitats. As a basic research tool, they are largely known to provide critical insights into the origin of life, photosynthesis, nitrogen fixation and primary metabolism. Marine organisms are a rich source of structurally novel and biologically active metabolites. Many chemically unique compounds of marine origin with various biological activities have been isolated to develop new pharmaceuticals [1]. The cell extracts and active constituents of various algae shown to have both antibacterial and antifungal activities [2,3]. The main objectives of the present investigation was to investigate the antimicrobial acitivity of isolated beta glucan from *Chroococcus turgidus*

MATERIALS AND METHODS

Cyanobacterial culture

Chroococcus turgidus, a cyanobacterium was obtained from the culture collection of Vivekananda Institute of Algal technology (VIAT) Chennai. Biomass was obtained by growing algal cultures in 20L of water and 0.25g / L of NPK fertilizer was added with a facility to pump the culture with aeration pump. The algae was grown for 20 days and harvested.

Extraction and Estimation of Beta-glucan

Extraction and Drying

The *Chroococcus turgidus* were air-dried at room temperature (30°C) for two weeks, after which it was ground to a uniform powder. The extracts of the dried samples were prepared in a sequential procedure by soaking 20 g of dried powder in 60 ml of 80%methanol for 48 h. procedure was repeated. At the end of each respective extraction, the extracts were filtered using Whatman filter paper. The filtrate was concentrated under reduced pressure in vacuum at 40°C for 25 min using a rotary evaporator (Super fit-rotavap, India). The percentage yield of extracts was calculated.

Antibacterial and Antifungal Activity

Agar Disc Diffusion Method

Preparation of inoculum:Stock cultures of bacteria were maintained at 4°C on slant of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes

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of nutrient broth for bacteria that were incubated at 24hrs at 37ºC. The Assay was performed by agar disc diffusion method.

Antibacterial activity of *Chroococcus turgidus*

Antibacterial activity of beta glucan sample was determined by disc diffusion method on Muller Hinton agar (MHA) medium. The Muller Hinton Agar medium was weighed as 3.8gms and dissolved in 100ml of distilled water and add 1gm of agar. Then the medium is kept for sterilization. After sterilization the media was poured in to sterile petriplates, these petriplates were allowed to solidify for twenty minutes. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistered with the bacterial suspension. The disc were placed in MHA plate and add 20 µl of sample [concentration : 1000µg, 500 µg, 250 µg, 125 µg, 62.5 µg]. The plates were incubated for 24 hrs, at 37ºc .Then the microbial growth was determined by measuring the diameter of zone of inhibition.

Antifungal activity of *Chroococcus turgidus*

Preparation of potato Dextrose Broth (PDB)

Antifungal activity of beta glucan sample was determined by antifungal susceptibility test. Prepare PDB Broth and inoculate the culture. Then it is kept in shaker for a day. The potato dextrose agar was weighed as 3.9gms and dissolved in 100ml of distilled water and add 1gm of agar. Then the medium is kept for sterilization. After sterilization the media was poured in to sterile petriplates, these petriplates were allowed to solidify for twenty minutes. After solidification, the inoculums were spread on the solid plates with sterile swab moistured with the fungal suspension. The discs were placed in PDA plate and add 20 µl of sample [concentration: 1000µg, 500 µg, 250 µg, 125 µg, 62.5 µg]. The plates were kept it at Room Temperature. Then the microbial growth was determined by measuring the diameter of zone of inhibition

RESULTS

Antimicrobial and antifungal activity of *Chroococcus turgidus*

Antibacterial activity of beta glucan sample was determined by disc diffusion method on Muller Hinton agar (MHA) medium. The Muller Hinton Agar medium was weighed as 3.8gms and dissolved in 100ml of distilled water and add 1gm of agar. Then the medium is kept for sterilization. After sterilization the media was poured in to sterile petriplates, these petriplates were allowed to solidify for twenty minutes. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistered with the bacterial suspension. Beta-glucan extract of *Chroococus turgidus* showed a good antibacterial activity against *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa*. The disc were placed in MHA plate and add 20 µl of sample [concentration : 1000µg, 500 µg, 250 µg, 125 µg, 62.5 µg/disc], against Standard (positive control): Streptomycin 10 µg/disc and DMSO (Dimethyl sulfoxide): Negative control. The plates were incubated for 24 hrs, at 37ºC .Then the microbial growth was determined by measuring the diameter of zone of inhibition. The antibacterial activity of extracts of *Chroococus turgidus* against test bacteria are shown in Figure 9. The results revealed that all the extracts of *Chroococus turgidus* had good activities against the tested bacteria (Plate 1). The maximum inhibition zone was observed against *Staphylococcus aureus* (13mm), *Pseudomonas aeruginosa* (8mm) and *Salmonella typhi* (8mm) in the higher concentration of algal sample 1000µg/disc.

Antifungal activity of *Chroococcus turgidus*

Antifungal activity of beta glucan extract of *Chroococus turgidus* was determined by antifungal susceptibility test. Prepare PDB Broth and inoculate the culture. Then it is kept in shaker for a day. The potato dextrose agar was weighed as 3.9gms and dissolved in 100ml of distilled water and add 1gm of agar. Then the medium is kept for sterilization. After sterilization the media was poured in to sterile petriplates, these petriplates were allowed to solidify for twenty minutes. After solidification, the inoculums were spread on the solid plates with sterile swab moistered with the fungal suspension. Beta-glucan extract of *Chroococus turgidus* showed a good antibacterial activity against *Aspergillus flavus*, *Penicillium notatum* and *Candia albicans*. The disc were placed in PDA plate and add 20 µl of sample [concentration: 1000µg, 500 µg, 250 µg, 125 µg, 62.5 µg/disc], Standard (positive control): Amphotericin -B 20 µg/disc and DMSO (Dimethyl sulfoxide): Negative control. The plates were kept it at Room Temperature. Then the microbial growth was determined by measuring the diameter of zone of inhibition. The antifungal activity of *Chroococus turgidus* against test fungi is shown in Figure 2. As the results revealed that *Chroococus turgidus* isolates produced a significant inhibition zones and thus antifungal activity. In our study we observed that methanol extracts of the *Chroococus turgidus* exhibited potential antifungal activity (Plate 2). The maximum inhibition zone was observed with *Aspergillus flavus* (5.5mm), *Penicillium notatum* (10mm) and *Candida albicans* (7mm).

DISCUSSION

Cyanobacteria are photosynthetic prokaryotes produces a high variety of secondary metabolites that often have potent biological activities. The aim of this study was to assess the effect of crude methanolic and hexane extracts of *Chroococcus turgidus* against gram positive and gram negative bacterial strains and to assess the antioxidant potential. In the preliminary antimicrobial study, hexane extracts outpaced beta-glucan extracts against all the seven bacterial strains. The study showed a

significant antagonistic activity against all tested bacteria in hexane extracts at different ranges. The largest zone of inhibition (13.3±1.84) was exhibited in *Vibrio parahaemolyticus*. The lipid peroxidation and iron chelating activity of *C. turgidus* assessed using TBARs and FRAP assays respectively and showed good antioxidant potential with increase of drug dose in both the assays [4]. A study reported that hexane extracts of 6 cyanobacterial strains exhibited more antibacterial potential as compared to methanol [5]. In another report *Chroococcus dispersus* collected from paddy fields showed antibacterial activity against *S. aureus, S. epidermidis* and *E.coli* showed approximately similar results. In contrast to our results it was reported that no antimicrobial activity was evidenced in hexane extracts and the study concluded, among all of the species studied for antibacterial and antifungal activity, *C. dispersus* exhibited widespread spectrum of antimicrobial activities [6].

Nowadays, there is a large availability of clinically useful antibiotics and also a continuous search for new anti-infective agents which remain indispensable. Some of the major antibiotics have indeed considerable drawbacks in terms of limited antimicrobial spectrum or serious side effects. The recent investigations with Cyanobacteria have demonstrated the antimicrobial and antifungal effects of *Phormidium* sp. [7]. These reports are in agreement with our present study, since the beta-glucan extract of *Chroococus turgidus* are much more effective when compared with contemporary antibiotics. The synthesis of highly active toxin is a defence option of Cyanobacteria in these environments against organisms like bacteria, fungi, viruses and eukaryotic micro algae [8,9]. It was observed that the methanol was the best solvent for extracting the antibacterial agents from *Chroococus turgidus*. An improved knowledge of the composition, analysis, and properties of cyanobacterial strains with respect to antimicrobial compounds would assist in efforts for the pharmaceutical application of blue green algae.

Antimicrobial activity of beta glucan sample was determined by antimicrobial susceptibility test. The results revealed that all the extracts of *Chroococus turgidus* had good activities against the tested bacteria. The maximum inhibition zone was observed against *Staphylococcus aureus* (13mm), *Pseudomonas aeruginosa* (8mm) and *Salmonella typhi* (8mm) in the higher concentration of algal sample 1000µg/disc. The antifungal activity of *Chroococus turgidus* against test fungi, the results revealed that *Chroococus turgidus* isolates produced a significant inhibition zones and thus antifungal activity.

In our study we observed that methanol extracts of the *Chroococus turgidus* exhibited potential antifungal activity. The maximum inhibition zone was observed with *Aspergillus flavus* (5.5mm), *Penicillium notatum* (10mm) and *Candida albicans* (7mm).

PLATE 1. ANTIBACTERIAL ACTIVITY OF *CHROOCOCCUS TURGIDUS*

Fig label: A - 1000µg, B – 500 µg, C - 250 µg, D – 125 µg, E – 62.5 µg, F – DMSO, G – Std

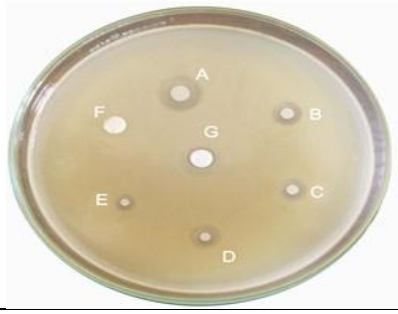
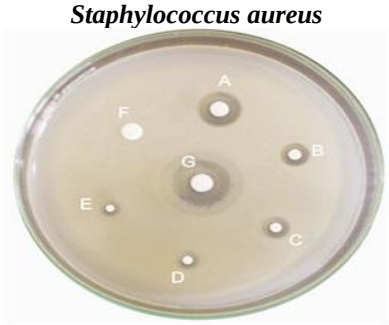
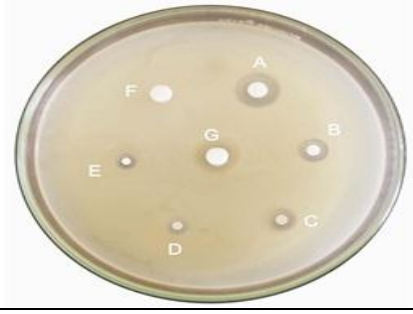
Pseudomonas aeruginosa	***Staphylococcus aureus***	***Salmonella typhi***
		

PLATE 2. ANTIFUNGAL ACTIVITY OF *CHROOCOCCUS TURGIDUS*

Fig label: A - 1000µg, B – 500 µg, C - 250 µg, D – 125 µg, E – 62.5 µg, F – DMSO, G – Std

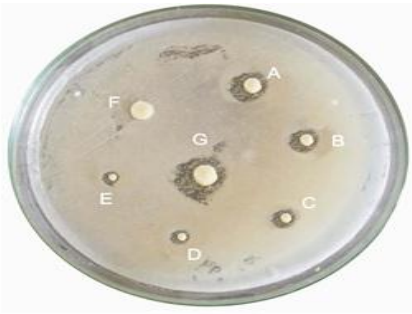
Aspergillus flavus	***Penicillium notatum***	***Candida albicans***
		

Figure 1. Antibacterial activity of *Chroococcus turgidus*

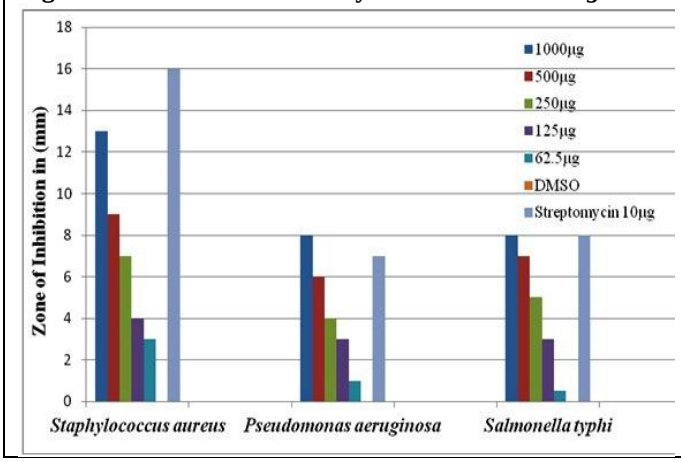
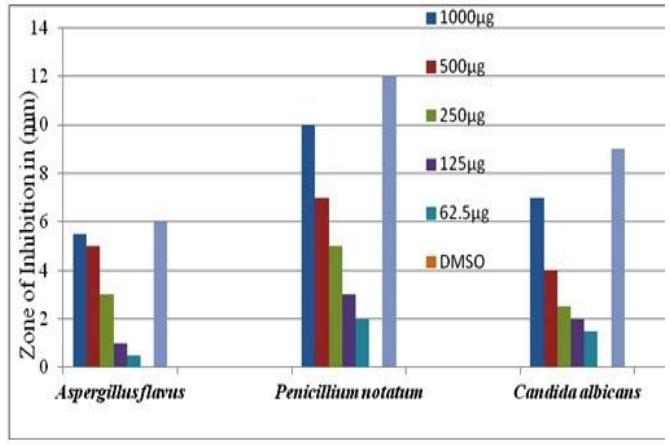


Figure 2. Antifungal Activity of *Chroococcus turgidus*



CONCLUSION

It can be concluded that the beta glucan extract obtained by *Chroococus turgidus* can used in this study had substantial antibacterial and antifungal activities and that other extract could be used for further studies against these microorganisms.

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