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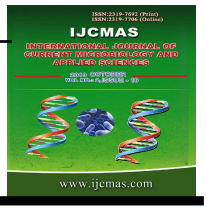

Original Research Article

Evaluation of antioxidant and antimicrobial potential of cyanobacteria, *Chroococcus turgidus (Kützing) Nägeli*

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Cyanobacteria; *Chroococcus turgidus*; antimicrobial study; TBARs and FRAP assays.

A B S T R A C T

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 methanolic 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 *V. parahaemolyticus.* The lipid peroxidation and iron chelating activity of *C. turgidus* assessed using TBARs and FRAP assays respectively. The results showed good antioxidant potential with increase of drug dose in both the assays. Gas chromatography-Mass spectroscopy analysis revealed the volatile organic compounds. This investigation revealed that the metabolites produced by *C. turgidus* can be a potential source of antimicrobial and antioxidant agents.

Introduction

Microalgae can be a very interesting natural source of new compounds with biological activity that could be used as functional ingredients (Carlucci *et al*., 1999). Microalgae contain numerous bioactive compounds that can be harnessed for commercial use. They have emerged as important sources of proteins and value added compounds with pharmaceutical and nutritional importance. Initially considered as laboratory curiosities or nuisances agents in water bodies, but now cyanobacteria or blue green algae form an important component of integrated nutrient management in agriculture and are exploited in commercial biotechnological ventures as a source of pigments, vitamins, phycocolloids, immuno-diagnostic agents and therapeutics and for biofuel production (Benemann, 1984). Cyanobacteria have become a new source of active compounds showing interesting and exciting biological activities ranging

from antibiotics, immunosuppressant, and anticancer, antiviral and anti-inflammatory to proteinase-inhibiting agents (Viviana and Vitor, 2011). The high degree of diversity in the bioactivities of cyanobacteria is due to the broad spectrum of its secondary metabolites and these bioactive molecules indicate the pharmaceutical potential of cyanobacteria. In the present investigation we have assessed the antimicrobial and antioxidant potential of *C. turgidus.*

Materials and Methods

Preparation of Algal extract

The cultures obtained from VIAT (Vivekananda Institute of Algal Technology) culture collection were washed successively with tap water and distilled water. Finally, they were dried in hot air oven at >40°c to prevent denaturation of biologically active compounds for 8 hr. The dried algal material was ground to fine powder. The grounded algal material taken in conical flask and placed in shaker for 24 hr at 200 rpm with methanol (1:10) and hexane (1:10). The extract was filtered through Buchner funnel with Whatman No.1 filter paper (90 mm). The solvent was evaporated from crude extract by rotary evaporator (40°c). The dried extracts were dissolved in Dimethyl Sulfoxide (DMSO) and stored at 4°c until use.

Antibacterial Assay

The target bacterial strains used for screening antibacterial activity are *Staphylococcus aureus (MTCC 3615), Vibrio parahaemolyticus (ATCC 17802), Escherichia coli (ATCC 25922), Enterococcus faecalis (ATCC 29212), Eubacterium lentum, Enterobacter aerogenes (ATCC 13048)* and *Staphylococcus epidermidis (ATCC 12228).* The bacterial strains were inoculated on nutrient broth and incubated for 24 h at 30±1°c. The sensitivity test of methanol and hexane extracts were determined using agar disc diffusion method (Bauer *et al*., 1966). The minimum inhibitory concentration tests were carried out according to National Committee for Clinical Laboratory Standards (1997).

Antioxidant Activity

TBARs assay - Lipid peroxidation quantification (Ottolenghi, 1959)

Two ml of 20% trichloroacetic acid and 2 ml of 0.67% 2-thiobarbituric acid was mixed with 0.1 ml of algal extract at various concentrations (0.012.5 to 0.2 mg/ml). The mixture was placed in a boiling water bath for 20 min. After cooling, the mixture was centrifuged at 3000 rpm for 20 min. Absorbance of supernatant was measured at 532 nm. The experiment was carried out in triplicates. Experimental data represents mean ± SD of each sample, unless otherwise stated.

FRAP assay - Iron chelating activity (Benzie *et al*, 1996)

The principle is based on the formation of O-Phenanthroline-Fe2+ complex and its disruption in the presence of chelating agents. The reaction mixture containing 1 ml of 0.05% O-Phenanthroline in methanol, 2 ml ferric chloride (200μM) and 2 ml of various concentrations ranging from 10 to 500μg was incubated at room temperature for 10 min and the absorbance of the same was measured at 510 nm. EDTA was used as a classical metal chelator. The experiment was carried out in triplicates. Experimental data represents

mean ± SD of each sample, unless otherwise stated.

Chromatographic analysis

Gas chromatography and mass spectrophotometer (GC-MS) analysis was performed using Agilent technologies (Gas chromatographic system) and Jeol GC Matell (Mass spectrometry) with parameters,

Injection temperature - 220°c
Column temperature - 70-250°c
Rate of temperature - 10°c/min
Carrier gas - Helium
Flow of gas - 15 ml/min
Column used - HP5 column.

Results and Discussion

In our study the antibacterial efficiency was studied using bacterial strains in 2 phases to screen better solvent for *C.turgidus* drug extract. Preliminary screening was done using methanol (highly polar) and hexane (non-polar) solvents. About 7 bacterial strains used, 4 gram-positive, *S. aureus, S. epidermidis, E. lentum, E. faecalis,* 3 gram-negative, *E. aerogens, E. coli, V. parahaemolyticus*. Preliminary screening was done against 7 bacterial strains using both methanol and hexane extracts. The results showed in all of the strains examined, gram-negative bacterial strains showed better inhibition zones in hexane extracts than the methanol extracts of *C.turgidus*. Gram-negative bacteria *S. epidermidis, E. aerogens* and *E. coli* showed no signs of inhibition in methanol extract. The results obviously made us to choose hexane extract to study zones of inhibition using the same bacterial strains in second phase.

In the second phase, 200µg/ml hexane extract impregnated discs placed for 24 h, the growth inhibition was assessed as diameter (mm) of the zone of inhibited microbial growth. MIC was read as the lowest algal extract concentration showing no visible bacterial growth after incubation for 24 h. Our study showed inhibition of all 7 bacterial strains by *C. turgidus* extracts. The largest zone of inhibition (13.3±1.84) was exhibited in *V. parahaemolyticus*, followed by *E. faecalis* (10.7±0.85). The least zone of inhibition was exhibited in *S. epidermidis* (6.9±1.13). The *C. turgidus* culture showed a good consistency of inhibition in all bacterial strains with an average rate of 9.65%. In average, the inhibition of gram-negative (9.83%) were little above than inhibition of gram-positive bacteria (8.98%) and this may be attributed to the presence of some active components in organic extract such as lipophilic and phenolic compounds. Minimum Inhibitory concentration (MIC) (Table 2) was carried out by serial dilution of drug extract against bacterial strains.

A study reported that hexane extracts of 6 cyanobacterial strains exhibited more antibacterial potential as compared to methanol (Shazia, 2011). 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 (Younes *et al*, 2007). It was reported among the several cyanobacterial strains isolated from river Nile *C. turgidus* exhibited 92.6% inhibition against *E. coli*

Table.1 Preliminary antibacterial screening. Experiments were repeated twice for each sample. '+' = Presence of inhibition, '-'= absence of inhibition

Bacterial strains	Methanol extract	Hexane extract	Positive control
S. aureus	+	+	+++
S. epidermis	-	+	+++
E. aerogens	-	++	+++
E. lentum	++	++	+++
E. coli	-	+	+++
V. parahaemolyticus	+	++	+++
E. faecalis	+	++	+++

Table.2 Zone of inhibition (cm) mean value with ± SD. Minimum Inhibitory Concentration by NCCLS, 1997. Experiments were repeated twice for each sample

Bacterial strains	*C.turgidus* zone of inhibition	Positive Control	MIC of *C.turgidus*	MIC of positive control
S. aureus	9.500±0.28	33	1	0.015
S. epidermis	6.900±1.13	39	0.5	0.015
E. aerogens	9.900±0.57	27	1	0.015
E. lentum	8.850±0.64	31	1	0.015
E. coli	7.450±0.64	30	1	0.015
V. parahaemolyticus	12.150±1.63	32	0.5	0.015
E. faecalis	10.700±0.85	36	0.5	0.015

Table.3 The results of antioxidant assays with inhibition % mean value ± SD. Experiments were repeated twice for each sample.

Assays	12.5µg	25 µg	50 µg	100 µg	200 µg
TBARs	12.080±0.933	16.084±4.66	26.410±2.63	30.692±2.88	33.2255±0.86
FRAP	20.410±3.87	32.960±3.96	45.680±6.46	61.785±2.88	78.920±3.54

Table.4 Results of GC-MS analysis of *C.turgidus*

S.No.	Compound name	Retention time	Area%
1	1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester	14.46	14.07
2	1,4-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	15.47	38.11
3	1,2,3-Benzenetricarboxylic acid	15.65	16.7
4	1,2-Benzenedicarboxylic acid, dipentyl ester	15.84	3.31
5	16-Octadecenoic acid, methyl ester	16.74	10.22
6	Hexadecanoic acid, 2-(octadecycloxy) ethyl ester	18.56	5.71
7	Ethyliso-allocholate	20.21	11.82

on methanolic extract and 74.4% of inhibition on aqueous extracts, which was also highest in aqueous extract against *E. coli* (Abdo *et al*, 2012). This was contrast to our study which showed negative inhibition in most of the methanolic extract against *E. coli*.

Antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reaction can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols (Hyoung, 1992). Oxidative attack on proteins result in site-specific aminoacid modifications, fragmentation of the peptide chain, aggregation of cross-linked reaction products, altered electrical charge and increased susceptibility to showed good scavenging potential proteolysis (Farr, 1991). Lipids oxidation is problematic as many oxidative chemical reactions are not controlled and constrained by enzymes and may show exponential reaction rates. Some of the products of the attack are highly reactive species that modify proteins and DNA (McIntyre *et al*., 1999). In TBARs study in which the extent of lipid peroxidation was estimated from the concentration of Malondialdehyde (MDA), a thiobarbituric acid reacting substance which is produced due to lipid peroxidation the results showed that in all of drug concentrations 33.22% of lipid peroxidation, which was the highest observed at 200 µg drug concentration. In TBARs drug extracts showed gradual increase in lipid peroxidation with increase in drug concentration. The FRAP assay showed a big leap forward in chelating activity with the increase in drug concentration. The leap was highest of 27.74% between 100 µg and 200 µg. At 200 µg iron chelating activity was highest at 78.9±3.54. It was reported, in-vitro antioxidant study on many green algae and cyanobacteria (Mannan *et al*, 2009; Nurul *et al*., 2011,

Shazia *et al*., 2011; Uma *et al*., 2011). The gas chromatography-mass spectroscopy analysis revealed a list of volatile organic compounds in *C.turgidus* with good abundance (38.11%) of 1,4-Benzenedicarboxylic acid, bis (2-methylpropyl) ester (Table 4).

It could be concluded from the results that crude extract of *Chroococcus turgidus* had good amount of active metabolites with antimicrobial and antioxidant potential. The finding of the current work appears to be useful for the further investigation of this cyanobacterium in the field of antimicrobial and antitumor investigation.

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