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GC-MS ANALYSIS OF SOME BIOACTIVE CONSTITUENTS FROM ISOLATED BETA GLUCAN FROM *CHROOCOCCUS TURGIDUS*

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ABSTRACT

The strain of cyanobacterial metabolites were extracted from effluent derived *Chroococcus turgidus* and cultivated in the laboratory condition at VIAT (Vivekananda Institute of Algal Technology) through improvised CFTRI medium. Then beta glucan was isolated and compounds were taken analysis for GC-MS. In this result showed that the presence of 12 metabolites as follows Payroll[1,2-c]oxazol-1(3H)-one, tee trihedral 7a-acetyl-3-(1,1-dimethylethyl), Proteomic acid, 2-phenyl-, 8-methy 1-8-azabicyclo[3.2.1]octan-3-yl ester, Phenol, 4,4'-(1-methylethylidene), Atropine, Octadecanoic acid, 2,3-dihydroxypropyl ester, Benzaldehyde, 2-nitro-, diaminomet hylidenhydrazone, 2,2'-(Alpha- methylbenzylidene) bis5-methylfuran), Trans-Traumatic acid, N,N-Dimethylacetoacetamide, Propenoic acid, 2-phenyl-, 8-methy 1-8-azabicyclo[3.2.1]octan-3-yl ester, 2-Hydroxy-dodecanoic acid, pyrrolidide and Bis(2-ethylhexyl) phthalate.

INTRODUCTION

Cyanobacteria are an ancient group of prokaryotic microorganisms and found in all ecological habitats. Cyanobacteria are gram-negative photoautotrophic prokaryotes having 'higher plant-type' oxygenic (Sinha, 1996, Stewart, 1980). Certain cyanobacteria differentiate a small fraction of their cells into heterocysts, the site of aerobic nitrogen fixation. Cyanobacteria are one of the most promising groups of organisms for isolation of novel and biochemically active natural products (Burja, 2001, Patterson, 1993). Antimicrobial compounds found in cyanobacterial exudates include polyphenols, fatty acids, glycolipids, terpenoids, alkaloids, and a variety of yet to be described bacteriocins. Although this species of *Oscillatoria* is nontoxic, it did produce an unattractive surface 'scum'. Lipids are the most effective source of storage energy, function as insulators of delicate internal organs and hormones and play an important role as the structural constituents of most of the cellular membranes. The present work is analysis of compounds from isolated beta glucan *Chroococcus turgidus*, isolated from the effluent

MATERIALS AND METHODS

Isolation of Cyanobacteria

The effluent collected periodically from the factory premises was subjected to laboratory examination and the cyanobacteria were isolated from the effluent using serial dilution, standard plating, colony isolation and culture techniques. The *Chroococcus turgidus*, a cyanobacterium was identified following the monograph of Desikachary (1959).

Laboratory growth conditions

The cultures were grown at $24 \pm 1^\circ\text{C}$ in a thermo-statically controlled environmental chamber illuminated with cool white fluorescent lamps (Philips 40w, cool daylight, 6500k) at an intensity of 2000 lux in a 12/12 h light/dark cycle. The Cyanobacteria were grown in various culture media. The Cyanobacterial nature of the culture was ascertained microscopically before cultivation and harvesting

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 1 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.

GAS CHROMATOGRAPHY-MASS SPECTROSCOPY

Preparation of extract:

1 µl of the methanolic extract was employed for GC/MS analysis.

Instruments and chromatographic conditions:

GC-MS analysis was carried out on a GC QP 2010 [SHIMADZU] comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25 mm ID × 1 µm df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 0.5 µl was employed (split ratio of 10:1) injector temperature 240°C; ion-source temperature 200°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C/min, then 5°C/min to 280°C/min, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da. Identification of components: Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS

Extraction of Beta-glucan from *Chroococcus turgidus*

Using the above mentioned procedure, the cyanobacterium *Chroococcus turgidus* dried biomass from was analyzed. The extract was used for further analysis. The percentage yield of extracts

was calculated. The yield beta-glucan obtained (0.0344g/10g) of 80% methanol extract from the *Chroococcus turgidus* algal dried biomass.

Compounds in *Chroococcus turgidus* identified using GC-MS

The Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained. The results showed that 12 molecular compounds were identified viz., Payroll[1,2-c]oxazol-1(3H)-one, tee trihedral 7a-acetyl-3-(1,1-dimethylethyl), Proteomic acid, 2-phenyl-, 8-methy l-8-azabicyclo[3.2.1]octan-3-yl ester, Phenol, 4,4'-(1-methylethylidene), Atropine, Octadecanoic acid, 2,3-dihydroxypropyl ester, Benzaldehyde, 2-nitro-, diaminomet hylidenhydrazone, 2,2'-(Alpha-methylbenzylidene)bis5-methylfuran), Trans-Traumatic acid, N,N-Dimethylacetoacetamide, Propenoic acid, 2-phenyl-, 8-methy l-8-azabicyclo[3.2.1]octan-3-yl ester, 2-Hydroxy-dodecanoic acid, pyrrolidide and Bis(2-ethylhexyl) phthalate with retention time viz., (18.849, 19.670, 20.567, 21.031, 21.483, 21.604, 22.030, 22.965, 23.010, 23.099, 23.163 and 23.411) and % of area like viz., (3.12, 2.35, 5.85, 15.40, 23.86, 3.62, 16.50, 4.00, 3.17, 3.61, 13.02 and 5.49) respectively (Table 1 and Figure 1).

The present study carried out on the isolated beta-glucan from *Chroococcus turgidus* revealed the presence of active constituents. In GC-MS analysis 12 bioactive phytochemical components were identified in the isolated beta-glucan from *Chroococcus turgidus*. The identification of phytochemical compounds is based on the peak area, molecular weight and molecular formula. The results were presented in figure 1. This study revealed the presence of 12 phytochemical compounds. The identification of the compounds from *Chroococcus turgidus* could be exploited for new potent anticancer agents. Table 1 & Figure 1 listed the major bioactive components and its biological activities obtained through GC-MS study of *Chroococcus turgidus*. It revealed the presence of 12 metabolites with retention time ranging from 18.849 to 23.411. The maximum peak was shown by Octadecanoic acid, 2,3-dihydroxypropyl ester (23.86%) followed by 2-Hydroxy-dodecanoic acid (13.02%), Trans-Traumatic acid (4.00%) and Bis(2-ethylhexyl) phthalate (5.49) respectively. The minimum peak was shown by Payroll[1,2-c]oxazol-1(3H)-one, tee trihedral 7a-acetyl-3-(1,1-dimethylethyl) (3.12%). GC-MS chromatogram of the beta-glucan

extract of *Chroococcus turgidus* is given in Table 1 and Figure 1. On comparison of the mass spectra of the constituents with the NIST library, fifteen peaks were obtained out of which five phytoconstituents were characterized and identified (Table 1). Retention time (RT) is in minutes.

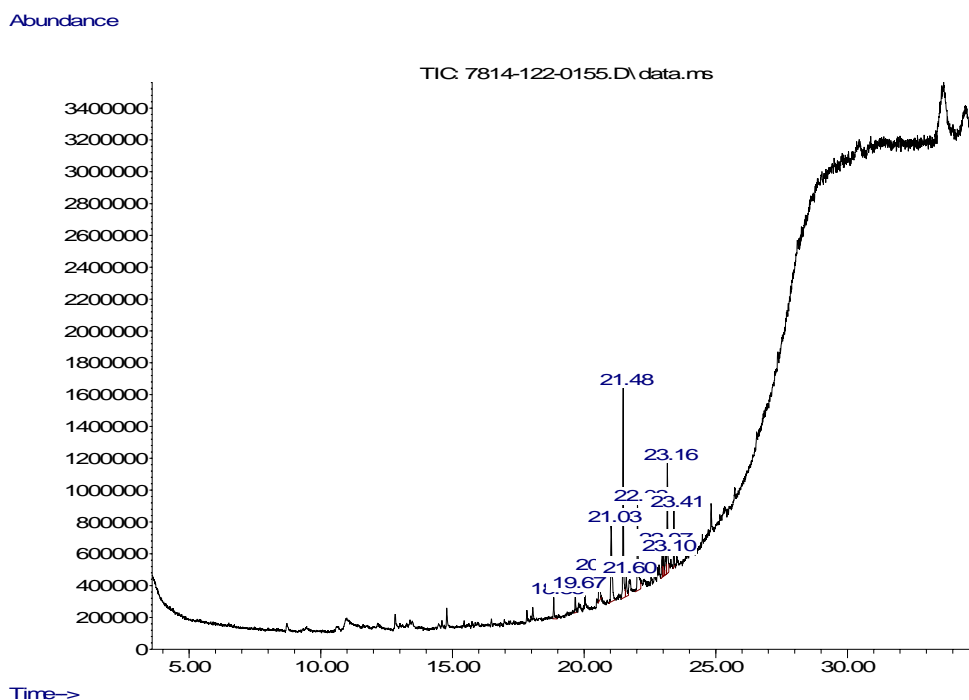
DISCUSSION

The present study carried out on the isolated beta-glucan from *Chroococcus turgidus* revealed the presence of active constituents. In GC-MS analysis 12 bioactive phytochemical components were identified in the isolated beta-glucan from *Chroococcus turgidus*. The identification of phytochemical compounds is based on the peak area, molecular weight and molecular formula. Compounds such as atropine, Octadecanoic acid, 2,3-dihydroxypropyl ester have antimicrobial and anticancer properties. Moreover, these compounds can contribute to the prevention of cancer by augmenting immunological responses against tumour cells in early stages of carcinogenesis. (Alarcon, 1984, Afshypuor, 1995 and Sheela, 2013).

CONCLUSION

In this study the isolated beta glucan from *Chroococcus turgidus* confirmed that the presence of the twelve different types of fatty acids compounds and these fatty acid compounds are useful to pharmaceutical industry. Further studies are required to assess the pharmaceutical properties.

FIGURE 1- GC-MS ANALYSIS OF *CHROOCOCCUS TURGIDUS*



**TABLE 1: AREA OF PERCENTAGE AND RETENTION TIME OF FATTY ACIDS
OBTAINED FROM GC – MS OF *CHROOCOCCUSTURGIDUS***

S.No	COMPOUNDS	Retention Time	Area (%)	Molecular formula
1	Payroll[1,2-c]oxazol-1(3H)-one, tee trihedral 7a-acetyl-3-(1,1-dimethylethyl)-	18.849	3.12	C ₁₂ H ₁₉ NO ₃
2	Proteomic acid, 2-phenyl-, 8-methy l-8-azabicyclo[3.2.1]octan-3-yl ester	19.670	2.35	C ₁₇ H ₂₁ NO ₂
3	Phenol, 4,4'-(1-methylethylidene)bus-	20.567	5.85	C ₂₈ H ₂₉ O ₅ P
4	Atropine	21.031	15.40	C ₁₇ H ₂₃ NO ₃
5	Octadecanoic acid, 2,3-dihydroxypropyl ester	21.483	23.86	C ₂₁ H ₄₂ O ₄
6	Benzaldehyde, 2-nitro-, diaminomet hylidenhydrazone	21.604	3.62	C ₂₀ H ₁₇ N ₃ O ₂
7	2,2'-(Alpha-methylbenzylidene)bis 5-methylfuran)	22.030	16.50	C ₁₈ H ₁₈ O ₂
8	trans-Traumatic acid	22.965	4.00	C ₁₂ H ₂₀ O ₄
9	N,N-Dimethylacetoacetamide	23.010	3.17	C ₆ H ₁₁ NO ₂
10	Propenoic acid, 2-phenyl-, 8-methy l-8-azabicyclo[3.2.1]octan-3-yl ester	23.099	3.61	C ₁₇ H ₂₁ NO ₂
11	2-Hydroxy-dodecanoic acid, pyrrolidide	23.163	13.02	C ₁₂ H ₂₄ O ₃
12	Bis(2-ethylhexyl) phthalate	23.411	5.49	C ₂₄ H ₃₈ O ₄

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