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STUDIES ON *VALUE ADDED BIO-CHEMICALS OF CHROOCOCCUS TURGIDUS*

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Keywords:

Chroococcus turgidus, Flavonoids, Polyphenols, Carbohydrates, Lipids, Protein

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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 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.

1. INTRODUCTION

Cyanobacteria, also known as blue-green algae include a highly diverse group of prokaryotic microorganisms exhibiting oxygenic photosynthesis. Oxygen released by this process gradually changed the original reducing atmosphere of the primitive earth to an oxidizing one (Olsen, 2006) triggering off a dramatic evolution of global biodiversity. The chloroplasts of eukaryotic algae and higher plants have originated from endosymbiotic relationships with cyanobacteria (Martin and Kowallik, 1999 and Raven and Allen, 2003) and this event in the early evolution of life has stimulated the advent of oxygen tolerant flora and fauna capable of aerobic respiration, a highly efficient mechanism of energy utilization. The rapid development of such organisms resulted in the predominance of oxygenic and aerobic species diversity on earth. Basically, many of the metabolites produced by the organisms are in low amounts. Hence, the present work was carried out; to study the biochemical characteristics such as amino acid content of grown cyanobacteria were investigated.

2. MATERIALS AND METHODS

Preparation of Cyanobacterial extract:

0.5g of dried *Chroococcus turgidus* was extracted in 20ml of acetone and methanol kept in an orbital shaker for overnight. The obtained extracts were filtered with Whatman no.1 filter paper and the filtrate was collected. The solvents were removed under reduced pressure at 50°C to yield a concentrated extract (12% and 11%) respectively.

2.1 Extraction and Estimation of chlorophyll 'a'

Extraction

Chroococcus turgidus cells were pelleted by centrifugation at 2000rpm (Remi-R-8-C) for 15 minutes. To the pellet, 10 ml of 90% acetone was added and sonicated for 15 minutes using ultrasonic processor (Vibronics P2). After complete extraction, it was again centrifuged at 2000 rpm for 15 minutes, clear supernatant was used for spectrophotometric estimation of chlorophyll pigments using double beam UV-visible spectrophotometer (Shimadzu UV–1650 PC).

Estimation

Chlorophyll 'a' was estimated from the extinction coefficients given by Jeffrey and Humphrey (1975).

Chlorophyll 'a' μg/ml = 11.85E664–1.54E647–0.08E630 .

2.2 Extraction and Estimation of β-Carotene (Shaish *et al*., 1992)

Extraction

Known quantity of *Chroococcus turgidus* culture was centrifuged at 2000 rpm for 5 minutes and 3ml of (2:1) ethanol: hexane mixture was added to the pellet. Then 2ml of double distilled water and 4ml of hexane was added, mixed well and centrifuged for 5 minutes. The hexane layer was aspirated and the absorbance was measured at 450 nm in a spectrophotometer.

$$\beta\text{-Carotene } (\mu g/ml): \quad A_{450nm} \times 25.2.$$

2.3. Extraction, estimation of total carbohydrates by anthrone method (Pons *et al.*, 1981).

Extraction

40 ml of *Chroococcus turgidus* culture was taken and centrifuged at 2000 rpm and the supernatants were discarded. The samples (pellets) were hydrolyzed with 5ml of 2.5 N-Hydrochloric acid by keeping it in a boiling water bath for three hours and then cooled to room temperature. After cooling, the samples were neutralized with solid sodium carbonate until the effervescence ceased. The volume was made up to 10ml and centrifuged. The collected supernatant was taken and 0.5 and 1ml.aliquots were used for analysis.

Reagents

a) 2.5 N-Hydrochloric Acid

b) Anthrone Reagent: Dissolve 200mg anthrone in 100ml of concentrated sulphuric Acid. Prepare fresh before use.

c) Standard Glucose.

Stock standard: Dissolve 100mg in 100ml double distilled water.

Working standard: 10ml of stock diluted to 100ml with double distilled water, Stored by refrigeration after adding a few drops of toluene.

Procedure

- Prepare the standards by taking 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml of the working standard. '0' serves as blank.
- Make up the volume to 1ml in all tubes including the test samples by adding distilled water.
- Then add 4ml of anthrone reagent.
- Heat for 10 minutes in a boiling water bath.
- Cool rapidly and read the green to dark green color at 620nm. in a spectrophotometer.
- From the graph calculate the amount of carbohydrate present in the samples.

Note: Cool the contents of all tubes on ice before adding anthrone reagent.

2.4. Extraction and estimation of total protein by Lowry et al., (1951) METHOD

Extraction

40 ml of algal cells were centrifuged and pellets were washed twice with tris HCl buffer (pH-7.0) and re-suspended in 5ml of the same buffer. The suspension was sonicated in an Ultra-sonicator for 15 minutes, centrifuged at 2000 rpm for 15 minutes. The supernatant was treated with 10% Trichloro Acetic Acid (TCA) and the precipitate is obtained by centrifugation at 5000rpm in a cooling microfuge for 15 minutes. Then the precipitate is neutralized in known quantity of 2N NaOH and analyzed for protein.

Reagents

A. 1N NaOH - 4g of NaOH was dissolved in 100ml of double distilled water.

B. 0.1N NaOH -10ml of 1N NaOH solution was made up to100ml with double distilled water.

C. 1% potassium sodium tartrate- 1g potassium sodium tartrate in 100 ml. of double distilled water.

Reagent A : 2% Sodium carbonate in 0.1N NaOH

Reagent B : 0.5% Copper sulphate ($CuSO_4.5H_2O$) in 1% potassium sodium tartrate. (Alkaline Copper Solution)

Reagent C : 1ml of Reagent B was mixed with 50 ml of reagent A.

Reagent D : Folin-Ciocalteau reagent.

Protein solution

Stock standard: Weigh accurately 100mg of Bovine Serum Albumin and dissolve it in double distilled water and make up to 100 ml.in a standard flask.

Working standard: Dilute 20ml of stock solution to 100 ml. of double distilled water in a standard flask. 1ml of this solution contains 200 □g of protein.

Note: All reagents to be freshly prepared prior to estimation.

Procedure

Pipette out standard BSA solution (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml) into a series of test tubes. Pipette out 0.5 and 1.0ml.of the sample extracts in other tubes. Make up the volume to 1ml. in all tubes. Distilled water is considered as blank. Add 5ml. of reagent C and allow to stand for 10 minutes. Add 0.5ml. of Folin's reagent, mix well and keep in dark at room

temperature for 30 minutes. Take readings at 650nm. Draw a standard graph and calculate the amount of protein in the test samples using the standard.

2.5. Estimation of Lipid (Bligh and Dyer 1959)

40 ml of algal cultures were pelleted by centrifugation at 2000 rpm for 15 min. The pellet was suspended in 10 ml. of double distilled water and sonicated for 30 min at 200 V using an ultrasonicator. Then, 2:1 chloroform-methanol was added and kept overnight at room temperature preferably in the dark condition. After the 12 hr standing time, 20 ml. of chloroform and 20 mL of double distilled water were added and mixed well, centrifuged at 2000 rpm for 15 min. A clear lower layer of chloroform containing all the lipid was obtained, which was aspirated carefully using a Pasteur pipette. After drying the solvent, the dry weight was measured using, a weighing balance.

2.6.Estimation of Polyphenols (Malick and Singh, 1980)

Phenols, the aromatic compounds with hydroxyl groups are widespread in plant kingdom. Phenols include an array of compounds like tannins, flavonols etc.Total phenol estimation can be carried out with Folin-Ciocalteau reagent. Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium and produce blue coloured complex (Molybdenum blue).

Reagents

1. 100% Methanol
2. Folin-Ciocalteau reagent
3. 20% sodium carbonate
4. Stock standard solution 100 mg catechol in 100 ml of distilled water.
5. Working standard solution: 10 ml of the stock solution diluted to 100ml with distilled water. 1.0ml of this solution contains 100 g of catechol.

Procedure

5 to 10 mg. of dry *Chroococcus turgidus* was taken and grounded with a mortar and pestle in 100% methanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The supernatant was preserved. The residue was re-extracted with five times the volume of 100% Methanol, centrifuged and supernatants pooled. The supernatant was evaporated to dryness. The residue was dissolved in a known volume of distilled water. Different aliquots (0.2 - 2.0 ml) were pipetted out into test tubes. The volume was made up to 3.0 ml in each tube, with water. 0.5ml of

Folin-Ciocalteau reagent was added. After 3 minutes, 2.0 ml of sodium carbonate solution was added to each tube. The tubes were shaken vigorously to allow mixing of the contents and then were placed in a boiling water bath for exactly 1 minute and cooled. Readings were noted at 650 nm against a reagent blank.

2.7. Estimation of Tannins

Reagents

1. Vanillin hydrochloride reagent: Mixed equal volumes of 8% HCL in methanol and 4% vanillin in methanol .The solution must be prepared freshly.
2. Catechin stock standard :1 mg/ml
3. Working Standard: 10ml of stock to 100ml with water.
4. Extract: Extracted 1g sample in 50ml methanol. Centrifuged and collected the supernatant after 20-28 hours.

Procedure

Pipetted 1.0 ml of the supernatant. Added 5.0 ml of vanillin hydrochloride reagent .Read in spectrophotometer at 500 nm after 20 minutes prepared a blank with reagent alone. Tannin in the sample is expressed as catechin equivalents.

2.8. Estimation of Flavonoids

***Reagents*:**

1. 5% Sodium nitrite
2. 10% Aluminum chloride.
3. 1M Sodium hydroxide
4. Stock Standard: Dissolved 10 mg of catechin in 10 ml of distilled water.

Procedure:

Added 0.5 ml of the sample to a test tube containing 1.25 ml of distilled water. Then added 0.075 ml of 5% sodium nitrite and allowed to stand for 5 minutes. Added 0.15 ml of 10% Aluminum chloride after 6 minutes. 0.5 ml of 1.0 M Sodium hydroxide was added and the mixture was diluted with another 0.275 ml of distilled water .The absorbance of the mixture at 510 nm was measured immediately .The flavonoid content was expressed as milligram catechin equivalents /g sample.

2.9. Estimation of alpha-tocopherol (Emmerie-Engel method 1938 as described by Rosenberg, 1992)

Reagents

1. Absolute alcohol
2. Xylene
3. 2,2'-dipyridyl
   1.2 gm in one liter of n-propanol
4. Ferric chloride solution: 1.2 gm of $FeCl_3.6H_2O$ in one liter ethanol. Stored in a brown bottle.
5. Standard solution of D, L-□-Tocopherol: 100 mg/100 ml of □-tocopherol in absolute alcohol. 91 mg of □- tocopherol equivalent to 100 mg of tocopherol acetate.

Procedure

1.5 ml of sample extract (test), 1.5 ml of standard (standard) and 1.5 ml of water (blank) were pipetted into three different stoppered centrifuge tubes. To the test and blank, 1.5 ml of ethanol was added and to the standard 1.5 ml of water was added. 1.5 ml of xylene was added to all the tubes. They were mixed well and centrifuged.1.0 ml xylene layer was transferred into another stoppered tube, taking care not to include any ethanol or protein. 2ml of 2, 2'-dipyridyl reagent was added to each tube and mixed. 1.5 ml of the mixture was pipetted out into spectrophotometer cuvette and extinction of the test and the standard against the reagent blank was read at 460 nm. Then, beginning with the blank, 0.33 ml of ferric chloride solution was added, mixed well and after exactly for 15 minutes, test and standard against reagent blank at 520 nm were read. The amount of vitamin E could be calculated using the formula,

Amount of tocopherol in µg	=	Reading at 520 nm – Reading at 460 nm / Reading of standard at 520 nm	x	0.24 x 15

2.10. Estimation of Ascorbic acid (Roe and Keuther, 1953)

Ascorbate is converted to dehydroascorbate by treatment with activated charcoal or bromine. Dehydroascorbic acid then reacts with 2,4 – dinitro phenyl hydrazine to form osazones, which dissolve in sulphuric acid to give an orange coloured solution whose absorbance can be measured spectrophotometrically at 540 nm.

Reagents

1. 4% TCA
2. 9 N Sulphuric acid
3. 2% 2,4 – Dinitro phenyl hydrazine (DNPH) reagent in 9N sulphuric acid
4. 10% Thiourea
5. 85% Sulphuric acid
6. Stock standard solution.
   100 mg of ascorbic acid in 100 ml of 4% TCA
7. Working standard solution.
   10 ml of stock solution diluted to 100 ml with 4% TCA

Procedure

1 gm. of the sample was ground and homogenized in 4% TCA. This was made up to 10 ml and centrifuged at 2000 rpm for 10 minutes. The supernatant was treated with a pinch of activated charcoal, shaken well and kept for 10 minutes. It was centrifuged once again to remove the charcoal residue. The volume of clear supernatant obtained was noted down.

0.5 and 1ml of aliquots of the supernatant were taken for the assay. The assay volume was made up to 2.0 ml with 4% TCA. 0.2 to 1.0ml of working standard solution containing 20-100 micro g of ascorbate respectively was pipetted out into clean dry test tubes and the volume was made up to 2.0 ml with 4% TCA.0.5ml of DNPH reagent was added to all the tubes followed by 2 drops of 10% thiourea solution and incubated at 37*C for 3 hours. The osazone formed was dissolved in 2.5 ml of 85% sulphuric acid, in cold, drop by drop with no appreciable raise in temperature. To the blank alone, DNPH reagent and thiourea were added after the addition of sulphuric acid. After incubation for 30 minutes at room temperature, the absorbance was read spectrophotometrically at 540 nm. The content of ascorbic acid in the algal samples was calculated by using the standard graph.

2.11. Content of Flavonoids by HPLC Method

HPLC analysis was performed using a LDC Milon Roy CM 4000 gradient pump coupled to a Hewlett Packard 1100 diode-array detector. Flavonoid separation was carried out in a 5 mm Chrompack C18 column 250 mm_4.6 mm, protected by a Chrompack C18 pre-column 3.0 mm_10 mm. Cyanobacterial extract were eluted at 1 ml/min20 µl injection volume) using as mobile phase a binary solvent system consisting in methanol, water, and phosphoric acid (100:100:1) equal volumes (about 20 µL) of each of the Standard solutions was injected and the Test solution into the chromatograph equal volumes (about 20 µL) of each of the Standard

solutions was injected and the Test solution into the chromatograph. Record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of each flavonoid in sample.

2.12. Quantification of amino acids by HPLC Method.

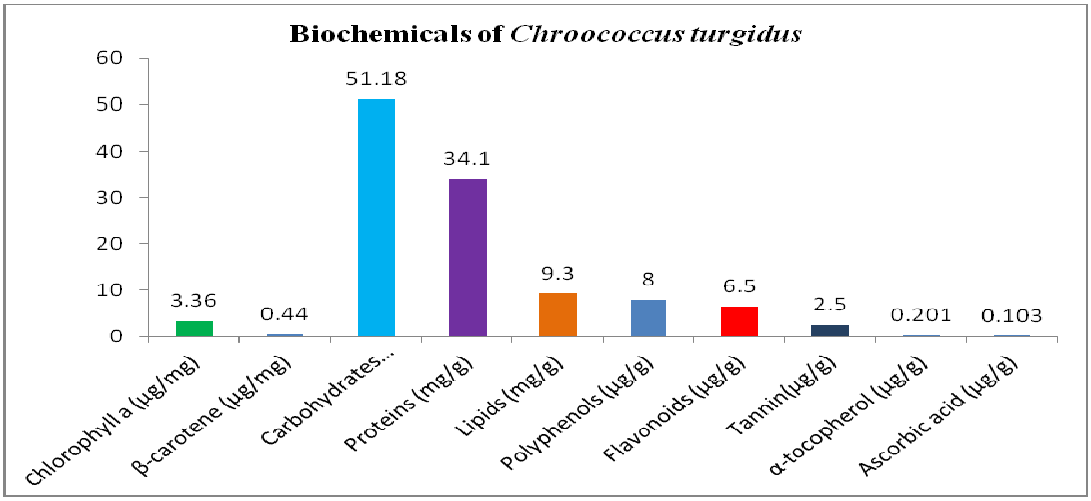
The peptides with N – terminal primary amines get derivated by orthopthaldehyde and the amino acid contents of the cyanobacteria are separated and quantified by reverse phase chromatography (Shimadzu – High Performance Liquid Chromatograph LC6A). Separation of the sample components occur on the column by interaction between the sample components of the stationary and the mobile phase.

RESULTS

3.1.Biochemical compositions of *Chroococus turgidus*

The *Chroococus turgidus* was analyzed for various value-added bio-chemicals such as pigments (Chlorophyll a and β-carotene); macromolecules (Carbohydrates, Proteins and Lipids);secondary metabolites (Polyphenols, Flavonoids, Tannin, α-tocopherol and Ascorbic acid) and amino acids contents. Experiments were conducted to identify useful bio-chemicals from the biomass of *Chroococus turgidus*. The Cyanobacteria was grown at 24 ± 1° C in a thermo-statically controlled room and with cool white fluorescent lamps (Philips 40 W, cool daylight, 6500K) at an intensity of 2000 lux in a 12:12 light dark regime.10 ml of *Chroococus turgidus* extract was taken, centrifuged at 4000 rpm for 10 minutes. Different procedures, from the methanolic extract of the *Chroococus turgidus*, the following value-added bio-chemicals were determined. Table 1 and Figure 1 show the levels of the following bio-chemicals.

FIGURE 1: HIGH VALUE BIO-CHEMICALS FOR *CHROOCOCCUS TURGIDUS*



3.2. Amino Acid Profiles of *Chroococus turgidus*

Chroococus turgidus was analyzed for amino acid profile by High Performance Liquid Chromatography (HPLC) and the results were tabulated (Table 3 and Figure 2). The essential amino acids and non –essential amino acids which are used as dietary ingredients were present in *Chroococus turgidus*. The result shows that essential amino acids were maximum level showed methionine (4.34mg/0.5g), phenyl alanine (2.98mg/0.5g) and leucine (2.43mg/0.5g) followed by other essential amino acids and very low level showed that lysine (0.1323mg/0.5g) respectively. The non- essential amino acids were maximum level showed cysteine (3.455mg/0.5g), proline and tyrosine (1.989mg/0.5g) and ascorbic acid (1.56mg/0.5g) followed by other non-essential amino acids and very low level showed that alanine (0.0054mg/0.5g) respectively.

3.3. HPLC Determination of flavonoids in *Chroococcus turgidus*

Chroococus turgidus was analyzed for flavonoids contents by Reversed phase High Performance Liquid Chromatography (RP-HPLC). Reversed-phase HPLC has been used in a number of occasions for the analysis of flavonoids in cyanobacteria, it was used to distinguish species based on the quantitative variation of flavonoids among them. It has been applied especially for the identification of flavonoids derivatives. The results showed that, flavonoids were quantified at 254nm using peak area by comparison to a calibration curve derived from the *Chroococus turgidus*, the main difference was in peak eluted at 3.336 min. The flavonoids content was found maximum unknown (1.20mg/ml), rutin (0.24mg/ml), quercetin (0.12mg/ml) and kaemferol (0.06mg/ml) respectively. The results are tabulated (Table 2 & Figure 3).

FIGURE 2: AMINO ACIDS PROFILES OF *CHROOCOCCUS TURGIDUS*

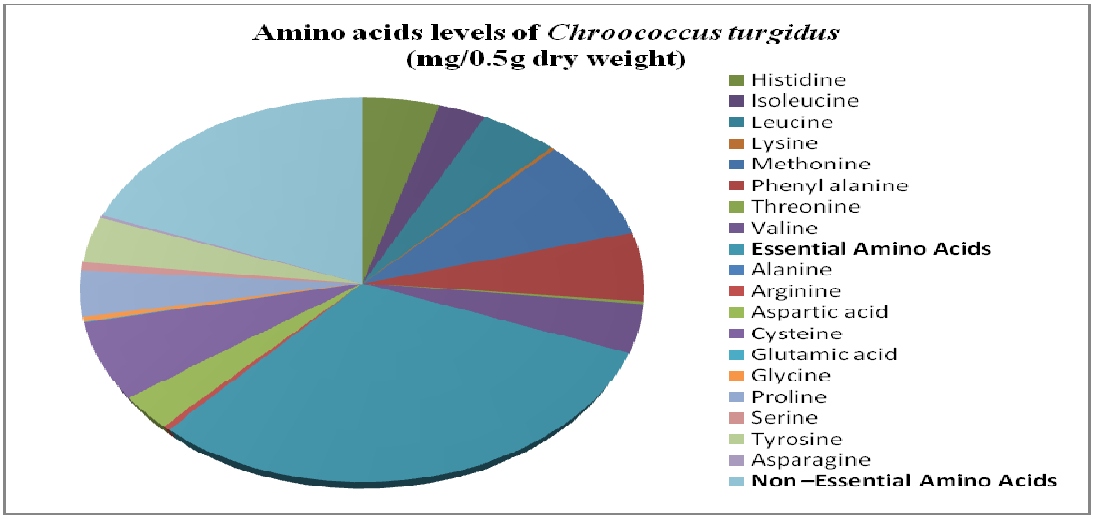


FIGURE 3: DETERMINATION OF FLAVONOIDS IN *CHROOCOCCUS TURGIDUS*

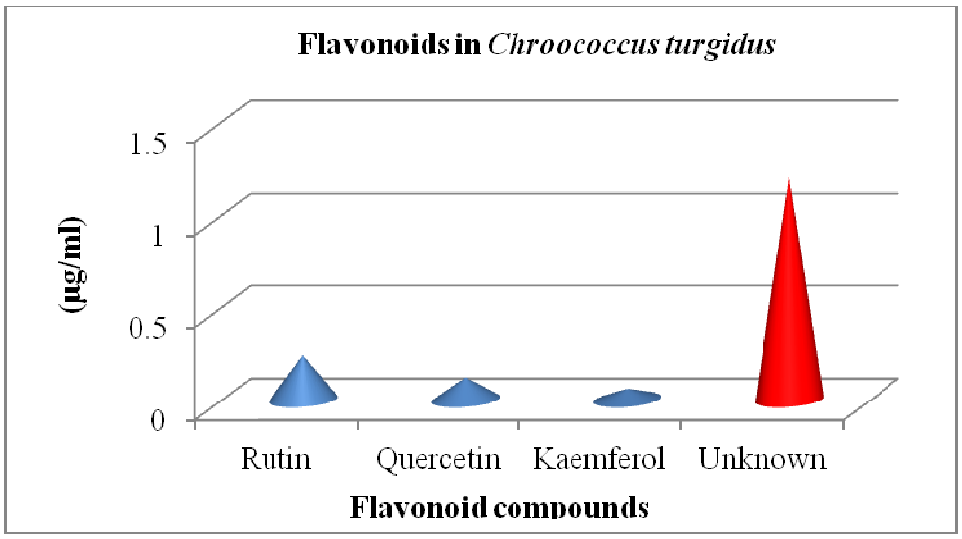


TABLE 1: HIGH VALUE BIO-CHEMICALS FOR *CHROOCOCCUS TURGIDUS*

S.No	Biochemicals	Amount
1	Chlorophyll a (µg/mg)	3.36
2	β-carotene (µg/mg)	0.44
3	Carbohydrates (mg/g)	51.18
4	Proteins (mg/g)	34.10
5	Lipids (mg/g)	9.30
6	Polyphenols (µg/g)	8.0
7	Flavonoids (µg/g)	6.5
8	Tannin(µg/g)	2.5
9	α-tocopherol(µg/g)	0.2010
10	Ascorbic acid(µg/g)	0.103

TABLE 2: DETERMINATION OF FLAVONOIDS IN *CHROOCOCCUS TURGIDUS*

S.No	Flavonoids(Compounds)	Quantity (µg/ml)
1.	Rutin	0.24
2.	Quercetin	0.12
3	Kaemferol	0.06
4	Unknown	1.20

TABLE 3: AMINO ACIDS PROFILES OF *CHROOCOCCUS TURGIDUS*

Amino Acids Contents	Amount in mg/0.5g dry weight
Histidine	2.340
Isoleucine	1.456
Leucine	2.43
Lysine	0.1323
Methonine	4.34
Phenyl alanine	2.98
Threonine	0.1145
Valine	2.13
Essential AminoAcids	**15.9228**

Alanine	0.0054
Arginine	0.2034
Aspartic acid	1.56
Cysteine	3.455
Glutamic acid	0.0314
Glycine	0.2134
Proline	1.989
Serine	0.3434
Tyrosine	1.989
Asparagine	0.114
Non –Essential Amino Acids	**9.904**

DISCUSSION

Chlorophyll A, Beta-carotene, Vitamins, carbohydrate, protein, and lipid content were determined in the *Chroococcus turgidus.* Chlorophyll is the pigment that allows plants (including algae) to use sunlight to convert simple molecules into organic compounds via the process of photosynthesis. Of the Several kinds of chlorophyll, chlorophyll a is the predominant type found in green plants and algae. Anti Carcinogenic: Chlorophyll protects against a whole host of carcinogens found in fungus-laden foods such as nuts and grains, the toxins from cooked meats, and air-borne carcinogens (from pollution). It blocks the metabolism in the body of harmful chemicals known as procarcinogens that damage DNA.

Studies published in the journals Carcinogenesis and Food and Chemical Toxicology clearly display that chlorophyll inhibits carcinogenesis. It Contains vitamin K, C, folic acid, iron, calcium, protein which are all also essential in building and repairing red blood cells and boosting our immune system

Beta-carotene

Beta-carotene belongs to a group of plant compounds called carotenoids. Beta-carotene is the most abundant carotenoid in human foods and is generally thought to be the most important carotenoid for humans. Beta-carotene, which is also known as pro-vitamin A, consists of two molecules of vitamin A linked together (A-A). Enzymes in the epithelial lining of the intestinal tract split beta-carotene into two molecules of vitamin A whenever the body needs it. (Nagao A *et al.,* 1996) .

Beta-carotene is the most abundant precursor of vitamin A in fruits and vegetables. Beta-carotene functions as a chain-breaking antioxidant. This means it does not prevent the initiation of lipid peroxidation, but rather, it stops the chain reaction by trapping free radicals, which halts the progression of free radical activity (Mathews-Roth *et al.,* 1990).

Chemotherapy stresses the antioxidant defense system and may lead to lower antioxidant levels which could cause an increase in the adverse side effects of the therapy. A study was conducted involving children with acute lymphoblastic leukemia who were undergoing chemotherapy were administered greater intakes of antioxidants. The increased consumption of beta-carotene at 6 months decreased the risk of toxicity (Kennedy *et al.*, 2004).

In *Chroococcus turgidus* ., carbohydrates content was more when compared with protein and lipids and in turn proteins were more than lipids (Table1).Cyanobacteria produce a wide variety of bioactive compounds, which include 40% lipopeptides,5.6%aminoacids,4.2%fatty acids, 4.2%macrolides and 9% amides. Cyanobacterial lipopeptides include different compounds like cytotoxic (41%), antitumor(13%), antiviral (4%), antibiotics (12%) and the remaining18% activities include antimalarial, antimycotics, multi-drug resistance reversers, antifeedant, herbicides and immunosuppressive agents;(besides the immune effect, blue-green algae improves metabolism(Beker *et al*., 1994).

It has been established that a wide range of cyanobacteria produced large amounts of extracellular polysaccharides which posses unique composition and structure (Parikh *et a*l.,2006,Mishra *et al*.,2009).It has also been shown that a number of these polysaccharides have antitumor activities (Gardeva *et al* ., 2009 , Umemura *et al.,* 2003*).*Cyanobacteria are a rich source of potentially useful natural products. Over 40 different Nostocales species, the majority of which are *Anabaena* and *Nostoc* sp. produce over 120 natural products (Secondary metabolites)having activities such as anti-HIV anticancer, antifungal, antimalarial and antimicrobial. Cyanovirin (CV-N, cyanoviorin-N), a 101 amino acid protein extracted from *Nostoc ellipsosporum* was found to have potent activity against all human immunodeficiency viruses such as HIV-1, M and T tropic strains of HIV-1, HIV-2, SIV (Simian), and FIV (Feline) (Burja *et al* 2001). Cyanobacteria have been identified as one of the most promising group of organisms from which novel and biochemically active natural products are isolated.Cyanobacteria such as *Spirulina, Anabaena, Nostoc and Oscillatoria* produce a great variety of secondary metabolites (Shalaby *et al*.,2010).The only comparable group is actinomycetes, which has yielded a tremendous number of metabolites. The rate of discovery from traditional microbial drug producers like actinomycetes and hyphomycetes, which are in the focus of pharmaceutical research for decades, is decreasing and it is time to turn to

cyanobacteria and exploit their potential. This is of paramount importance to fight increasingly resistant pathogens and newly emergent diseases (Hehmann *et al*., 2002).

Methanolic extract of *Chroococcus turgidus* was used to for the analysis of phytocompound like phenols, flavonoids and tannin which was quantitatively estimated.

Phenolic Compounds

Total Phenolic compounds in *Chroococcus turgidus* was 8.0 mg/g. Phenolic compounds are known to be powerful chain breaking antioxidants and are important constituents of plants. Phenolic compounds may contribute directly to antioxidative action. It is suggested that phenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans.

Total Flavonoids contents in *Chroococcus turgidus was* 6.5mg/g .Many researchers have conducted in vitro studies on the potential anticancer activity of flavonoids in diverse cell systems.Hirano and co-workers examined anticancer efficacy of 28 flavonoids on human acute myeloid leukemia cell line HL-60, and compared differences between antiproliferative activity and cytotoxicity of these compounds with those of four clinical anticancer agents. Eight of the 28 flavonoids showed considerable suppressive effects on HL-60 cell growth with IC50s ranging from10–940 ng/ml. The flavonoid genistein had the strongest effects almost equivalent to the effects of current anticancer agents with little cytotoxicity against HL-60 cells, whereas the regular anticancer agents had potent cytotoxicity. Kuntz *et al* (1999)screened more than 30 flavonoids for their effects on cell proliferation and potential cytotoxicity in human colon cancer cell lines Caco-2 and HT-29. Almost all compounds displayed antiproliferative activity without cytotoxicity.

There was no obvious structure-activity relationship in the antiproliferative effects either on basis of the subclasses (i.e., isoflavones, flavones, flavonols, and flavonones) or with respect to kind or position of substituents within a class.

Total concentration of Tannin in *Chroococcus turgidus* was 2.5mg/g.Tannic acid has numerous food and pharmacological applications. It is an additive in medicinal products, and is used as a flavouring agent and as an anti-oxidant in various foods and beverages. Tannic acid in the presence of Cu (II) causes DNA degradation through generation of reactive oxygen species. On the other hand, it exhibits antimutagenic and anticarcinogenic activities, and induces apoptosis in animal cells. It is known that most plant-derived polyphenolic anti-oxidants also act as pro-oxidants under certain conditions.

Vitamin E (α-tocopherol) is probably the most important lipid-soluble antioxidant protecting membranes, lipids and lipoproteins (VanBakl *et al.*, 2000). Vitamin E is one of the few nutrients for which supplementation with higher than recommended levels have been shown to enhance immune response and resistance to diseases (Bendich A *et al*., 1997).

Ascorbic acid or "vitamin C" is a monosaccharide antioxidant found in both animals and plants. As it cannot be synthesized in humans and must be obtained from the diet, it is a vitamin (Smirnoff *et.al*., 2001).Most other animals are able to produce this compound in their bodies and do not require it in their diets. In cells, it is maintained in its reduced form by reaction with glutathione, which can be catalyzed by protein disulfide isomerase and glutaredoxins (Meister *et al*., 1994). Ascorbic acid is a reducing agent and can reduce and thereby neutralize ROS such as hydrogen peroxide (Padayatty *et al.,*2003) In addition to its direct antioxidant effects, ascorbic acid is also a substrate for the antioxidant enzyme ascorbate peroxidase, a function that is particularly important in stress resistance in plants.(Shigeoka *et al*.,2002).

Quantification of Flavonoids in *Chroococcus turgidus*

The weight of the epidemiological evidence for a protective effect of flavonoids against cancer is impressive. A growing number of epidemiological studies suggest that high flavonoid intake may be correlated with a decreased risk of cancer. The present study indicated that rutin **is** the most prominent flavonoid in *Chroococcus turgidus.* The flavonoid analysis of methanolic extract of *Chroococcus turgidus by* HPLC revealed that the main flavonoid compounds in *Chroococcus turgidus were* Quercetin, Kaemferol, Rutin and Unknown. The levels of major flavonoids were Rutin (0.2.4mg), Quercetin (0.12 mg), Kaemferol (0.006 mg), and unknown flavonoid (1,20 mg).

Amino acid profile of *Chroococcus turgidus*

Chroococcus turgidus was analyzed for amino acid contents by High Performance Liquid Chromatography (HPLC). The essential amino acids and non - essential amino acids in the Cyanobacterium were estimated. Studies were undertaken to explore the presence of useful biochemicals in *Chroococcus turgidus*

CONCLUSION

Thus, result shows that cyanobacterium isolated from effluents has also very high nutritional value. *Chroococcus turgidus was* analyzed for amino acid and flavonoids contents by High

Performance Liquid Chromatography (HPLC). Studies were undertaken to explore the presence of value added biochemicals in the *Chroococcus turgidus.*Further research is recommended for these compounds.

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REFERENCES

Beker EW. Microalgae. Cambridge, NY: Cambridge University Press 1994.

Burja AM, Banaigs EB, Abou-Mansour, Burgess JG, Wright PC: Marine cyanobacteria-a prolific source of natural products. Tetrahedron 2001; 57: 9347–9377.

Bendich A. Vitamin C safety in humans, In: L. Packer and Fuchs, J. (eds.) Vitamin C in health and disease,NewYork:MarcelDekker Inc;1997.p.367-379.

Bligh, E.G., Dyer. W.J. (1959). A rapid method for total Lipid extraction and purification. Can. J. Biochem. physiol. 37: 911 – 917

Emmerie A., Engel C., 1938. Colorimetric determination of tocopherol (vitamin E). Rec. Trav. Chim. 57, 1351.

Hehmann A, Kaya K, Watanabe MM. Selective control of Microcystis sp using an amino acid a laboratory assay. J Appl Phycol 2002; 14:85-9.

Jegan, G, S.Mukund, N.S.Senthilkumar and RamaRajaVallinayagam V.Sivasubramanian. Aminoacid content and Biochemical analysis of Methanolic extract of *Oscillatoria terebriformis* IJPRD, 2013; Vol 5(7); September 2013,ISSN: 0974 – 9446.

Hirano T, Gotoh M, Oka K. Natural flavonoids and lignans are potent cytostatic agents against human leukemic HL-60 cells. Life Sci 1994;55:1061–1069.

Kennedy DD, Tucker KL, Ladas ED, Rheingold SR, Blumberg J, Kelly KM. Low antioxidant vitamin intakes are associated with increases in adverse effects of chemotherapy in children with acute lymphoblastic leukemia. Am J Clin Nutr. Jun2004;79(6):1029-36.

Kuntz S, Wenzel U, Daniel H. Comparative analysis of the effects of flavonoids on proliferation,cytotoxicity, and apoptosis in human colon cancer cell lines. Eur J Nutr 1999;38:133–142/

Lowry, L.H. Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). J. Biol. Chem. 265

Malick, CP. &Singh, MB. (1980)PlantEnzymology and HistoEnzymology. KalyaniPublishers, New Delhi, pp. 286.

Mathews-Roth MM. Carotenoid functions in photoprotection and cancer prevention. J Environ Pathol Toxicol Oncol. Jul1990;10(4-5):181-92.

Martin, W. and Kowallik, K. V. (1999).Annonated English translation ofMereschkowski□s 1905 paper. Euro. Jou.rPhycol. 34 (3): 287 – 295.

Meister A. Glutathione-ascorbic acid antioxidant system in animals. J Biol Chem. 1994;269:9397–400.

Meister A, Anderson A. Glutathione. Annu Rev Biochem. 1983;52:711–60.

Mishra A, Jha B. Isolation and characterization of extracellular polymeric substances from micro-algae Dunaliella salina under salt stress. Bioresour Technol 100, 2009, 3382-3386.

S.Mukund, V.Sivasubramanian and N.S.Senthil kumar, In vitro antioxidant activity of the Methanolic Extract of Oscillatoria terebriformis C.A. Agardh ex Gomont J. Algal Biomass Utln. 2013, 4 (1): 17–25 ISSN: 2229- 6905.

Nagao A, et al. Stoichiometric Conversion of All Trans-beta-carotene to Retinal by Pig Intestinal Extract. Arch Biochem Biophys. Apr1996;328(1):57-63.

Olson, John M.(2006).Photosynthesis in the Archean Era.Photosynthesis Research 88:109–17

Parikh A, Madamwar D. Partial characterization of extracellular polysaccharides from cyanobacteria. Bioresour Technol 97, 2006, 1822-1827.

Padayatty S, Katz A, Wang Y, Eck P, Kwon O, Lee J, et al.Vitamin C as an antioxidant: Evaluation of its role in disease prevention Am Coll Nutr. 2003;22:18–35.

Pons, A., Rola, P., Agvilo, C., Garcia, F.J., Alemarry, M., Paloo, A., (1981), J. Biochemical and Bio Physical methods, Volume 4, Issue 3-4, pp. 227-231

Roe JH, Kuether CA (1943) The determination of ascorbic acid in whole blood and urine through 2, 4 - dinitrophenyl hydrazine derivative of Dehydroascorbic acid. J Biol Chem 147:399–407

Shalaby EA, Shanab SMM, Singh V. Salt stress enhancement of antioxidant and antiviral efficiency of Spirulina platensis. J Med Plants Res 2010; 4:2622-32.

Shigeoka S, Ishikawa T, Tamoi M, Miyagawa Y, Takeda T, Yabuta Y, et al. Regulation and function of ascorbate peroxidase isoenzymes. J Exp Bot. 2002;53:1305–19.

Smirnoff N. L-ascorbicacid biosynthesis. Vitam Horm. 2001; 61:241–66.

Sadasivam, S. and A. Manickam. 1992. Biochemical method for agricultural science, Willey, Eastern Ltd. pp. 105.

Shaish, A., Ben-Amotz, A., Avron M. (1992). Biosynthesis of -carotene in Dunaliella. methods in Enzymology. 213: 439-444**.**

Umemura K, Yanase K, Suzuki M, Okutani K, Yamori T, Andoh T.Inhibition of DNA topoisomerases I and II, and growth inhibition of human cancer cell lines by a marine microalgal polysaccharide. Biochem Pharmacol 66, 2003, 481-487.

R.Uma, V. Sivasubramanian and S.Niranjali Devaraj.2011. Evaluation of in vitro antioxidant activities and antiproliferative activity of green microalgae, *Desmococcus olivaceous* and *Chlorococcum humicola*. J. Algal Biomass Utln. 2011, 2 (3): 82– 93

Van Bakl, Weisman UN. Antioxidant and thyroid hormone status in selenium-deficient phenyl ketonuric and hyperphenyl alaninemic patienys. Am. J. Clin. Nutr 2000;976-981.