SCREENING OF PHYTOCHEMICALS CONSTITUENTS AND ANTIBACTERIAL ACTIVITY OF *Scytonematopsis* SPECIES AGAINST SELECTED HUMAN PATHOGENIC BACTERIA

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Date: 4th June 2017

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CERTIFICATE – I

This is to certify that the thesis entitled **"Screening of phytochemicals constituents and antibacterial activity of *Scytonematopsis* species against selected human pathogenic bacteria**" submitted **by Miss Vijaylakshmi Satapathy, bearing Roll no. - 15BOT/15** for the degree of Masters of Science in the subject of Botany of College of Basic Science and Humanities, Orissa University of Agriculture and Technology , Bhubaneswar is the result of bonafied research work done under my supervision. This thesis conforms to standard envisaged by the regulations of the university in my judgment. The work is an original peace of research undertaken by the candidate and the work has not been submitted earlier for any other Degree/Diploma. The thesis presented is adequate and merits consideration for the award of the degree for which it is submitted.

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Date: 4th June 2017

CERTIFICATE – II

This is to certify that the thesis entitled "**Screening of phytochemicals constituents and antibacterial activity of *Scytonematopsis* species against selected human pathogenic bacteria**" submitted by Miss Vijaylakshmi Satapathy, bearing Roll no. 15Bot/15 to the College of Basic Science and Humanities, Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfilment of the requirements for the degree of Masters of Science in the subject of Botany, has been approved by the Students' Advisory Committee and the External Examiner.

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DECLARATION

I do here by declare that the thesis entitled "**Screening of phytochemicals constituents and antibacterial activity of *Scytonematopsis* species against selected human pathogenic bacteria**" submitted to the College of Basic Science and Humanities, Orissa University of Agriculture and Technology, Odisha for the degree of Master of Science in the subject Botany is an original piece of Research Work, and no part of this thesis had been submitted for any degree or diploma to any other University / Institution.

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Place: Bhubaneswar

Date: 4th June 2017 (Vijaylakshmi Satapathy)

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ABBREVIATIONS AND SYMBOLS USED

Approx	-	Approximately
et al.	-	et alebi (and others)
etc	-	et cetra
Fig	-	Figure
g	-	gram
g/l	-	gram per litre
mg	-	miligram
ml	-	mililitre
µl	-	microlitre
$\mu g.ml^{-1}$	-	microgram per mililitre
pH	-	negative logarithm of hydrogen ion concentration
rpm	-	revolution per minute
sp	-	Species
$^{\circ}C$	-	degree celcius
%	-	Percent
±	-	plus or minus

ABSTRACT

Cyanobacteria are the gram negative photosynthetic prokaryotic have emerged as a promising biosources for the production of biologically active molecules. In present investigation effort had been made to screen sub-aerial cyanobacteria biochemical analysis and for the production of antibacterial compounds and GC analysis for chemical composition. The growth rate determination and biochemical analysis of chlorophyll a (chl-a), total soluble protein (PRT), total cellular carbohydrates (CHO) and exopolysaccharides (EPS), total carotenoids content (TCC) and Phycobiliproteins are carried out . Further, cyanobacteria extracts extracted from two different organic solvent acetone and chloroform were subjected to TLC plates. the TLC spot obtained were bio assayed for antibacterial activities by Agar well diffusion array of different concentration (Control, 50µl, 150µl, 300µl, 400µl and 500µl) per well and incubated at 37±2ºC against four selected human pathogenic bacterial strains (*Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*). The isolate cyanobacteria *Scytonematopsis* strains had higher chlorophyll-a, soluble protein, cellular carbohydrate and exo- polysaccharides and Allophycocyanin during log phase and moderate at the lag phase, but in case of Phycocyanin and phycoerythrin was found to be more during early stationary growth phase. The crude extracts extracted in acetone and chloroform of *Scytonematopsis* strains were analysis by using TLC and the spots obtained were further tested for antibacterial activity against selected human pathogenic bacteria. Incubation zone are measured in centimetre (cm) after 18-24 hours. *Scytonematopsis* strains were found to produce antibacterial component in both acetone and chloroform extracts. The chloroform extracts was observed to inhibit *Escherichia coli* and *Pseudomonas aeruginosa* while *Bacillus subtilis* and *Staphylococcus aereus* were found resistant whereas in acetone extracts showed moderate effect particularly against *Escherichia coli*. GC analysis detected distinct group may have a distinct active group metabolite which are promising sources of antibacterial compounds.

Keywords: Cyanobacteria, Biochemical analysis, Crude extracts, Thin Layer Chromatography, Antibacterial compound, Gas chromatography.

Chapter – 1

INTRODUCTION

1. INTRODUCTION

Infectious diseases are one of the main causes of high morbidity and mortality in Human being around the world, especially in developing countries (Waldvogel, 2004). The severity of the diseases have increased in recent year significantly due to heavy infectious and the pathogenic bacteria agents including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Proteus vulgaris* is a major public health problem and becoming resistant to drug is common due to indiscriminate use of antibiotics. Recent emergence of antibiotic resistance and related toxicity issues limited the use of antimicrobial agents and is prompting a revival in research of the antimicrobial role of cyanobacteria against resistant strains due to comparable safety and efficacy.

Cyanobacteria are prospective source have been traditionally used for either food or medicinal purposes in different parts of the world for centuries (Chapman and Gellenbeck, 1998). In the last twenty years this ancients group of phototrophic organisms was targeted by many researches as a sources of drugs and other commercially valuable products. At present, cyanobacteria have a significant attraction as natural source of low molecular weight secondary metabolites with a broad range of biologically activities including antibacterial, antifungal, algicidal, antiviral, anticancer, antioxidant and anti-inflammatory effect (Tuney *et al*., 2006; Tan, 2007; Patra *et al*., 2009). Some of these compounds are recognized toxins (Wiegand and Pflugmacher, 2005). The active compounds isolated from cyanobacteria include fatty acids, bromophenols, peptides, polysaccharides, alkaloids and alcohols; however, the precise chemical composition for most of them is unknown.

Scytonematopsis is a genus of aerophytic or subaerophytic heterocystous cyanobacteria and belongs to the order Nostocales. They are generally considered to be cosmopolitans; however, it contains numerous species, which grow only in tropical and ecologically distinctly habitats, such as lateritic soil, dripping rocks, reservoirs with water vegetation or sometimes from hot springs (http://www.cyanodb.cz). Few species grow epiphytic on mosses; several species are incrusted by calcium carbonate and represent dominant members of microphytes in travertine springs. These strain is morphologically characterized by filamentous, cylindrical trichomes along the whole length, more or less quadratic cells in the main trichomes, creeping on the substrate or with erected branches, commonly falsely branched, both branches grow parallel aside or in crossing

position, cells pale or olive-green, usually with solitary, irregularly disposed granules or with granular content, rarely yellowish or pinkish coloured; apical cells sometimes with large vacuoles. Heterocytes intercalary, solitary, rarely in pairs and relatively narrow sheath (Komárek & Hauer, 2014). *Scytonematopsis* species has been reported for the presence of various secondary metabolites including antimicrobial compounds, i.e., Scytophytin from *Scytonema pseudohofmanii* (Ishibashi *et al*., 1986), Didehydromirabazole from *Scytonema mirabile (*Stewart *et al*.,1988), Tolytoxin and 6- hydroxy-7-O-Methylescytophycine from Scytonema sp. (Carmeli *et al*., 1990) and Scytophycin from *Scytonema* sp. HAN3/2 (KP701039) (Shishido *et al*., 2015). These diverse bioactive molecules have been shown to possess antibacterial and antifungal activity.

Earlier studies have highlighted that tropical region of Odisha state having good Cyanobacterial diversity and having biologically active metabolites (Samad and Adhikary, 2008). During our hunt for potential cyanobacteria with the wide spectrum antimicrobial activity of pharmaceutical interest, the present study is step ahead to assess the antibacterial potential of an axenic culture locally isolated sub-aerial cyanobacteria *Scytonematopsis* species against selected human pathogenic bacteria after bioassay of crude extracts as well as TLC purified ones and subjected to spectroscopic analysis for identification of Biomolecules.

Objectives:

1. Survey, isolation, identification and documentation of the collected sub-aerial cyanobacteria from College building facades, Bhubaneswar, Odisha.
2. Quantitative Biochemical analysis of the selected cyanobacteria isolates (i.e *Scytonematopsis* sp.).
3. Phytochemicals screening test of the above cyanobacteria isolates for their secondary metabolites using two different organic extracts i.e. Acetone and Chloroform.
4. Antibacterial activity of the isolates against four selected human pathogenic bacteria (*Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*).

Chapter – 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Cyanobacteria, the blue-green algae, are the ancient and diverse group of photosynthetic microorganisms that have evolved to inhabit many different and extreme environments (i.e., in terrestrial, freshwater and marine environments). The origin of these organisms dates back to 3 billion or 4 billion years ago. Their adaptability to different niches is because of their ability to produce a unique range of defensive metabolites. The advantages of these are that a sustainable supply of a desired material can be achieved, which is not always possible from a product derived from a microorganism source. Investigations by several workers revealed that cyanobacteria are a rich source of potentially useful natural products like novel antitumor, antifungal, and, to a lesser extent, antibacterial compounds (Patterson, *et al*., 1994). Many compounds isolated from cyanobacteria such as dolastatins, curacins, and cryptophycins have shown antitumor activity (Simmons *et al*., 2005). Among these, dolastatin 10 and cryptiphycin 52 have entered into clinical trials for solid tumors (Jonge *et al*., 2005) and lung cancer, respectively (Edelman *et al*., 2005). There are numerous reports on biological active compounds being isolated from both freshwater and marine environment, however there is an indication that terrestrial cyanobacteria might possess even greater biological activity than those originating from aquatic environments.

Svircev *et al*., (2008) isolated 9 strains of *Anabaena* and 9 strains of *Nostoc* strains from various soil types of Serbia and tested their both extracellular product and cellular crude lipophilic extracts against 13 bacterial strains and 8 fungal strains. Of all cyanobacterial strains tested, 52% showed some antifungal and 41% antibacterial activity. *Anabaena* strain S12 showed strong cytotoxin with antibacterial activity and *Anabaena* strain S20 as a potent antifungal compound.

Raina *et al*., (2008) reported 3 cyanobacteria i.e *Anabaena oryzae, Tolyprothix ceytonica* and *Spirulina platensis* and 2 green microalgae i.e *Chlorella pyrenoidosa* and *Scenedesmus quadricauda* and studied their antibacterial and antifungal agent production on various organisms that incite diseases of human and plants like *E. coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Aspergillus niger, Aspergillus flavus, Penicillium herquei, Fusarium moniliforme, Helminthosporium sp. Alternaria brassicae, Saccharomyces cerevisiae* and *Candida albicans*. They found that *Spirulina platensis* and *Anabaena oryzae* with highest antibacterial and antifungal activity towards the tested bacteria and fungi.

Ghasemi *et al*., (2009) studied the antifungal and antibacterial activity of the microalgae collected from paddy fields in the south of Iran. They screened the methanolic and hexane extract of 60 strains of microalgae against 6 strains of bacteria and four strains of fungi. i.e *Staphylococcus aureus* PTCC 1112, *Staphylococcus epidermidis* PTCC 1114 *Bacillus subtilis* PTCC 1023, *E.coli* PTCC 1047, *Salmonella typhi* PTCC 1609 *Pseudomonas aeruginosa* PTCC 1074, *Candida kefyr* ATCC 38296 , *Candida albicans* ATCC

14053 *Aspergillus niger* PLM 1140 and *Aspergillus fumigates* PLM 712. The results represented that culture supernatants of 21 strains of microalgae and methanolic extracts of 8 strains exhibited significant antibacterial effect and 17 strains showed anti fungal effect. No antimicrobial activity was detected in the hexane extract and no methanolic extract inhibited the growth of fungi. *Chroococcus disperses Chlamydomonas reinhardtiii* and *Chlorella vulgaris* excreated a broad spectrum of antimicrobial substances in the culture medium. *Chroococcus* disperses PTCC 1677 indicated widespread spectrum of antimicrobial activities.

Bharadwaj *et al*., (2010) screened 5 thermophilic cyanobacteria for the production of antibacterial compound. The 5 cyanobacteria were *Synecoccus elongatus* HRCC T02, *P. frigidum* HRCC T07, *P. cebernense* HRCC T 08, *P. bohneri* HRCC T09i and *Mastigocladus laminosus* HRCC T13. Among these 5 thermophilic cyanobacteria, *Mastigocladus laminosus* HRCC T13 were found to produce antibacterial component in methanolic extract to inhibit *E. coli*, *Pseudomonas aeruginosa*, *P. syringe*, *Enterobacter sp.* and *Enterococcus fecali*.

Madhumati *et al*., (2011) studied the 46 strains of cyanobacteria isolated from fresh water of Samuthiram Lake, Tamilnadu. Out of these 46 strains, 5 species namely *Oscillatoria latevirns*, *Phormidium corium*, *Lyngbya martensiana*, *Chroococcus minor* and *Microcystis aeroginosa* were selected for the production of antimicrobial agents against organisms like *Basillus subtilis*, *Staphylococcus aureus*, *Streptococcus mutants*, *E. coli*, *Micrococcus mutans*, *Klebsiella pneumonia*, *Saccharomyces cerevisiae* and *Candida albicans*. Antimicrobial activity of acetone extracts on Gram negative bacteria, methanol extracts on Gram positive bacteria and ethanol extracts on both Gram negative and Gram positive organisms were observed. They found that species like *Oscillatoria latevirns*, *Chrococcus minor* and *Microcystitis aeroginosa* showed antifungal activity on *Candida albicans*.

Suhail *et al*., (2011) studied the antimicrobial activity of several cyanobacterial strains like *Anabaena variabilis*, *Oscillatoria* sp., *Chroococcus* sp., *Nostoc* sp., *Plectonema boryanum* and *Scytonema* sp. by disc diffusion method against *Staphylococcus epidermis* bacterium. The results showed that hexane extracts of all above cyanobacterial strains exhibited more antibacterial potential as compared to methanol extracts. They also studied the free radical scavenging activity of all above cyanobacterial strains by using 2,2-diphenyl-1-picrylhydrazyl(DPPH) and represented that the maximum antioxidant potential were showed of *P. boryanum* followed by *Scytonema* sp., whereas *Nostoc* sp. Showed least activity .

Tiwari and Sharma (2012) explored the bloom forming cyanobacterial strains for their antibacterial activity. They isolate the *Anabaena variabilis* and *Synechococcus* elongates from natural blooms, purified the strains and studied it thoroughly. They concluded that the diameter of inhibition zone depends mainly on the type the algal species, type of solvent used and the tested bacterial species. They analyses and reported that the antibacterial effects of ethanol: acetic acid extracts of *Anabaena variabilis* and methanol

extracts of *Synechococcus elongates* were with the highest antimicrobial activity against *E. coli* (17mm inhibition zone) and *Enterococcus sp.* (18mm inhibition zone).

Bharat *et al*., (2013) screened 10 Cyanobacterial extracts for antimicrobial activity. They observed that cyanobacterial extracts significantly inhibited gram-positive bacteria, whareas selectively inhibited gram negative bacteria. They observed that extracts inhibited all tested *Staphylococcal* isolates. Based on their results they concluded that fresh water strains of cyanobacteria are abundant sources of several pharmaceutically important compounds which can be used as sources of new and useful antimicrobial chemical entities against bacteria. The chemicals present in these extracts are cytotoxic against cervix cancer cell lines which can be potentially explored further.

Shrivastava (2014) studied cyanobacteria like *Anabaena iyengerii*, *Nostoc sphaerica, Microcystis aeruginosa, Oscillatoria boryana* and *Phormidium fragile* against 3 pathogenic bacteria like *E. coli*, *Mycobacterium ulcerance* and *Staphylococcus epidermidis* and fungi like *Aspergillus niger*, *Candida albicans* and *Rizotonia solani*. Crude extracts of 2 heterosystous and 3 non-heterocystous cyanobacteria were examined and positive responses were observed. They found that non - heterocystous cyanobacteria showed great toxicity comparable to standard antibiotics and fungicides.

Sundaramanickam *et al*., (2015) studied on 4 marine cyanobacteria extracts i.e *Lyngbya* sp., *Nostoc* sp., *Phormidium* sp. and *Calothrix* sp. against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *E. coli*, *Aspergillus niger and Penicillium* sp. The extracts showed significant inhibitory bioassay on the bacterial pathogens except *Phormidium* sp. which showed inhibition only on 2 strains *S. aureas and P. aeruginosa*, no inhibition activity were seen against *B. subtilis* and *E. coli.*

Yadav *et al*., (2016) studied the antibacterial, and in addition, antifungal activity of an axenic culture extract of locally isolated aquatic (benthic) *Nostoc calcicola* against human pathogenic bacteria and fungi. The results concluded that ethanolic extract of *N. calcicola* inhibited all the tested bacteria and fungi. The antimicrobial potency on dry weight basis (5 µg equivalent dry weight in most cases) was better than the 100 µg pure ampicillin and 30 µg miconazole. The activity was pronounced against *Staphylococcus aureus* ATCC 25923 and *Candida albicans* ATCC 10231. The active compound was stable for 6 months at 2-8°C. They represented that cyanobacterial antimicrobial compounds being structurally different may have different mechanisms of action and hence can be used as a source of antibiotics and antifungal compounds for various diseases.

Chapter – 3

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Collection, culture, isolation, identification and maintenance

Blackish-dark greenish biofilms were collected from Cement wall surface of Basic science College, building, Bhubaneswar (Temperature: 21°C, Latitude /Longitude: **20 25 36 82^0 N 85 48 10^0 E**) of Odisha and stored in pre-sterilized sampling bottles. The biofilms were soaked in sterile distilled water and incubated under fluorescent light for up to 72 h and observed microscopically. Since the morphologically features needed for identification were not distinct even after prolonged soaking (up to 7 to 10 days), a small amount of sample were transferred to BG -11 medium with and without nitrogen (Rippka *et al*., 1979) and to agar plates (1.5%w/v agar in same medium) with the help of inoculation needle. Cultures were incubated at 25 ± 1^{o}C under continuous light from fluorescent tubes at an intensity of 7.5 W/ m^2. After a period of 10 to 14 days of incubation, cyanobacteria appeared in the culture were isolated and maintained in the laboratory. Microphotographs were taken using a inverted microscope (model – Leica EC3), and identified by following standard monographs as described by T. V. Desikachary (1959) and (Komárek & Hauer, 2014). The successful axenic cultures were diluted and sub-cultured in 150ml of culture media in 250mL conical flasks for biomass production to be used for further biochemical analysis.

3.2. BIOCHEMICAL CHARACTERIZATION

The successful axenic strains *Scytonematopsis* species was monitored for growth by the method of (Sfriso et al., 2014). A total of 3 culture tubes (18×150 mm), containing 9 ml aliquots of sterile BG – 11 liquid medium were inoculated with 1ml stock culture of the isolated strain. Each tube was incubated for 35 days on the culture rack at 25 ± 1^{o}C under continuous light from fluorescent tubes at an intensity of 7.5 W/m^2 in light/dark cycles of 16:8 hours at an average room temperature of 24 °C, with proper shaking. At 5 days time interval during growth, specific amount of culture was withdrawn from each tube and were used for quantification of chlorophyll-*a* (Chl-*a*), total carotenoids contents(TCC) and exopolysaccharides(EPS), total proteins(PRT) and total carbohydrates(CHO) having three biological replicates for analyses. The biochemical parameters, Chl*a*, TCC, CHO, and EPS were estimated on the same day of sample withdrawal till the end of 35th day. Growth was estimated by measuring the absorbance of the homogenized culture suspension at 750 nm in a Systronics 105 spectrophotometer with reference to blank containing BG -11 medium.

3.2.1. Test for chlorophyll-*a* and carotenoids pigments

The presence of Chlorophyll - *a* in the isolated strains were determined by using Mackinney (1941) test method. Known volume of cell suspension (5ml) was taken in a graded centrifuge tube and was cooling centrifuged in “Remi” C-24 laboratory centrifuge for 20 minutes at 3000 rpm. The supernatant was discarded and the chlorophyll-*a* and carotenoid pigments of the

pellets were extracted using acetone and 90% chloroform. This whole setup was kept at 4°C, for 30 minutes in the refrigerator followed by keeping at room temperature. Then the extracts was heated gently in a boiling water bath (60°C), ideal for two minutes, and was again brought to room temperature. The supernatant was recovered by centrifugation at 3000 rpm for 15 minutes. The process was repeated until the entire acetone and chloroform soluble pigments were removed. The methanol extracts were combined and the final volume was recorded. The absorbance of the supernatant was measured at A_{665nm} and A_{750nm} wavelength using Systronics UV-visible spectrophotometer. The amount of chlorophyll-*a* was estimated according to Mackinney (1941). Absorbance of the acetone and methanol extracts was also measured at 450nm and amount of total carotenoids pigment was estimated according to the method of Jenssen (1978).

3.2.2. Test for cellular and extra cellular-carbohydrates

Cellular and extracellular carbohydrate (EPS) content of the cyanobacteria cultures was estimated using Anthrone reagent (Herbert *et al*., 1971). One ml of the supernatant of the culture (free from cyanobacteria cells) and 1ml of pellets was taken in a test tube and to this 4ml of freshly prepared Anthrone (LOBA) reagent (250mg Anthrone dissolved in 100ml of concentrated H_2SO_4) was added slowly keeping in an ice bucket and was shaken continously. After 10 minutes, this setup was kept in boiling water-bath (60°C) for 15 minutes by keeping marble ball on the mouth of each tube to prevent evaporation. Then it was brought to room temperature for cooling. Absorbance of the emerald-green colour was read at 620nm against the reference, each containing 1ml of distilled water along with 4ml of Anthrone reagent in a Systronics UV – visible Spectrophotometer.

The amount of extracellular carbohydrates of the samples was determined from the standard curve prepared from a known sugar, glucose (BDH, AR) solution within the range 10-100µg. ml^{-1} and represented as amount in glucose equivalent.

3.2.3. Test for soluble protein

Protein content was determined using Folin Ciocalteau's phenol reagent following Lowry *et al*., (1951). A known value of the uniform cell suspension was taken and centrifuged at 3000 rpm for 20 min. Then the supernatant was discarded. The pellet was again washed with distilled water at 3000 rpm for 10 min. To the pellet, 3ml of 10% TCA was added and then kept in boiling bath for 30 min. After bringing the whole setup to room temperature, it was again centrifuged at 3000 rpm for 5 min and the supernatant was discarded. To the pellet, 1ml of 1 N NaOH was added and from this solution, 0.1 ml was collected and was made up to 1 ml with distilled water. Five ml of freshly prepared alkaline reagent was added to each tube and was allowed to stand for 3 minutes, followed by addition of 0.5 ml of Folin's reagent. After keeping at room temperature for 30 min the absorbance was recorded at 750nm. The amounts of protein were calculated taking Bovine Serum Albumin as standard.

3.3 PREPARATION OF CRUDE EXTRACTS

After a period of 25 days of incubation of the biomass were harvested through Buchner funnel with Whatman No.1 filter paper and shade dried for 30 minutes in hot air oven and ground to fine powder with the help of glass homogenizer and finally weighed. 3gms of the powdered samples of *Scytonematopsis* were extracted with two different organic solvents, chloroform (210ml) and acetone (210ml) using soxhlet apparatus for 48 hours maintaining at temperature 45^{o}C. The extract was collect in air tight container and stored at 4^{o}C for further phytochemicals analysis.

3.4. PRELIMINARY PHYTOCHEMICALS CHARACTERIZATION

Preliminary phytochemicals characterizations of two different organic extracts (i.e Acetone and chloroform) of the cyanobacteria culture were subjected to different phytochemicals tests to detect different phytoconstituents present in extracts.

3.4.1. Test for Alkaloids: About 5 ml of the extracts was dissolved in 5ml of dilute hydrochloric acid and filtered. The filtrate was tested for presence of alkaloids as follows.

i. Dragendorff's test: To the 1ml filtrate extract add Dragendorff's reagent, reddish brown precipitate indicates presence of alkaloids.
ii. Mayer's Test: Two drops of Mayer's Reagent were added to 1ml of the filtrate in a test tube. Occurrence of white or creamy precipitate indicates the test as positive
iii. Wagner's Test: Two drops of Wagner's Reagent were added to 1 ml extracts in a test tube. Appearance of reddish brown precipitate indicates the test as positive
iv. Hager's test: 2 ml of Hager's Reagent (saturated aqueous solution of picric acid) were added to 1 ml extract. Appearance of prominent yellow precipitate indicates the presence of Alkaloid.
v. Tannic acid test: To the 1ml filtrate extract add tannic acid solution, buff coloured precipitate indicates presence of alkaloids.

3.4.2. Detection of Phenolic compounds and Flavonoids

i. Ferric chloride test: About 1ml of extract was dissolved in 5ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. Appearance of dark green colour indicates the presence of phenolic compounds
ii. Gelatin test: About 1ml of extract was dissolved in 5ml of distilled water and 2ml of 1% solution of gelatin containing 10% sodium chloride were added to it. Occurrence of White precipitate indicates the presence of phenolic compounds.
iii. Lead Acetate test: About 1ml of extract was dissolved in 5ml of distilled water and 3ml of 10% solution of lead acetate were added to it. Occurrence of bulky white precipitate indicates the presence of phenolic compounds.

iv. Alkaline Reagent test: About 1ml of extract was treated with 10% ammonium hydroxide solution. Appearance of Yellow fluorescence indicates the presence of flavonoid.

v. Zinc hydrochloride test: To the 1ml extract add a mixture of zinc dust and conc. Hydrochloric acid. It gives red colour after few minutes indicates the presence of flavonoid.

3.4.3. Test for Glycosides:

i. Borntrager's test: Boil the extract with 1ml of sulphuric acid in a test tube for 5minutes. Filter while hot. Cool the filtrate and shake with equal volume of dichloromethane or chloroform. Separate the lower layer of dichloromethane or chloroform and shake it with half of its volume of dilute ammonia. A rose pink to red colour is produced in the ammoniacal layer.

ii. Legal's test: Treat the extract with pyridine and add alkaline sodium nitroprusside solution, blood red colour appears.

3.4.4. Test for Saponins:

The 5ml of extracts was diluted with distilled water and volume was made up to 10 ml. The suspension is shaken vigorously in a graduated cylinder for 15 min. Occurrence of a 2 cm layer of foam indicates the presence of saponins.

3.4.5. Test for Steroids and Triterpenoids:

i. Libermann Burchard test: Treat the extract with few drops of acetic anhydride, boil and cool. Then add concentrated sulphuric acid from the side of the test tube, brown ring is formed at the junction two layers and upper layer turns green which shows presence of steroids and formation of deep red colour indicates presence of triterpenoids.

ii. Salkowski test: Treat the extract with few drops of concentrated sulphuric acid red colour at lower layer indicates presence of steroids and formation of yellow coloured lower layer indicates presence of triterpenoids.

iii. Sulfur powder test: Add small amount of sulphur powder to the extract, it sinks at the bottom.

3.4.6. Test for Fixed Oils and Fats:

i. Spot test: A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oil.

ii. Saponification Test: A few drops of 0.5N alcoholic potassium hydroxide solution are added to a small of extract along with a drop of phenolphthalein. The mixture is heated on hot water bath for 2hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oil and fats.

3.5. ANTIBACTERIAL BIOASSAY

3.5.1. Purification of crude extracts of isolates by thin layer chromatography

TLC was performed with crude extracts of isolates for purification using TLC silica plates (TLC 60 Merck, Germany). Separations of crude extracts were done using ethyl acetate and methanol (9: 1) as mobile phase. Spots developed on such TLC plates were observed under UV illumination. The illuminated orange spots were eluted separately and dissolved in 3 ml acetone and chloroform (25%). Each elutes again subjected to TLC purification using hexane: ethyl acetate (1: 1). Now all spots obtained at second stage were bio assayed for antibacterial activities.

3.5.2. Screening of cyanobacteria isolates for antibacterial activity

The designate spots in 2nd TLC were concentrated in rotary evaporator. These spots were redissolved in 25%. Antibacterial bioassays of such samples were performed. Antibacterial activities were performed using human pathogenic bacteria strains such as against *Bacillus subtilis* (MTCC 2191), *Escherichia coli* (MTCC 723), *Pseudomonas aeruginosa* (MTCC 741) *Staphylococcus aureus* (MTCC 902), and by agar well diffusion method. In this test 3.5 % Mueller Hinton Agar (MHA) plates were prepared; each MHA plates were swabbed with 0.1 ml of each target bacterium aseptically. The wells of 6mm diameter were made with sealing off bottom by soft agar (1%). The spots elutes obtained after second TLC were dissolved in 25% acetone and chloroform and prepared a different concentrations of each (i. e 50, 150, 300, 400 & 500µl) and filled in well respectively along with respective solvents and standard antibiotic Ciprofloxacin (5µg/ml) as controls, and incubated at 37°C for 24 h thereafter. The diameter of the inhibition zone was measured in mm in triplicates. Data are reported as means ± standard deviation (SD).

3.5.3. Screening and identification of prospective compounds

UV spectra of antibacterial purified bands were recorded in methanol over scanning range of 195 – 500nm using Gas chromatography. Cyanobacteria extracts were subjected to Gas chromatography analysis on a Clarus680GC-HS+AS (LABORATORY FOR ADVANCED RESEARCH IN POLYMERIC MATERIALS (LARPM), CIPET, BHUBANESWAR) comprising an auto injector and RTX – 5 column. Nitrogen was used as the carrier gas (1ml/min). The column temperature was programmed at 60°C for 1min and then raised to 180°C for the total analysis time 60 min. Sample (1ml) was injected in the split less mode. The chemical component of the extracts were identified by comprising the retention time of the chromatographic peaks with that of authentic compounds using the Software Version: 6.3.2.0646

3.6. STATISTICAL ANALYSIS

All tests were conducted in triplicate. Data are reported as means ± standard deviation (SD).

Chapter – 4

RESULTS AND DISCUSSIONS

4. RESULTS AND DISCUSSIONS

4.1. Characterization and identification of cyanobacteria isolates

In most of the cases, blackish-dark greenish biofilms tightly adhering to the upper surface of the cement wall were generally colonized by cyanobacteria. In present study, the isolate was identified as a filamentous cyanobacteria strain. Thallus was gelatinous; solitary filaments, clusters with parallel or irregularly arranged filaments or mats on the substrate. Filaments free or densely coiled, creeping on the substrate, or joined to the substrate by middle parts and free ends of branches, sparsely or commonly falsely branched, usually with two (rarely with one) branches. The vegetative cells were cylindrical with rounded terminal cells, Cells shorter or longer than wide, variable in length, olive-green. Heterocyst found present, somewhat cylindrical or barrel-shaped with intercalary in position. Sheaths firm, limited and yellowish in colour (Fig. 1).

Based on the description of the morphology provided by Desikachary (1959) and (http://www.cyanodb.cz) this cyanobacterium was identified as a strain of *Scytonematopsis* belongs to the order Nostocales and Family Scytonemataceae.

4.2. Biochemical characterization of cyanobacteria isolates

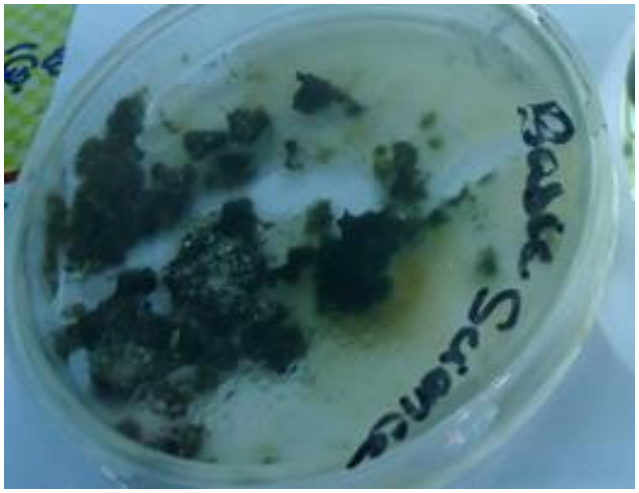
Biochemical analysis revealed the presence of total chlorophyll – *a*, soluble protein, cellular and extracellular carbohydrates (EPS), carotenoids, and Phycobiliproteins were presented in Graph from Fig. 2 – 4. It showed a specific growth rate of the isolates with a lower generation time. Total available chlorophyll – *a* were recorded maximum during 25th day of growth and showed highest chlorophyll - *a* content with comparison to other days of growth is presented in fig-2. Similar results were obtained from the rice field of Dima Hasau district in Assam, North East India where in two strains of *Nostoc* commune were characterised on the basis of their pigments and biochemical analysis in addition to molecular studies(Borah et.al, 2014). Total soluble protein was also recorded maximum during 25th day of growth (Fig. 2). Available of carbohydrates and extracellular carbohydrates was presented in Fig. 3. Cellular carbohydrate and extra cellular carbohydrates recorded maximum during 30th days of growth in *Scytonematopsis* sp. isolated from building facades of basic science Bhubaneswar Odisha.

Carotenoids content was also found maximum during 25th day of growth (Fig. 4), whereas available of Phycobiliproteins showed slight difference in their amount. The isolates produced maximum amount of Phycocyanin and Allophycocyanin respectively on 15th days of the growth as compared to phycoerythrin showed in figure 5. Since these isolates were found to be potent isolates, they can be commercialized. The can also be further modified for better yield. Our findings reported in this paper are comparable to other previous workers (Borowitzka, 1994). Apart from the use of Phycobiliproteins as foods grade dyes, they are also tool for basic research and medical diagnostics. Phycocyanin the major Phycobiliproteins also exhibited anticancer activity, stimulation of immune system and ability to treat ulcers and haemorrhoidal bleeding. A large array of natural products of economic potentials may be

produced from cyanobacteria. Cyanobacteria represent an attractive source of natural pigments such as Phycobiliproteins such as Phycocyanin, phycoerythrin and Allophycocyanin etc. Among them, Phycocyanin and phycoerythrin are commercially valuable. Phycocyanin is also obtained in a water insoluble form from *Spirulina* and used in eye-shadow, eye-liner and lipstick preparations (Dainippon patent 1980) and phycoerythrin from *Spirulina* and other cyanobacteria is used as a food colour for products like ice-cream, yoghurt and it could also be used in cosmetics (Borowitzka 1994).

A variety of carotenoids have important commercials uses. Since carotenoids are non-toxic, they are desirable as colouring agents in the food industry as well as vitamins A precursors (Bauernfeind 1981). The spectrum of carotenoids in *Anabaena variabilis* and three species of *Phormidium* showed β – carotene as the majors pigments (Healey 1968).

Samples collected from cement wall surfaces



Isolation of cultures in BG -II media

Cultivation and maintenance of cultures

Microphotographs of isolated species

Fig. 1. Sampling sites, isolation and preservation of cyanobacteria cultures

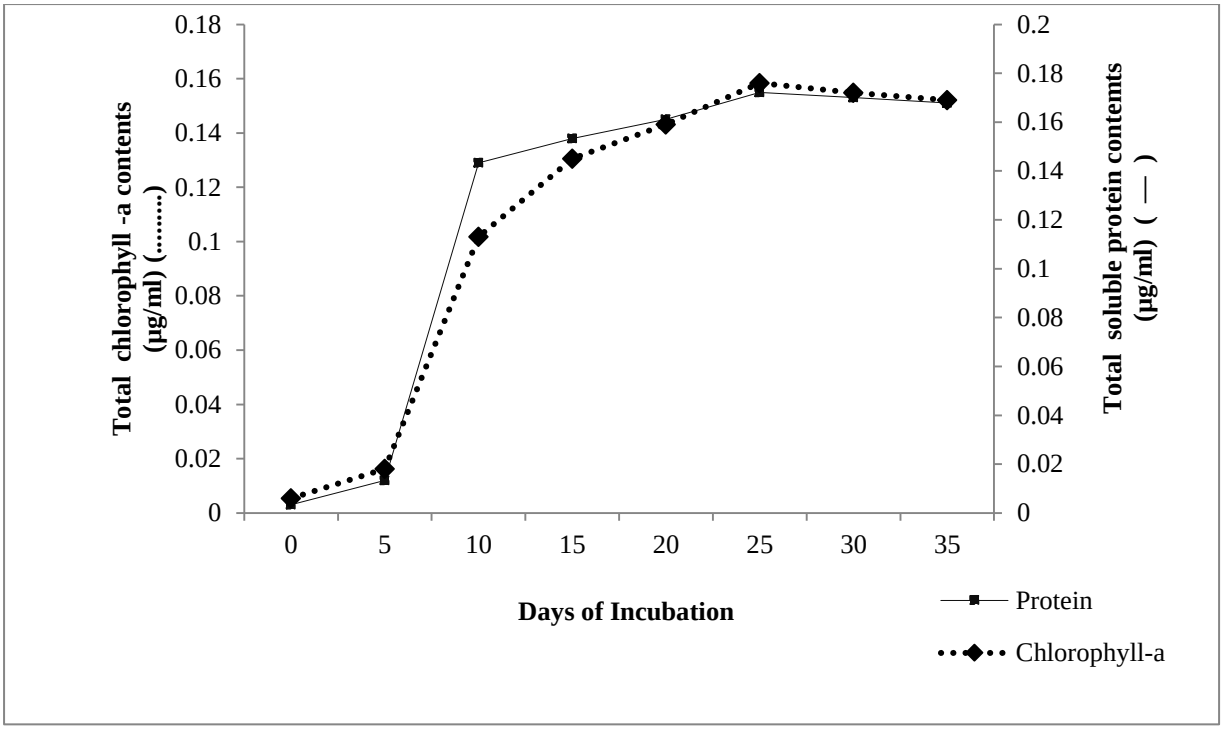


Fig. 2. Total chlorophyll - *a* and soluble protein contents of *Scytonematopsis* sp. isolated from cement wall surface of College of Basic Science building, O.U.A.T, Bhubaneswar, Odisha. Data are mean values ± SD (n = 3).

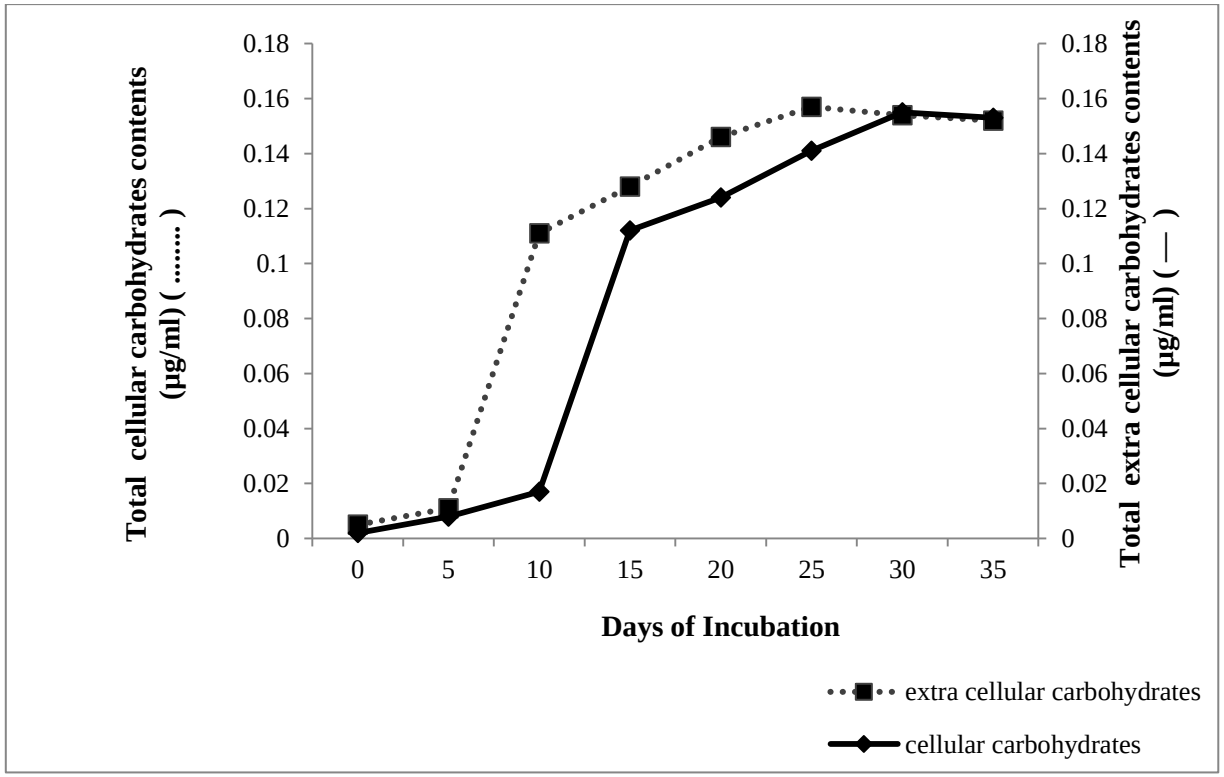


Fig. 3. Total cellular carbohydrates and extra-cellular carbohydrates content of *Scytonematopsis* sp. isolated from cement wall surface of College of Basic Science building, O.U.A.T, Bhubaneswar, Odisha. Data are mean values ± SD (n = 3).

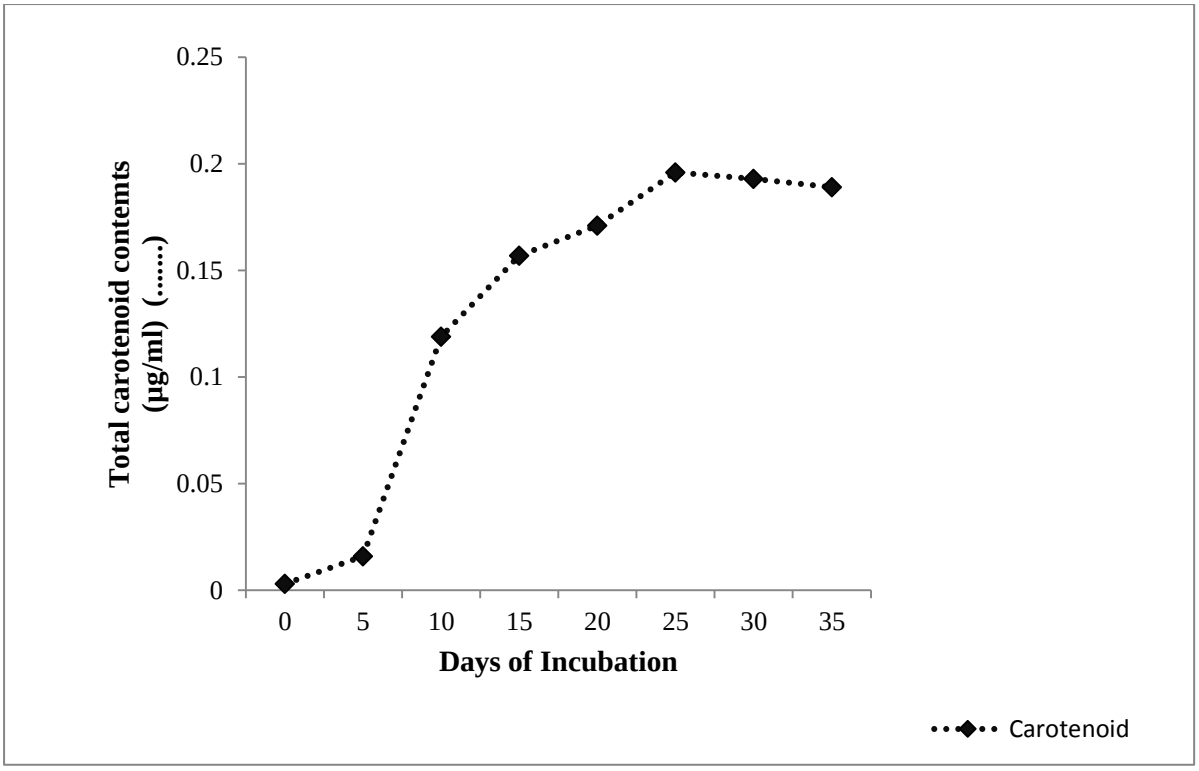


Fig. 4. Total carotenoids contents of *Scytonematopsis* sp. isolated from cement wall surface of College of Basic Science building, O.U.A.T, Bhubaneswar, Odisha. Data are mean values ± SD (n = 3).

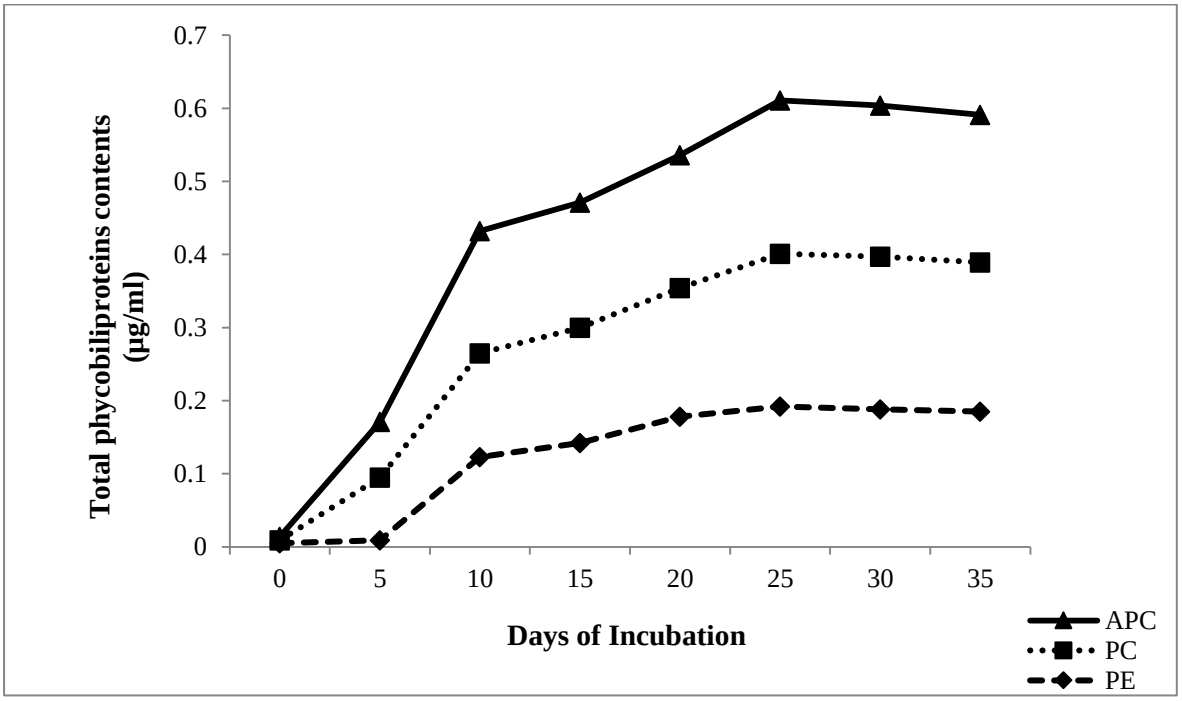


Fig. 5. Total phycobiliproteins contents of *Scytonematopsis* sp. isolated from cement wall surface of College of Basic Science building, O.U.A.T, Bhubaneswar, Odisha. Data are mean values ± SD (n = 3).

In the present study, chlorophyll- *a*, total soluble proteins, carbohydrates, carotenoids and Allophycocyanin were found to be less during lag phase and more during log phase. This showed that during log phase, metabolic activity will be at the peak. But in case of Phycocyanin and phycoerythrin, it was found to be more during early stationary growth phase i. e. cells starts excreting metabolites during this stage only. The analysis clearly revealed the need for a morpho-physiological and molecular approach for cyanobacteria characterization and their utilization in agriculture and industry. Data generated during present investigations could be useful in understandings of a commercial or biotechnological potential of blue green algae. Nowadays scientists are motivated to search for more potential species available in nature for exploiting them in a variety of ways to meet the demands. Its needs an extensive screening which expected to results in the discovery of better cyanobacteria isolates of industrials interest. Higher growth rates and nutrients profile of cyanobacteria make them a potentially valuable source of nutrients (Cannel 1989).

4.3. Preliminary phytochemicals characterization of the isolates

The Phytochemicals constituents such as alkaloids, carbohydrates, glycosides, proteins, amino acids, Phenolics and flavonoid, steroids, terpenoids, fixed oils and fats contains of isolates are shown in Table – 1. The acetone and chloroform extracts *Scytonematopsis* species isolated from cement wall surface of Building facades of Basic Science, Bhubaneswar, Odisha showed positive results for the presence of alkaloids and phenolic compounds. These extracts also showed positive results for the presence of Saponins, fixed oils and fats. The presence of alkaloids in the isolates genus indicates that it can be commercialized particularly for pharmaceuticals applications and the phenolic compounds particularly the complex flavonoid are important class of antioxidants (Roya and Fatemeh, 2013) can be used for industrial applications. Zeeshan *et al.*, 2010 reported the presence of certain metabolites as tannin, alkaloids, protein and flavonoid in the extracts of cyanobacteria.

Table - 1. Preliminary Phytochemicals screening of *Scytonematopsis* sp. **isolated from cement wall surface of Building facades of Basic Science, Bhubaneswar, Odisha.**

Phytochemicals tests	**Acetone**	**Chloroform**
Detection of Alkaloids		
Dragendorff's test	+++	+++
Mayer's test	+++	+++
Wagner's test	+++	+++
Hager's test	+++	+++
Tannic acid test	++	+
Detection of Phenolic compounds (Tannin) and flavonoid		
Ferric chloride test	-	-
Gelatin Test	-	-
Lead acetate test	++	++
Alkaline reagent test	+++	+++
Zinc hydrochloride test	+++	+++
Detection of Glycosides		
Borntrager's Test	-	-

Legal's Test		
Detection of Saponins	++	++
Detection of Steroids and Triterpenoids Libermann Burchard test	-	-
Salkowski test	-	-
Sulfur powder test	-	-
Detection of Fixed oils and fats Spot test	++	++
Saponification test	++	++

"+" – presence of phytochemicals
"-'' – absence of phytochemicals

4.4. Assaying *Scytonematopsis* strain for TLC spots and antibacterial activity

In the present course of investigation one band observed from *Scytonematopsis* strain. These potent bands were subjected to second TLC and illuminated designate band were bioassay against human pathogenic bacteria strains such as *Bacillus subtilis* (MTCC 219), *Escherichia coli* (MTCC 723), *Pseudomonas aeruginosa* (MTCC 741) and *Staphylococcus aureus* (MTCC 902) by agar well diffusion method. The bioassay revealed that band A1 from *Scytonematopsis* strain were potent antibacterial at 400µl and 500µl concentrations showed in table -2. The significant result was observed in evaluating antibacterial property of cyanobacteria extracts particularly in two different concentrations i. e 400µl and 500µl as evidence from the table - 2, in general to other concentrations (Control, 50µl, 150µl and 300µl). The chloroform extracts of *Scytonematopsis* strain at the concentration 400µl and 500µl had a highest antibacterial effect on *E. coli* than *Pseudomonas aeruginosa.* The inhibition zone diameter of *Scytonematopsis* strain in the concentration 500µl was 4.33 ± 0.05cm against *E. coli* and 1.6 ± 0.05cm against *Pseudomonas aeruginosa* and in 400µl concentration inhibition zone diameter was 3.86 ± 0.05cm against *E. coli* and 0.86 ± 0.05cm against *Pseudomonas aeruginosa* whereas, acetone extract of *Scytonematopsis* strain inhibited a good extent of *E. coli* i. e 1.76 ± 0.05cm in 500µl and 5.1 ± 0.05cm in 400µl in contrast with synthetic antibiotic disc against *E. coli* (6.8 ± 0.68cm) and *Pseudomonas aeruginosa* (6.4 ± 0.06cm) were presented in Table - 3. No allelopathic effect against *Bacillus* and *Staphylococcus*. The solvents control revealed no activity.

Extract from *Oscillatoria* principles were active against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Brucella bronchiseptica* (Gupta and Shrivastava, 1965). The chloroform extract of *Anabaena circinalis* and *Synedra ulva* recorded appeared to be the most effective by showing maximum antibacterial activities against the selected bacterial pathogen *E. coli* (Sivakami *et al*., 2013). In present study, chloroform extracts have some effect against *E. coli* and *Pseudomonas aeruginosa* (Fig. 6B, C). The antibacterial activity of the extract could be due to the presence of different chemical that may include flavonoid and triterpenoids besides phenolic compounds and free hydroxyl group (Yu *et al*., 2009), amides and alkaloids (Ghasemi *et al*., 2004), metabolites such as tannin, alkaloids, protein and flavonoid (Zeeshan *et al*., 2010) etc.

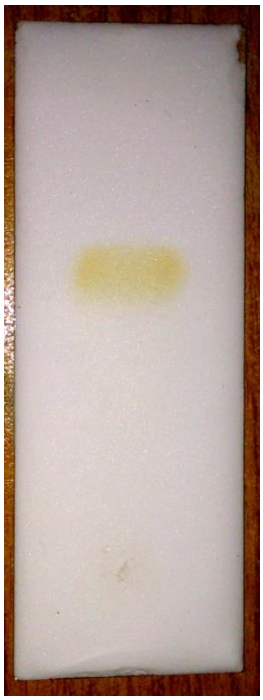
Acetone extract of cyanobacteria show maximum inhibition zone against *Escherichia coli, Bacillus subtilis* and *Pseudomonas aeruginosa* (De Mule *et al*., 1991 and Ishida *et al*., 1997). Acetone and

methanol extracts from *Spirulina plantensis* showed more or less similar inhibition zones against *Staphylococcus aureus* and *Staphylococcus typhimurium* (Kumar *et al*., 2011). Diether and acetone extract of *Spirulina plantensis* had highest antibacterial and antifungal activity (Ozdemir *et al*., 2004). In the present study also acetone extracts of Scytonematopsis showed minimum inhibition zone against *Escherichia coli* (Fig. 6A).

The results obtained in the study indicate that the chloroform extracts of *Scytonematopsis* strain showed excellent inhibition activity against two pathogenic bacteria whereas acetone extracts showed moderate effect particularly against *Escherichia coli.* So these both extracts might have a potential as antibiotic compound against the growth of pathogenic bacteria *Escherichia coli* and *Pseudomonas aeruginosa.*

The cyanobacteria *Scytonematopsis* sp. highlights the potential of cyanobacteria as a resource of secondary metabolites. Asthana *et al*., 2006 isolated and identified new antibacterial compound from *Nostoc CCC* which were similar to anthraquinone and indane derivatives of a diterpenoid.

Thus, results proved that chloroform and acetone was the best solvent for extracting the antibacterial agents from *Scytonematopsis* (fig. 6 B, C). Many investigators mentioned that the acetone extract of cyanobacteria revealed antibacterial activity on *E. coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* (De Mule *et al*., 1991 and Eshida *et al*., 1997). The present study also supported that acetone extract showed moderate activities against *E. coli* (Fig. 6A and Table - 2).



Thin layer Chromatography of *Scytonematopsis* strain extracts with one band designated as A1.

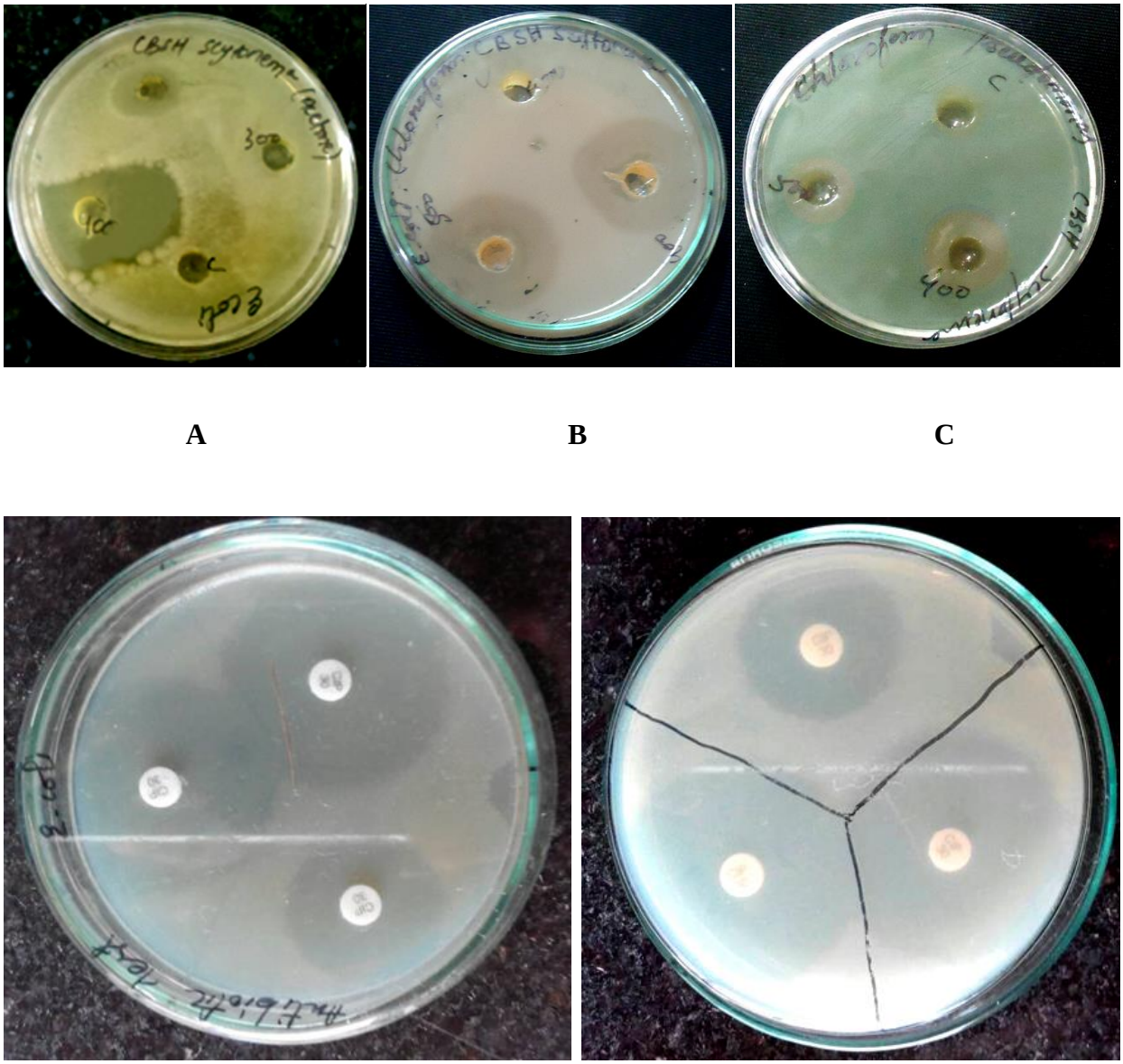
Table - 2. Antibacterial activity of two different extracts of *Scytonematopsis* strain.

Test Organisms	TLC Spots	Different Concentration of extracts	Effective zone of inhibition (cm)		
			Organic Extracts		Standard Antibiotics Ciprofloxacin (5µg/ml)
			Acetone	Chloroform	
Bacillus subtilis (MTCC 219)	A1	Control	-	-	5.37 ± 0.08
		50µl	-	-	
		150µl	-	-	
		300µl	-	-	
		400µl	-	-	
		500µl	-	-	
Escherichia coli (MTCC 723)	A1	Control	-	-	6.8 ± 0.68
		50µl	-	-	
		150µl	-	-	
		300µl	-	-	
		400µl	**5.10 ± 0.05**	**3.86 ± 0.05**	
		500µl	**1.76 ± 0.05**	**4.33 ± 0.05**	
Pseudomonas aeruginosa (MTCC 741)	A1	Control	-	**0.23 ± 0.05**	6.4 ± 0.06
		50µl	-	-	
		150µl	-	-	
		300µl	-	-	
		400µl	-	**0.86 ± 0.05**	
		500µl	-	**1.60 ± 0.05**	
Staphylococcus aureus (MTCC 902)	A1	Control	-	-	8.5 ± 1.1
		50µl	-	-	
		150µl	-	-	
		300µl	-	-	
		400µl	-	-	
		500µl	-	-	

4.5. GC analysis of *Scytonematopsis* strain and bioactive compounds

Gas chromatography analysis revealed the presence of one peak in two different extract of *Scytonematopsis strain* Fig. 7 & 8. The peaks 1.98 and 2.18 led the possibility of a compound in the sample of two different extracts. Thus data from GC analysis of these two extracts confirmed presence of one or two compounds in the isolates strains show antibacterial activity against the growth of two pathogenic bacterial strains (*Escherichia coli* and *Pseudomonas aeruginosa*) possessed broad spectrum antibiotic activity.

Thus selected cyanobacteria strains have potency to produce one compound. Further researches should be made to identify and purify natural Biomolecules from these cyanobacteria against antibacterial activity. Improvement knowledge of the composition, analysis and the properties of these cyanobacteria with respect to antibacterial compounds would assist in efforts for the pharmaceutical application.



A **B** **C**

D **E**

Fig. 6. Zone of inhibition exhibited by acetone and chloroform extracts of *Scytonematopsis* species against *Escherichia coli* and *Pseudomonas aeruginosa*. A - Acetone extract against *E. coli*, B - Chloroform extracts against *E. coli*, C - Chloroform extracts against *Pseudomonas aeruginosa*, D - Standard Antibiotics Ciprofloxacin against *E. coli* and E - Standard Antibiotics Ciprofloxacin against *Pseudomonas aeruginosa*.

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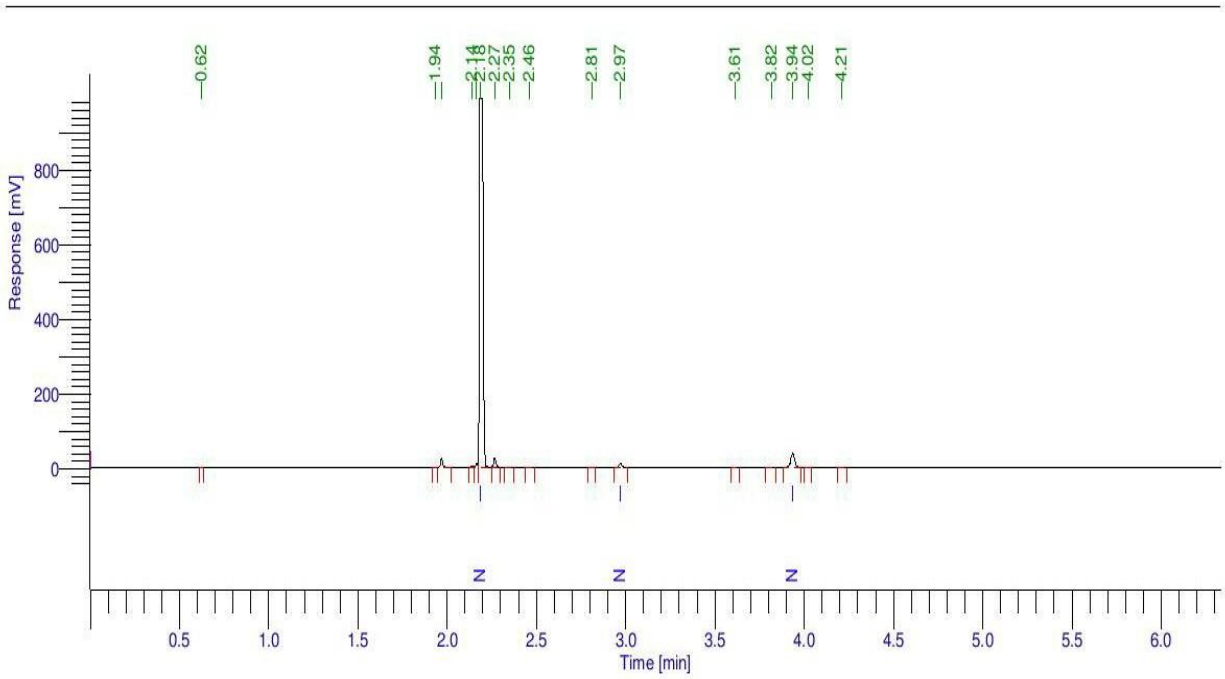

Fig. 7. GC analysis of the TLC spots of the acetone extracts of *Scytonematopsis* sp.

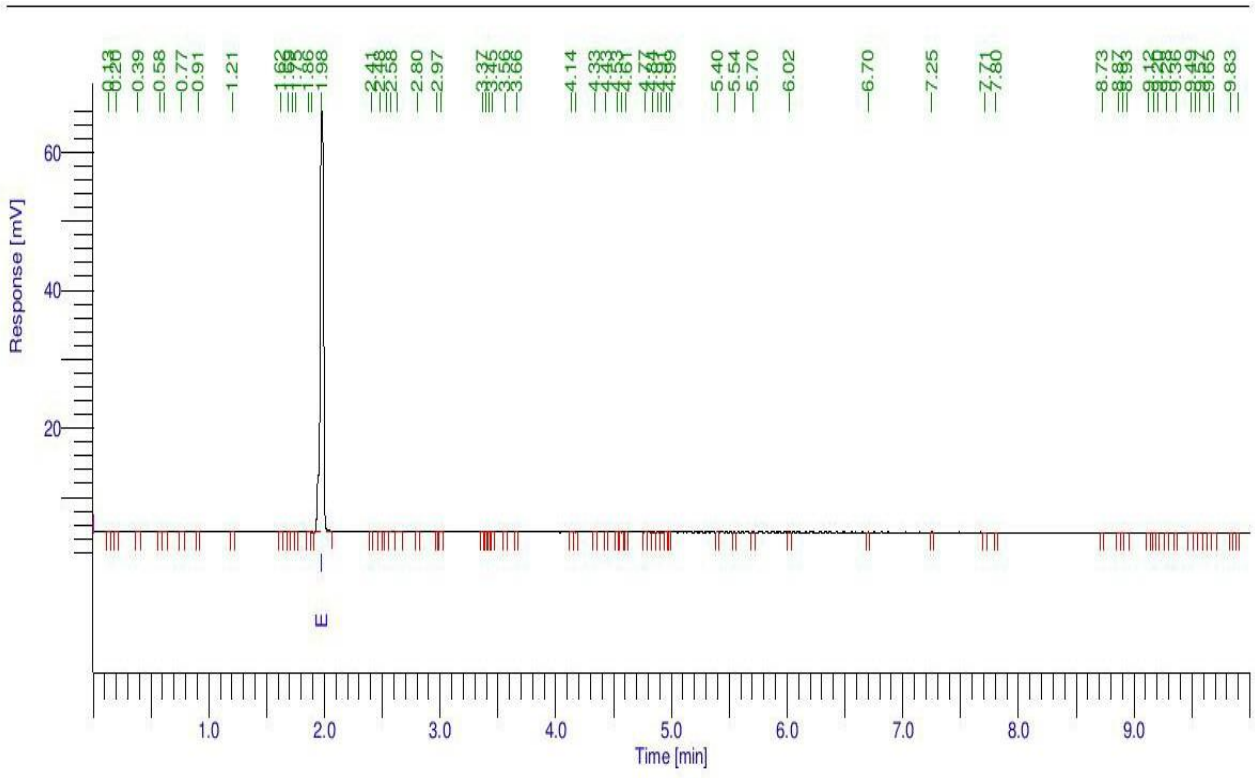

Fig. 8. GC analysis of the TLC spots of the chloroform extracts of *Scytonematopsis* sp.

Chapter – 5

SUMMARY AND CONCLUSION

5. SUMMARY AND CONCLUSION

Cyanobacteria are photoautotrophic prokaryotes; they are capable of producing a variety of Secondary metabolites that exhibited as antibiotics, allele-chemicals, Hormones and toxins etc. Besides their specific feature of nitrogen fixation, toxic behaviour has attracted to biologists and various strains have been extensively studied by several workers and advocated their antimicrobial nature such as antibacterial, antifungal, antialgal, as well as allele-pathic and antagonistic properties. In present study, one of the isolates *Scytonematopsis* species isolated from cement wall surface of Basic science college building, Bhubaneswar, OUAT were cultured into pure culture for further investigation. These species were grown in N- free BG – 11 media for culture and their doubling time was determined. The organisms were slow growing. The doubling time at 7.5 w/m² light intensities under 28 ± 2º C in still culture was 172 h^{-1}. The organism were cultured for 35 days under 7.5 w/m² light intensity to understand their biochemical composition, chlorophyll-*a* content and the macromolecules like carbohydrates, exopolysaccharides and soluble cell, and the pigment like carotenoids and Phycobiliproteins content in addition to growth was measured at 5 days interval up to 35 days of culture. The amount of chlorophyll – *a*, soluble cell protein, carbohydrates, EPS, carotenoids as well as Allophycocyanin was higher in the organism till 25 days of the growth phase. But in case of Phycocyanin and phycoerythrin it was found to be more during early stationary growth phase. Chlorophyll – *a* as well as the content of macromolecules was further increased at higher light intensities.

Further screenings of phytochemical test of the isolates have also been done to know the presence of certain secondary metabolites of the two different organic solvents i.e acetone and chloroform. The results showed that the isolates cyanobacteria species has positive response for the presence of alkaloids and phenolic compounds. These extract also showed positive results for the presence of Saponins, and phenolic compounds in the isolates suggesting the presence of certain secondary metabolites commercialised particularly for pharmaceutical applications.

The crude extracts of the isolates *Scytonematopsis* sp. extracted in acetone and chloroform organic solvents were purified using TLC plates and the spots developed on such TLC plates were observed under illumination. The illuminated orange spots were eluted separately and dissolved in respective solvents. Each elutes again subjected to TLC purification using hexane: ethyl acetate (1:1). Now a single spots obtained at second stage were bio assayed for antibacterial activities against four human bacterial pathogens. The designate single spots obtained in second TLC were redissolved in respective solvents and filled in well with different concentrations (50, 150, 300, 400, and 500µl) along with respective solvents (i.e acetone and chloroform) and Ciprofloxacin (5µg/ml) as controls, and incubated at 37°C for 24h thereafter. The diameter of the zones of inhibition were measured in cm. The results showed that at a concentration of 400µl and 500µl , the inhibition zone of diameter of isolates *Scytonematopsis* sp. were recorded (3.86 ± 0.05cm and 4.33 ± 0.05cm) against *E. coli* and (0.86 ± 0.05cm and 1.6 ± 0.05 cm)

against *Pseudomonas aeruginosa*. Both acetone and chloroform extracts of isolated cyanobacteria have been examined and positive response was observed with different variation in the diameter of the inhibition zone especially against *E. coli* and *Pseudomonas aeruginosa*. It is clear from the study that the diameter of the inhibited zone depends mainly on the types of the algal species, types of the solvent used and the tested bacterial organisms. The observation revealed that the cyanobacteria strains posse's broad spectrum antimicrobial properties.

It is concluded from this study that selected *Scytonematopsis* species belongs to the order Nostocales and Family Scytonemataceae from building facades and its produces antibacterial activity against the certain pathogens used in the present investigation. Through purification of acetone and chloroform extract, it also presented antibacterial activity. GC analysis indicates the presence of single peak in both solvents which is responsible for the antibacterial activity. Thus selected cyanobacteria strains have potency to produce one compound. Further researches should be made to identify and purify natural Biomolecules from these cyanobacteria against antibacterial activity. Improvement knowledge of the composition, analysis and the properties of these cyanobacteria with respect to antibacterial compounds would assist in efforts for the pharmaceutical application.

Though this kind of investigation creates quite a general view of cyanobacteria possibilities to produce biologically active compounds still it points out the necessicity of exploring cyanobacteria strains as potentially excellent sources of these substances and reveal the most prospective strains for further scientific investigation.

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