

Original Research Article

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## Morphological and Biochemical Characterization of Sub-Aerial Cyanobacteria Isolated from Building Facades and Monuments of Odisha

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### ABSTRACT

Several phototrophic microorganisms, principally cyanobacteria species predominantly sheathed forms occur as biofilms on the exposed surface of stone temples, monument as well as building facades receiving areas. A study was conducted to analyse the potential of six sub-aerial cyanobacteria species belonging to three genera, i.e. *Scytonema*, *Tolypothrix* and *Westiellopsis* which were recorded as the main components in the biofilms isolated from 10 different sub-aerial sites (six building facades, three stone temples and one mortar monument) of Bhubaneswar and Puri, Odisha under laboratory culture conditions. The sub-aerial cyanobacteria strains were morphological characterized and changes in their biochemical constituents were analyzed in terms of total chlorophyll- *a*, total soluble protein, total carotenoids, total phycobiliproteins including phycocyanin, phycoerythrin and allophycocyanin, total cellular carbohydrate and extracellular polysaccharides in response to different time interval culture in BG-11 media. The analysis showed that maximum amount of total chlorophyll- *a* in *Scytonema hyalinum* ( $8.98\mu\text{gml}^{-1}$ ) and minimum in *Westiellopsis prolifica* ( $3.78\mu\text{gml}^{-1}$ ). On other hand, total soluble protein content increased with incubation time and maximum amount were recorded in two species *Tolypothrix rechingeri* and *Scytonema bohneri* ( $95.76\mu\text{gml}^{-1}$  and  $90.76\mu\text{gml}^{-1}$ ), while species *Westiellopsis prolifica* showed minimum content of soluble protein ( $27.90\mu\text{gml}^{-1}$ ) as compared to other strains examined. Maximum amount of total carotenoids were recorded in *Tolypothrix rechingeri* ( $2.54\mu\text{gml}^{-1}$ ) and total phycobiliproteins in *Scytonema bohneri* ( $5.99\mu\text{gml}^{-1}$ ). The results of phycobillin analysis of investigated sub-aerial cyanobacteria showed phycocyanin (PC) to be dominant phycobiliproteins with maximum value recorded in three species *Tolypothrix rechingeri*, *Scytonema bohneri*, *Scytonema hyalinum* and lowest in *Scytonema hofman-bangii*. On the contrary, *Scytonema hofman-bangii* recorded highest phycoerythrin ( $2.28\mu\text{gml}^{-1}$ ). Total cellular carbohydrate were recorded maximum during 28<sup>th</sup> day growth in *Tolypothrix rechingeri* ( $78.76\mu\text{gml}^{-1}$ ) and minimum in *Scytonema hyalinum* ( $44.9\mu\text{gml}^{-1}$ ) whereas, the total extracellular polysaccharides contents were found to be higher in *Tolypothrix rechingeri* and *Scytonema ocellatum* on 32<sup>th</sup> days growth ( $64.54\mu\text{gml}^{-1}$  and  $58.98\mu\text{gml}^{-1}$  respectively). Study showed that Qualitative phytochemical screening of sub-aerial cyanobacteria revealed its bioactive phytochemical constituents to be alkaloids, phenol and Flavonoid etc. Acetone, methanol and chloroform are better and more efficient solvent extractors of the bioactive phytochemical constituents which vary from species to species. However, the overall response during the investigation it was observed sub-aerial cyanobacteria are prolific producers of primary and secondary metabolite(s) that may act as the antimicrobial drug resource in addition to their much explored marine and freshwater counterpart. This study is expected to contribute to data base for the drugs discovery as raw material for both biotechnological applications as well as in industrial uses for benefit of future generation.

#### Keywords

Cyanobacteria, Sub-aerial, Biochemical and phytochemical characterization

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## Introduction

Cyanobacteria are considered as one of the potential source of both primary and secondary metabolites. They are cosmopolitan in nature and are found in both filamentous and coccoid forms occupying mostly tropical climate than temperate zones. These autotrophic organism can withstand harsh and extreme conditions like extreme temperature, drought, U.V radiations etc. thus can grow on exposed walls of building facades, temples and monuments. During last decades, these gram negative, photosynthetic prokaryotes are considered to be the pioneer microorganisms inhabiting the exposed surface of most building facades and monuments across the length and breadth of the world (Rossi *et al.*, 2012; Keshari and Adhikary, 2014; Adhikary *et al.*, 2015). Cyanobacteria secrete some exopolymeric substances (EPS) which help them to grow in association with other microorganisms like bacteria, fungus, actinomycetes by forming biofilms on the exposed walls of building facades and monuments (Rossi *et al.*, 2012). They play an important role in reducing the a number of microorganisms like bacteria, fungus and viruses thus showing antimicrobial activity against them (Gutierrez *et al.*, 2008; Asthana *et al.*, 2009). Cyanobacteria are rich source of a number of primary metabolites like proteins, carbohydrates, amino acids, pigments which can be used as dietary supplements, as well as industrial application. Among microorganisms, cyanobacteria are rapidly proving to be an extremely important source of biologically active secondary metabolites like alkaloids, phenol compounds, saponin, tannins etc. have wide spread applications in pharmaceutical industries against many human diseases. Recent trends in drug research from natural sources suggest that cyanobacteria are excellent sources to provide novel biochemically active constituents produced during primary and

especially secondary metabolism (Skulberg, 2000) with varied application which are useful to mankind in various ways.

In spite of having such wide spread importance in the field of biotechnology, cyanobacteria have received less importance as compared to viruses, bacteria and fungus. Therefore, the objective of our study was to make an exploratory analysis about biochemical and phytochemical composition of six sub-aerial cyanobacteria possessing well developed sheath layers around their trichome found typically in sub-aerial habitats. In addition, we compared our results with other studies in an attempt to contribute to current researches about economic value of sub-aerial cyanobacteria and, if warranted, to recommend its use for human activities.

## Materials and Methods

Microbial biofilms were collected from horizontal and vertical surfaces of 10 sub-aerial sites including six building facades, three stone temples and one mortar monuments of Orissa state but principally from Bhubaneswar (Temperature: 21°C, Latitude /Longitude: 20°25'36.82"N and 85° 48' 10"E) and Puri cities (Temperature: 21°C, Latitude /Longitude: 20°25'36.82"N and 85° 48' 10"E) during monsoon and post monsoon period. Sample collection included substrates like composites (limestone, sandstone, stone, cement, concrete, brick and rock), painted surfaces (lime-washed over concrete or mortar) and dimensional stone of both calcareous and siliceous types of building including monuments and temple soon after monsoon rain and post monsoon seasons, and stored in pre-sterilized sampling bottles. The biofilms were soaked in sterile distilled water and incubated under fluorescent light for up to 72h and observed microscopically. Since the morphological 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 agar plates medium with and without nitrogen (Rippka *et al.*, 1979) (1.5%w/v agar in same medium) with the help of inoculation needle. Cultures were incubated at  $28 \pm 2^{\circ}\text{C}$  under continuous light from fluorescent tubes at an intensity of 2000lux. After a period of 10 to 14 days of incubation, cyanobacteria appeared in the culture were isolated and maintained in the laboratory. The cultures were microscopically examined using inverted microscope, and identified by following standard monographs (Desikachary, 1959; Komárek and Hauer, 2015). Total six sub-aerial cyanobacteria strains were isolated i.e *Scytonema ocellatum* (cement surface, Building facades), *Scytonema hofman-bangii* (stone, Building facades), *Scytonema hyalinum* (rock surface, Temple), *Scytonema bohneri* (mortar, monument), *Westellopsis prolifica* (cement and stone surface, Building facades and temples) and *Tolypothrix rechingeri* (stone carving, Temple), and sub cultured to 50ml of culture media in 100ml conical flasks, and incubated under illumination of 2000lux with 16:8h light and dark regime, using cool fluorescent tubes. Cultures were uniformly stirred twice for 3-5 minutes every day to prevent cell clumping.

Three replicas of each test tubes containing 9ml aliquots of sterile BG – 11 liquid medium (with and without nitrogen) were inoculated with 1ml stock culture with inoculums of the isolated strain and were maintained for zero day, 4<sup>th</sup>, 8<sup>th</sup>, 12<sup>th</sup>, 16<sup>th</sup>, 20<sup>th</sup>, 24<sup>th</sup>, 28<sup>th</sup>, 32<sup>th</sup>, 36<sup>th</sup> and 40<sup>th</sup> day's analysis. Cells were harvested at 4 day interval during growth, specific amount of culture was withdrawn from each test tube and analysed for the chlorophyll-*a* by the standard methods of McKinney (1941), carotenoids pigment by (Jensen, 1978) and phycobiliprotein including PC, PE and APC were estimated by the methods of Bennett and Bogorad (1973). Total soluble protein was

estimated by the method of Lowry *et al.*, (1951). Total cellular carbohydrates and released extracellular polysaccharides (EPSs) were estimated by anthrone reagent (Herbert *et al.*, 1971). All the experiments were conducted in triplicates. Mean value of 3 replicates of each experiment  $\pm$  S.D has been presented in the text. The absorbance was measured using Systronics UV – visible spectrophotometer.

After 28days of incubation, biomass was harvested through Buchner funnel with Whatman No.1 filter paper and dried in shade for 30 minutes in hot air oven and grinded to fine powder with the help of glass homogenizer and finally weighed. 1gms of the powdered samples of each isolates were extracted with aqueous and using different organic solvents such as hexane, benzene, chloroform, methanol and acetone using soxhlet apparatus for 48 hours maintaining at temperature  $45^{\circ}\text{C}$ . The each crude extracts were collected separately in air tight containers, and finally were stored at  $4^{\circ}\text{C}$  for phytochemical analysis. To detect different phytoconstituents present in the extracts, experiments were conducted following the standard of Dragendorff's test and Mayer's test; Ferric chloride test and Lead Acetate test; Alkaline Reagent test; Quinone test; Libermann Burchard test, Borntrager's test, Terpenoids test and Coumarin test etc.

## Results and Discussion

Bhubaneswar ( $20^{\circ}15' \text{N}$  and  $85^{\circ}50' \text{E}$ ) and Puri ( $19^{\circ} 38' 59. 63'' \text{N}$  and  $85^{\circ} 50' 23.52'' \text{E}$ ) of Odisha has occupied a unique position among different states of India for ancient sandstone temples, old and modern monuments as well as building facades. The sides exposed to direct sunlight were colonized by microorganisms that have produced microbial biofilms appeared with blackish-brown/blackish green/ brownish or bluish-

green etc., were mainly dominated with cyanobacteria as compared to other organisms. Such sub-aerial cyanobacteria occurring in the biofilms on the horizontal as well as vertical surface of 10 different sub-aerial sites were sampled during monsoon and post monsoon period. Totally, six species of cyanobacteria were recorded as major component in biofilms/crusts possess well developed sheath layers around their trichome given in Table 1 and Figure 1. Out of the 6 species of cyanobacteria, three species of cyanobacteria were isolated from building facades e.g. *Scytonema ocellatum*, *Scytonema hofmanbangii* and *Westellopsis prolifica*, whereas two species from temple e.g. *Scytonema hyalinum*, *Tolypothrix rechingeri* and other from monument *Scytonema bohneri* identified using different monographs and research publications: Desikachary (1959), Komárek and Hauer (2015), Keshari and Adhikary (2014).

Six isolates from three genera viz-a-viz, *Scytonema ocellatum*, *Scytonema hofmanbangii*, *Scytonema hyalinum*, *Scytonema bohneri*, *Tolypothrix rechingeri* and *Westellopsis prolifica* are summarised in (Table 1 and Fig. 1) were grown in nitrogen free BG – II media at  $28 \pm 2^\circ\text{C}$  under continuous light provided by day light fluorescent tube at a light intensity of 2000lux and harvested at 4 day interval up to 40<sup>th</sup> day for biochemical analysis such as chlorophyll-*a* and soluble protein, carotenoid, total PBPs including phycocyanin, phycoerythrin and allophycocyanin, cellular carbohydrate and released extracellular polysaccharides content shown in Figure 2-4(a – f).

Cyanobacteria are recognized as an excellent source of natural pigments (Dufossé *et al.*, 2005). The chlorophyll is the primary photosynthetic pigments in all microalgae species and they also produce various accessory or secondary pigments, such as

PBPs and a wide range of carotenoids (Gouveia *et al.*, 2008). In the present study, highest chlorophyll-*a* and soluble protein contents increased with increased incubation time in all species. The maximum chlorophyll-*a* and soluble protein content was accumulated from 8<sup>th</sup> to 28<sup>th</sup> day in all the sub-aerial cyanobacteria strains. Results showed that total chlorophyll- *a* content of the isolates ranged from (1.67 – 7.78 $\mu\text{gml}^{-1}$ ; 1.19 – 7.66 $\mu\text{gml}^{-1}$ ; 1.30 – 8.98 $\mu\text{gml}^{-1}$ ; 1.26 - 6.24 $\mu\text{gml}^{-1}$ ; 2.72 – 6.84 $\mu\text{gml}^{-1}$  and 1.19 – 3.78 $\mu\text{gml}^{-1}$ ) with incubation time as it attained maximum peak value on 28<sup>th</sup> day, only minor reduced amount were noted on 40<sup>th</sup> day showed in Figure 2(a-f). *Scytonema hyalinum* Gardner (8.98 $\mu\text{gml}^{-1}$ ) showed highest chlorophyll-*a* content with comparison to other on 28<sup>th</sup> day of growth (Fig. 2c), followed by *Scytonema ocellatum*, *Scytonema hofmanbangii* and *Tolypothrix rechingeri*. The chlorophyll-*a* content of *Scytonema tolyprothrichoides* isolated from rice field reported by Deb *et al.*, (2015) were higher than the value obtained in the present study which was 4.94 $\text{mgml}^{-1}$ . The chlorophyll-*a* content of all isolates showed equal value or falls within the ranges as recorded from freshwater, marine and terrestrial habitats (Becker, 1994; Rai and Rajashekhar, 2015; Paul and Rout, 2017). The study conducted by Balder *et al.*, (2006) and Ferruzzi and Blakesee, (2007) has provided evidence linking chlorophyll consumption to a decreased risk of colorectal, and its derivatives can exhibit health-promoting activities and these compounds have traditionally used in medicine due to its wound healing and anti-inflammatory properties. Total soluble protein content showed a gradually increase from their initial incubation day to 28<sup>th</sup> day and then decreased as nitrogen became limiting. It was *Tolypothrix rechingeri* followed by *Scytonema bohneri* which exhibit maximum amount of soluble protein content (95.76 $\mu\text{gml}^{-1}$  and 90.76 $\mu\text{gml}^{-1}$  respectively), followed by



*Scytonema ocellatum*, *S. hofman-bangii*, *S. hyalinum* and *Westiellopsis prolifica* ( $77.98\mu\text{gml}^{-1}$ ;  $76.90\mu\text{gml}^{-1}$ ;  $72.09\mu\text{gml}^{-1}$  and  $27.90\mu\text{gml}^{-1}$ ). Upon exposure to 40<sup>th</sup> day of incubation, the value of soluble protein showed slightly decreased by  $28.12\mu\text{gml}^{-1}$ ,  $67.23\mu\text{gml}^{-1}$ ,  $62.90\mu\text{gml}^{-1}$ ,  $82.12\text{gml}^{-1}$ ,  $91.12\mu\text{gml}^{-1}$  and  $17.23\mu\text{gml}^{-1}$  in four species of *Scytonema*, *Tolypothrix* and *Westiellopsis*. In *Tolypothrix* strain including four species of *Scytonema*, there were tendencies toward increased soluble protein content which could be due to the fact that they adapt to such habitats exposed to extreme temperature or deprivation of nutrients resulting in signaling the synthesis of new proteins which contribute to the survival of the organisms (Pandey, 2006). However, soluble protein content varied significantly with increase incubation time as well as amount content of chlorophyll. However, all of them could grow and synthesis the pigments and protein at a reduced rate than that of the control. The studies reported by Tonetto *et al.*, (2014) showed total lipid, protein, carbohydrate and gross fibres values for *L. majuscula*, *S. delicatula* and *Oedogonium* sp. *L. majuscula* displayed the highest values of protein and gross fibre content ( $264.73\text{ g Kg}^{-1}$  and  $194.40\text{ g Kg}^{-1}$ , respectively).

High protein content was registered for other cyanobacteria species (Gantar and Svircev, 2008). Some studies reported *Spirullina* and *Nostoc commune* contains highest amount of protein and fibre that make it an important component of human diet. *Scytonema* and *Tolypothrix*, although its lower protein content in comparison to *L. majuscula*, *Spirullina* and *Nostoc commune* isolated from stream system but it presented almost equal values of protein in ranges than those found for freshwater algae and marine red algae (Ciferri and Tiboni, 1985; Rajeshwari and Rajashekhar, 2011; Gressler *et al.*, 2011), which have been used in a variety of foods throughout the

world (Gantar and Svircev, 2008). Thus, *Tolypothrix* and *Scytonema* followed by other species examined could be more studied to become an option of fibres source to healthy food industry after proper scientific investigation. The changes in the pigment composition and soluble protein of all the six organisms with incubation duration followed almost a similar trend like that of their growth response. This result shows that the sub-aerial cyanobacteria have a great potential for economic application and deserve more attention.

Apart from chlorophyll, two major groups of secondary pigments (carotenoids and phycobiliproteins) were also examined in all six strains isolates. The study of Hertzberg *et al.*, (1971) reported that carotenoids composition of *Anabaena flos-aquae* and three other species of *Phormidium* showed the presence of  $\beta$ -carotene as the major carotenoids in all studied. Hence, in cyanobacteria the most abundant carotenoids are carotenes (e.g.,  $\beta$ -carotene), act in prevention of photo-oxidative damage (Nishiyama and Murata, 2014) and also protect against age related diseases (Takaichi, 2011).

Some studies reported a variety of carotenoids and their important commercial uses such as colouring agents in food industry, dietary supplements in the field of poultry and aquaculture, as well as for use in cosmetics due to non-toxic in nature (Enzing *et al.*, 2014). In present study, total carotenoids content was found maximum during 28<sup>th</sup> day of growth. *Tolypothrix rechingeri* ( $2.54\mu\text{g ml}^{-1}$ ) showed highest carotenoids content with comparison to other on 28<sup>th</sup> day followed by *Scytonema bohneri* ( $2.12\mu\text{gml}^{-1}$ ). The carotenoids production in the remaining four species examined, carotenoids content ranged from  $1.05 - 1.98\mu\text{gml}^{-1}$  respectively represented in Figure 2a-f.

**Table.1** Detail of cultural studies and their taxonomical characterization of the sub-aerial cyanobacteria isolates

Sl. No.	Major Organisms in Biofilms	Habitat with Geographical details	Taxonomical Studies	Voucher no.
1.	<i>Scytonema ocellatum</i> (Lyngbye) Bornet & Flahault [Synonym: <i>Scytonema cinereum</i> Meneghini 1837; <i>Scytonema murale</i> Zeller 1873]	Occurred as brownish crust on sandstone, Building facades, Bhubaneswar, Odisha Temperature: 21°C Latitude /Longitude: <b>20°25' 36.82<sup>0</sup> N 85° 48' 10<sup>0</sup> E</b>	Thallus expanded, greyish blue; filaments 10–16.5 µm broad, erect, false branched, less constricted at the cross walls; sheath firm; trichome 6–14 µm broad; apical cell rounded; heterocyst sub-quadrate or cylindrical to discoid, yellowish, 12–13.2 µm broad, 9.9–12 µm long.	BOT/ UAT - 82
2.	<i>Scytonema hofman-bangii</i> Agardh ex Silva [Synonym: <i>Scytonema hofmanni</i> C. Agardh ex Bornet and Flahault 1887]	Occurred as brownish crust on stone surface, Building Facades, Bhubaneswar, Odisha Temperature: 21°C Latitude /Longitude: <b>20° 25' 36.82" N 85° 48' 10" E</b>	Thallus olive to blue green; filament long, false branched, aggregated in vertical fascicles; cells unequal in length, 7.7 – 14.3 µm broad, 4.0 - 5.5 µm long.; heterocyst oblong.	BOT/UAT - 81
3.	<i>Scytonema hyalinum</i> Gardner 1927	Occurred as blackish mats on rock surface, Temple, Bhubaneswar, Odisha Temperature: 31°C Latitude /Longitude: <b>20° 15' 86.88" N 85° 84' 39" E</b>	Thallus blue-green; filaments densely entangled, cylindrical 10 - 15µm broad, 1.5 - 2.5 µm long, young branches usually constricted at cross walls; trichomes mostly 4.6 - 12µm wide; cells shorter than wide up to rarely isodiametric, cell at the apex slightly barrel shaped; sheath firm, thin and slightly lamellated; terminal cell yellowish; heterocyst intercalary, cylindrical or barrel shaped with rounded ends, yellowish, 8.5 - 12µm broad.	BOT/ UAT - 72
4.	<i>Scytomema bohneri</i> Schmidle	Occurred as greenish mats on mortar, Monument, Bhubaneswar, Odisha Temperature: 34°C Latitude /Longitude: <b>20° 28' 90.28" N 85° 81' 33" E</b>	Thallus blue-green; filaments long, 7.5 - 15 µm broad, 2.5 - 9 µm long, cells shorter than wide up to rarely isodiametric, cell at the apex slightly barrel shaped; binary false branching; sheath slightly thickened; heterocyst intercalary, cylindrical or barrel shaped, yellowish, 8.5 - 10 µm broad.	BOT/ UAT - 62
5.	<i>Tolypothrix rechingeri</i> (Wille) Geitler	Occurred as blackish crusts on Stone carving, Temple, Bhubaneswar and Puri, Odisha Temperature: 27.4°C Latitude /Longitude: <b>20° 24' 34" N 85° 84' 35" E</b> Puri, Odisha Temperature: 27.4°C Latitude /Longitude: <b>20° 24' 34" N 85° 84' 35" E</b>	Thallus brownish, wooly; filaments straight, sometimes curved, false branched; false branch short, many; cells barrel shaped, broader than long, 9.2- 16 µm broad, 6.9 – 9.2 µm long, trichome constricted at the cross walls; heterocyst both basal and intercalary, sub-spherical to barrel shaped, sub-spherical when board and barrel shaped when intercalary, 11.5 – 13.8µm broad, 4.6–11.5 µm long; sheath yellowish-brown, 2.3 – 4.6µm thick.	BOT/UAT - 07 BOT/UAT - 46 & 48
6.	<i>Westiellopsis prolifica</i> Janet	Occurred as greenish patch on cement wall surface and stone surface, Building facades and Temple, Bhubaneswar and Puri, Odisha Temperature: 31°C Latitude /Longitude: <b>20° 15' 86.88" N 85° 84' 39" E</b>	Thallus blue green; filament with prostrate and erect branches, lateral branch thinner than main branch, 6 - 7 µm broad, 9 - 12 µm long; cell spherical or elliptical 5 - 6 µm diameter; heterocyst intercalary, light yellow, rectangular, 4 - 6 µm broad, 8 - 10 µm long.	BOT/ UAT - 08 BOT/ UAT - 73

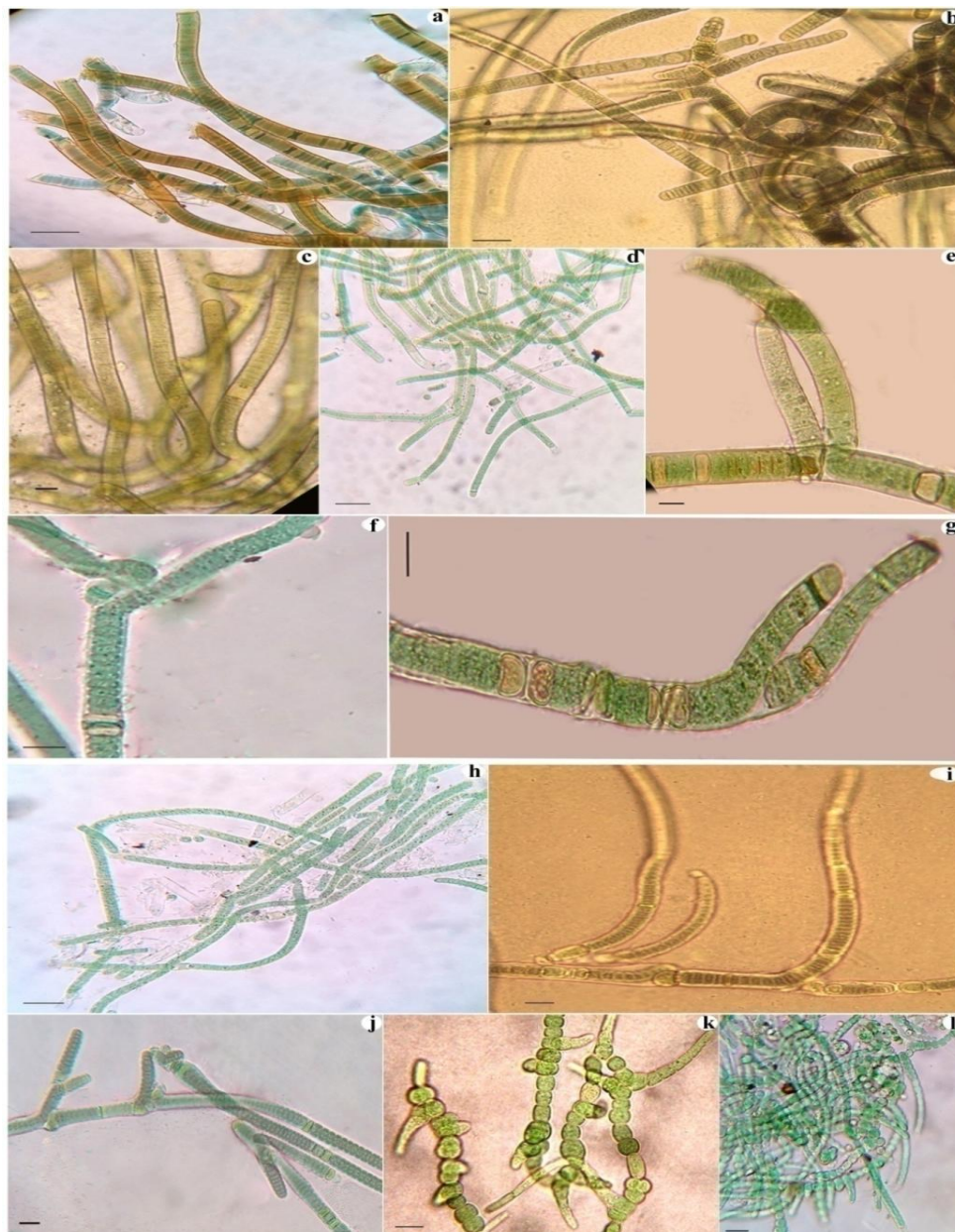
**Table.2** Clearly indicate the presence of phytochemical constituents present in the aqueous and five different extracts

Major Organisms in the Biofilms	Different Organic Extracts	Phytochemical constituents								
		Alkaloids	Phenolics	Flavonoid	Quinones	Glycosides	Terpenoids	Tannin	Steroids	Coumarins
1.	Aqueous									
	Hexane	+	+	+						
	Benzene	+	+	+	+					
	Chloroform	++	++	+		+				
	Acetone	++	++	+	+	+				
	Methanol	++	++	+						
2.	Aqueous									
	Hexane	+	+	+		+				
	Benzene	+	+	+	+	+				
	Chloroform	++	+	++						
	Acetone	++	++	++	++	+				
	Methanol	+++	++	+	++					
3.	Aqueous									
	Hexane	+	+	+						
	Benzene	+	+	+						
	Chloroform	++	++	+	+					
	Acetone	+++	++	++	+	+				
	Methanol	++	++	++	++					
4.	Aqueous									
	Hexane	+	+	++						
	Benzene	+	+	++						
	Chloroform	++	++	++						
	Acetone	+++	++	++	+					
	Methanol	+++	++	++	+					
5.	Aqueous									
	Hexane	+	+	++						
	Benzene	+	+	+						
	Chloroform	+	+++	++		+				
	Acetone	+++	++	++	+	+				
	Methanol	+++	+++	+++	+					
6.	Aqueous									
	Hexane	+	+	+						
	Benzene	+	+	+						
	Chloroform	+++	+++	+++						
	Acetone	+++	++	++	+					
	Methanol	+++	+++	+++						

Major Organisms in Biofilms: 1. *Scytonema ocellatum*, 2. *Scytonema hofman-bangii*, 3. *Scytonema hyalinum*, 4. *Scytonema bohneri*, 5. *Tolypothrix rechingeri* and 6. *Westiellopsis prolifica*.

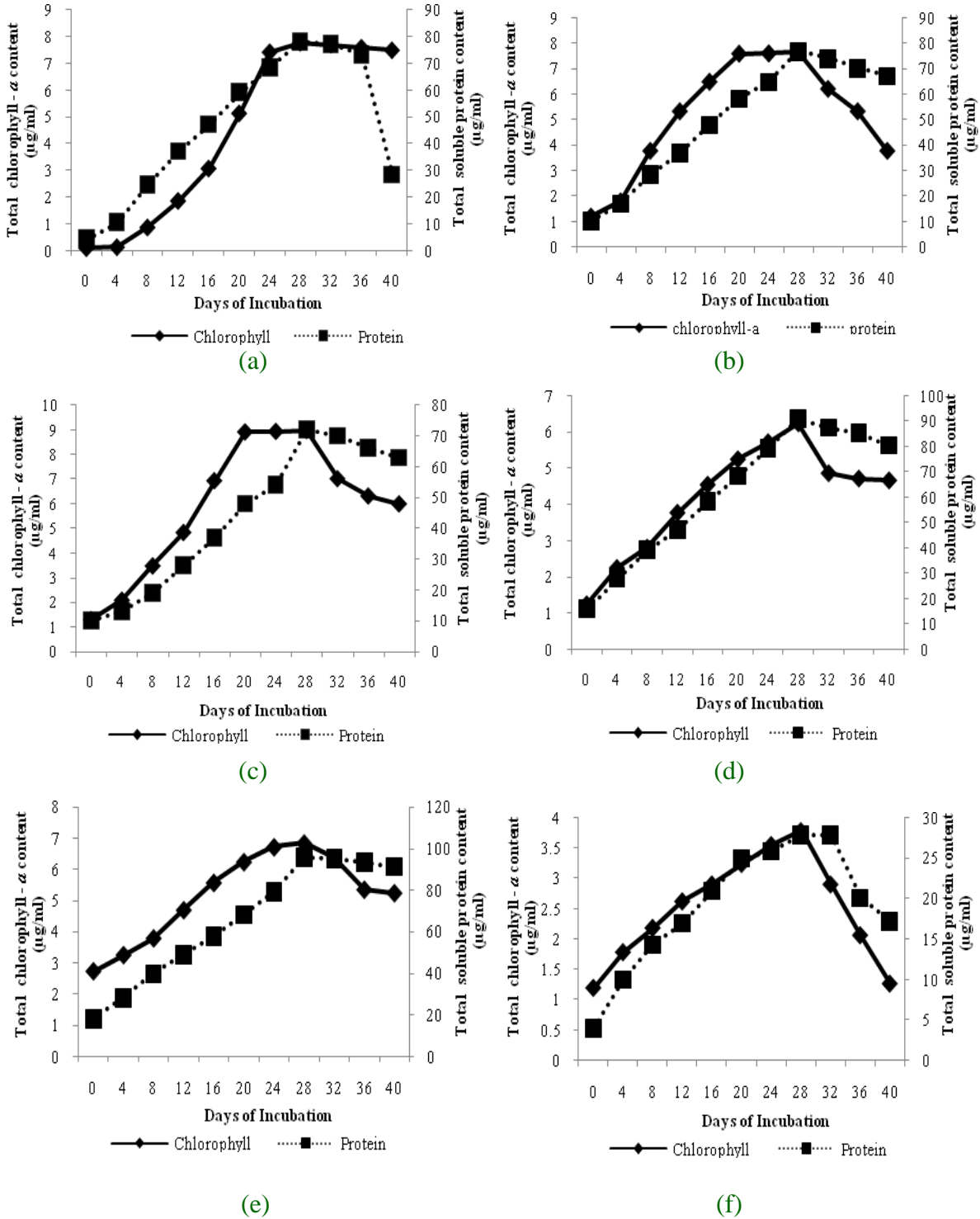
Keys: +++ = indicate positive response with highest concentration; ++ = indicate positive response with high concentration and + = indicates presence with low concentration.

**Fig.1** Major species of sub-aerial cyanobacteria isolated from the exterior surface of the ten different sub-aerial sites (building facades, temple and monuments) in Bhubaneswar and Puri, Odisha. Building facades: (a) within 72h of wetting the biofilm; (b) *Scytonema ocellatum*, which appeared in a culture of the same biofilm sample; Building facades: (c) within 72h of wetting the biofilm, (d) *Scytonema hofman-bangii*, which appeared in a culture of the same biofilm sample; Temple: (e) within 72h of wetting the biofilm; (f) *Scytonema hyalinum*, which appeared in a culture of the same biofilm sample. Monument: (g) within 72h of wetting the biofilm; (h) *Scytonema bohneri*, which appeared in a culture of the same biofilm sample. Temple: (i) within 72h of wetting the biofilm; (j) *Tolypothrix rechingeri*, which appeared in a culture of the same biofilm sample. Building facades and Temple: (k) within 72h of wetting the biofilm; and (l) *Westiellopsis prolifica*, which appeared in a culture of the same biofilm sample. Scale bars=20µm

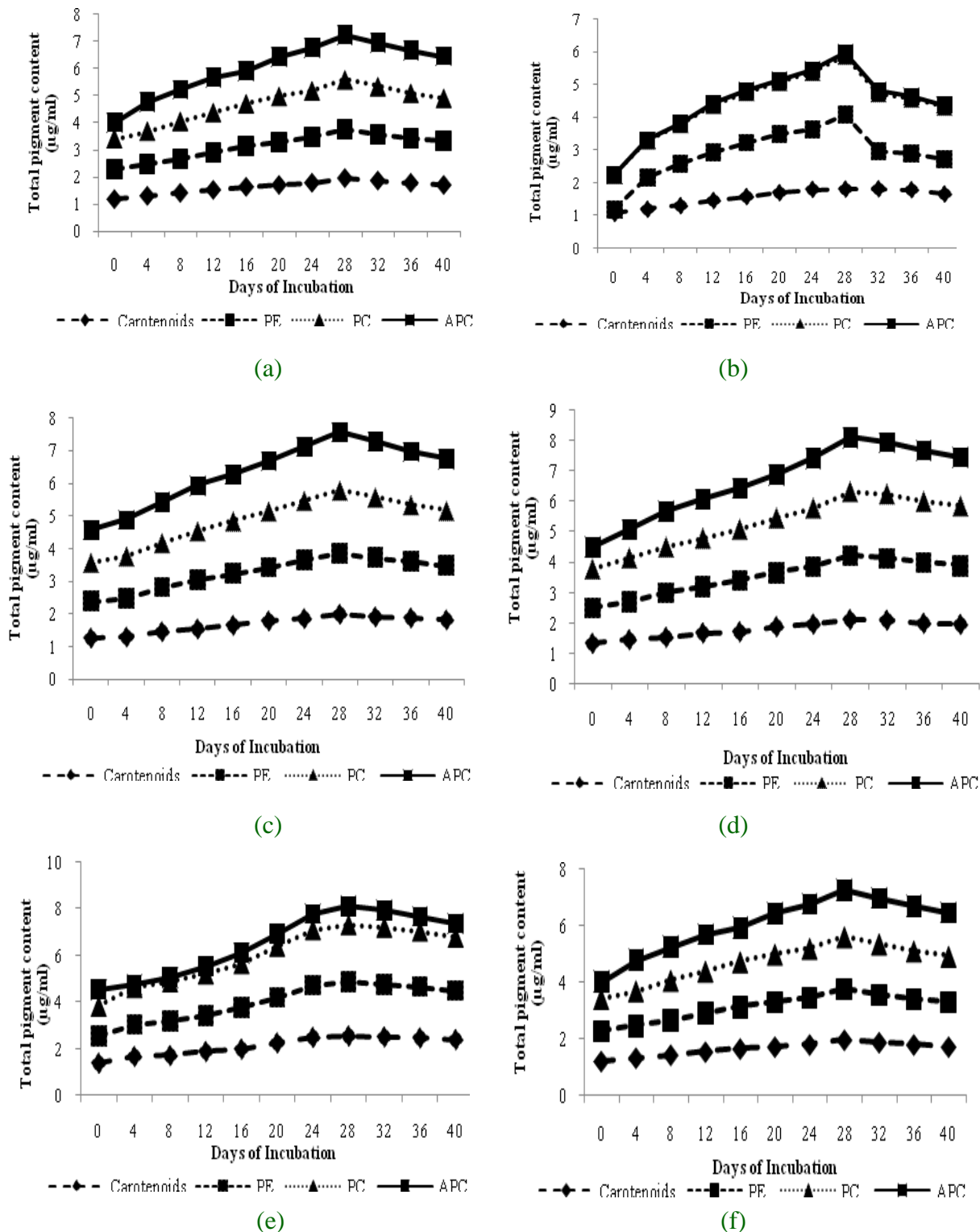




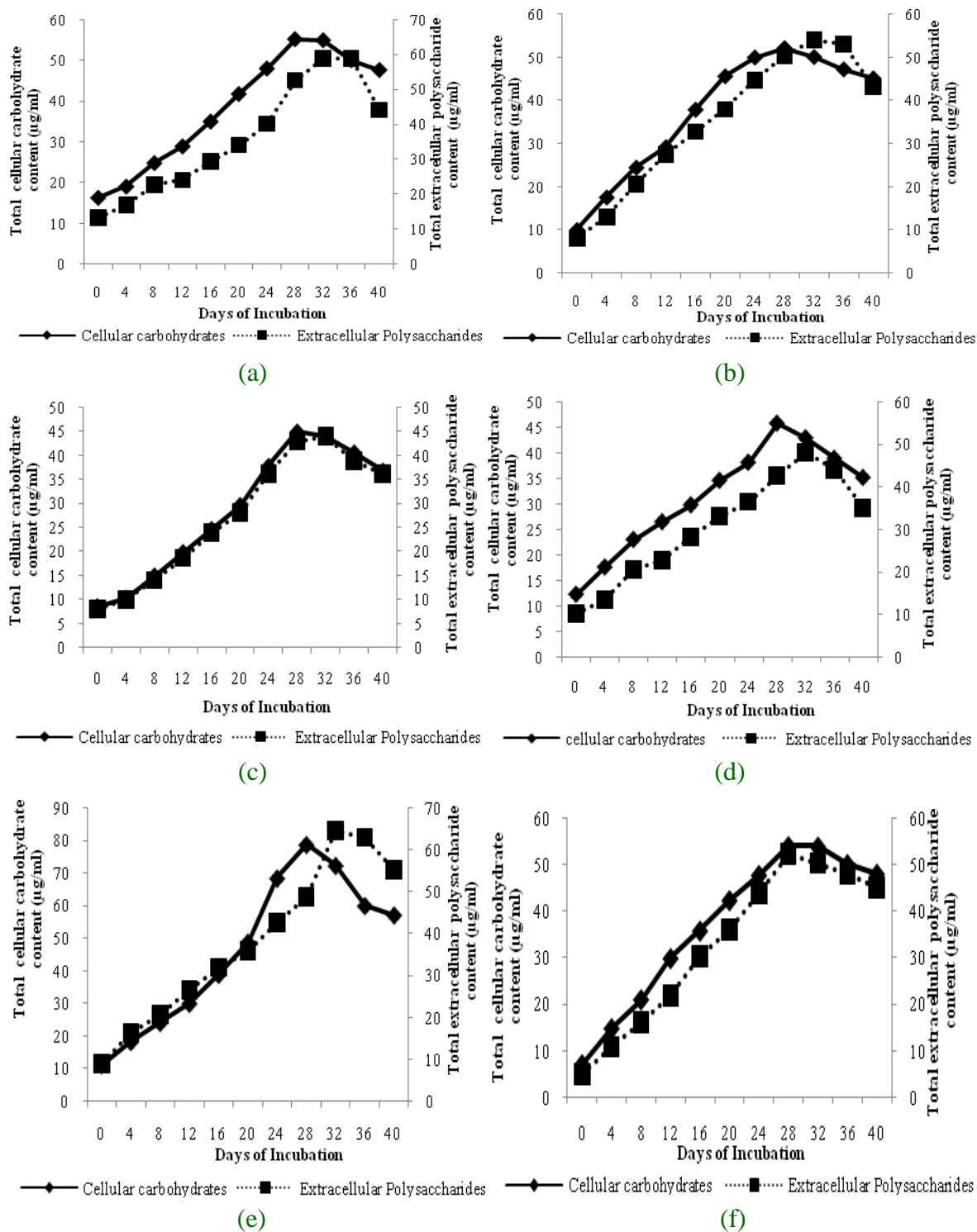
**Fig.2** Graph showing temporal variations of chlorophyll and soluble protein content in following sub-aerial Cyanobacteria species (a) *Scytonema ocellatum*; (b) *Scytonema hofman-bangii*; (c) *Scytonema hyalinum*; (d) *Scytonema bohneri*; (e) *Tolypothrix rechingeri*; and (f) *Westiellopsis prolifica*. Data are mean values  $\pm$  SD (n = 3)



**Fig.3** Graph showing temporal variations of carotenoids, phycoerythrin, phycocyanin and allophycocyanin content in following sub-aerial Cyanobacteria species (a) *Scytonema ocellatum*; (b) *Scytonema hofman-bangii*; (c) *Scytonema hyalinum*; (d) *Scytonema bohneri*; (e) *Tolypothrix rechingeri*; and (f) *Westiellopsis prolifica*. Data are mean values  $\pm$  SD (n = 3)



**Fig.4** Graph showing temporal variations of cellular carbohydrate and extracellular polysaccharide content in following sub-aerial Cyanobacteria species (a) *Scytonema ocellatum*; (b) *Scytonema hofman-bangii*; (c) *Scytonema hyalinum*; (d) *Scytonema bohneri*; (e) *Tolypothrix rechingeri*; and (f) *Westiellopsis prolifica*. Data are mean values  $\pm$  SD (n = 3)



Cyanobacteria in general possess phycobiliproteins, water soluble pigments that can be divided into three main groups according to their structures: PC, PE and APC (Abd El Baky and El Baroty, 2013). Among them, phycocyanin (PC) and phycoerythrin (PE) are commercially important over synthetic colour as they are environmental friendly, non-toxic and non-carcinogenic as recorded from extracts extracted from *Spirulina* sp., *Synechococcus* sp., *Oscillatoria quadripunctulata* and *Anabaena* (Borowitzka, 1994) etc. These natural photosynthetic pigments have already been commercialized as products in the food industry, as natural dyes, in fluorescence microscopy, cosmetics and in pharmaceuticals applications. In this view, present investigation were carried out to analyze the total phycobiliproteins including PC, APC and PE in six sub-aerial cyanobacteria strains isolated from different sub-aerial sites. The results of phycobilin analyses of investigated sub-aerial cyanobacteria showed total PBPs ranged from 2.28 – 5.28  $\mu\text{gml}^{-1}$  in *Scytonema ocellatum*, 1.16 – 4.15  $\mu\text{gml}^{-1}$  in *Scytonema hofman-bangii*, 3.29 – 5.58  $\mu\text{gml}^{-1}$  in *Scytonema hyalinum*, 3.15 – 5.99  $\mu\text{gml}^{-1}$  in *Scytonema bohneri*, 3.14 – 5.59  $\mu\text{gml}^{-1}$  in *Tolypothrix rechingeri* and 2.28 – 2.28  $\mu\text{gml}^{-1}$  in *Westiellopsis prolifica* respectively have been depicted in Figure 3(a – f). The major PBPs were found in *Scytonema* and *Tolypothrix* followed by *Westiellopsis*. Total six isolates, three species showed phycocyanin to be the most dominant phycobiliproteins with maximum value recorded in *Tolypothrix rechingeri* (2.46  $\mu\text{gml}^{-1}$ ), *Scytonema bohneri* (2.10  $\mu\text{gml}^{-1}$ ) and lowest in *Scytonema hofman-bangii*. On the contrary, *Scytonema hofman-bangii* recorded highest phycoerythrin (2.28  $\mu\text{gml}^{-1}$ ). Low contents of PC and APC was recorded in *Scytonema hofman-bangii*. The high content of APC content were found in *Scytonema bohneri* and *Westiellopsis prolifica*. The

species namely, *Tolypothrix rechingeri*, *Scytonema bohneri* and *Scytonema hyalinum* showed similar trends in increase in the contents of PC followed by APC and PE respectively. The study conducted by Patel *et al.*, (2005) showed that the contents of three PBPs on three species of cyanobacteria namely, *Spirulina* sp., *Phormidium* sp., and *Lyngbya* sp. Decreased in the order of PC, APC and PE. Pandey and Pandey (2013) reported the high content of PC followed by APC and PE in *Oscillatoria proboscidea*, *O. Limosa*, *O. Irrigua*, *Phormidium foveolarum*, *P. Tenue*, *Anabaena oryzae*, *Rivularia aquatica* and *Hapalosiphon* sp. This kind of similar variation in the individuals was observed in present study. In study of Sekar and Chandramohan (2008), PBPs have been described as the strong antioxidants, and PC and APC were reported for antiviral and antifungal activities. Several investigators showed anti-inflammatory activities of PBPs of phytoplankton (Abd El Baky and Baroty, 2013; Jensen, 2015). The results of this investigation revealed that sub-aerial Cyanobacteria are able to synthesis photosynthetic pigment which can be used in various biotechnological applications.

The total cellular carbohydrates content analysis showed that the highest content in *Tolypothrix rechingeri* (78.76  $\mu\text{gml}^{-1}$ ) on 28<sup>th</sup> day of incubation followed by *Scytonema ocellatum*, *Westiellopsis prolifica* and *Scytonema hofman-bangii* (55.23  $\mu\text{gml}^{-1}$ , 54.1  $\mu\text{gml}^{-1}$  and 52.12  $\mu\text{gml}^{-1}$  respectively) while the species *Scytonema bohneri* and *S. hyalinum* species exhibit low cellular carbohydrate content in comparison to control (45.76  $\mu\text{gml}^{-1}$  and 44.9  $\mu\text{gml}^{-1}$ ). However, released extracellular polysaccharides (EPS) in medium were recorded after 28<sup>th</sup> day. Extracellular polysaccharides (EPS) content was found maximum during 32<sup>th</sup> day growth. *Tolypothrix rechingeri* and *Scytonema ocellatum* (64.54  $\mu\text{gml}^{-1}$  and 58.98  $\mu\text{gml}^{-1}$ )



showed highest EPS content with comparison to other on 32th day of growth (Fig. 4b, c, d, f). Increased development of pigments are indicative of high photosynthetic activity and consequently, probable high accumulation of carbohydrate content in all the six organisms. During physical stress, microalgae tend to use available nutrients to preferentially produce carbohydrates instead of proteins (Rodolfi *et al.*, 2009). According to studies reported by Shah *et al.*, (2000), intracellular and extracellular carbohydrates of cyanobacteria can be used in food packing and metal removal industries.

### **Phytochemical characterization**

Phytochemical characterization of the six sub-aerial Cyanobacteria isolates were screened for its photochemistry constituents using aqueous and different organic solvents like hexane, benzene, chloroform, acetone and methanol based on polarity. The presence of phytochemical compounds such as alkaloids, glycosides, phenol, flavonoid, Quinones, terpenoids, tannin, steroids coumarins in the six different extracts of sub-aerial cyanobacteria is shown in following Table – 2. The results indicated that among all the six strains, the species which belong to genus *Scytonema* isolated from different substratum of building facades and monument showed the highest presence of alkaloids and phenolic contents as compared to other phytochemical constituents in hexane, benzene, chloroform, acetone and methanol extracts shown in Table – 2. The presence of alkaloids in the genus indicates that it can be commercialized particularly for pharmaceutical application and the phenolic compounds particularly the complex flavonoid are important class of antioxidants can be used for industrial application.

In general, cyanobacteria species investigated here displayed a potential use for humans as

other species has already demonstrated. However, more attention must be given for sub-aerial cyanobacteria, commonly found in extreme environment. Amongst the strains analyzed, *Tolypothrix rechingeri* (Wille) Geitler, *Scytonema ocellatum* (Lyngbya), *Scytonema bohneri* Gardner, *Scytonema hofman-bangii* Agardh ex Silva and *Scytonema hyalinum* showed higher production of the pigments, proteins, carbohydrate and EPS content but variation was observed from species to species. Thus, further exploratory studies should be done with sub-aerial cyanobacteria species through genetic approaches and mass cultivation technology to improve their potential for industrial and biotechnological applications towards human welfare. In addition, other investigations with more precise analyses of biochemical composition of them should also be made in order to create more detailed information and, thus, favour future applications.

The present study highlights the importance of combined morphological, biochemical and phytochemical characterization of the potent isolates from Bhubaneswar and Puri of Odisha. The species isolated in the present work was found to possess a higher phycocyanin content compared to other phycobiliproteins pigments. High soluble protein and cellular carbohydrate content found in these sub-aerial cyanobacteria contribute to the nutritional value of the species. The present findings indicate the positive response in case of alkaloids, phenolic and flavonoids in these isolates which possess immense antimicrobial activity for application towards pharmaceutical industry. However, few species is popularized in worldwide due to limitation in the economic production of cyanobacteria products in the industrial and pharmaceutical scale. Thus, intensive research is needed for the need of potent cyanobacteria from these

extreme habitats towards large production of the products with non-toxic from these isolates which could be used for further studies.

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