Investigation of the value-added potential of some selected freshwater cyanobacteria

Tharangika K. Bowange[1], Md. Fuad Hossain[1, 2, 3], Kavinda A. Wijesekera[1], K.L. Wasantha Kumara[2], R.R. Ratnayake[1, *]

[1] *National Institute of Fundamental Studies, Hantana Road, Kandy, Sri Lanka*

[2] *Department of Agricultural Biology, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya 81100, Sri Lanka*

[3] *Department of Biochemistry and Molecular Biology, Gono Bishwabidyalay, Savar, Dhaka, Bangladesh*

[*] *Corresponding Author: renuka.ra@nifs.ac.lk*

Abstract

Ever growing population and environmental degradation lead to a rapid deterioration of global health, causing malnutrition and Ultra Violet radiation-induced skin damages to be more prevalent. It is critical to address these health issues quickly and sustainably. Compared to natural botanicals, cyanobacteria could be more promising due to their superior photosynthetic capabilities, rapid growth, low space and simple nutrients requirements, low capital investment, and zero environmental pollution. Therefore, this study explores the value added potential of freshwater cyanobacteria in addressing the above health issues sustainably. Eight cyanobacteria strains, isolated from freshwater reservoirs in the dry zone of Sri Lanka were analyzed for total carbohydrate, protein, macro and micro minerals using Dubois' method, Lowry method, and Inductively Coupled Plasma Optical Emission Spectrometry. Mansur equation was applied to determine the Sun Protection Factor (SPF). Total carbohydrate and protein contents were in the range of 7.08 ± 0.32% - 53.08 ± 0.32% and 15.27 ± 0.90% - 49.77 ± 9.62%, respectively. Oscillatoriales had the highest total carbohydrate content (53.08 ± 0.32%), higher than the carbohydrate content of other previously reported *Oscillatoria* species. Calcium and iron were the most abundant macro and micro minerals, respectively. Oscillatoriales recorded the highest SPF (1.57 ± 0.002), whereas all the other strains had considerably greater or similar SPFs compared to other previously reported herbal extracts. Cyanobacteria with rich nutrition profiles and high SPF values may thus represent interesting alternatives for offering sustainable and ecofriendly solutions to significant health challenges associated with population growth.

Keywords: Cyanobacteria; food insecurity; global health; macronutrients; sun protection factor

1. Introduction

Uncontrollable population growth has caused the rapid reduction of arable lands thus limiting the crop-based food production (Elmi *et al*., 2019). Insufficient food production and uneven distribution of nutritious food create nutritional inequality among communities. Malnutrition has become a more prevalent, serious health issue especially among children and adolescents from the poorest and developing countries, causing frequent deaths, severe deficiencies and infections (UNICEF, 2019). For instance, micronutrient deficiencies of Iron, Iodine and Zinc are the most

prevalent, causing major public health issues in a considerable portion of the world population. However, this issue is comparatively unnoticed (Müller and Krawinkel, 2005).

Due to the rapid deteriorating pattern of global health and nutrition status (UNICEF, 2019), introducing dietary diversification through easily available, accessible, natural, low cost and nutritionally rich alternatives would be an important and immediate requirement. Most importantly their utilization should be sustainable, since, the unsustainable utilization would create many other irreversible environmental problems such as deforestation, water scarcity and ecological imbalance. Productivity limit reached by traditional crops due to seasonal limitations, specific growth requirements, climatic changes and unsustainable utilization of resources has made cyanobacteria more attractive as a natural alternative food source. This is evident by the usage of few certain cyanobacteria such as *Spirulina* spp., *Nostoc* sp. and *Anabaena* sp. as food and feed additives in countries such as Chile, Mexico, Peru, and Phillipines (Hoseini *et al*., 2013; Singh *et al*., 2016).

Rapid environmental degradation has also led to poor health status of global population. In the event of UV radiation, a rapid increase in undesirable skin problems, such as mutagenicity, accelerated skin aging, and photodermatoses, occurs (Dubey and Venkatesh, 2021; Mishra *et al*., 2011; Nohynek and Schaefer, 2001). Therefore, people are more conscious of skin protecting agents and sunscreens which are natural or synthetic chemicals with a variety of immunosuppressive effects of sunlight by absorbing and blocking UV rays (Nohynek and Schaefer, 2001). However, applications of synthetic sunscreens in cosmetic industry are limited, due to the known potential toxicity of certain synthetic sunscreens to humans (Chanchal and Saraf, 2009).

In contrast, natural sun protecting agents discourage skin carcinogenesis and are known to have higher level of safety over synthetic sunscreens. Among them, natural botanicals, including many plant extracts (Wagemaker *et al*., 2011) and lichens had been studied for photo protection (Radice *et al*., 2016). Active compounds extracted from microalgae also show a significant effect in preventing blemishes, repairing skin damages, inhibiting inflammation, accelerating healing process and maintaining skin moisture (Mourelle *et al*., 2017). *Chlorella*, *Spirulina*, *Nostoc* and *Nannochloropsis* are among the most studied species in cosmetics production (Mourelle *et al*., 2017). Therefore, identifying and introducing natural botanicals with efficient sun protecting properties together with beneficial mineral profiles, would be important in finding solutions for UV induced skin damages.

Higher photosynthetic ability, simple, rapid and ubiquitous growth, equal or better nutritional quality compared to some traditional crops, the ability to utilize the same space for regrowth and continuous production of quality harvest throughout the year with less capital investment and zero environmental pollution, make cyanobacteria more promising and sustainable to be used in food and cosmetics industry to ensure good health status in global population.

Diversity of cyanobacteria in tropics is remarkably high and tropical islands such as Sri Lanka provide habitats for a diverse collection of cyanobacteria (Hossain *et al*., 2020; Senanayake and Yatigammana, 2017; Wanigatunge *et al*., 2014) with an unexplored industrial potential, specifically in food and cosmetic industries. Therefore, the major objective of the study

was to evaluate the value-added potential of some selected Sri Lankan freshwater cyanobacteria in providing sustainable solutions for malnutrition and UV induced skin damage. We hypothesized that cyanobacteria could provide a sustainable and environmentally friendly solution to some of the most prevalent health issues such as malnutrition and UV radiation induced complications on skin in the global population.

2. Materials and Methods

2.1 Research Materials

Hydrochloric Acid (37%; Germany), Sulfuric Acid (95% - 97%; 30743; Germany), Nitric Acid (69%; extra pure AR grade; 30702; Germany), Phenol (GC; 16017; UK), Ammonium Ferrous Sulphate (99%; 215406; Japan), Ammonium Molybdate Tetrahydrate (A7302; USA), Folin–Ciocalteu reagent (F9552), Bovine Serum Albumin (A9056), Sodium Dodecyl Sulfate salt (L4509, GC; ≥ 98.5%) and Copper II Sulfate Pentahydrate (209198; ACS reagent; ≥ 98.0%) were purchased from Sigma Aldrich for media preparation and nutrient analysis. Sodium Carbonate (7541-4405), Potassium Sodium Tartrate Tetrahydrate (6618-4405), Citric Acid monohydrate (2562-4405), and Sodium Nitrate (7599-4405) were of extra pure quality and purchased from Daejung, Korea. D-Glucose (101174Y), Sodium Hydroxide (ACS reagent; 28244.262) used for standard preparation, media preparation and nutrient analysis were purchased from VWR BDH PROLABO, Belgium. Absolute ethanol (ACS reagent, 20821.321, France) was used for DNA extraction and SPF analysis. Magnesium Sulphate heptahydrate (A546586; Merck, Germany) was used for media preparation. The following reagents were prepared in distilled water for total protein analysis. Lysis buffer: (5 mLL^{-1} of TritonX-100 (437002A; EC), 0.3722 gL^{-1} of Ethylenediaminetetraacetic Acid Disodium salt (AR grade; 20301.186), and 0.0348 gL^{-1} of Phenyl Methyl Sulfonyl Fluoride (P-470-10, US)), 5% SDS solution: (0.05 gL^{-1} of Sodium Dodecyl Sulfate salt), Reagent A: (4.0 gL^{-1} of Sodium Hydroxide and 20.0 gL^{-1} of Sodium Carbonate), Reagent B1: (0.001 gL^{-1} of Copper II Sulfate pentahydrate), Reagent B2: (0.002 gL^{-1} of Potassium Sodium Tartrate tetrahydrate (6618-4405, Korea) and Reagent C: (100 mL of reagent A, 1 mL of reagent B1 and 1 mL of reagent B2 prepared just prior to use). Folin–Ciocalteu reagent (1:1 v/v Folin reagent/distilled water) prepared just prior to use. CTAB buffer: (100 mM of Tris.HCl [pH 8]; Promega Corporation, USA), 20 mM of Ethylenediaminetetraacetic Acid, 1.4 M Sodium Chloride (ACS reagent, 152575, MP Biomedicals, France) and 2% Cetyl Trimethyl Ammonium Bromide (Janssen Chimica, Belgium)) and TE buffer: (10 mM of Tris.HCl [pH 8] and 1 mM of Ethylenediaminetetraacetic Acid) were prepared for DNA extraction. Whatman No: 42 (ashless, 90 mm, 1442090) and syringe filters (cellulose acetate, hydrophilic, 0.45 µm and 25 mm diameter) were used for sample filtration in ICP-OES analysis. Risheng- RS-2800 ultra-quiet air oxygen pump (50 Hz; 2.5w; China) was used for aeration of cultures. Compound light microscope (Euromex BioBlue.Lab BB. 1153-PLi, Euromex Microscopen BV, Netherlands, equipped with Euromex DC.5000C CMEX microscope USB Camera) was used for microscopic imaging. Microcentrifuge (Ortoalresa Bioprocen 22R, Spain) was used for DNA extraction. Thermal cycler (Techne TC 3000 Thermal Cycler, USA) was used for PCR amplification and PCR clean up system PCR purification kit (Promega Corporation, USA) was used for PCR product purification. Water bath (YCW-010E, Germany) was used in DNA extraction and nutrient analysis. Agilent UV-Vis Cary 60 spectrophotometer (G68 60A; USA) was used to obtain

absorbance of colorimetric analyses. Inductively Coupled Plasma Optical Emission Spectrometry (ICPA 7000, Thermo Fisher Scientific) was used for macro and micro mineral analysis.

2.2 Isolation, purification and culturing of cyanobacteria isolates

Water samples were collected from the photic zone of freshwater reservoirs in the Dry zone of Sri Lanka (5° 54′ N - 9° 52′ N latitude and 79° 39′ E - 81° 53′ E longitude) (Hossain *et al*., 2020) using a Ruttner sampler. Ten mL of the sample which was filtered through 20 µm mesh size planktonic net was transferred into 100 mL conical flasks with 40 mL of BG11 medium with 7.5 pH (Table 1). The cultures were incubated at room temperature, under 2000 lux, on a shaker at 200 rpm. Once the initial bluish green color growth was observed, 100 µL of the sample was sub cultured into agar plates containing BG 11 medium solidified with 1.5% (w/v) bacteriological agar. To establish pure monocultures, isolated cyanobacteria in streak or spread plates were repeatedly sub cultured, and numerous microscopic examinations were performed. After isolation, monocultures were maintained in 250 mL Erlenmeyer flasks filled with 200 mL of BG-11 medium (Hossain *et al*., 2020).

Table 1. BG 11 media components, their concentrations to prepare stock solutions, and the volume required to prepare 1 L of the medium (Stanier *et al*., 1971).

Component	**Stock solution concentration (g/L)**	**Volume required to prepare 1L of BG11 (mL)**
$NaNO_3$	150 g/L	10 mL
K_2HPO_4	40 g/L	1 mL
$MgSO_4.7H_2O$	75 g/L	1 mL
$CaCl_2.2H_2O$	36 g/L	1 mL
Citric Acid	6 g/L	0.5 mL
Ferric Ammonium Citrate	6 g/L	0.5 mL
EDTA	1 g/L	1 mL
Na_2CO_3	20 g/L	1 mL
A5 Trace Metal Solution		
H_3BO_3	2.86 g/L	1 mL
$MnCl_2.4H_2O$	1.81 g/L	
$ZnSO_4.7H_2O$	0.222 g/L	
$Na_2MoO_4.2H_2O$	0.390 g/L	
$CuSO_4.5H_2O$	0.079 g/L	
$Co(NO_3)_2.6H_2O$	0.049 g/L	

2.3 Morphological characterization of the isolates

Microscopic images observed under the compound light microscope (Euromex BioBlue.Lab BB. 1153-PLi equipped with Euromex DC.5000C CMEX microscope USB Camera, Euromex Microscopen BV, Netherlands) were photographed using Image Focus 04 software.

Morphological characterization was done based on the morphological characteristics described by Desikachery (1959) and Mcgregor (2013 and 2018).

2.4 DNA extraction, 16S rRNA region amplification and DNA sequencing

DNA extraction of the selected cyanobacteria isolates was carried out using the method by Smoker and Barnum (1988) with slight modifications.

One milliliter aliquot of cyanobacteria culture was centrifuged at 2000 rpm for 5 min and the BG11 medium was decanted. The pellet was suspended in a 2 mL microcentrifuge tube with 700 µL of CTAB extraction buffer and incubated in a water bath at 60°C for 1 hour. The contents were mixed by inverting the tubes at regular intervals. Then 700 µL of chloroform/isoamylalcohol (24:1) solution was added to the tube and mixed for 2 min by repeatedly inverting the tube. The tube was centrifuged at a speed of 10000 rpm for 15 min. Next 300 µL of the aqueous supernatant was carefully transferred to a fresh 2 mL microcentrifuge tube. Total genomic DNA was precipitated by adding 600 µL of absolute ethanol followed by centrifugation at 10000 rpm for 15 min. Then the supernatant was decanted and the DNA pellet was washed with 70% ice cold ethanol. The tubes were centrifuged briefly in between washes to prevent the DNA pellet getting disturbed. Then the pellet was dried in the laminar flow chamber for 10 min, allowing any residual ethanol to evaporate. Finally, the DNA pellet was suspended in 50 µL of TE buffer and was stored at -20°C for further analysis (Figure 1).

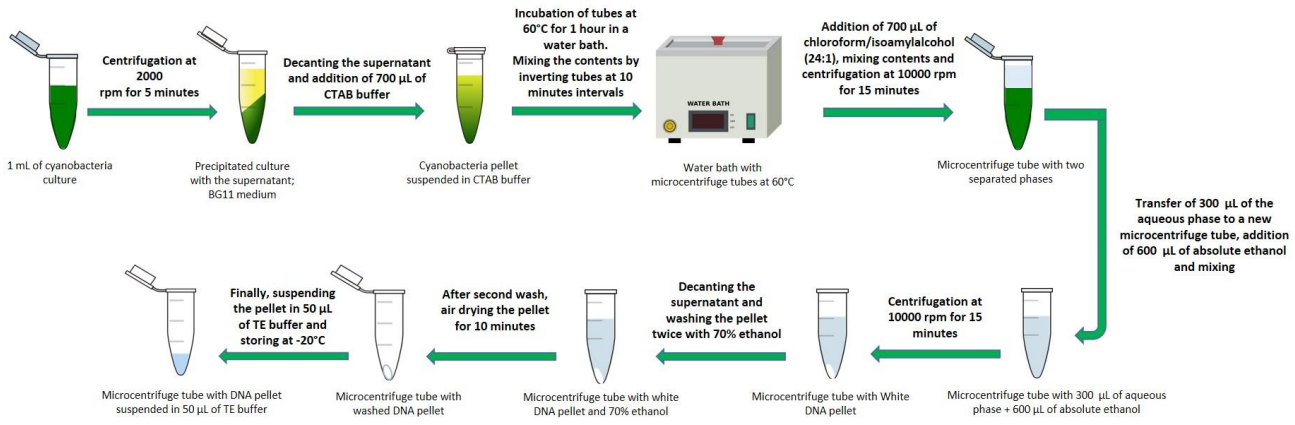

Fig. 1. Steps in DNA extraction of cyanobacteria isolates using the method described by Smoker and Barnum (1988) with slight modifications.

PCR amplification of the 16S rRNA gene region was carried out using two primer sets; CYA106F and CYA359F as forward primers and CYA781Ra and CYA781Rb as reverse primers (Hossain *et al*., 2020) (Table 2).

Table 2. Primers used in the PCR amplification and their information

Primer name	Primer sequence	Number of bases
CYA106F	5′-CGG ACG GGT GAG TAA CGC GTG A-3′	22
CYA781Ra	5′-GAC TAC TGG GGT ATC TAA TCC CAT T-3′	25
CYA359F	5′-GGG GAA TYT TCC GCA ATG GG-3′	20
CYA781Rb	5′-GAC TAC AGG GGT ATC TAA TCC CTT T-3′	25

The PCR amplification cycle of 16S rRNA region including an initial denaturation step of template DNA at 94°C for 5 min, 40 cycles of 94°C for 1 min, annealing step at 60°C for 1 min, elongation at 72°C for 1 min and the final elongation at 72°C for 15 min, was carried out in the Thermal Cycler (Techne TC 3000 Thermal Cycler, USA) (Hossain *et al*., 2020). Then PCR product purification was carried out using a Wizard SV gel and PCR clean up system PCR purification kit (Promega Corporation, USA) and DNA sequencing of the amplified PCR products for forward and reverse primers was carried out at Macrogen, South Korea using a ABI 3730XL DNA sequencer (Hossain *et al*., 2020). The strains were identified at the molecular level using BLAST software and the partial DNA sequences obtained were compared and multiple-aligned with the reference sequences from GenBank using Clustal Omega and the phylogenetic tree was constructed (Supplementary Figure 1). The information of the identified strains was then deposited in the GenBank through BankIt submission tool under the accession numbers: KX962076, KX962083, KX96208, KX962090, KX962091, KX962093, KX962095 and KX962098 (Table 3) (Hossain *et al*., 2020.)

2.5 Evaluation of value-added potential of cyanobacteria strains

Mass culturing of cyanobacteria was carried out in 1/5th strength of BG-11 (Table 1) at a pH of 7.5, in 100 L large fish tanks, under natural greenhouse light and temperature conditions and the system was agitated using aerators (Risheng- RS-2800 ultra-quiet air oxygen pump; 50 Hz; 2.5w; China) (Figure 2).

Fig. 2. Mass culturing of cyanobacteria strains under greenhouse conditions.

The fresh biomass was harvested in the 5th week after initial culturing and the biomass of filamentous strains was harvested using continuous filtration while unicellular strains were

harvested through centrifugation at 2000 rpm for 5 min at 27°C. The harvested fresh biomass was then oven dried at 50°C to obtain 10-15 g of dry biomass and the powdered dry biomass was stored in the freezer for further analysis.

2.5.1 Total carbohydrate content analysis

Twenty-five mg of dry biomass powder was hydrolyzed with 2.5 N HCl at 100°C for one hour. Then the samples were neutralized with sufficient amounts of Na_2CO_3 (7541-4405), filtered with Whatman No 42 (ashless, 90 mm, 1442090) filter papers and diluted in 25 mL volumetric flasks. One mL of the extract was analyzed by Dubois' method (Dubois *et al*., 1956). In brief, 5% Phenol (GC grade, Sigma Aldrich, UK) was added to each tube with 1 mL of the extract. Then 5 mL of Conc. H_2SO_4 (95-97%, ACS reagent, Sigma Aldrich, Germany) was added and the tubes with the content were vortex-mixed and kept for color development in a water bath (YCW-010E, Germany) at 25°C for 30 min. After 30 min, the absorbance of each sample was measured at 490 nm using UV spectrophotometer (Agilent UV-Vis Cary 60 spectrophotometer, G68 60A, USA). Total carbohydrate concentration (in mg/mL) was calculated based on the standard curve of D-Glucose (101174Y).

2.5.2. Total protein content analysis

Twenty mg of the dry biomass powder was lysed with 10 mL of lysis buffer and the lysate was centrifuged at 4800 rpm for 5 min. Then 0.5 mL of the supernatant was analyzed using Lowry method (Lowry *et al*., 1951). In brief, 0.5 mL of 5% SDS (L4509, GC; ≥ 98.5%) solution was added to 0.5 mL of the extract and the contents were mixed using a vortex. Then 5 mL of the Reagent C was added and the contents were mixed again. After 10 min, 0.5 mL of Folin reagent was added to each tube and the tubes were vortex-mixed again. The tubes were then kept for 30 min for color development and the absorbance was measured at 750 nm, using UV spectrophotometer (Agilent UV-Vis Cary 60 spectrophotometer, G68 60A, USA). Total protein concentration in mg/mL was calculated based on the standard curve of Bovine Serum Albumin (A9056).

2.5.3 Analysis of macro and micro minerals

Hundred mg of dried biomass powder was digested with 3 mL of 69% HNO_3 (extra pure AR grade; 30702; Germany) for 25 min. The digested volume was filtered using Whatman No 42 filter papers (ashless, 90 mm, 1442090) and diluted in 10 mL volumetric flask. The filtrate was filtered twice using 0.45 μm syringe filters (cellulose acetate, hydrophilic, and 25 mm diameter). Final filtrate was analyzed by Inductively Coupled Plasma Optical Emission Spectrometry (ICPA 7000, Thermo Fisher Scientific).

2.5.4 Determination of Sun Protection Factor (SPF)

Sun Protection Factor of the ethanol extracts of dry biomass was determined by the method described by Dutra *et al*. (2004). Cyanobacteria dry biomass powder (1000 mg) was transferred into a 100 mL volumetric flask, diluted with absolute ethanol (ACS reagent, 20821.321, France) followed by ultra-sonication for 5 min. The content was then filtered using Whatman No 42 filter

papers (ashless, 90 mm, 1442090). Five mL aliquot from the filtrate was diluted fifty times with absolute ethanol (ACS reagent, 20821.321, France) and the absorbance of the samples were obtained at 5 nm intervals in the range of 290-320 nm using a UV spectrophotometer (Agilent UV-Vis Cary 60 spectrophotometer, G68 60A, USA). Mansur equation (Kaur and Saraf, 2010; Mishra *et al.*, 2012) was applied to calculate the SPF value of the product as per Equation (1).

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda) \qquad (1)$$

Where;
CF = Correction Factor (10), EE (λ) = Erythmogenic Effect of radiation with wavelength λ, Abs (λ) = spectrophotometric absorbance values at wavelength λ. The values of EE x λ are constants.

2.6 Statistical Analysis

Three replicates were carried out per sample in each analysis. Results were statistically analyzed using One-way ANOVA in Minitab 17 (Hossain *et al*., 2016).

3. Results and Discussion

3.1 Morphological characterization of the isolates

As a basic step towards value addition of the isolates, their characterization was confirmed through a polyphasic approach. Observable morphological features of each isolate are described below.

Limnothrix sp. (Fig. 3a.) was green, having non-branching, thin, straight or slightly curved filaments without sheath or with very fine, colorless, facultative sheath. The filaments were solitary, free floating or arranged in separate bundles (Desikachery, 1959; Mcgregor, 2013 and 2018).

Croococcidiopsis sp. (Fig. 3b.) was unicellular; spherical cells, sometimes gathered in free-living irregular agglomerations or forming somewhat spherical or irregular colonies. Cells or small groups of cells are enveloped by thin, firm, colorless, sometimes slightly layered sheaths (Desikachery, 1959; Mcgregor, 2013 and 2018).

Calothrix sp. (Fig. 3c.) was simple, green heteropolar filaments and found as solitary or in small groups, separated from one another. Filaments were rarely with single, lateral, false branches. Cells were cylindrical or barrel-shaped. Basal cells were funnel shaped and wide, while the filaments were tapering. Sheaths were always present, usually firm, sometimes lamellated and yellow-brownish colored (Desikachery, 1959; Mcgregor, 2013 and 2018).

Limnothrix sp. (Fig. 3d.) showed similar morphological features as shown in Fig. 3a. However, these filaments were slightly curved, smaller and thin.

Geitlerinema sp. (Fig. 3e.) was observed as simple, bluish green, thin, straight or slightly wavy filaments with rounded or conical apical cells, while the filaments were arranged in bundles and showed slight gliding movements. Cells were longer than wide and sheaths were absent (Desikachery, 1959; Mcgregor, 2013 and 2018).

Oscillatoriales (Fig. 3f. and 3h.) were observed as green, simple, unbranched filaments (trichomes) with cylindrical cells. Heterocyst formation or false branching was not observed. Filaments were solitary or arranged in bundles.

Synechocystis sp. (Fig. 3g.) was green to yellowish green, unicellular, solitary, spherical or widely oval cells containing narrow, fine colorless mucilaginous envelopes (Mcgregor, 2013 and 2018).

However, identification of isolates based solely on classical morphological features would be impossible as some cyanobacteria can be detectable with unclear morphological features which are not distinguishable in microscopic observations (Komárek, 2016). Therefore, for the strains which cannot be identified with distinguishable morphological features, molecular characterization is essential. On the other hand, there are some strains which are detectable with clearly distinguishable morphological markers. Morphological characterization of these strains would be useful in more reliable identification (Komárek, 2016). Hence, a polyphasic approach where both morphological and molecular characterizations can be used together would be useful in more accurate identification of any strain.

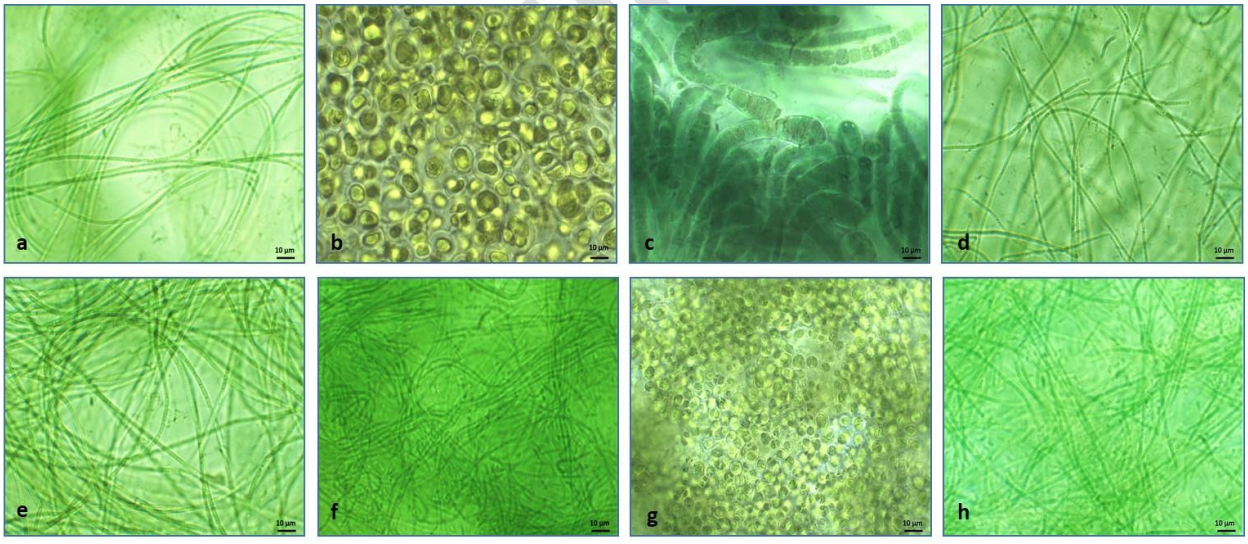

Fig. 3. Microscopic images of some purified strains obtained under oil immersion (1000x) a: *Limnothrix* sp. (U03), b: *Croococcidiopsis* sp. (U13), c: *Calothrix* sp. (U15), d: *Limnothrix* sp. (U33), e: *Geitlerinema* sp. (U36), f: Oscillatoriales (U40), g: *Synechocystis* sp. (U42) and h: Oscillatoriales (U55).

3.2 DNA extraction, 16S rRNA region amplification and DNA sequencing

All isolates were identified up to the genus or order level, based on the molecular characterization (Table 3).

Table 3. Molecular identification of the strains

Strain ID	Molecular identification	Query cover	Identity	Genbank accession no
U03	*Limnothrix* sp.KW3	100%	100%	KX962076
U13	*Chroococcidiopsis* sp.UW13	100%	91%	KX962083
U15	*Calothrix* sp.KK15	97%	94%	KX962084
U33	*Limnothrix* sp.UW33	100%	96%	KX962090
U36	*Geitlerinema* sp.KK36	100%	100%	KX962091
U40	Oscillatoriales.PK40	99%	92%	KX962093
U42	*Synechocystis* sp.MW42	99%	98%	KX962095
U55	Oscillatoriales.DL55	99%	92%	KX962098

Morphological characteristics, together with molecular characterization (Hossain *et al*., 2020) confirmed the identity of the isolates (Figure 3 and Table 3). Based on the molecular characterization, four strains (U03, U33, U36 and U42) showed ≥ 95% similarity with both query cover and identity, while other four strains (U13, U15, U40 and U55) showed ≥ 95% similarity with either query cover or identity (Hossain *et al*., 2020). The identity of the morphologically characterized strains was confirmed with the closest matching data through molecular characterization (Table 3).

3.3 Total carbohydrate content

In this study, the highest total carbohydrate content was recorded in Oscillatoriales (53.08 ± 0.32%) while the lowest content was in *Limnothrix* sp. (7.08 ± 0.32%) (Figure 4).

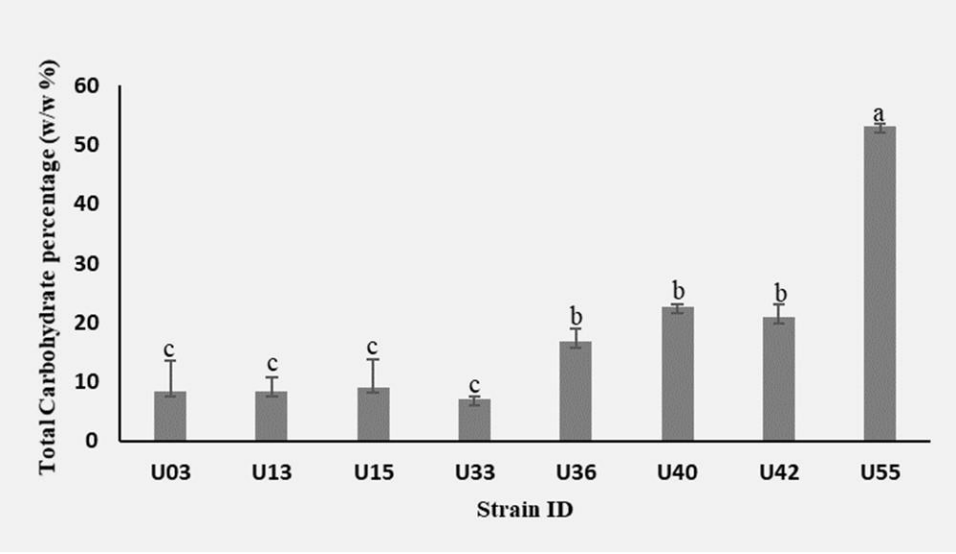

Fig. 4. Total carbohydrate content (% w/w) of eight selected strains namely; *Limnothrix* sp. (U03), *Croococcidiopsis* sp. (U13), *Calothrix* sp. (U15), *Limnothrix* sp. (U33), *Geitlerinema* sp. (U36), Oscillatoriales (U40), *Synechocystis* sp. (U42) and Oscillatoriales (U55). (a, b and c show the significant difference among mean values; One Way Analysis of Variance: Tukey pairwise comparison at $P< 0.05$ and 95% confidence level).

Results were comparable with some previous studies. Total carbohydrate content of two Oscillatoriales (U40 - 22.62 ± 0.32% and U55 - 53.08 ± 0.32%) were higher, compared to previously reported values of 12.05% in *O. formosa* and 16.23% in *O. salina* from Vethalai coastal regions, India (Kanimozhi *et al*., 2017). They were also significantly higher than *O. accuminata* (14%), *O. foreaui* (8%) and *O. calcuttensis* (9.6%) isolated from effluent waters (Rajeshwari and Rajashekhar, 2011). Carbohydrate contents reported for some cyanobacteria including *Anabaena cylindrica*, *Spirulina platensis*, *Spirulina maxima* and *Synechococcus* sp. were also within the range of 8% - 30% (Koyande *et al*., 2019) which were comparable for many recorded carbohydrate contents in this study. *Synechocystis* sp. (20.92 ± 1.2%) recorded in this study was higher than previously recorded *Synechocystis* sp. (9.8%) (Patel *et al*., 2018). The total carbohydrate content recorded in Oscillatoriales (53.08 ± 0.32%) in this study was significantly higher compared to all the previously reported species mentioned above.

Compared to eukaryotic algae, cyanobacteria are easily digestible and more acceptable for human consumption as cyanobacteria cell wall lacks polysaccharides such as cellulose, and other monosaccharaides such as xylose, and mannose (Richmond and Preiss 1980). Therefore, easily digestible cyanobacteria strains with higher carbohydrate contents would be one of the best, easily available food alternatives to fulfill the energy requirement of the malnourished communities.

3.4 Total protein content

The highest and the lowest total protein contents were recorded in *Limnothrix* sp. (49.77 ± 9.62%) and Oscillatoriales (15.27 ± 0.90%) respectively. Total protein contents of other strains were in the range of 19.3 ± 2.69% to 44.33 ± 1%. Out of 8 strains, five contained more than 30% of total protein contents (Figure 5).

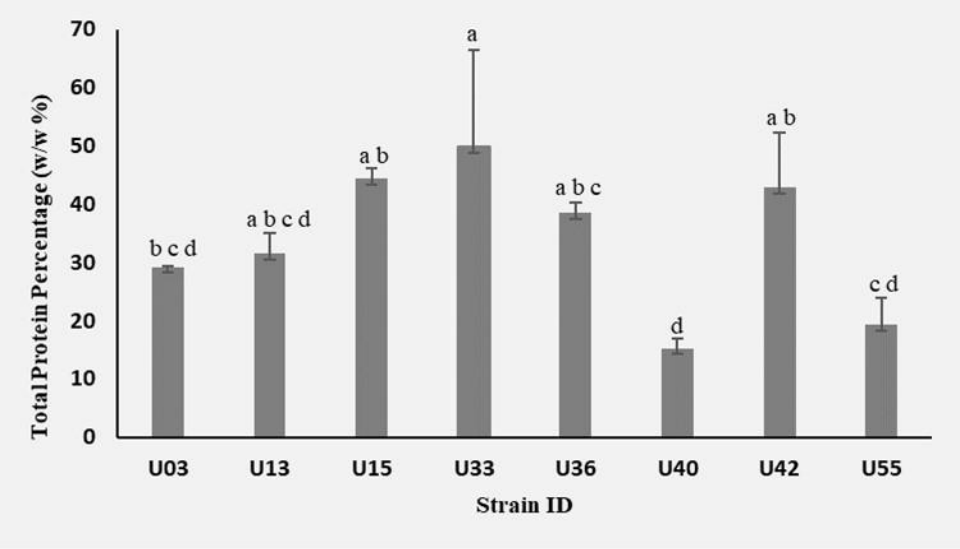

Fig. 5. Total protein content (% w/w) of eight selected strains namely; *Limnothrix* sp. (U03), *Croococcidiopsis* sp. (U13), *Calothrix* sp. (U15), *Limnothrix* sp. (U33), *Geitlerinema* sp. (U36), Oscillatoriales (U40), *Synechocystis* sp. (U42) and Oscillatoriales (U55). (a, b and c show the significant difference among mean values; One Way Analysis of Variance: Tukey pairwise comparison at $P < 0.05$ and 95% confidence level).

Five strains showed more than 30% of total protein content similar to the previously reported protein contents (30% - 55%) of the microalgae and cyanobacteria including *Synechocystis aquatilis* and *Arthrospira platensis* (López *et al*., 2010). However, the highest total protein content recorded for *Limnothrix* sp. (49.77 ± 9.62%) in the study was lower than that of *Spirulina platensis* (63%) previously recorded by Tokus¸oglu and Ünal (2006). The protein contents of the two studied *Limnothrix* sp.; (U03 - 29.23 ± 0.08% and U33 - 49.77 ± 9.62%) were significantly different. Though both of them were from freshwater reservoirs in the dry zone of Sri Lanka, they were isolated from two distant locations where the environmental conditions were considerably different. Thus, their physiology could be differently adapted to survive under the environmental conditions of their natural habitats. Variations of the environmental conditions of the two natural habitats of these *Limnothrix* sp. therefore could be a major reason for the significant difference of their total protein contents (Billi and Potts, 2000; Muhetaer *et al*., 2020).

Strains reported in this study with considerable amounts of total protein contents, highlight the potential for eradication of protein related malnutrition by introducing diverse dietary options.

There is an inverse relationship between the total carbohydrate content and the total protein content of the tested strains. Strains with higher total protein contents were recorded with lower total carbohydrate contents. This inverse relationship of higher total protein contents and low carbohydrate contents can be commonly observed in majority of microalgal species (Markou *et al*., 2012). However, the total protein and carbohydrate contents can be dependent on several

factors such as the nutrient availability in the growth medium, light intensity and temperature (Markou *et al*., 2012).

In this study, many tested strains with comparatively and significantly higher total protein and carbohydrate contents would be promising food alternatives to promote dietary diversification among poor communities. It would provide a sustainable, low cost and natural solution to global protein-energy malnutrition, ensuring nutrition equity. Therefore, crop based agriculture should be transformed by introducing modern agricultural practices where these nutrient alternatives are incorporated.

3.5 Macro and micro minerals contents

Cyanobacteria are rich sources of macro and micro minerals as they contribute to the formation of cyanobacterial internal cellular structures (Rajeshwari and Rajashekhar, 2011). Significantly high amounts of Ca in all strains highlight their suitability as a supplement of Ca. Ca of the tested strains ranged between 1145.33 ± 58.25 – 10456.00 ± 32.35 ppm and these contents were closely comparable with the recorded Ca contents in *Spirulina* (1300-14000 ppm), considered to be comparable to the amounts found in milk (Falquet and Hurni, 1997). Thus, tested cyanobacteria could be promising in fulfilling Ca mineral requirements with a similar potential of Ca sources such as milk.

Table 4. Macro and micro mineral profiles of the cyanobacteria strains

Strain ID	Cd [ppb]	Ni [ppb]	Zn [ppb]	Mn [ppb]	Cr [ppb]	Pb [ppb]	Co [ppb]	Cu [ppb]	As [ppb]	Fe [ppm]	Mg [ppm]	Ca [ppm]	K [ppm]	Sr [ppb]
U03	0.89 ± 0.09	167.61 ± 2.20	1587.06 ± 22.17	28529.31 ± 57.95	184.73 ± 3.49	31.24 ± 0.68	174.19 ± 4.84	894.45 ± 43.11	ND	104.82 ± 2.01	910.12 ± 7.54	4026.52 ± 2.91	474.23 ± 7.91	215.33 ± 8.84
U13	1.16 ± 0.01	18.24 ± 0.62	1481.67 ± 8.27	5580.98 ± 87.55	28.87 ± 2.74	1052 ± 0.85	125.55 ± 2.96	104.78 ± 3.62	5.47 ± 0.70	10.56 ± 0.03	52686 ± 4.64	4421.78 ± 28.34	57.11 ± 1.52	420.94 ± 9.32
U15	1.48 ± 0.02	23.87 ± 1.34	1106.03 ± 9.01	7995.11 ± 42.93	35.09 ± 11.94	10.41 ± 1.93	105.86 ± 0.31	110.77 ± 0.62	ND	5.93 ± 0.04	474.74 ± 1.15	3253.21 ± 51.57	53.44 ± 0.64	97.00 ± 0.22
U33	1.14 ± 0.05	81.13 ± 2.60	990.22 ± 3.22	674089 ± 474.59	503.13 ± 8.11	2357 ± 2.46	97.97 ± 6.44	400.43 ± 51.93	ND	20.05 ± 0.98	559.43 ± 5.69	4080.83 ± 78.48	27302 ± 18.71	57.38 ± 2.73
U36	0.74 ± 0.13	540.75 ± 16.16	1309.01 ± 1142	4281.91 ± 94.59	34.89 ± 2.31	12.42 ± 2.07	69.81 ± 1.56	115.11 ± 0.75	ND	9.53 ± 0.83	313.57 ± 2.41	2758.40 ± 6.10	67.29 ± 0.93	58.54 ± 3.35
U40	1.55 ± 0.27	106.95 ± 5.30	1775.08 ± 27.06	16325.27 ± 343.03	239.54 ± 17.67	24.42 ± 0.86	114.57 ± 0.36	352.12 ± 1.91	ND	45.25 ± 0.88	807.87 ± 5.90	506667 ± 45.32	246.56 ± 10.15	156.03 ± 5.83
U42	1.18 ± 0.08	30.04 ± 1.03	1645.97 ± 67.34	6578.31 ± 150.18	76.20 ± 0.88	23.01 ± 1.03	89.61 ± 1.26	159.45 ± 1.98	11.62 ± 0.73	20.93 ± 0.61	412.27 ± 1.91	1145.33 ± 58.25	62.92 ± 1.44	611.65 ± 6.83
U55	1.58 ± 0.01	31.51 ± 0.26	1442.17 ± 101.64	4464.89 ± 27.13	59.38 ± 1.30	17.59 ± 0.59	102.09 ± 1.00	114.52 ± 1.31	18.18 ± 0.14	19.31 ± 0.12	348.65 ± 1.55	10456.00 ± 32.35	44.11 ± 0.19	461.14 ± 10.53

(Concentration (mean concentration ± standard error of mean); Concentrations are given in ppb and ppm; ND: Not Detectable)

Iron deficiencies are common and widely spread in all communities, particularly among children and pregnant women (Müller and Krawinkel, 2005). However, iron rich food sources are rare. Average iron content in cereals and grains is within the range of 25 - 80 ppm (Moreira *et al*., 2013). However, the bioavailability of iron in some cereals could be limited while iron supplements may sometimes cause toxic effects (Johnson and Shubert, 1986). In this study, iron showed the highest amounts, followed by Mn, Zn, Cu, Co and Ni, respectively, among all micro minerals (Table 4). Comparatively, the highest iron content recorded was 104.82 ± 2.01 ppm in *Limnothrix* sp. and the iron content of five more cyanobacteria strains was within the range of

10.56 ± 0.03 – 45.25 ± 0.88 ppm range. Thus, tested cyanobacteria strains could be far more promising representative sources of iron (Table 4).

Sufficient amount of Zinc is essential for the activity of many enzymes and its deficiency can cause major health issues including pneumonia. Zinc content of tested strains ranged between 0.990 ± 3.22 ppm - 1.775 ± 27.06 ppm (Table 4) and these results were comparable with the recorded zinc contents in commercialized *Spirulina* products (0.533-6.255 ppm), considered as safe food (Al- Dhabi, 2013). Out of 25 *Spirulina* products, 24 showed >1.200 ppm of Zn contents which was comparable to this study where 6 out of 8 strains showed >1.200 ppm of Zn contents (Table 4). Furthermore, these amounts are below the recommended daily intake of heavy metal elements for Zn (13 mg/daily) (Iyengar, 1985) suggesting higher potential of improving these strains into alternative sources of Zn for safe consumption. According to Table 4, *Limnothrix* sp. had the highest Mg (910.12 ± 7.54 ppm), K (474.23 ± 7.91 ppm), Fe (104.82 ± 2.01 ppm), Cu (894.45 ± 43.11 ppb) and Mn (28529.31 ± 57.95 ppb) contents, whereas Ni (540.75 ± 16.16 ppb) was the highest in *Geitlerinema* sp. The presence of greater amounts of many minerals in *Limnothrix* sp. nominates itself as the most promising alternative source of essential macro and micro elements. Thus, few grams of these mineral rich cyanobacteria biomass could be easily sufficient to meet the recommended daily intakes of major macro and micro minerals for both adults and children. Cd, Cr, Pb, As, Co and Sr quantities were below the accepted maximum level recommended by the World Health Organization, eliminating all risk levels of their utilization (Table 4). These recommendations for developing cyanobacteria as a commercial product will improve global public health and economy.

3.6 Sun Protection Factor

The mean SPF values of the strains are shown in Figure 6.

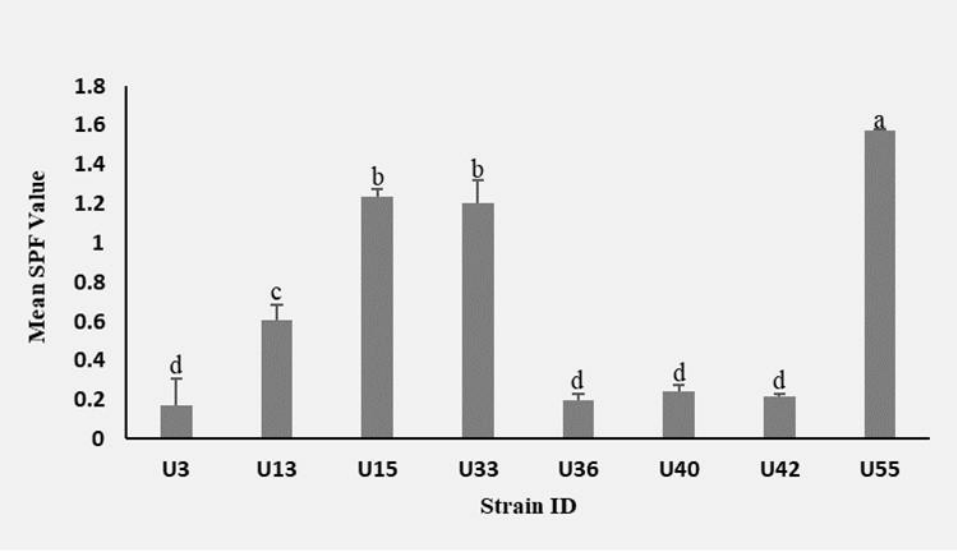

Fig. 6. Mean Sun Protection Factor (SPF) of eight selected strains namely; *Limnothrix* sp. (U03), *Croococcidiopsis* sp. (U13), *Calothrix* sp. (U15), *Limnothrix* sp. (U33), *Geitlerinema* sp. (U36), Oscillatoriales (U40), *Synechocystis* sp. (U42) and Oscillatoriales (U55). (a, b and c show the

significant difference among mean values; One Way Analysis of Variance: Tukey pairwise comparison at $P < 0.05$ and 95% confidence level).

The mean SPF of the cyanobacteria biomass ranged between 0.17 ± 0.091 in *Limnothrix* sp. (U03) and 1.57 ± 0.002 in Oscillatoriales (U55). There was no significant difference among the mean SPF of *Limnothrix* sp. (U03), *Geitlerinema* sp. (U36), Oscillatoriales (U40), and *Synechocystis* sp. (U42) all of which showed comparatively very low mean SPF. SPF of *Calothrix* sp. (U15) and *Limnothrix* sp. (U33) were comparatively higher with no significant difference between them.

Compared to previous studies, results of this study suggest that cyanobacteria could be more effective over many plant extracts (Table 5).

Table 5. Comparison of Sun Protection Factor of different plant extracts and tested cyanobacteria strains

Cyanobacteria strain/ Plant species	Sun Protection Factor	Reference
Limnothrix sp. (U03)	0.17 ± 0.091	
Chroococcidiopsis sp. (U13)	0.61 ± 0.057	
Calothrix sp. (U15)	1.24 ± 0.026	
Limnothrix sp. (U33)	1.21 ± 0.078	The present study
Geitlerinema sp. (U36)	0.20 ± 0.024	
Oscillatoriales (U40)	0.24 ± 0.021	
Synechocystis sp. (U42)	0.22 ± 0.007	
Oscillatoriales (U55)	1.57 ± 0.002	
Oil extracts of coffee beans of;		
C. canephora	0.35	
C. congensis	1.08	Wagemaker *et al*., 2011
C. kapakata	0.06	
C. arabica	1.50	
C. racemosa	1.59	
C. liberica var. dewevrei 'Abeokutae'	0.42	
C. liberica var. dewevrei 'Excelsa'	0.88	
C. liberica var. liberica	0.48	
C. liberica var. liberica 'Passipagore'	0.29	
Aqueous herbal extracts of;		
Aloe vera	1.28	Malsawmtluangi *et al*., 2013
Carrot	1.34	
Coconut	7.38	
Cucumber	1.45	
Watermelon	0.97	

The highest SPF reported in Oscillatoriales (U55) was comparatively higher than many previously reported coffee species namely, *C. canephora* (0.35), *C. congensis* (1.08), *C. kapakata* (0.06) and four varieties of *C. liberica* (Wagemaker *et al*., 2011). However, the most cultivated coffee species around the world, *C. arabica* (1.50) bearing many important cosmetic properties, and *C. racemosa* (1.59) (Wagemaker *et al*., 2011) (Table 5) showed similar SPF values as Oscillatoriales (1.57 ± 0.002).

The SPF reported for seven herbal extracts from aloe vera, carrot, coconut, cucumber, papaya, strawberry, and watermelon by Malsawmtluangi *et al*. (2013) ranged between 0.97 and 7.38. Oscillatoriales (U55) showed a higher SPF (1.57 ± 0.002) than aloe vera (1.28), carrot (1.34), cucumber (1.45) and watermelon (0.97) (Malsawmtluangi *et al*., 2013), highlighting its effectiveness in sun protection over many previously reported herbal extracts. As seen in Table 5, *Calothrix* sp. (1.24 ± 0.026) and *Limnothrix* sp. (1.21 ± 0.078) had similar SPF values as aloe vera (1.28) (Malsawmtluangi *et al*., 2013). In the cosmetics industry, the use of natural botanical ingredients is well-known to be safe and has gained widespread customer acceptability. Many studies have proved that different extracts, vitamins (Schaeffer and Krylov, 2000) and secondary metabolites (Priyadarshani and Rath, 2012) isolated from cyanobacteria have effective sun screening properties. This study further reports many freshwater cyanobacteria strains with higher or similar UV filtering properties compared to many other plant extracts. Thus, cyanobacteria could be more promising as better natural botanical alternatives with effective UV screening properties to provide effective solutions to health issues emerged with environmental destructions such as UV induced skin damages.

4. Conclusion

Rapid deterioration of global health with increased malnutrition and UV induced skin mutagenesis needs to be addressed immediately with natural, sustainable alternatives such as cyanobacteria, for which everyone has easy access. With the aim of evaluating the potential of cyanobacteria in providing sustainable solutions, especially for malnutrition and UV induced skin mutations, selected freshwater cyanobacteria strains from freshwater reservoirs in the tropical Asian region, Sri Lanka, were analyzed for macro nutrients, mineral profiles and sun screening properties. Nutrient profiles and SPF values reported for the selected cyanobacteria were higher compared to nutrient profiles and SPF values of many previously reported strains and herbal/plant extracts, emphasizing potential applicability in food and cosmetic industries. Thus, they can be used as potential protective resources for UV induced skin problems and can be improved to be used as nutrient supplements to address the malnutrition developed through macro and micro nutrient deficiencies. Their commercially beneficial characteristics over plant material, such as higher photosynthetic ability, rapid growth, requirement of lesser area and simple nutrients for the growth and higher production with less capital investment and zero environmental pollution, make cyanobacteria more promising in providing sustainable and ecofriendly solutions to major health issues arisen with population growth.

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Running Title: Cyanobacteria for a healthy globe.

Supplemetary documents

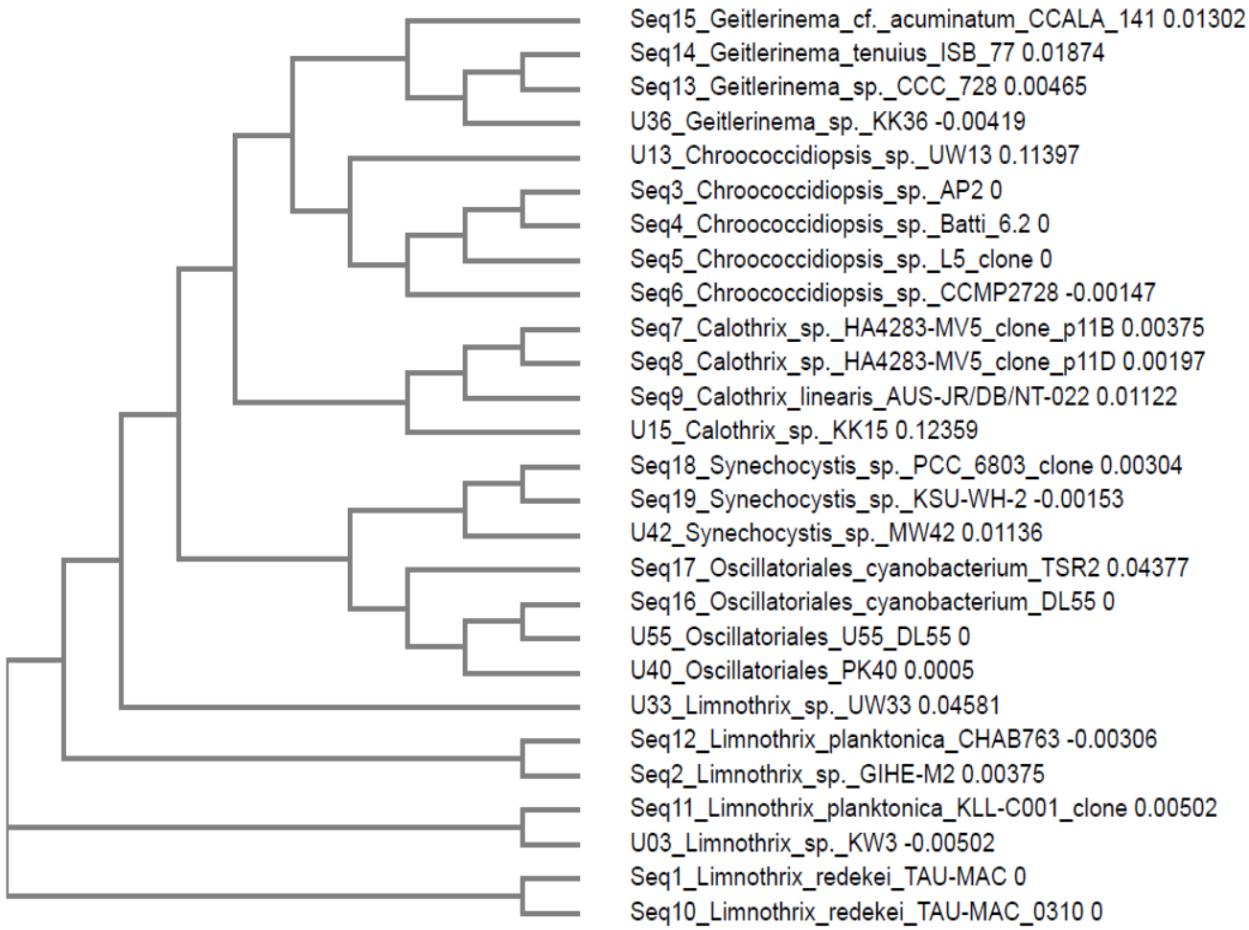

Fig. 1. Phylogenetic tree constructed using Clustal Omega, showing the relationship between the of 16S rRNA gene sequences of the isolates obtained from this study (denoted by 'U') and the closely related reference sequences (denoted by 'Seq') obtained from the National Center for Biotechnology Information (NCBI) database.