Biochemical composition, nutraceutical profile, and GC-MS analysis of novel euryhaline microalgal cultures from Punjab, India.

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Biochemical composition, nutraceutical profile, and GC-MS analysis of novel euryhaline microalgal cultures from Punjab, India

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(a) Abstract

Microalgae have gained recognition in the scientific community as a potential source of nutraceuticals because it is a sustainable reservoir having the capability to substitute the industrial production of ordinary organic chemical based health supplements. The biomass of microalgae incorporates various functional biomolecules like chlorophyll, carotenoids and phenols that exhibit therapeutic properties and serve as nutraceuticals. In spite of the higher production of the microalgal biomass, their biochemical composition differs, and lack of characterization for nutraceutical properties are the prime hindrance in upscaling these bio-factories. Nutraceuticals are nutrient components of the food that not only exhibit many health benefits but also prevent the occurrence of various health disorders. Two euryhaline microalgal strains BGLR8 and BGLR16, isolated from water logged areas of Punjab, India were screened for biomass production and biochemically characterized for the nutraceutical values. Results indicated that BGLR8 produced maximum amount of nutraceutical compounds such as lipid (86 mg/g), chlorophyll (29.42 mg/g), carotenoid (28.82 mg/g), phenols (4.46 mg/g), phycocyanin (52 mg/g), astaxanthin (19.27 mg/g) and β-carotene (5.6 mg/g) and anti-oxidant activity (31.73%). The results of Gas Chromatography-Mass Spectrometry (GC-MS) revealed the presence of 8 therapeutic compounds *viz.* Dimethyl (*E*)-but-2-enedioate, Hexasilacyclododecane, Heptasilacyclotetradecane, Methyl (*Z*)-pentadec-8-enoate, Methyl octadec-13-enoate, Methyl hexadecanoate, Methyl octadecanoate, Methyl-octadeca-9,12-dienoate in BGLR8. Molecular identification through 18S rRNA gene sequencing confirmed BGLR8 to be a member of *Coelastrella*. (GenBank accession no.- MW443083.1).

(b) Keywords: Microalgae, nutraceutical, β-carotene, total phenol, phycocyanin.

(c) Introduction

Owing to the economic inequality and nutritional inadequacy, a large section of the global population is facing malnutrition that needs to be addressed *via* a multi-dimensional strategy through nutritional awareness, knowledge, self-efficacy, food fortification and supplementation. Novel and innovative ways to fulfill the nutritional demands have received increased attention in current times. Currently, there has been an emphasis on searching for medicinally and nutritionally novel functional foods known as nutraceuticals, which are derived from natural sources. Nutraceuticals are gaining massive popularity in the food industry as they can serve as a cost-effective nutritional source that can also provide therapeutic benefits and fulfill the global nutritional requirements. Using microalgal biomass for nutraceutical production is deemed suitable due to high yield and simultaneous elimination of the competition with food production [1].

Various microalgal pigments (like chlorophyll and carotenoids) are endowed with cardioprotective, anti-mutagenic, anticarcinogenic, anti-inflammatory and anti-oxidant properties. *Chlorella*, *Chlamydomonas, Dunaliella, Micractinium*, *Oscillatoria*, *Spirulina* and *Scenedesmus*

are indeed protein-rich that comprises most of the essential amino acids needed by heterotrophs, as well as being a substantial source of chlorophyll. *Chlorella vulgaris* possess a lipid content of up to 50%, thereby implying a valuable source of lipids. Extracts of *Chlorella* have exhibited the qualities that are anti-tumor and antioxidant activities. It can also regulate blood pressure and cholesterol levels, speeds tissue repair, boosts immunity and helps to prevent hypertension, malnutrition, gastric ulcers, constipation, and diabetes mellitus [1]. *Spirulina* is a good source of active oleic acid and other fatty acids like docosahexanoic acid (DHA), and c-linolenic acid. As a result, it produces prostaglandins, thromboxans and leukotrienes, which are involved in the control of immunological, inflammatory and coronary heart disease. Owing to its poor purine level, it is among the few palatable microorganisms that reduces the danger of uric acid build-up inside the body [2]. *Spirulina* sp. has also been shown to have beneficial benefits on weight reduction, hypertension, diabetes, as well as antiviral, antibacterial, anti-inflammatory, cancer-fighting, and antioxidant characteristics [3]. Because of these benefits, it is now used as nutritional supplements as tablet or powder, either alone or in combination with plant extracts, for human and livestock usage [4].

This study is the first to re-affirm that the euryhaline microalgae cultures isolated by Dar [5] can be a reliable source of carbohydrates, proteins, lipids, chlorophyll, β-carotene, astaxanthin, phycocyanin, phenols and flavonoids. Although multiple reports have emerged lately, regarding the nutraceutical potential of freshwater algal species, similar studies involving euryhaline microalgae strains are still scarce. Euryhaline cultures can adapt to diverse environmental conditions by synthesizing secondary metabolites. The goal of this study was to find out more about the nutraceutical prospect of the two euryhaline microalgae cultures, BGLR8 and BGLR16, sequestered from the flooded parts of Punjab, India, by using some innovative methodologies, providing for a comparative evaluation. This involved determining the biomass, carbohydrates, lipid, protein, chlorophyll, carotenoids, phenols, astaxanthin, phycocyanin, β-carotene and anti-oxidant activity, opening the way for future nutraceutical importance unique to each microalgal species. This work proposes a novel strategy for using the hitherto underutilised euryhaline microalgal biomass for innovative nutraceutical/functional food applications.

(d) Material and methods

Microalgae production and maintenance

The microalgal cultures BGLR8 and BGLR16 were previously sequestered from flooded parts of Punjab, India by Dar [5]. Sample collection was done from area at outskirts of Sri Muktsar Sahib, Punjab (30°32'47.0"N 74°40'00.0"E). The two cultures were microscopically examined for their purity (Olympus, 528293 Magnus Icon Freedom Model). The microalgal cultures BGLR8 and

BGLR16 were enriched in Bold's basal medium [6] and BG-11 medium [7] respectively. Cultivation of microalgae was carried out in a 2 l flask by adding 1 l medium and 10% inoculum. Each microalgal culture was incubated at 25±2°C and exposed to a light intensity of 8000 lux. After 30 days of growth, cultures were retained as stock cultures in low light conditions and maintained at room temperature. The stock cultures were maintained by sub-culturing into fresh culture broth at least bimonthly.

Screening of the microalgal isolates by estimation of dry cell biomass

The BGLR8 and BGLR16 microalgal cultures were screened for the biomass production. 1 l of broth was dispensed in Erlenmeyer flasks of 2 l capacity (in triplicate) and each flask was inoculated with 10% of fresh microalgal culture. The cultures were incubated for 35 days under the standard laboratory conditions (25±2°C, 8000 lux, and 12:12 h light: dark photoperiod). Centrifugation of the cells for 10 minutes was performed at 10,000 rpm, and the obtained biomass pellet was stored overnight in a hot air oven at 60°C. The dried cell pellet was weighed to estimate dry cell biomass produced from each culture.

Analysis of Biochemical composition

Estimation of carbohydrates

The total carbohydrate amount of microalgae was estimated by DuBois et al [8] method. Ten ml of 70% ethanol was added in 1 ml of microalgal culture to prepare the extract for carbohydrate estimation. Subsequently, in a boiling water bath, the mixture was heated, till ethanol evaporates. Extraction of carbohydrate was completed by reiterating the above step with 5 ml of 80% ethanol. After mixing 0.9 ml of distilled water to the 0.1 ml of produced extract, the absolute volume was increased to 1 ml, and then 5 ml of 5% phenol was added. The mixture was vortexed completely to guarantee legitimate mixing. In this way, 5 ml of concentrated H_2SO_4 was added, followed by vortexing and cooling. A Spectrophotometer (Hitachi U-2800, Japan) was utilized to observe the absorbance of the last solution at 490 nm.

Estimation of proteins

The protein content was determined using the Lowry et al [9] technique. To make the protein extracts, two ml of homogenised microalgae sample were mixed with one ml of 1 N NaOH. After that, the mixture was cooked for 15 minutes in a hot water bath at 100°C before chilling under running tap water. In a 1 percent sodium potassium tartarate solution, 0.1 ml of the generated extract was combined with five ml of 0.5 percent $CuSO_4.5H_2O$. 0.5 ml of 1:1 Folin Ciocalteu's reagent was added after 10 minutes. The absorbance was measured using a UV-Vis Spectrophotometer (Hitachi U-2800, Japan) at 620 nm after the reaction mixture was kept at room temperature for 30 minutes. The protein content was determined using a standard calibration curve generated from Bovine Serum Albumin (BSA) at a concentration of 100 g/ml.

Estimation of lipids

The lipid content was estimated using Bligh and Dyer's [10] technique. Five gram of dried microalgal biomass were combined and homogenised in a solution containing 25 millilitres of methanol, 12.5 millilitres of chloroform, and 5 millilitres of water, then ultrasonicated for 40 minutes. After that, 12.5 mL chloroform and 12.5 mL sodium sulphate (1.5%, w/v) were added to the mixture. The resulting mixture was ultrasonicated for another 20 minutes. The extraction was carried out at room temperature (25°C). The following equation was used to compute the total lipid content:

$$\text{Total Lipid} = \frac{\text{Weight of liquid in aliquot} \times \text{Volume of chloroform layer}}{\text{Volume of aliquot}}$$

Analysis of algal pigments

Estimation of chlorophyll

The chlorophyll composition was assessed utilizing the protocol of El-Baky et al [11]. 20 ml of microalgal culture was centrifuged for 10 minutes at 6,000 rpm at 25°C. The pellet was dissolved in 20 ml of 100 percent acetone and held overnight at 4°C in the dark for full chlorophyll extraction. The mixture was centrifuged at 10,000 rpm for 5 minutes. The resulting supernatant was used to calculate total chlorophyll concentration (T-Chl) using a spectrophotometer (UV-Vis spectrophotometer, Hitachi U-2800, Japan) using the following Lichtenthaler equations [12]:

$$\text{Total chlorophyll} = 7.05 \times A_{661.6} + 18.09 \times A_{644.8}$$

Where, $A_{661.6}$ = Absorbance taken at wavelength 661.6 nm

$A_{644.8}$ = Absorbance taken at wavelength 644.8 nm

Estimation of carotenoid

The carotenoid content was determined using El-Baky et al [11] standard procedure and standard equation as follows [12]:

$$\text{Chl a} = A_{661.6} \times 11.24 - A_{644.8} \times 2.04$$

$$\text{Chl b} = A_{664.8} \times 20.13 - A_{661.6} \times 4.19$$

$$\text{Total Carotenoid} = \frac{1000 \times A_{470} - 1.82 \times \text{Chl a} - 85.02 \times \text{Chl b}}{198}$$

Where, $A_{661.6}$, $A_{644.8}$, $A_{664.8}$ and A_{470} represent the optical density at wavelength 661.6, 644.8, 664.8 and 470 nm. Chl a and Chl b represent Chlorophyll-A and Chlorophyll-B respectively.

Estimation of flavonoids

The flavonoids were calculated utilizing the method of Eom et al [13]. An aliquant of 0.5 ml of microalgae culture was mixed with 0.1 ml of 10% aluminium chloride and 0.1 ml of 1M potassium acetate solution to make a final volume of 5 ml, and then 4.3 ml of 80 percent Methanol was added. After the mixture was vortexed, the absorbance was measured at 415 nm with a UV-Vis Spectrophotometer (Hitachi U-2800, Japan). The observed absorbance was used to estimate flavonoid content using a standard curve for Quercetin generated with values ranging from 20-100 g/ml.

Quality determination of astaxanthin

Dried microalgae biomass (10 mg) was heated at 70°C in a test tube after being treated with 1 ml of 4M hydrochloric acid. After cooling, the mixtures were re-suspended in 1 ml of 100% acetone and centrifuged at 3500 rpm for 6 minutes at 4°C [14]. A Hitachi UV-Vis Spectrophotometer U-2800 was used to measure the absorbance of the samples at 480 nm.

Estimation of phycocyanin

The amount of phycocyanin was calculated utilizing the inorganic acid extraction method [15]. In a test tube, wet microalgae biomass (0.5 gm) was mixed with 11.6 M hydrochloric acid in a 1:2 (biomass:acid) ratio, then incubated at room temperature for 24 hours. A UV-Vis spectrophotometer (Hitachi U-2800, Japan) was used to detect absorbance at 620 nm and 652 nm the next day. The following equation was used to calculate the amount of phycocyanin in the microalgae samples:

$$\mathrm{PC} = \frac{\mathrm{A}_{620} - 0.474 \times A_{652}}{5.34}$$

Where, PC = Concentration of Phycocyanin in mg/ml

A_{620}= Absorbance of the sample at 620 nm

A_{652}= Absorbance of the sample at 652 nm

Estimation of β-carotene

The content of β-carotene was measured utilizing the protocol of Herrero-Martnez et al [16]. In a centrifuge tube, dry microalgal powder (0.5 gm) was combined with 0.05 gm of Na_2CO_3 then, 4 ml tetrahydrofuran was added. At room temperature, centrifugation of the mixture was done at 5000 revolutions per minute for 5 minutes. For extraction, the supernatant was combined with 3 ml dichloromethane and 3 ml 10% NaCl. For 2 minutes, the test tube was vortexed adequately. Using a UV-Vis spectrophotometer, the bottom organic layer was measured for absorbance at 461 nm (Hitachi U-2800, Japan). The measured absorbance was used to estimate β-carotene with the help of a standard curve for β-carotene with concentrations ranging from 10-60 μg/ml.

Estimation of total phenol

The methodology of Taga et al [17] devised for determining total phenols was used. In triplicates, 0.1 ml of methanolic microalgae extract was combined with 2 ml of 2% Na_2CO_3. After 2 minutes, 0.1 ml of Folin Ciocalteu's reagent (50%) was added. The mixture was adequately vortexed and incubated for 30 minutes at room temperature in the dark. A UV-Vis spectrophotometer was used to detect the absorbance at 720 nm (Hitachi U-2800, Japan). With the use of a reference curve for gallic acid concentrations ranging from 20-100 μg/ml, the obtained absorbance was utilised to estimate total phenol content.

Determination of anti-oxidant capacity by DPPH radical scavenging activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging technique was used to determine the anti-oxidant activity of microalgal extracts [18]. 1 ml of 0.1 mM DPPH solution in methanol was combined with 3 ml of 100 g/ml methanolic extract. The absorbance was measured at 517 nm using a UV-Vis Spectrophotometer (Hitachi U-2800, Japan). To calculate DPPH free radical scavenging, use the following formula:

$$\text{DPPH scavenging effect (\% inhibition)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 represents the absorbance of the standard reaction and A_1 represents the absorbance in the presence of all extract samples and the reference.

Qualitative determination of valuable compounds by Gas chromatography-mass spectrometry (GC-MS) analysis of microalgal biomass

Microalgae culture BGLR8 and BGLR16 were analyzed by GC-MS. Polyphenolic compounds were extracted *via* a modified method of refluxing [19]. Methanol (80% v/v) was used as a solvent for the extraction, which was carried out for 4 hours at 30°C. Fifty ml of solvent was used to reflux one gram of microalgae dry powder. After refluxing for 2 hours, filtration was performed. The remaining sample from the filter paper was again refluxed for two hours with 50 ml of solvent thrice. Centrifugation of the extracts was done at 6000 rpm, followed by vacuum evaporation at 45°C. The vacuum evaporated tests were separated through a 0.45 µm layer channel and disintegrated in the methanol in the ratio of 1:10 followed by collection in amber glass bottles.

Following processing, the concentrates were broken down using a Thermo Trace 1300 gas chromatograph coupled to a Thermo TSQ 800 Triple Quadrupole mass spectrometer with BP5MS section (30 m x 0.25 mm x 0.25 m). Helium gas (99.9%) was used as the transporter gas at a constant flow rate of 1.0 ml/min, with a 1.0 l infusion volume. The column oven temperature was adjusted from 50°C to 280°C at a rate of 50°C/min for 1.0 minute, then from 250 to 280°C at a rate of 15°C/min for 18 minutes, with the injector temperature held at 280°C. The findings of the GC-MS were translated using the National Institute of Standards and Technology's (NIST) database.

Molecular Identification by 18SrRNA sequencing

The culture was collected in the developmental phase for molecular identification of the putative microalgal culture BGLR8, and DNA was recovered utilizing the DNA isolation kit and PCR reagents provided by Chromous Biotech Pvt. Ltd., Bangalore, India. Amplification of 18S rRNA (1.8 kb) by Polymerase Chain Reaction (PCR) utilizing the following set of specific primers: 18S forward-‘5–GTAGTCATATGCTTGTCTC–3’ and 18S reverse- ‘5–GAAACCTTGTTACGACTT–3’. 100 ng of genomic DNA sample as a template, 400 ng of each primer, 4 µl of each dNTPs mixture, and 1 µl of Taq DNA Polymerase (3U/L). In the Thermal Cylcer ABI 3500 Genetic Analyzer, denaturation at 96°C for 5 minutes, denaturation at 96°C for 30 seconds, hybridization at 50°C for 30 seconds, and polymerization at 60°C for 1.3 minutes were employed. The sequences after amplification were

compared with a DNA ladder consisting of 1500 bp. The PCR results were sequenced using an ABI 3500 XL Genetic Analyzer. The data was analysed with Seq Scape-v 5.2 software. The sequences to be used in the Phylogenetic Tree Builder were aligned using the System Software aligner. A distance matrix was created using the Jukes-Cantor corrected distance model [20]. The distance matrix was generated using just alignment model sites; alignment inserts were excluded, and the minimum comparable position was 200. For comparative research and homologous sequence search, the National Centre for Biotechnology Information's (URL- http://www.ncbi.nlm.nih.gov/blast) Basic Local Alignment Search Tool (BLAST) method was used. The ribosomal 18S sequences were aligned using the ClustalW Multiple Alignment programme (URL- http://www.ebi.ac.uk/clustalw/). The neighbor-joining (NJ) strategy was employed to generate a phylogenetic tree using MEGA X 5.05 software. Bootstrap analysis with 1000 replications was used to examine the relative support for the branches formed by NJ analysis.

Statistical Analysis

Multiple comparisons with a t-test were conducted to examine the significance of the difference between mean values of different microalgae functional properties using SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA).

(e) Results and discussion:

Screening for biomass production

The euryhaline microalgae cultures namely BGLR8 and BGLR16 were cultivated and screened for biomass production. BGLR16 produced significantly higher dry biomass (8.3 g/L) ($p < 0.001$), followed by BGLR8 with 7.0 g/l (Figure 1(A)). Statistical tests showed significant differences in total biomass among the three microalgal strains ($p < 0.001$). The highest dry biomass obtained as 0.06 g/L in *Dunaliella tertiolecta*, which is lower than the biomass obtained in this study [21]. The biomass in various microalgal species varies because of the variation in the photosynthetic and carbon utilization efficiency depending upon the chemical kinetics of various light- dependent reactions, Calvin-Benson-Bassham (CBB) cycle, tri-carboxylic acid (TCA) cycle, Embden-Meyerhof Parnas (EMP) pathway and the pentose phosphate (PP) pathway of different microalgal species [22].

Analysis of Biochemical composition

BGLR16 had the highest concentration of carbohydrates (191 mg/g of microalgae dry weight) followed by BGLR8 that produced 124 mg/g of microalgae dry weight (Figure 1(B)). The carbohydrate content in BGLR16 is much higher than other algae like *Scenedesmus almeriensis, Chlorella sorokiniana,* and *Nannochloropsis gaditana,* which were previously reported to have 88, 128, and 129 mg/g of carbohydrates, respectively [23]. These results are of great significance as complex carbohydrates like pectic-oligosaccharides, fructo-oligosacharides, xylo-oligosaccharide, and galacto-oligosaccharide that are derived from various microalgae species act as prebiotic compounds

and promote the growth of specific gut microbes like *Bifidobacteria* and *Lactobacillus.* Fructo-oligosaccharide is also known to contain anti-inflammatory activity [24]. The results also indicate that BGLR16 had the highest protein content (538 mg/g) among the two strains evaluated ($p < 0.001$) followed by BGLR8 (511 mg/g) (Figure 1(B)). The protein content in these microalgae cultures surpassed 50% (dry weight), which conforms to the protein supplement guidelines accepted by Food Safety and Standards Authority of India (FSSAI). Furthermore, these microalgal cultures tested were rich (>2% dry weight) in lipids (Figure 1(B)), with substantial quantities of lipids present in BGLR8 (86 mg/g). Statistical tests showed significant differences in total lipids among the two microalgal strains ($p < 0.001$). A previous study by Prabakaran and Ravindran [25] had shown that other algal species like *Nostoc* sp., *Chlorella* sp. and *Tolypothrix* sp. produce 13.27, 17.61, and 17.75 mg/g of lipids, respectively, which is less than the results reported in this study.

Algal Pigments

The total chlorophyll content varied from 8.23 mg/g to 29.42 mg/g for different microalgal species (Figure 1(C)). The results show that BGLR8 produced the highest chlorophyll content (29.42 mg/g) followed by BGLR16 (8.23 mg/g). The chlorophyll content significantly differed among the two microalgal strains ($p < 0.001$). These results corroborate with the previously reported data indicating that *C. pyrenoidosa* can possess total chlorophyll upto 28 mg/g [26]. The biological activities associated with chlorophyll include anti-oxidant, anti-mutagenic activity, and induction of apoptosis in cancerous cells [27]. Epidemiological evidences indicated that increasing chlorophyll intake leads to a decrease in the risk associated with colorectal cancer [28].

The other economically sought-after pigments are carotenoids that have importance in food industries. Carotenoids content in BGLR8 was highest (28.8 mg/g) followed by BGLR16 (23.3 mg/g), both having >20 mg/g carotenoid (Figure 1(D)). Carotenoids are produced in the event of excessive free-radical generation. They protect the cells from photo-damage by quenching the excited state of chlorophyll and maintain the continuation of the growth [29]. Data from the study of other algal species like *Dunaliella* sp. and *Isochrysis* sp. suggests that their carotenoid content is much less (10.8 and 6.1 mg/g carotenoids, respectively), than BGLR8 and BGLR16 [30], making the latter a suitable choice for high quantity carotenoid source. The various carotenoids obtained from microalgae have numerous therapeutic properties. Zeaxanthin has anti-tumour and anti-oxidant activity, and fucoxanthin possesses hepato-protective and anti-diabetic activity [31]. Similarly, Lutein prevents cataracts, atherosclerosis, and retinitis, while lycopene is known to show anti-ulcer activity and strengthens the immune system [31].

The flavonoid content of the two microalgal cultures differed significantly from each other. BGLR16 had the highest level of flavonoids (9.75 mg/g) as compared to BGLR8, respectively (6.79 mg/g) (Figure 1(E)). Compared to other algal species like *Nannochloropsis gaditana,* that has flavonoid content of 5.18 mg/g [32], BGLR16 had nearly two-fold higher levels of flavonoids. Flavonoids have

been reported to show anti-oxidant activity and anti-microbial activity against *Salmonella typhi* and *Bacillus subtilis* [33]. The deep blue water-soluble pigment, phycocyanin synthesized by microalgae, has been used as a food additive. We found that BGLR 8 synthesized the highest amount of phycocyanin (52 mg/g) (Figure 1 (F)). Other algal species like *Galdieria sulphuraria* have been reported to yield a nearly comparable concentration of 22 mg/g of Phycocyanin [34]. Phycocyanin is a potential nutraceutical compound for cancer treatment as it can inhibit the proliferation of cancer cells and destroy cancer cells [35]. Figure 1(G) demonstrates that at a level of 19.27 mg/g dry weight, astaxanthin content of BGLR8, found in this study nears the level of 19.8 mg/g astaxanthin achieved in *Haematococcus pluvialis* [36], supporting the idea that BGLR8 could be a prominent source of this pigment. Astaxanthin strengthens immune response, possesses anti-inflammatory, anti-oxidant activity, anti-tumor activity, ocular protective effect, anti-diabetic activity [31]. Another carotenoid pigment that is a treasure in the nutraceutical industry is β-carotene which is desirable for its coloring and vitaminic properties. Figure 1(H) illustrates that BGLR8 was able to accumulate β-carotene upto 5.6 mg/g on a dry basis. These results suggest that BGLR8 is rich in β-carotene than other algal species like *Tetraselmis* sp. CTP4 synthesizes 3.21 μg/g of β-carotene [37]. β-carotene has anti-oxidant activity, anti-inflammatory activity, anti-diabetic activity, anti-tumor activity and benefits cognitive function and atherogenesis [31].

Anti-oxidant activity

The therapeutic actions of phenols are primarily attributed to their anti-oxidant activity, ability to scavenge free radicals, redox metal ion chelation, gene expression modification, and involvement with cell signalling pathways. We found that the phenolic content of BGLR8 (4.46 mg/g) was higher than that of BGLR16 (3.62 mg/g) (Figure 1(E)). This value is also higher than the phenolic content of methanolic and ethanolic extract of *Daphniphyllum neilgherrense*, containing 2.12 mg/g and 1.90 mg/g of total phenol, respectively [38]. Furthermore, we quantified the anti-oxidant activity and found that the highest anti-oxidant activity was demonstrated in BGLR8 (31.73%) followed by BGLR16 (9.90%) (Figure 1(I)). The excessive anti-oxidant activity of the BGLR8 is attributed to the hydroxyl functional groups on phenols that are potent hydrogen donators. The redox potential of phenolic compounds plays an important role in adsorbing free radicals and, as a result, quenching the O^{-1} and O^{-3} radicals. Apart from anti-oxidant activity, polyphenols are also involved in other functions. Methanolic extract rich in polyphenols obtained from *Ecklonia stolinifera* showed anti-diabetic activity as it significantly reduced the plasma glucose level in Diabetes Mellitus-induced rats. The anti-proliferative action of algae is determined by their total polyphenolic content [39].

Gas chromatography-mass spectrometry (GC-MS) analysis of microalgal biomass

The chromatographs for the methanolic extract of BGLR8 and BGLR16 had multiple peaks. The peak area and retention time for compounds detected in methanolic extract of BGLR8 and BGLR16, which are valuable as nutraceuticals, are presented in Table 1 & 2; Figures 2 & 3. The 8 volatile compounds

identified by GC-MS of BGLR8 are Dimethyl (*E*)-but-2-enedioate, 2,2,4,4,6,6,8,8,10,10,12,12-dodecamethyl-1,3,5,7,9,11-hexaoxa-2,4,6,8,10,12-hexasilacyclododecane, 2,2,4-1,3,5,7,9,11,13-heptaoxa-2,4, ,4,6,6,8,8,10,10,12,12,14,14-tetradecamethyl 6,8,10,12,14-heptasilacyclotetraecdane, Methyl (*Z*)-pentadec-8-enoate, Methyl hexadecanoate, Methyl (9*E*,12*E*)-octadeca-9,12-dienoate, Methyl octadec-13-enoate and Methyl octadecanoate. While, 4 volatile compounds identified from BGLR16 are 2,2,4,4,6,6,8,8,10,10,12,12,14,14-tetradecamethyl-1,3,5,7,9,11,13-heptaoxa-2,4,6,8,10,12,14-heptasilacyclotetradecane, [4-[1,2-bis(trimethylsilyloxy)ethyl]-2 trimethylsilyloxyphenoxy]-trimethylsilane and Methyl 14-methylpentadecanoate, (*E*,7*R*,11*R*)-3,7,11,15-tetramethylhexadec-2-en-1-ol.

Molecular identification

BGLR8 was found to contain the highest amount of lipids, chlorophyll, carotenoids, phenols, astaxanthin, phycocyanin, β-carotene and anti-oxidant activity than BGLR16. It was, therefore, selected for molecular identification through 18S rRNA sequencing. The 18S rRNA sequence analysis of BGLR8 through the phylogenetic tree construction is shown in Figure 4. The BGLR8 isolate was identified as the member of *Coelastrella.* (GenBank accession no. MW443083.1).

Conclusion

Microalgae are lesser micro-organisms, as 97% of euryhaline microalgal species remain unexplored and uncharacterized. This lacuna highlights the demand for extensive research in bioprospecting, including isolation, identification, and growth optimization of native microalgal strains. This study provides evidence that the member of *Coelastrella* (GenBank accession no.- MW443083.1) exhibits potentially valuable biochemical profile rich in high-quality protein, lipids, carotenoids (such as phycocyanin, astaxanthin and β-carotene), and anti-oxidant pigments. Our results suggest that these microalgae are promising candidates for use as nutraceuticals. The member of *Coelastrella* sp. BGLR8 is favorable as a nutritional supplement due to its high lipids, chlorophyll, carotenoids, phenols, astaxanhin, phycocyanin, β-carotene and anti-oxidant activity.

Moreover, various potential bioactive components identified by GC-MS analysis showed propitious therapeutic activities. Therefore, the microalgae analyzed in this study could prove to be effective in manufacturing nutraceuticals. These miniature biofactories might give rise to the revolution in the energy, pharmaceutical and food industries in the future.

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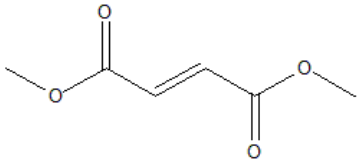
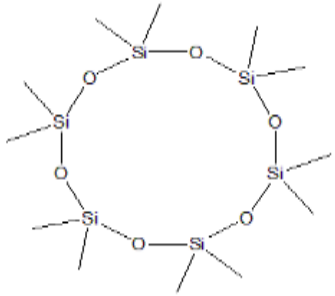
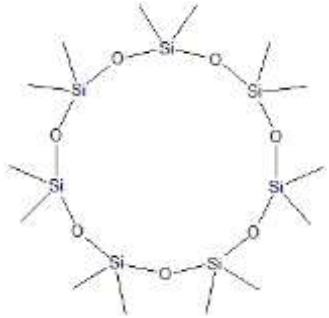
Table 1 Composition and therapeutic application of different volatile compounds in BGLR8 analysed through GC-MS

Sr. No.	Chemical Name	Structure	Structural formula	Retention time	Peak area %	Therapeutic properties	References
1.	Dimethyl (*E*)-but-2-enedioate (PubChem CID: 87864520)		$C_6H_8O_4$	12.3	0.59	Used for treatment of multiple sclerosis	[40]
2.	2,2,4,4,6,6,8,8,10,10,12,12-dodecamethyl-1,3,5,7,9,11-hexaoxa-2,4,6,8,10,12-hexasilacyclododecane (PubChem CID: 10911)		$C_{12}H_{36}O_6Si_6$	21	7.84	Antimicrobial	[41]
3.	2,2,4 -1,3,5,7,9,11,13-heptaoxa-2,4, ,4,6,6,8,8,10,10,12,12,14,14-tetradecamethyl 6,8,10,12,14-heptasilacyclotetraecdane (PubChem CID: 7874)		$C_{14}H_{42}O_7Si_7$	25.41	2.12	Antioxidant, flavor, hypocholesterolemic	[42]

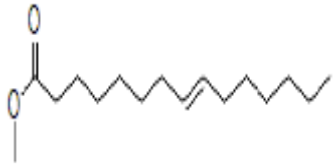
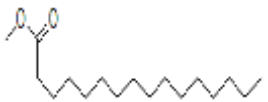
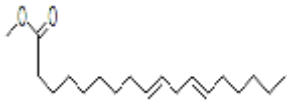
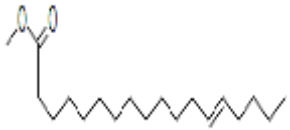
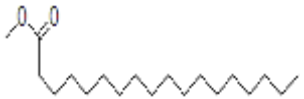
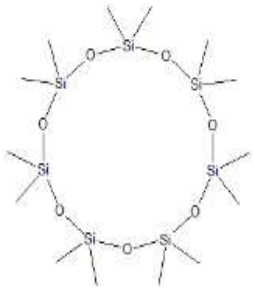
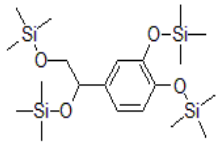
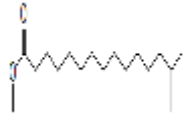
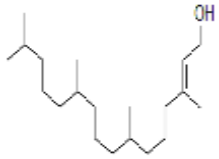
4.	Methyl (*Z*)-pentadec-8-enoate (PubChem CID: 21629438)		$C_{17}H_{32}O_2$	34.78	0.86	Antioxidant, insecticide, hemolytic, hypo – cholesterolemic	[43]
5.	Methyl hexadecanoate (PubChem CID: 6482596)		$C_{17}H_{34}O_2$	35.32	36.08	Antibacterial and antifungal	[44]
6.	Methyl (9*E*,12*E*)-octadeca-9,12-dienoate (PubChem CID: 6149093)		$C_{19}H_{34}O_2$	38.53	14.67	Anti-inflammatory, antibacterial, antiarthritic, hepatoprotective, anti-histaminic, anti-coronary	[43]
7.	Methyl octadec-13-enoate (PubChem CID: 5364506)		$C_{19}H_{36}O_2$	38.61	30.82	Anti-inflammatory, antiandrogenic, cancer preventive, dermatitigenic, irritant, antileukotriene—D4, hypocholesterolemic, 5-alpha reductase inhibitor, anemiagenic, insectifuge, flavor	[45]
8.	Methyl octadecanoate (PubChem CID: 8201)		$C_{19}H_{38}O_2$	39.17	2.04	GABA aminotransferase inhibitor, anti-inflammatory, intestinal lipid metabolism regulator gastrin inhibitor antihelmintic (nematodes) antinociceptive	[45]

Table 2 Composition and therapeutic application of different volatile compounds in *Chlorella* BGLR16 analysed through GC-MS

Sr. no.	Chemical Name	Structure	Structural formula	Retention time	Peak area %	Therapeutic properties	References
1.	2,2,4,4,6,6,8,8,10,10,12,12,14,14-tetradecamethyl-1,3,5,7,9,11,13-heptaoxa-2,4,6,8,10,12,14-heptasilacyclotetradecane (PubChem CID: 7874)		$C_{14}H_{42}O_7Si_7$	25.37	2.58	Antimicrobial, antiseptic, hair conditioning agent, skin- conditioning agent-emollient; solvent	[46]
2.	[4-[1,2-bis(trimethylsilyloxy)ethyl]-2-trimethylsilyloxyphenoxy]-trimethylsilane (PubChem CID: 530365)		$C_{20}H_{42}O_4Si_4$	29.31	0.47	Dentistry	[47]
3.	Methyl 14-methylpentadecanoate (PubChem CID: 21205)		$C_{17}H_{34}O_2$	35.29	8.68	Catechol-o-methyl-transferase-inhibitor, methyl-guanidine-inhibitor	[48]
4.	(*E*,7*R*,11*R*)-3,7,11,15-tetramethylhexadec-2-en-1-ol (PubChem CID: 145386)		$C_{20}H_{40}O$	38.84	4.97	Antimicrobial, anti-inflammatory anticancer, diuretic	[49]

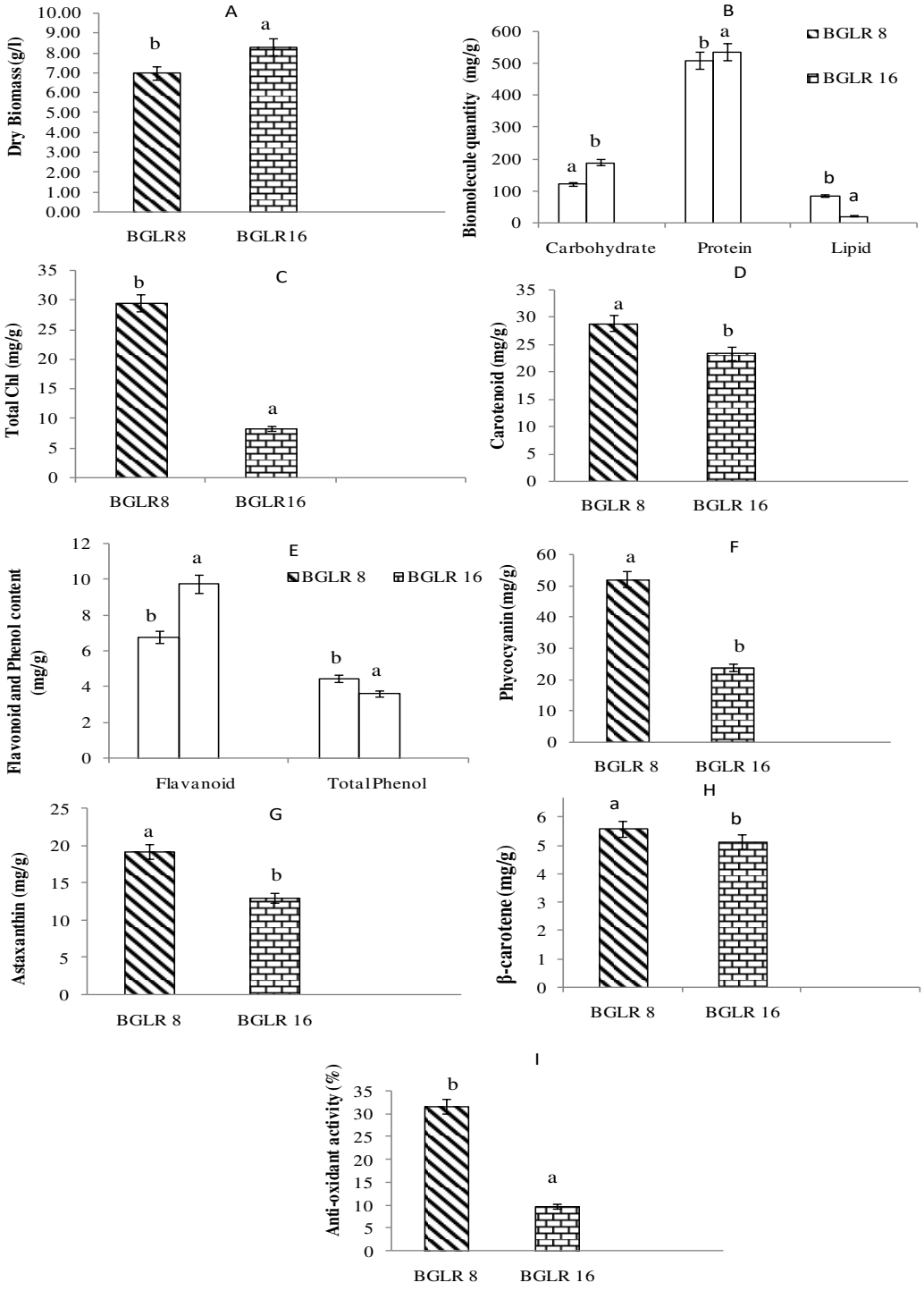


Fig. 1(a.) Dry biomass; (b.) carbohydrate, protein and lipid; (c.) total chlorophyll; (d.) total carotenoids; (e.) flavanoids and total phenols; (f.) phycocyanin; (g.) astaxanthin; (h.) β-carotene; (i.) anti-oxidant activities. The mean and standard error of the mean (n = 3) are used to express the data. Significant variations between samples (p 0.05) are shown by different letters (a–b), whereas letters shared in common between or among samples indicate no significant differences.

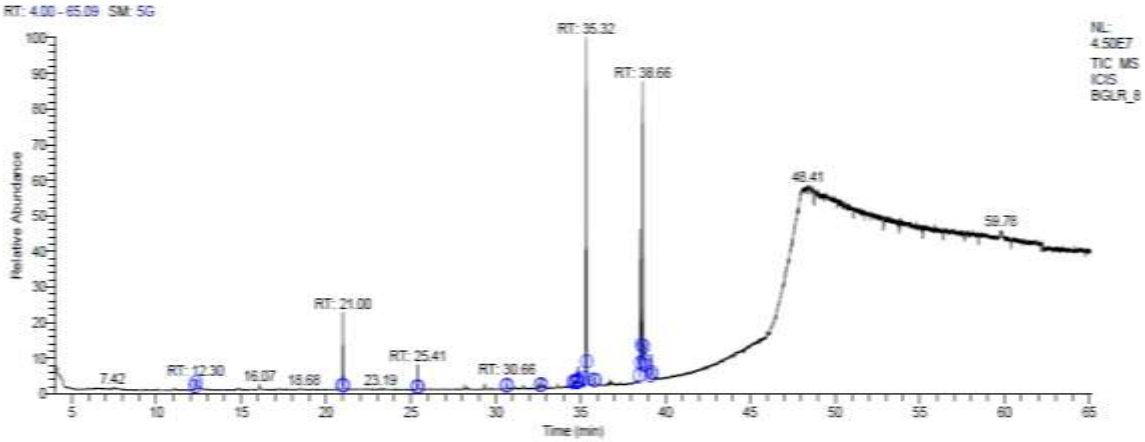


Fig. 2 Peaks for various compounds revealed in GC-MS analysis of *Coelastrella* sp. BGLR8.

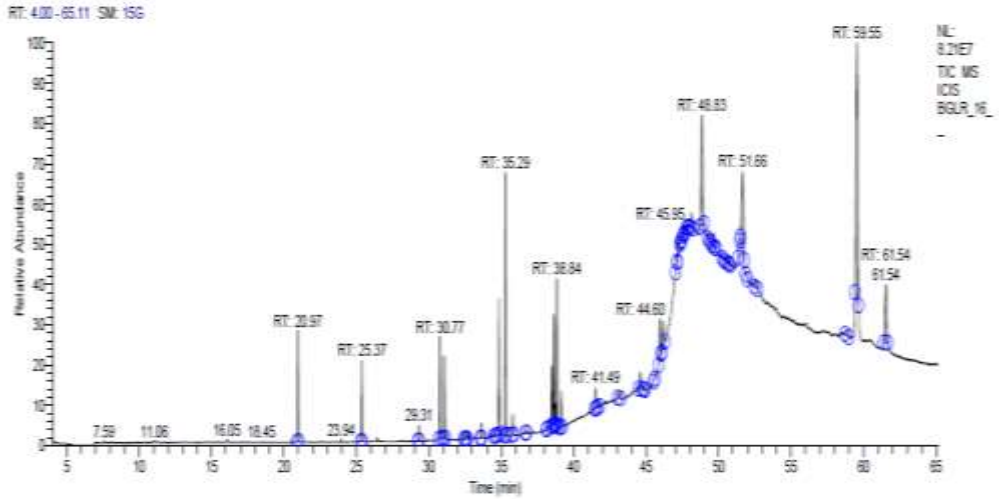


Fig. 3 Peaks for various compounds revealed in GC-MS analysis of microalgal culture BGLR16.

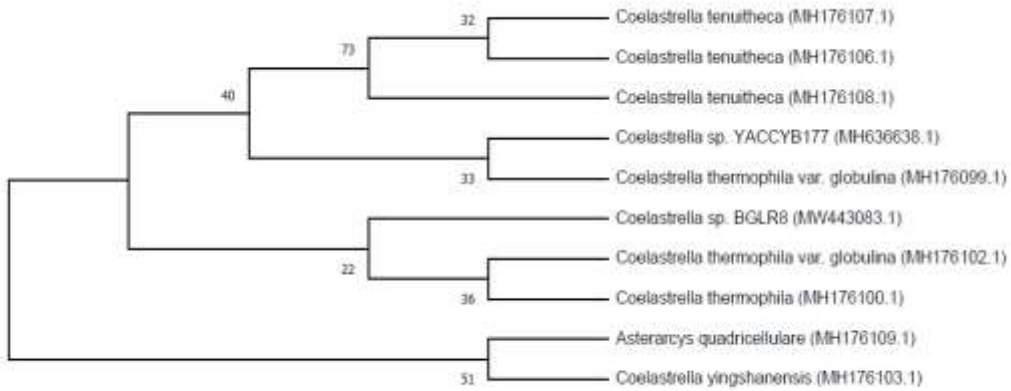


Fig. 4 The 18S rDNA sequences of BGLR8 were analysed for their phylogenetic relationships. Weighbor (a weighted form of Neighbor Joining that gives the longer distances in the distance matrix much less weight) and 100 rounds of bootstrap resampling were used to create the tree are expressed as the mean and standard error of the mean (n = 3).