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Exploration of antioxidant activities, microstructural properties, and fatty acid composition of three cyanobacteria species

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ABSTRACT

Cyanobacteria have gained increasing attention in the food and pharmaceutical industries as a natural source of antioxidants. However, more knowledge is needed regarding the antioxidant and nutritional properties of cyanobacteria found in water bodies worldwide, including those in Sri Lanka. This study investigated the antioxidant activities and microstructural properties using FTIR-ATR (Fourier Transform Infrared Spectroscopy-Attenuated total reflectance) analysis and the fatty acid composition of three cyanobacterial species: *Chroococcidiopsis* sp., *Gleocapsa* sp., and *Merismopedia* sp., which were isolated from water bodies in Sri Lanka. The water extract of *Chroococcidiopsis* sp. Exhibited the highest ($p \leq 0.05$) phenolic content (2.26 ± 0.09 mg GAE/g DW) and ABTS activity (13.28 ± 0.36 mmol TE/g DW) among the tested species. The methanol extract of *Merismopedia* sp. Displayed the highest ($p \leq 0.05$) DPPH activity (IC_{50}-15.21 mg/mL) and notable ($p \leq 0.05$) ORAC values (112.50 ± 5.79 mmol TE/g DW). Analysis by FTIR-ATR revealed the presence of nutritional components, including proteins, lipids, and polysaccharides, as well as functional compounds such as phenolics and amides in the studied cyanobacterial species. The fatty acid composition indicated that the studied cyanobacteria contain twenty different saturated and unsaturated fatty acids, with compositions ranging from 60 to 70% and 29–39%, respectively. Saturated fatty acids, caprylic, capric, pentadecanoic, heptadecanoic, palmitic, and lignoceric acids, were detected in all species, while elaidic, palmitoleic, and *Cis*-10- heptadecanoic acids were identified as prominent monounsaturated fatty acids among them. *Gleocapsa* sp. Had the highest ($p \leq 0.05$) saturated fatty acid content, while *Merismopedia* sp. Showed the highest ($p \leq 0.05$) unsaturated fatty acid content. Based on the results, *Merismopedia* sp. Has demonstrated promising potential as a rich source of antioxidants and unsaturated fatty acids.

1. Introduction

Cyanobacteria, a fascinating group of microorganisms with characteristics bridging algae and bacteria, are widely acknowledged as the most abundant oxygenic photosynthetic organisms, thriving across an array of ecosystems on our planet (Zahra et al., 2020). These versatile microorganisms exhibit remarkable adaptability to diverse environmental conditions, and they possess the unique

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ability to produce an array of secondary metabolites endowed with diverse bioactive properties (Ismaiel et al., 2014). For millennia, cyanobacteria have been an integral part of the human diet, owing to their exceptional nutritional profile, offering a rich source of proteins, essential amino acids, fatty acids, phytosterols, carbohydrates, and vitamins (Tabarzad et al., 2020). Nevertheless, the significance of cyanobacteria extends far beyond basic nutrition; they are known for a range of other health benefits, including potent antioxidant activities (Safafar et al., 2015).

Antioxidants, which are abundantly present in cyanobacteria, play a pivotal role in shielding living organisms from the detrimental effects of oxidative stress, a state characterized by an imbalance between free radicals and the body's ability to counteract their harm (Banskota et al., 2019). The overproduction of reactive oxygen species (ROS) can lead to cellular damage, inflammation, and the development of various chronic diseases (Liu et al., 2018). Cyanobacteria have been recognized as a promising source of natural antioxidants, exhibiting the capacity to produce a diverse array of bioactive molecules, including carotenoids, phycobiliproteins, and polyphenols. Furthermore, their natural antioxidants have proven valuable as preservatives in the food industry, effectively extending the shelf life of products, and also hold promise for applications in the pharmaceutical and cosmetic sectors (Hossain et al., 2016; Safafar et al., 2015).

Fatty acid composition, a critical component of cyanobacteria biomass, is not only significant for microbial physiology but also bears industrial relevance. Cyanobacteria are known to synthesize various lipids, including essential polyunsaturated fatty acids (PUFAs) such as linoleic acid and α-linolenic acid. These PUFAs are of great interest for their potential application in producing biofuels, functional foods, and pharmaceuticals, making cyanobacteria a compelling resource for sustainable bioproducts (Barone et al., 2023). Although a large number of studies have reported the nutritional properties of cyanobacteria species, their potential as a fatty acid substrate has not been extensively studied (Stebegg et al., 2023).

While the exploration of various cyanobacterial species has expanded our understanding, studies investigating the properties of three specific cyanobacteria species, namely *Chroococcidiopsis* sp., *Gleocapsa* sp., and *Merismopedia* sp., cultivated in Sri Lanka, remain relatively scarce. Previous research has shed light on *Chroococcidiopsis* sp., a remarkably versatile and primitive photosynthetic cyanobacterium that thrives in extreme environments (Antonopoulou et al., 2005). The biomass of *Chroococcidiopsis* sp. Exhibits substantial quantities of proteins, lipids, nucleic acids, ashes, and notably higher carbohydrate levels compared to health-food supplements resembling *Spirulina platensis* (Montero-Lobato et al., 2020). *Gloeocapsa* sp., on the other hand, possesses the remarkable ability to thrive aerobically in illuminated conditions without requiring a nitrogen source (Mishra et al., 2018).

The main aim of this study is to enhance the utilization of cyanobacteria species in various fields, including the food, pharmaceutical, and cosmetic industries, by emphasizing the significance of this study through the evaluation of antioxidant properties, FTIR-ATR microstructural availabilities, and fatty acid profiling of three isolated cyanobacterial species: *Chroococcidiopsis* sp., *Gleocapsa* sp., and *Merismopedia* sp. From Sri Lankan freshwater bodies. The findings of this study may significantly contribute to the growing body of knowledge concerning cyanobacteria and underscore their pivotal role in shaping a sustainable and healthier future.

2. Materials and methods

2.1. Materials; chemicals and reagents

Methanol, ferric ammonium citrate, citric acid, calcium chloride, magnesium sulphate, dipotassium hydrogen phosphate, boric acid, magnesium chloride, zinc sulphate, copper sulphate, sodium molybdate, phosgene, hexane, sodium methoxide, glacial acetic acid, dichloromethane, folin–Ciocalteu reagent, sodium carbonate, sodium hydroxide, aluminium chloride, sodium nitrite, ferrous sulphate, ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), DPPH (2,2-Diphenyl-1-picrylhydrazyl), APPH (2,2′-azobis (2-amidinopropane) dihydrochloride), and phosphate buffer, sodium methoxide, hexane.

2.2. Methods

2.2.1. Cyanobacterial primary cultures and incubation

For this research, three cyanobacterial strains were selected, including *Chroococcidiopsis* sp. (KF321949), *Gleocapsa* sp. (ON870362.1), and *Merismopedia* sp. (GU815987.2). These strains were taken from the culture collection in the Molecular Microbiology and Human Diseases Project (MM&HD) at the National Institute of Fundamental Studies, Sri Lanka, which had been isolated during previous studies.

To cultivate the samples, BG11 medium was employed (Rippka et al., 1979; Wanigatunge et al., 2014b). The samples were exposed to a 25 μmol m^{-2} s^{-1} photon flux to ensure controlled growth conditions. A well-regulated photoperiod of 14 h of light and 10 h of darkness was maintained.

Further, isolated sources, morphological characteristics and molecular confirmation details are presented in the following table (Table 1), and the light microscopic view of these three species is shown in Fig. 1.

2.2.2. Semi-mass scale cultivation of cyanobacteria

2.2.2.1. Preparation of the stock solutions and BG11 medium for cyanobacteria. Four stock solutions and BG11 medium for mass culturing were prepared (the composition is given in supplementary material) as described previously (Jena et al., 2011).

2.2.2.2. Sub-culturing of cyanobacteria. The selected cyanobacterial cultures were scaled up to 2 L flasks containing 500 mL of BG11 medium. A 500 μL inoculum of cyanobacteria cultures was introduced in a controlled environment with continuous aeration at a temperature of 28 ± 2 °C, using a fluorescent light source (25 μmol m^{-2} s^{-1} photon flux) at a well-regulated photoperiod of 14 h of light and 10 h of darkness.

Table 1
Isolated sources, morphological characteristics, and molecular confirmation details of three cyanobacterial species.

Cyanobacterial species	Isolated source	Latitude of the source	Morphological characteristics	Gene Bank accession number	Reference
Chroococcidiopsis sp.	A reservoir in Girandurukotte	(7°46′29″N, 81°1′74″E)	Spherical cyanobacteria with a colorless homogeneous sheath and found as unicellular colonies with closely packed cells of dark green color	(KF321949)	(Wang et al., 2019; Liyanage, 2021)
Gleocapsa sp.	Mahapelassa hot springs	(6°15′12.84″N, 80°58′53.66″E)	Unicellular or in aggregates or packets; spherical to hemispherical shaped cells; binary fission central to form equal size daughter cells; fission occurs in 2–3 planes	(ON870362.1)	Samarasinghe (2022)
Merismopedia sp.	Water tank in Kurunegala	(7°29′45.68″N, 80°21′30.29″E)	Bright green color spherical and oval-shaped cells. Comprise regular colonies of free-living, plate-shaped, flat, and slightly wavy with prominent intercellular mucilage connections	(GU815987.2)	(Palinska et al., 1996; Wanigatunge, 2014)

Sub-culturing was performed in 1500 mL of BG11 medium with 100 mL of the aforementioned cyanobacteria cultures, following similar conditions (Pulz and Gross, 2004).

2.2.3. Harvesting and separation of biomass

All the Cyanobacterial samples were harvested approximately 40–45 days after culturing. The growth rate was slow and all strains reached the mid-exponential growth stage after 40–45 days of culturing, which was monitored microscopically by the Inverted fluorescence microscope (Olympus CKX41SF, Philippines) equipped with and Olympus DP73 universal camera (Olympus DP73, Japan). According to the observations, all species exhibited a lag phase of about 3 weeks, followed by an exponential phase of another 4 weeks, and a stationary phase of 3 weeks, before the death phase. The harvested biomass were centrifuged using a centrifuge (5340 R, Germany) at 5000 rpm for 20 min at a temperature of 27 °C until the entire cell content was obtained. The dry cell mass content was weighed, frozen at −80 °C, and freeze-dried (CHRIST™, ALPHA 1-4LD Plus, Germany).

2.2.4. FTIR-ATR analysis

Powdered cyanobacteria samples were analyzed by Fourier Transform Infrared Spectroscopy with attenuated total reflectance (FTIR-ATR). An NICOLET iS50 F T-IR analyzer equipped with a GladiATR diamond ATR module (Thermo Fisher Scientific, Madison, WI, USA) was used to record data, which was mounted on an FTIR spectrometer. Spectral resolution of 4 cm^{-1} was used to acquire absorbance spectra across the wavenumber range of 4000 to 400 cm^{-1}. Absorbance data were collected over 32 scans. For the primary analysis, a triplicate of each sample from each dataset was employed, following the method outlined by Ozer et al. (2019).

2.2.5. Fatty acid composition

The lipids of the cyanobacterial samples were extracted, and derivatization was carried out according to the method of (Wickramasinghe et al., 2023) with slight modifications. In brief, 2 g of dried and crushed cyanobacterial samples were shaken with 50 mL of hexane for 30 min on a wrist-action shaker (BURRELL™, USA) at room temperature. The ultra-sonication process (CL-188, USA) was carried out for 30 min, and the supernatant was obtained by centrifugation (1500 rpm for 10 min). The crude oil content was obtained using a rotary evaporator (Heidolph™, 200,003,264, Germany) at 37 °C under vacuum conditions.

Fatty acid methyl esters (FAME) were prepared using an oil sample in a 15 mL screw-capped methylation tube. The oil was mixed with 0.3 mL of dichloromethane and 2 mL of 0.5 M sodium methoxide. The resulting mixture was then placed in a hot water bath set at 50 °C for 30 min and allowed to cool to room temperature. After cooling, 0.1 mL of glacial acetic acid and 0.5 mL of hexane were added and mixed well, followed by the slow addition of 5 mL of distilled water.

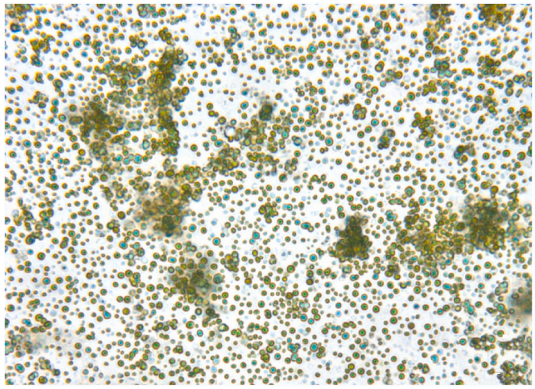
The top layer of hexane was separated into a 2 mL GC vial after the contents were held at room temperature for 30 min. Subsequently, the vials were sealed with Parafilm and stored immediately at −20 °C until GC analysis (GC system, US 16443037, USA). Finally, the vials were capped and sealed further with Parafilm and kept immediately at - 20 °C until analysis by GC (GC system, US 16443037, USA). Running conditions in GC were as follows: injection volume (1 μL), carrier gas (hydrogen), pressure mode (constant); inlet: split/spitless 260 °C, split ratio 50:1; oven conditions: 100 °C (5 min), 8 °C/minute to 180 °C (9 min), 1 °C/minute (15 min). The FID was adjusted for 260 °C, and the airflow was as follows: hydrogen 40 L/min, air 400 mL/min, makeup gas 25 mL/min. The column used was an Agilent J&W *C*P-Sil 88 for FAME (100 m, 250 μm, 0.2 μm).

2.2.6. Water and methanolic extractions

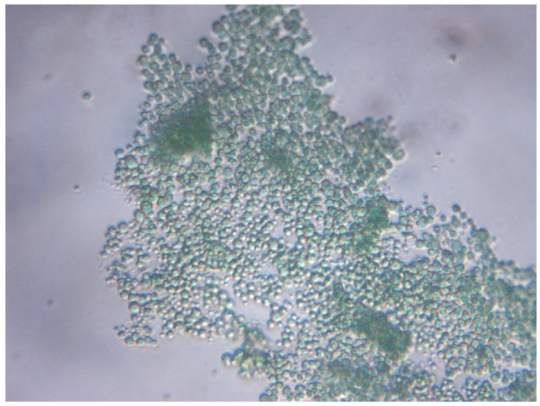
A 30 mg freeze-dried cell mass was separately extracted into 1 mL of distilled water and methanolic (water: methanol = 2:1) solution, where methanol and water were used separately. The extraction involved sonication (CL-188, USA) at a controlled temperature and 40 kHz for 20 min. Subsequently, the sonicated samples were centrifuged (5340 R, Germany) at 6000 rpm for 15 min, and the resulting supernatant was employed to assess antioxidant activities.

2.2.7. Total phenolic content (TPC)

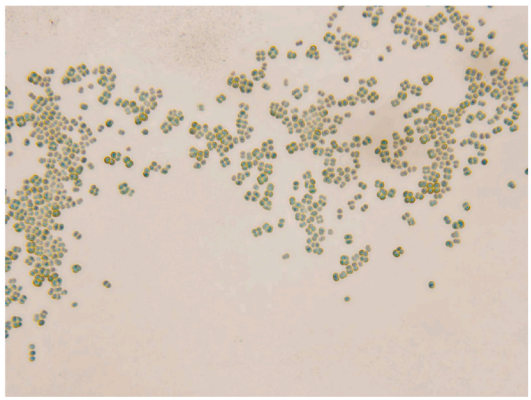
This assay was conducted using the method of Liyanage et al. (2016) with slight modifications. In summary, 20–50 μL of the extracted sample and 105 μL of 10 % Folin-Ciocalteu's reagent were mixed to prepare the reaction mixture. After 3 min, 80 μL of Na_2CO_3 (7.5 %, w/v) was added to the mixture and the reaction mixture was incubated for 30 min at room temperature. A UV–visible



Chroococcidiopsis sp. (40x)



Gleocapsa sp. (40x)



Merismopedia sp. (40x)

Fig. 1. Microscopic structures of cyanobacteria species **(a)** *Chroococcidiopsis* sp. (40*x*) **(b)** *Gleocapsa* sp. (40*x*) **(c)** *Merismopedia* sp. (40*x*).

microplate spectrophotometer (Omega 415–3441, Germany) was used to measure the absorbance at 760 nm. All tests were carried out in triplicate, and the final results were expressed in milligrams of gallic acid equivalents (mg of GAE) per gram dry weight (DW).

2.2.8. Total flavonoid content (TFC)

This assay was carried out according to the method of Agbo et al. (2015). First, the sample extract (50 μL) was mixed with 20 μL of NaNO2 (5%, w/v), and the mixture was incubated for 6 min. Subsequently, 20 μL of AlCl3 (10%, w/v) was added, and the mixture was incubated for an additional 6 min. Finally, 200 μL of NaOH (4%, w/v) was introduced. After a total incubation period of 15 min, the absorbance was measured in triplicate at 510 nm using a UV–visible microplate spectrophotometer (Omega 415–3441, Germany). Total flavonoid content (TFC) was expressed in milligrams of catechin equivalents (CE) per gram of dry weight (DW).

2.2.9. DPPH radical-scavenging capacity

Different volumes (30–90 μL) of methanol and water extracts of samples were mixed with 100 μL of DPPH radical solution. Afterward, the reaction mixture was left to stand for 30 min at room temperature in the dark before determining absorbance at 517 nm. Finally, sample concentrations with 50% inhibition (IC_{50}) were calculated, and values were expressed (Sanjeevkumar et al., 2016).

2.2.10. ABTS radical-scavenging capacity

Briefly, 2.5 mM ABTS solution and 5.0 mM $K_2S_2O_8$ were mixed and kept in the dark at room temperature for 12 h to form the radical solution. After 10 min, the ABTS radical solution (150 μL) was added to 50 μL of the extract, and the absorbance was measured in triplicate at 734 nm (Liyanage et al., 2016). The final results were expressed as millimole Trolox equivalents (TE) per gram of dry weight (DW).

2.2.11. Oxygen radical antioxidant capacity (ORAC)

This assay was carried out according to the method of Price et al. (2006). Freshly prepared reagents, including phosphate buffer (10 mM, pH 7.4), were used for this analysis. Briefly, 25 μL of the sample (100–1000 ppm) and 150 μL of fluorescein (10 nM) were mixed and incubated at 37 °C for 30 min. The auto-injector on board was used to inject 25 μL of 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) (240 mM) into the microplate. The area under the curve (AUC) was calculated for each sample using the MARS software. The net AUC of the sample was calculated by subtracting the AUC of the blank. The ORAC values were expressed as μmol Trolox equivalents/g of a sample using a previously established standard (Trolox) curve.

3. Results

3.1. Growth characteristics

During the growth, *Merismopedia* sp. Showed floating colonies, *Gleocapsa* sp. Covered the entire surface, and *Chroococcidiopsis* sp. Displayed a comparatively fast growth forming dense colonies.

3.2. Biomass content of cyanobacteria

The dry biomass content is an indicator of the growth rate and survival capabilities of cyanobacterial species. The dry biomass content (Fig. 2) of the three species is presented in the supplementary material. *Chroococcidiopsis* sp. Exhibited the highest ($p \leq 0.05$) weight as 0.65 ± 0.02% while *Gleocapsa* sp. And *Merismopedia* sp. Showed relatively low ($p \leq 0.05$) 0.11 ± 0.00 % dry weights. The possible reason for this observation could be the unique ability of *Chroococcidiopsis* sp. To survive in extreme conditions than other species (Fewer et al., 2002).

3.3. FTIR-ATR analysis

According to the graph (Fig. 3), all three species of cyanobacteria show bands near the wavenumbers of 3600–3380 cm^{-1}, 3000-2855 cm^{-1}, 1720 cm^{-1}, 1660 cm^{-1}, 1520 cm^{-1}, 1450 cm^{-1}, 1420 cm^{-1}, 1250 cm^{-1}, 1190-1170 cm^{-1}, and 1500-1000 cm^{-1}. *Gleocapsa* sp. And *Merismopedia* sp. Showed more peaks near 4000-3000 cm^{-1} than *Chroococcidiopsis* sp. Further, there are several of differences in bands around 1500-500 cm^{-1}.

3.4. Fatty acid composition

The cellular fatty acid contents in the cyanobacteria samples were analyzed by gas chromatography. According to the results (Table 2), all three cyanobacterial species exhibited the presence of 20 different fatty acids. Significant differences ($p \leq 0.05$) in the fatty acid profiles were observed among the three species. When considering the fatty acid profiles of these three groups of cyanobacteria, several distinctions emerged. The detected fatty acids ranged in length from 4 to 22 carbons. The total fatty acids with unsaturated: saturated fatty acid ratios (U: S) varied from 0.42 (*Gleocapsa* sp.) to 0.65 (*Chroococcidiopsis* sp).

Saturated fatty acid content in these three species ranged from 60% to 70%, with *Gleocapsa* sp. Displaying the highest content ($p \leq 0.05$). Unsaturated fatty acid content ranged from 29% to 39%, and *Merismopedia* sp. Exhibited the highest value ($p \leq 0.05$). Variations ($p \leq 0.05$) in the composition of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were ob-

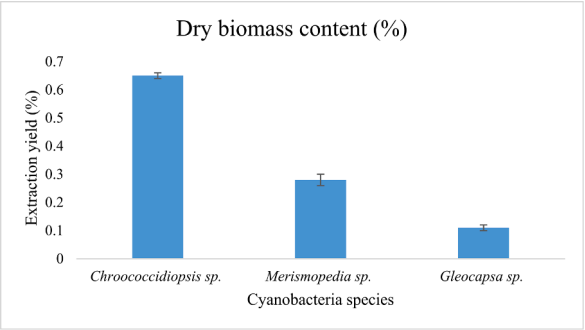


Fig. 2. Dry biomass content (%) in three cyanobacteria species. Data represent the mean ± SD ($n = 3$) of three independent experiments.

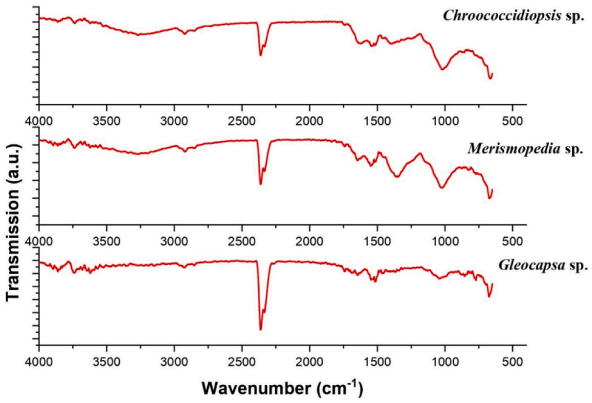


Fig. 3. FTIR-ATR spectra of the three different cyanobacteria species between 4000 and 400 cm^{-1} wavenumber.

Table 2
Fatty acid composition (percentage (%)) of three cyanobacteria species.

	Component (methyl esters)	*Merismopedia* sp.	*Gleocapsa* sp.	*Chroococcidiopsis* sp.
1	Caproic acid C6:0	ND	3.43 ± 0.01^{e}	ND
2	Caprylic acid C8:0	5.71 ± 0.02^{e}	14.82 ± 0.10^{b}	18.14 ± 0.03^{a}
3	Capric acid C10:0	0.75 ± 0.00^{i}	2.07 ± 0.03^{f}	1.95 ± 0.00^{h}
4	Lauric acid C12:0	ND	ND	0.81 ± 0.00^{i}
5	Tridecanoic C13:0	ND	ND	ND
6	Myristic	1.66 ± 0.00^{h}	ND	2.11 ± 0.01^{g}
7	Pentadecanoic acid C15:0	4.94 ± 0.13^{f}	ND	2.19 ± 0.00^{g}
8	*Cis*-10-Pentadecanoic acid C15:1	2.16 ± 0.00^{g}	12.86 ± 0.01^{c}	11.26 ± 0.15^{b}
9	Palmitic acid C10:0	10.71 ± 0.06^{c}	3.20 ± 0.17^{e}	5.14 ± 0.01^{d}
10	Palmitoleic acid C16:1	0.85 ± 0.00^{i}	3.00 ± 0.08^{e}	3.45 ± 0.18^{e}
11	Heptadecanoic acid C17:0	14.03 ± 0.09^{b}	3.38 ± 0.01^{e}	5.65 ± 0.00^{d}
12	*Cis*-10- Heptadecanoic acid 17:1	1.29 ± 0.01^{g}	12.51 ± 0.05^{c}	9.88 ± 0.11^{c}
13	Stearic acid C18:0	16.34 ± 0.11^{a}	ND	ND
14	Elaidic acid C18:1trans (n9)	1.21 ± 0.02^{h}	11.50 ± 0.07^{d}	5.01 ± 0.03^{d}
15	Linolelaidic acid C18:2 trans (n6)	9.13 ± 0.30^{d}	ND	9.50 ± 0.02^{c}
16	γ-Linoleadic acid C18:3n6	13.52 ± 0.01^{b}	ND	ND
17	*Cis*-8,11,14- Eicosatrienoic acid C20:3n6	ND	ND	2.91 ± 02^{f}
18	Erucic acid C22:1n9	9.00 ± 0.01^{d}	ND	ND
19	Lignoceric acid C24:0	4.40 ± 0.00^{f}	27.31 ± 0.11^{a}	22.00 ± 0.21^{a}
20	Nervonic acid C24:1	4.30 ± 0.03^{f}	ND	ND
	SFA (saturated fatty acids)	60.70 ± 0.33^{b}	70.45 ± 0.46^{a}	69.25 ± 0.51^{a}
	UFA (unsaturated fatty acids)	39.30 ± 0.21^{a}	29.55 ± 0.20^{b}	30.75 ± 0.21^{b}
	MUFA (monounsaturated fatty acids)	16.65 ± 0.41^{c}	27.01 ± 0.15^{a}	18.34 ± 0.11^{b}
	PUFA (polyunsaturated fatty acids)	22.65 ± 0.07^{a}	2.54 ± 0.10^{c}	12.41 ± 0.10^{b}

ND: not detected. Data represent the mean ± SD (n = 3) of three independent experiments. Means followed by the same.

served in all three species. *Chroococcidiopsis* sp. And *Gleocapsa* sp. Had higher ($p \leq 0.05$) MUFA contents compared to PUFA, while *Merismopedia* sp. Showed the opposite pattern. Saturated fatty acid contents in all three species were higher ($p \leq 0.05$) than the unsaturated fatty acid contents.

Caprylic, capric, pentadecanoic, heptadecanoic, palmitic, and lignoceric acids were available as saturated fatty acids in all species. Elaidic, palmitoleic, and *Cis*-10- heptadecanoic acids were prominent as MUFA. The presence of palmitic acid in all the studied species agrees with the previous observations on some cyanobacterial species (Anahas and Muralitharan, 2015, 2018). Nevertheless, all species did not show the same PUFA types.

3.5. Total phenolic content

As shown in Table 3, water extract of *Chroococidiopsis* sp. Had the highest ($p \leq 0.05$) phenolic content (2.26 ± 0.09 mg GAE/g), which was higher ($p \leq 0.05$) than that in its methanol extract. *Chroococciodpsis* sp. And *Merismopedia* sp. Showed significantly different TPC values ($p \leq 0.05$), while *Gleocapsa* sp. Had similar values ($p \leq 0.05$) for methanol and water extracts (1.16 ± 0.07 and 1.17 ± 0.06 mg GAE/g.

3.6. Total flavonoid content

The highest ($p \leq 0.05$) flavonoid content (Table 3) was observed in the methanol extract of *Merismopedia* sp. (0.87 ± 0.18 mg CE/g), while the lowest ($p \leq 0.05$) was found in *Gleocapsa* sp. (0.07 ± 0.00 mg CE/g). When considering all three species, the methanol

Table 3
Total phenolic content of the three species of cyanobacteria.

Species	TPC in water (mg GAE/g)	TPC in methanol (mg GAE/g)	TFC in water (mg CE/g)	TFC in methanol (mg CE/g)
Chroococcidiopsis sp.	2.26 ± 0.09^{a}	1.59 ± 0.06^{b}	0.29 ± 0.54^{cd}	0.57 ± 0.01^{b}
Gleocapsa sp.	1.17 ± 0.06^{c}	1.16 ± 0.07^{c}	0.07 ± 0.00^{d}	0.17 ± 0.05^{d}
Merismopedia sp.	0.54 ± 0.01^{d}	1.47 ± 0.16^{b}	0.43 ± 0.09^{bc}	0.87 ± 0.18^{a}

Data represent the mean ± SD (n = 3) of three independent experiments. Means followed by the same letters in a row are not significantly different (P < 0.05).

extract exhibited higher ($p \leq 0.05$) TFC than the water extracts. Although a significant ($p \leq 0.05$) difference in flavonoid content was observed between water and methanol extracts of *Chroococcidiopsis* sp. And *Merismopedia* sp., there was no significant difference ($p \leq 0.05$) in flavonoid content between the water and methanol extracts of *Gleocapsa* sp.

3.7. DPPH activity

Among all the tested cyanobacteria species (Table 4), *Gleocapsa* sp. Exhibited the highest IC_{50} value in its water extract ($p \leq 0.05$), while the methanol extract of *Gleocapsa* sp. Showed the lowest IC_{50} value ($p \leq 0.05$). With the exception of *Chroococidiopsis* sp., the other two species demonstrated lower IC_{50} values ($p \leq 0.05$) in their methanol extracts, indicating higher antioxidant activities.

3.8. ABTS activity

As shown in Table 4, the methanol extract of *Chrooccocidiopsis* sp. Exhibited the highest ($p \leq 0.05$) ABTS antioxidant activity, while the water extract of *Gleocapsa* sp. Displayed the lowest ($p \leq 0.05$) activity. Both water and methanol extracts of *Chrooccocidiopsis* sp. And *Gleocapsa* sp. Showed a significant difference ($p \leq 0.05$), but this difference was not observed in the case of *Merismopedia* sp.

3.9. ORAC activity

As shown in Table 4, *Merismopedia* sp. Exhibited the highest ($p \leq 0.05$) ORAC for both water and methanol extracts. A significant ($p \leq 0.05$) difference was observed between the ORAC values obtained for the water and methanol extracts of all three species. The water extract of *Gleocapsa* sp. Displayed the lowest ($p \leq 0.05$) ORAC value.

3.10. Correlation

When considering the total phenolic and flavonoid contents, all three species exhibited a negative correlation with IC_{50} values, indicating a weak positive correlation ($p \leq 0.05$) with DPPH and ABTS antioxidant activities, as shown in Table 5. ORAC values exhibited a strong positive correlation ($p \leq 0.05$) with the flavonoid content in studied cyanobacteria species.

4. Discussion

Our analysis, including FTIR-ATR examination, assessment of antioxidant properties, and determination of fatty acid composition, revealed notable differences among studied three species of cyanobacteria. The biomass content of cyanobacterial species serves as an indicator of their growth rate and survival capabilities. The highest biomass content observed in *Chroococcidiopsis* sp. May be attributed to its unique ability to thrive in extreme conditions, including its recent recognition as a candidate for Martian exploration

Table 4
Antioxidant activities of the three species of cyanobacteria.

Species	Extract	DPPH activity IC_{50} (mg/mL)	ABTS activity (mmol TE/g)	ORAC activity (mmol TE/mg)
Chroococcidiopsis sp.	Water	19.71 ± 0.93^{cd}	12.65 ± 0.96^{ab}	16.88 ± 0.70^{e}
	Methanol	44.51 ± 0.04^{b}	13.28 ± 0.36^{a}	45.75 ± 5.61^{c}
Gleocapsa sp.	Water	88.84 ± 10.26^{a}	2.38 ± 0.11^{d}	8.00 ± 0.52^{f}
	Methanol	15.91 ± 1.76^{cd}	7.60 ± 0.18^{c}	22.84 ± 1.76^{d}
Merismopedia sp.	Water	27.32 ± 2.83^{c}	12.03 ± 0.27^{b}	70.28 ± 5.00^{b}
	Methanol	15.21 ± 5.65^{d}	7.89 ± 0.53^{c}	112.50 ± 5.79^{a}

Data represent the mean ± SD (n = 3) of three independent experiments. Means followed by the same letters in a row are not significantly different (P < 0.05) for each antioxidant activity.

Table 5
Correlation of TPC and TFC with antioxidant assays.

	DPPH	ABTS	ORAC
TPC	−0.209	0.268	−0.227
TFC	−0.456	0.381	0.902

Each value is expressed according to the Pearson correlation coefficient (r) significantly different ($p \leq 0.05$).

(Fewer et al., 2002; Assuncao et al., 2021). Nonetheless, the potential applications of these cyanobacteria species in the realms of food, feed, pharmaceuticals, and cosmetics have been slow to progress (Assuncao et al., 2021).

The FTIR-ATR analysis confirmed the presence of distinct functional compounds within these studied cyanobacteria species. Typically, cyanobacterial spectra are characterized by the prevalence of proteins and lipids, with carbohydrates being less abundant. The nutritional availability and the proportions of these macromolecular components vary among species (Ozer et al., 2019). In accordance with research studies, bands within the range of 3600–3380 cm^{-1} indicate *O*–H and *N*–H stretching in water, proteins, and phenolic groups, which are responsible for antioxidant activities. The spectral bands in the region of 3000–2855 cm^{-1} represent the stretching of CH_2 and CH_3, primarily in lipids and carbohydrates, while around 1720 cm^{-1}, there is C = O stretching in esters found in phospholipids and fatty acids such as palmitic, linoleic, linolenic, stearic, capric, and palmitoleic acids (Ozer et al., 2019).

Research findings from Montero-Lobato et al. (2020) suggest that cyanobacteria contain a substantial amount of proteins. Moreover, according to Ozer et al. (2019), bands around 1660 cm^{-1} indicate C = O stretching in the amide I, 1520 cm^{-1} represent *N*–H bending in amide II, and *C*–N stretching in proteins, while 1450 cm^{-1} corresponds to CH_2 and CH_3 bending in proteins and CH_2 bending in lipids. Bands around 1420 cm^{-1} are associated with CH_2 and CH_3 bending in proteins and *C*–O of COO groups in a carboxylic acid.

The spectrum around 1250 cm^{-1} is associated with nucleic acids, other compounds containing phosphate, and the P = O stretching of phosphodiesters. Cyanobacteria are known for their abundant production of polysaccharides (Singh et al., 2013), and the spectra observed at 1190-1170 cm^{-1} correspond to *C*–*O*–C bonds present in these polysaccharides (Ozer et al., 2019).

Among the three species examined (Fig. 2), *Chroococcidiopsis* sp. And *Merismopedia* sp. Exhibited spectra that were quite similar to each other, with most of the bands mentioned earlier. In contrast, *Gleocapsa* sp. Displayed distinct results, particularly in the 1500-1000 cm^{-1} range of the spectrum, which could be attributed to variations in the composition of proteins, lipids, and polysaccharides among these species. Additionally, all three cyanobacteria species demonstrated *O*–H and *N*–H stretching, potentially indicative of their antioxidant activities. In light of these findings, all three of these species displayed bonds related to a variety of nutritional and functional compounds, which could prove valuable for food and pharmaceutical production.

The unsaturated-to-saturated fatty acid ratios (U: S) obtained for these three cyanobacteria species align with a previous study by Caudales et al. (2000). Unsaturated fatty acids are crucial for maintaining heart health and preventing chronic heart diseases (Martinez-Frances and Escudero-Onate, 2018). Caprylic, capric, pentadecanoic, heptadecanoic, palmitic, and lignoceric acids were present as saturated fatty acids in all species, while elaidic, palmitoleic, and *cis*-10-heptadecanoic acids were notable as monounsaturated fatty acids (MUFA). The presence of palmitic acid in all species was corroborated by a previous study that reported the presence of palmitic acid in 29 cyanobacteria strains isolated from hot springs in Indonesia (Prihantini et al., 2018). However, not all species exhibited the same types of polyunsaturated fatty acids (PUFA). These findings indicate that saturated fatty acids predominate over unsaturated fatty acids, a trend supported by the findings of Caudales et al. (2000) and Prihantini et al. (2018). Additionally, Aboim et al. (2016) found variations in the availability of MUFA and PUFA in different cyanobacteria species, which further supports these observations. Our results for palmitic and caproic acids closely align with the findings of Aboim et al. (2016), who conducted a study on cyanobacteria species including *Microcystis aeruginosa*, *Synechocystis* sp., *Cyanobium* sp., and *Leptolyngbya* sp. *Gleocapsa* sp. Had the highest ($p \leq 0.05$) saturated fatty acid content, while *Merismopedia* sp. Showed the highest ($p \leq 0.05$) un saturated fatty acid content and the differences in fatty acid availability among the three species in our study are likely influenced by intrinsic factors. According to the literature, fatty acid synthesis is affected by species, growth stage, culture age, as well as external parameters such as pH, medium composition, nitrogen source, degree of aeration, temperature, and incident light intensity (Tonon et al., 2002). Moreover, these intrinsic and extrinsic factors depend on the mode of cultivation, be it continuous, semi-continuous, or batch-wise (Pernet et al., 2003). Excessive light can lead to photo-inhibition of biosynthesis, potentially reducing the fatty acid content, although extended growth stages may result in increased eicosapentaenoic acid (EPA) production (Guedes et al., 2011). Despite the extensive research on the nutritional aspects of cyanobacteria species, their potential as a viable source for fatty acid substrates remains an understudied area (Ronald et al., 2023). These findings have the potential to be applied in the production of fatty acid supplements and other food products, enhancing their nutritional value.

Phenolic are aromatic compounds with one or more hydroxyl groups, which can include flavonoids (flavonols, flavanones, flavones, flavonols, and anthocyanidins), isoflavonoids, and phenolic compounds such as phenolic acids, hydroxycinnamic acids, hydroxybenzoic acids, gallic acid, protocatechuic acid, homogentisic acid, tannins, oxidized polyphenols, and more (Carbonell-Capella et al., 2014). These compounds may act as antioxidants, metal chelates, peroxide decomposers, and oxygen scavengers in biological systems. While *Gleocapsa* sp. Exhibited similar values ($p \leq 0.05$) for methanol and water extracts, *Chroococcidiopsis* sp. And *Merismopedia* sp. Displayed different values ($p \leq 0.05$). Previous studies have shown that the total phenolic content (TPC) of various cyanobacteria species (e.g., *Phormidium* sp, *Synechocystis* sp., *Nodosilinea* sp., *Cyanobium* sp., and *Tychonema* sp.) falls within the range of 1.07–2.45 mg GAE/g in ethanolic extracts, consistent with our results (Morone et al., 2020). The values obtained for TPC and total flavonoid content (TFC) in *Chroococcidiopsis* sp. Closely match those found by Del Mondo et al. (2021). However, Hossain et al. (2016) reported higher TPC values in water extracts of four cyanobacteria species (*Oscillatoria* sp., *Lyngbya* sp., *Microcystis* sp., and *Spirulina* sp.) compared to those obtained in this study (ranging from 1.78 mg GAE/g to 5.02 mg GAE/g). Jeevanantham et al. (2019) found lower values for phenolic content in 80% methanolic extracts of *Synechococcus* sp., *Oscillatoria* sp., and *Phormidium* sp. (0.316 mg GAE/g - 0.772 mg GAE/g) compared to our results. These discrepancies may arise from variations in phenolic compounds between species and differences in extraction methods. Notably, the water-soluble phenols were found to be more abundant ($p \leq 0.05$) in these species compared to methanol-soluble phenols.

Flavonoids found in cyanobacteria have been recognized for their beneficial health properties, including antimicrobial and anti-inflammatory activities. However, the results presented here diverge from recent studies by Hossain et al. (2016), potentially owing

to the greater solubility of flavonoids in methanol compared to water. There were significant variations in the flavonoid content among the samples, which may be attributed to species-specific differences.

Cyanobacteria have been identified as sources of natural color pigments known as phycobiliproteins, which include phycoerythrin, phycocyanin, and allophycocyanin. Additionally, common carotenoids such as β-carotene, zeaxanthin, echinenone, ketocarotenoid, and myxoxanthophyll play a role in the bioactivities of these organisms (Prasanna et al., 2010). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is employed to assess the radical scavenging capacity of antioxidants. Singh et al. (2017) reported antioxidant activities in methanol extracts of 20 cyanobacteria species using the DPPH method, with IC_{50} values ranging from 0.91 mg/mL to 8.91 mg/mL. These values were lower than those reported by Hossain et al. (2016). However, the values obtained by this study were found to be somewhat in agreement with the findings by Hossain et al. (2016). IC_{50} values ranged from 15.21 to 88.84 mg/mL for the studied three species. Coulombier et al., (2020) studied IC_{50} values for *Dunaliella* sp and *Cylindrotheca closterium* were as 0.89 mg of dry extract·mL^{-1} and *Nitzschia* sp. 0.49 mg of dry extract·mL^{-1} which showed higher DPPH activities than our three species. Babic et al., 2016 showed the DPPH activities as 30.72, 47.62, 50.54, 102.47, 93.25 IC_{50} (μg mL^{-1}) *in Calothrix* M2, *Phormidium* M1, *Anabaena* M2, *Nostoc* M1, *Oscillatoria* M2 respectively. These activities were higher than our study. Singh et al., (2017) studied species also had *Cylindrospermum* sp.; 1.27 mg/mL cell-free extract and *Microcheate tenera*; 4.28 mg/mL cell-free extract values.

The ABTS assay is another important measure of antioxidant potential, which evaluates a sample's ability to scavenge the 2,2′-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) radical cation in comparison to a Trolox standard. Shanab et al. (2012) demonstrated higher ABTS activity in water extracts than in 70% methanol extracts of *Oscillatoria* sp., which aligns with the results for *Merismopedia* sp. In this study. However, studies by Assuncao et al. (2021) and Lomakool et al. (2021) on marine *Chroococcidiopsis* sp. Yielded differing values for ABTS activity. ABTS activities for the studied three species were at the range of 7.60–13.28 mmol TE/g. Coulombier et al., (2020) have conducted studies to find the ABTS activities in several cyanobacteria species. *Schizochlamydella* sp. And *Nitzschia* sp. Have shown IC_{50} values as >1000 in μg of dry extract·mL^{-1}. In *Nephroselmis* sp. 311.08 μg of dry extract·mL^{-1} and *Tetraselmis* sp. As 193.17 μg of dry extract·mL^{-1}. Singh et al., (2017) studied *Anabaena constricta* and *Cylindrospermum* sp. Showed 0.23 and 0.32 ABTS antioxidant power IC_{50} as mg mL^{-1} cell-free extract while *Oscillatoria acuta* has shown 2.63 (IC_{50}) mg mL^{-1} cell-free extract.

The Oxygen Radical Absorbance Capacity (ORAC) assay is used to evaluate the effect of potential antioxidants by measuring fluorescence quenching, detecting both hydrophilic and hydrophobic antioxidant activities in aqueous extracts (Schaich et al., 2015). Reported ORAC data in *Nostoc lobatus* were consistent with our study, but Lopez-Rodriguez et al. (2021) presented contrasting results. ORAC activities for studied species ranged from 8.00 to 112.50 mmol TE/mg DW. Pandey and Pandey (2008) have mentioned *Nostochopsis lobatus* has shown 27.20–59.07 μmol TE/g of fresh wt for ORAC assay which values were at the range of studied species. Lopez-Rodriguez et al., (2021) studied ORAC capacities for *Arthrospira maxima* and *Arthrospira platensis* as 0.37 and 0.26 mmol TE/g dry extract and they were lower than our values. Some of the species studied by Coulombier et al., (2020) showed variation in activities for different species; *Nephroselmis* sp. (188.32 μg TE/·mg of dry extract), *Chaetoceros* sp. (190.3 μg TE/·mg of dry extract) *Thalassiosira weissflogi* (27.71 μg TE/·mg of dry extract) *Picochlorum* sp. (55.17 μg TE/·mg of dry extract).

The growth rate and survival abilities are higher in *Chroococcidiopsis* sp. Than the other two species, while it has the highest ($p \leq 0.05$) phenolic content. Overall, methanolic extracts of *Merismopedia* sp. Appeared to constitute a more significant ($p \leq 0.05$) antioxidant source than the other two species. However, it is noteworthy that previous studies have indicated the presence of toxins in some *Merismopedia* isolates (Jakubowska and Szelag-Wasielewska, 2015), specifically microcystin, and further studies are needed to investigate the presence of toxins in these Cyanobacteria species. *Gleocapsa* sp. Also possessed significant ($p \leq 0.05$) antioxidant properties.

These antioxidant effects are of great importance in human and animal bodies, offering protection against oxidative stress, which is critical in preventing non-communicable diseases such as diabetes mellitus, cancers, and cardiovascular diseases (Thecla et al., 2022). While *Chroococcidiopsis* sp. Exhibited the highest total phenol content, methanolic extracts of *Merismopedia* sp. Appear to serve as a more substantial source of antioxidants than the other two species, with *Gleocapsa* sp. Also displaying noteworthy antioxidant properties.

The findings of this study further emphasize the potential of cyanobacteria for extracting nutritional compounds, antioxidants, and short-chain fatty acids. Out of all the investigated cyanobacteria species, *Merismopedia* sp. Stands out as a promising source of antioxidants and unsaturated fatty acids, positioning it as a compelling choice for utilization in the food, cosmetic, and pharmaceutical sectors. Certain cyanobacterial species produce cyanotoxins which could be within the cyanobacterial cells. These toxins are hazardous and according to the literature, *Merismopedia* species have the potential to produce cyanotoxins, specifically microcystin. Therefore, before utilizing these in the industry, screening is necessary to determine the potential cyanotoxin production.

5. Conclusion

Significant associations ($p \leq 0.05$) were observed between the bioactive compounds of these cyanobacterial species and their antioxidant activities. *Chroococcidiopsis* sp. Was identified as a valuable source of phenolic compounds. However, among all the species, the methanol extract of *Merismopedia* sp. Displayed the most potent antioxidant activities. All these studied species stood out as an excellent source of unsaturated fatty acids, which could have notable benefits for the food and pharmaceutical industries. Therefore, exploring the unveiling of novel and unique molecules in these cyanobacterial species that have not yet been explored for commercial purposes is crucial. However, the some of these Cyanobacterial isolates may contain toxins which are detrimental to human health and further studies are needed before exploiting them for bioactive or pharmaceutical properties.

Ethical approval and consent to participate

Not applicable.

Consent for publication

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon a reasonable request.

CRediT authorship contribution statement

Malmi Wickramasinghe: Writing – original draft. **Kashmi Katyana:** Formal analysis. **Kaushalya Sewwandi:** Formal analysis. **Isuri Rathnayaka:** Data curation, Formal analysis. **Dhammika Magana-Arachchi:** Investigation, Project administration, Resources, Writing – review & editing. **Barana Jayawardana:** Supervision, Writing – review & editing. **Ruvini Liyanage:** Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Abbreviations Used

TPC	Total phenolic content
TFC	Total flavonoid content
DPPH	2,2-diphenyl-1-picrylhydrazyl
ABTS	2,2′ azino-bis (3-ethylbenzothiazoline-6-sulfonate)
ORAC	Oxygen radical antioxidant capacity assay
Trolox	6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
DW	Dry weight
UV	Ultraviolet
TE	Trolox equivalent
GAE	Gallic acid equivalent
CE	Catechin equivalent
GC	Gas Chromatography
SFA	Saturated Fatty Acids
UFA	Unsaturated Fatty Acids
PUFA	Poly Unsaturated Fatty Acids
MUFA	Mono Unsaturated Fatty Acids
FTIR-ATR	Fourier Transform Infrared Spectroscopy with attenuated total reflectance

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2023.103015.

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