

Extraction and Characterization of Bioactive Metabolites from an Axenically Cultured Cyanobacteria *Microcystis Aeruginosa*

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Abstract

Cyanobacteria are recognized as promising sources of diverse metabolites with potential applications in pharmaceutical, nutraceutical and biotechnological fields. The present study investigated the bioactive compound profile of an axenically cultured isolate *Microcystis aeruginosa* through solvent extraction, quantitative estimation and gas chromatography–mass spectrometry (GC-MS) analysis. The polyphenol and phenolic contents were estimated as 0.31 and 0.24 µg/mg extract, respectively, whereas the flavonoid, alkaloid, terpenoids and saponin contents were 9.84, 110.52 and 6.6 and 165 µg/mg extract, respectively. GC-MS analysis revealed a diverse chemical profile comprising hydrocarbons, fatty acid derivatives, terpenoid-related compounds and other organic constituents. Among the identified constituents were isopropyl myristate, phytol, n-hexadecenoic acid, hexadecane, heptadecane, octadecane and other hydrocarbon derivatives. The findings indicate that the axenically cultured *M. aeruginosa* isolate contains diverse extractable metabolites and measurable quantities of several bioactive compound groups. The study contributes to the chemical characterization of an axenically cultivated *M. aeruginosa* isolate and provides a basis for further investigation and structural characterization of its bioactive constituents.

Keywords: *Microcystis aeruginosa*, Cyanobacteria, Phytochemicals, Methanolic extract, GC–MS, Bioactive metabolites, Secondary metabolites, Metabolite profiling.

1. Introduction

Cyanobacteria are photosynthetic prokaryotic microorganisms distributed across freshwater, marine and terrestrial environments. In addition to their ecological importance as primary producers, cyanobacteria have attracted considerable scientific interest because of their ability to synthesize structurally diverse primary and secondary metabolites. These metabolites include pigments, fatty acids, peptides, alkaloids, terpenoid compounds, polysaccharides and numerous specialized metabolites with biological activities of potential pharmaceutical and biotechnological relevance [1], [2], [3], [4].

The chemical diversity of cyanobacteria is particularly notable among members of the genus *Microcystis*. *Microcystis* is widely distributed in freshwater environments and is well known for forming dense blooms. Although *microcystis* have historically received considerable attention because of their toxicological importance, current research demonstrates that the secondary metabolism of *Microcystis* is substantially broader than microcystin production alone [5], [6]. Other metabolite classes reported from *Microcystis*

include aeruginosins, cyanopeptolins, microginins, microviridins, cyanobactins, anabaenopeptins and other peptide-related metabolites [6], [7], [8], [9], [10].

The biosynthetic potential of *Microcystis* is associated with numerous biosynthetic gene clusters distributed throughout its genome. Recent studies indicate that the presence and expression of these biosynthetic pathways vary among strains, contributing to substantial chemical diversity within the genus [6]. Consequently, metabolite composition observed in one *Microcystis* strain cannot necessarily be assumed to represent another strain. This strain-dependent chemical variability provides an important rationale for investigating individual axenic isolates under controlled culture conditions.

Several analytical approaches have been used for characterization of cyanobacterial metabolites. Gas chromatography coupled with mass spectrometry (GC–MS) is particularly useful for the analysis of volatile and semi-volatile compounds and derivatized metabolites, while liquid chromatography coupled with mass spectrometry (LC–MS and LC–MS/MS) is widely used for larger, less volatile and thermolabile secondary metabolites such as cyanopeptides [6], [11]. The complementary nature of these techniques allows different portions of the chemical space of cyanobacteria to be investigated.

GC–MS-based investigations have demonstrated that *M. aeruginosa* contains a broad range of volatile metabolites. A study using headspace solid-phase microextraction coupled with GC–MS reported 210 volatile metabolites from *M. aeruginosa*, demonstrating the chemical complexity detectable by GC–MS [11]. Another investigation of *M. aeruginosa* biomass from the Muttukadu estuary used methanolic extraction and chromatographic and spectroscopic approaches to characterize secondary metabolites, further demonstrating the usefulness of organic solvent extraction for cyanobacterial metabolite studies [12].

In contrast, LC–MS/MS studies have revealed extensive diversity among peptide-based metabolites of *M. aeruginosa*. McDonald et al. reported 82 cyanopeptides from five *Microcystis* strains, including cyanopeptolins, microviridins, microginins, cyanobactins, anabaenopeptins, aeruginosins and microcystins. Importantly, the metabolite profiles differed among strains [13]. Similarly, Stewart et al. used mass-spectrometry-guided metabolomics of a cultured *M. aeruginosa* strain to discover five microginin peptides, whose structures were subsequently investigated using spectroscopic and chemical methods [14]. These studies demonstrate that mass spectrometry can provide valuable evidence of chemical diversity, while also emphasizing the need for appropriate structural confirmation when individual compounds are reported.

Phytochemical screening provides another relatively simple approach for obtaining an initial overview of the chemical groups present in biological extracts. Colorimetric methods have been widely applied for estimation of total phenolic compounds, flavonoids, tannins and related constituents [15], [16], [17], [18], [19]. Although such assays do not establish the identity of individual molecules, they provide useful quantitative information regarding broad classes of extractable compounds and can support subsequent chromatographic characterization.

The choice of extraction solvent is an important factor in metabolite profiling because solvent polarity strongly influences the classes of compounds recovered. Aqueous methanol is frequently used for extraction of cyanobacterial metabolites because it can recover a broad range of moderately polar compounds. However, extraction yield alone does not necessarily reflect total cellular metabolite content, since extraction efficiency depends on biomass condition, solvent composition, extraction time, temperature and the physicochemical properties of individual metabolites [20], [21].

The present investigation therefore focused specifically on an axenically cultured *M. aeruginosa* isolate maintained under controlled laboratory conditions. The study aimed to obtain an 80% methanolic extract of the dried biomass, determine its extraction yield, quantitatively estimate selected phytochemical groups and characterize the extract by GC–MS. The objective was not to assign a complete metabolome or to establish definitive structures for individual GC–MS features, but rather to generate an initial chemical profile that could guide further isolation, characterization and application-oriented studies.

2. Materials and Methodology

2.1 Isolation, characterization and Biomass production of Cyanobacterial Isolate

An axenically cultured cyanobacterial isolate designated C-16 was used for the present investigation. The isolate was originally collected from a freshwater canal at Eru Gam Canal, Eru Road, Jalalpore, Navsari, Gujarat, India. Following isolation and purification, the culture was maintained in BG-11 medium under controlled laboratory conditions. The culture was incubated at room temperature under a 16:8 h light photoperiod. A working culture volume of 500 mL was maintained and allowed to grow for 20 days prior to biomass harvesting. Molecular identification was performed based on 16S rRNA gene sequencing, and the corresponding sequence was deposited in the NCBI GenBank database.

Axenic culture conditions were maintained throughout the experimental period to minimize interference from associated heterotrophic microorganisms and to obtain biomass suitable for metabolite characterization. Following the 20-day cultivation period, the cyanobacterial biomass was separated from the culture medium by centrifugation. The recovered biomass was collected and subjected to drying. Initial drying was performed under shade, followed by drying in a hot-air oven at 45 °C until a suitable dry biomass was obtained. The dried biomass was subsequently weighed and used for extraction.

2.2 Preparation of Methanolic extract and determination of extraction yield

For extraction, 2 g of dried *Microcystis aeruginosa* biomass was transferred to a suitable extraction vessel and mixed with 40 mL of 80% methanol. The biomass-solvent mixture was subjected to maceration followed by sonication to facilitate disruption of the biomass and improve the release of extractable metabolites. After extraction, the mixture was separated from the residual biomass, and the resulting extract was concentrated under suitable laboratory conditions. The recovered crude extract was weighed and stored appropriately until further analysis. The use of methanolic extraction is consistent with previous investigations of secondary metabolites from *M. aeruginosa* and other cyanobacteria [12], [21], [22], [23]. The extraction yield was calculated based on the mass of dried crude extract obtained relative to the initial mass of dried biomass using the following equation:

$$\text{Extraction yield (\%)} = (\text{Mass of dried extract} \div \text{Mass of dried biomass}) \times 100 \quad (1)$$

2.3 Quantitative estimation of selected bioactive compound groups

The methanolic extract of *Microcystis aeruginosa* was subjected to quantitative spectrophotometric estimation of selected bioactive compound groups, including total phenolics, polyphenols, flavonoids, alkaloids, terpenoids and saponins. Appropriate reagent blanks and calibration standards were used for the respective assays. The results were expressed as micrograms of the corresponding standard equivalent per milligram of crude extract ($\mu\text{g}/\text{mg}$ extract), unless otherwise specified.

2.3.1 Determination of Total Phenolic & Total Polyphenolic Content

Total phenolic content was determined using the Folin–Ciocalteu colorimetric method reported by Singleton and Rossi [15]. Briefly, appropriate dilutions of the methanolic extract were reacted with diluted

Folin–Ciocalteu reagent and sodium carbonate and allowed to stand for 15 min. Absorbance was measured at 760 nm. Gallic acid was used as the reference standard, with a calibration range of 0–50 µg/mL. The results were expressed as µg gallic acid equivalents per mg extract (µg GAE/mg extract).

Total polyphenolic content was estimated using a Folin–Ciocalteu-based colorimetric assay. Appropriate dilutions of the extract were reacted with diluted Folin–Ciocalteu reagent and sodium carbonate and incubated for 15 min. Absorbance was measured at 630 nm. Gallic acid was used as the reference standard, and a calibration range of 25–300 µg/mL was employed. The results were expressed as µg gallic acid equivalents per mg extract (µg GAE/mg extract).

2.3.3 Determination of Total Flavonoid Content

Total flavonoid content was determined using the aluminium chloride colorimetric method described by Chang et al. [16], [17]. Appropriate dilutions of the extract were reacted with 10% aluminium chloride, ethanol and sodium acetate and incubated for 30 min at room temperature in the absence of direct light. Absorbance was measured at 415 nm. Quercetin was used as the reference standard, and the results were expressed as µg quercetin equivalents per mg extract (µg QE/mg extract).

2.3.4 Determination of Total Alkaloid Content

Total alkaloid content was determined using the bromocresol green (BCG) complexation method described by Ajanal et al. [18] with atropine as the reference standard. Appropriate extract dilutions were acidified with hydrochloric acid, followed by removal of interfering constituents, neutralization and formation of the alkaloid–BCG complex. The complex was extracted into chloroform and the resulting fraction was subjected to spectrophotometric measurement at 470 nm. The alkaloid content was calculated from the atropine calibration curve and expressed as µg atropine equivalents per mg extract (µg AE/mg extract).

2.3.5 Determination of Total Terpenoid Content

Total terpenoid content was estimated using the colorimetric procedure described by Ghorai et al. [19]. Briefly, the biomass was extracted with hexane (1:1, v/v), and the pooled extract was concentrated. Chloroform and concentrated sulfuric acid were subsequently added and the reaction mixture was incubated in the dark, followed by addition of methanol. Absorbance was measured at 538 nm. Linalool was used as the reference standard, and the results were expressed as µg linalool equivalents per mg dry weight (µg LE/mg DW).

2.3.6 Determination of Total Saponin Content

Total saponin content was estimated using the vanillin–sulfuric acid colorimetric reaction [24]. Appropriate sample dilutions were reacted with vanillin reagent and concentrated sulfuric acid, followed by heating at 60–70 °C for 5 min and subsequent cooling. Absorbance was measured at 492 nm. The saponin content was calculated from the corresponding calibration curve and expressed as µg standard equivalent per mg of sample.

2.4 Estimation of Photosynthetic Pigments

Photosynthetic pigments were estimated spectrophotometrically using a DMSO-based extraction procedure following Wellburn et al. [20]. An accurately weighed portion of *M. aeruginosa* biomass was homogenized in DMSO and centrifuged at 10,000 rpm for 15 min at 4 °C. The supernatant was collected, and 0.5 mL was diluted with 4.5 mL DMSO. Absorbance was measured at 480, 649 and 665 nm. Chlorophyll-a, chlorophyll-b and total carotenoids were calculated using the following equations [20]:

$$\text{Chlorophyll-a } (\mu\text{g/mL}) = 12.47 \times A_{665} - 3.62 \times A_{649} \quad (2)$$

$$\text{Chlorophyll-b } (\mu\text{g/mL}) = 25.06 \times A_{649} - 6.5 \times A_{665} \quad (3)$$

$$\text{Total carotenoids } (\mu\text{g/mL}) = (1000 \times A_{480} - 1.29 \times \text{Chl-a} - 53.78 \times \text{Chl-b}) \div 220 \quad (4)$$

The calculated pigment concentrations were expressed relative to the dry biomass used for extraction.

2.5 Gas chromatography–mass spectrometry analysis

The methanolic extract was subjected to GC–MS analysis for characterization of volatile and semi-volatile constituents. The chromatographic separation generated individual peaks corresponding to compounds eluting at different retention times. The mass spectra associated with the detected chromatographic peaks were compared with entries in the NIST mass spectral library for tentative compound assignment. NIST provides extensive mass spectral and retention-index databases for analytical compound identification [22], [23].

The chromatographic parameters recorded included retention time, peak area, peak area percentage, peak height, height percentage, molecular formula, molecular weight and availability of compound name/library assignment. Because the compound assignments were based primarily on mass spectral library matching, the identified compounds were treated as tentative or putative identifications rather than definitive structural confirmations. Confirmation of individual compounds would require additional evidence such as authentic standards, retention-index agreement, high-resolution accurate mass and/or spectroscopic characterization [25].

3. Results

3.1 Morphological characterization, Culture establishment and Biomass preparation

The cyanobacterial isolate designated C-16 was successfully purified and maintained as an axenic culture in BG-11 medium. Morphological observations were consistent with the characteristics of the genus *Microcystis* and characterized as *Microcystis aeruginosa*. The established axenic culture was subsequently cultivated for biomass production. Following the 20-day cultivation period, the biomass was harvested by centrifugation and subjected to shade drying followed by drying at 45 °C. The resulting dried biomass was used for methanolic extraction and subsequent quantitative and GC–MS analyses.

3.2 Methanolic Extract Recovery

Extraction of 2 g of dried *M. aeruginosa* biomass with 40 mL of 80% methanol yielded approximately 15 mg of crude extract. The calculated extraction yield was therefore 0.75%.

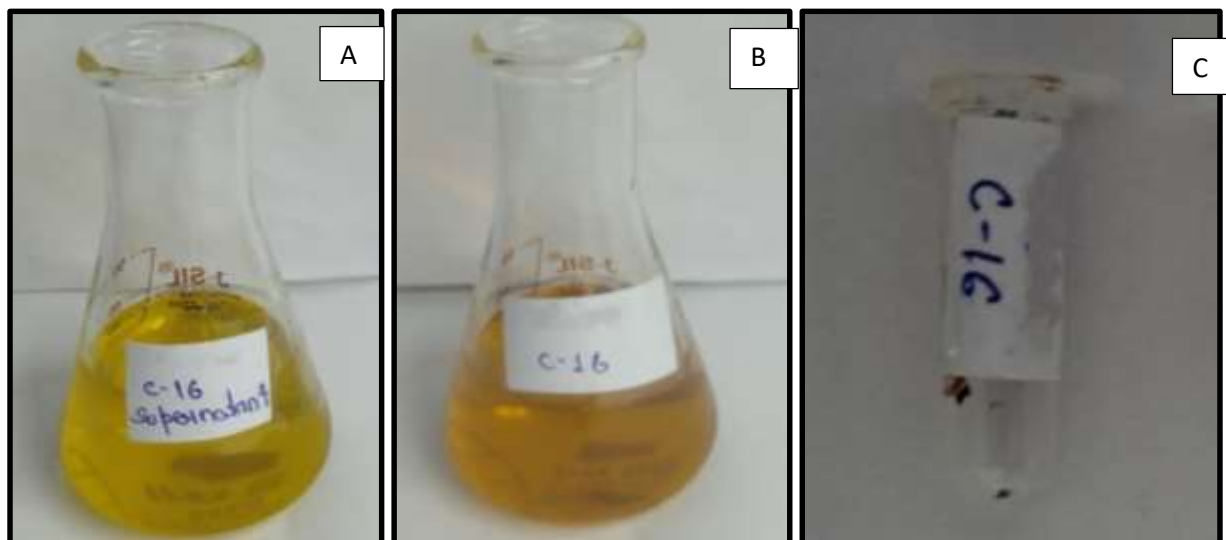
Table 1. Extraction yield of the 80% methanolic extract of *Microcystis aeruginosa*

No	Parameter	Value
1.	Initial dry biomass	2.0 g
2.	Extraction Solvent	80% Methanol
3.	Solvent Volume	40 mL
4.	Biomass-to-solvent ratio	1:20 (w/v)
5.	Crude extract obtained	15 mg
6.	Extraction yield	0.75%

The extraction yield represents the proportion of dried biomass recovered as crude methanolic extract and should not be interpreted as the total percentage of cellular metabolites.

Figure 1. Preparation of the 80% methanolic extract from *Microcystis aeruginosa* C-16.

(A) Extract after first centrifugation; (B) Extract after second centrifugation; (C) Concentrated crude methanolic extract.



3.3 Quantitative phytochemical composition

Quantitative analysis demonstrated measurable levels of the selected bioactive compound groups in the 80% methanolic extract (Table 2). The highest measured value among the reported groups was observed for saponins, followed by alkaloids, flavonoids, terpenoids, polyphenolics and phenolics.

Table 2. Quantitative phytochemical composition of *M. aeruginosa* methanolic extract

No.	Bioactive compound group	Reference standard	Expression	Content (mean $\pm$ SD)
1.	Total phenolics	Gallic acid	$\mu\text{g GAE/mg extract}$	0.24 ± 0.05
2.	Total polyphenolics	Gallic acid	$\mu\text{g GAE/mg extract}$	0.31 ± 0.06
3.	Total flavonoids	Quercetin	$\mu\text{g QE/mg extract}$	9.8 ± 0.05
4.	Total alkaloids	Atropine	$\mu\text{g AE/mg extract}$	110.52 ± 0.3
5.	Total terpenoids	Linalool	$\mu\text{g LE/mg dry weight}$	6.6 ± 2.37
6.	Total saponins	Dexamethasone	$\mu\text{g [standard equivalent]}/\text{mg sample}$	165 ± 2.1

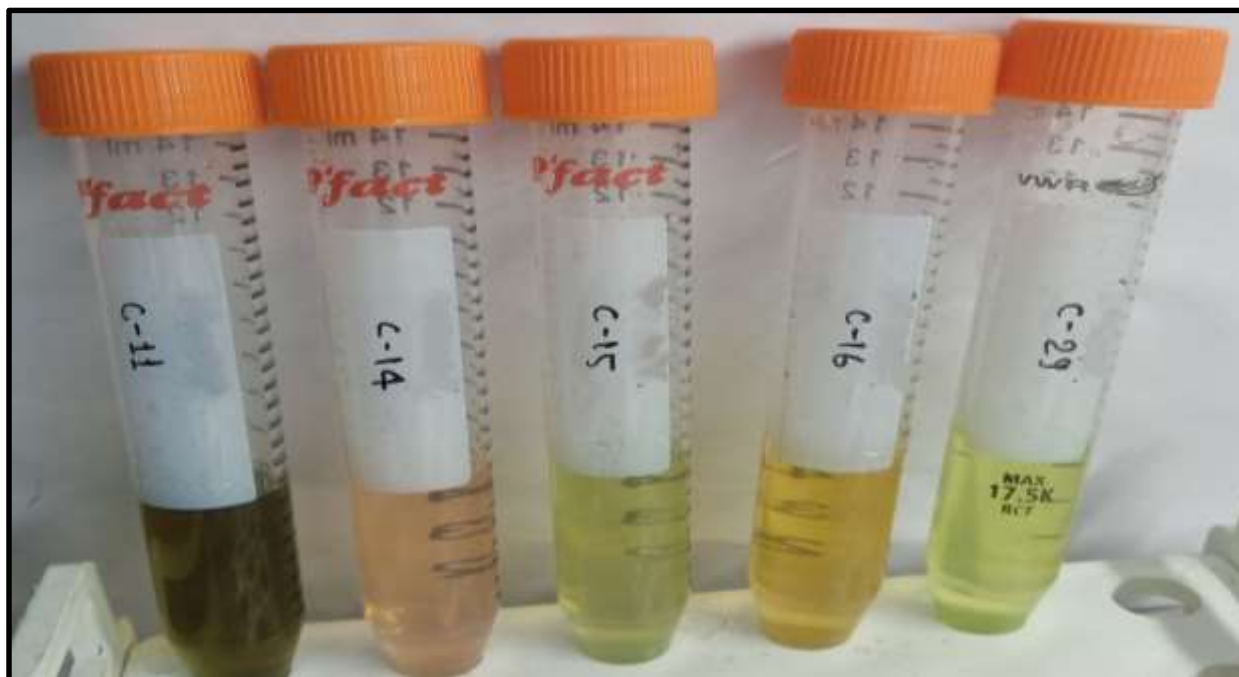
The measured values indicate that the methanolic extract contained appreciable amounts of compounds reacting with the respective phytochemical assays. However, these colorimetric estimations represent compound classes and do not establish the identity of individual molecules.

3.4 Photosynthetic pigment estimation

Spectrophotometric analysis of the methanolic/cell extract demonstrated the presence of photosynthetic pigments in *M. aeruginosa* C-16. Chlorophyll-a, chlorophyll-b and total carotenoids were estimated from their respective absorbance values using the equations described by Wellburn et al. [20]. The calculated pigment concentrations are presented in Table 3.

Table 3. Photosynthetic pigment content of *Microcystis aeruginosa* C-16

No	Pigment	Content (mean $\pm$ SD) (μ g/ml)
1.	Chlorophyll-a	2.30 $\pm$ 0.001
2.	Chlorophyll-b	0.23 $\pm$ 0.006
3.	Total carotenoids	1.50 $\pm$ 0.001

Figure 2. Photosynthetic pigment extract obtained from *Microcystis aeruginosa* C-16 biomass.


3.5 GC–MS profile

GC–MS analysis demonstrated the presence of multiple chromatographic components in the ethyl acetate extract with tentative library assignments to hydrocarbons, fatty-acid-related compounds and terpenoid/diterpenoid compounds.

Representative library assignments included isopropyl myristate, phytol, n-hexadecanoic acid, hexadecane, heptadecane and octadecane.

Table 3. Tentatively assigned compounds detected by GC-MS in the ethyl acetate extract of *Microcystis aeruginosa*

No	Retention time	Peak area (%)	SI	Molecular formula	Molecular weight	Retention index	Tentative compound
1.	3.516	19.5185	88	C ₃ H ₆ N ₂	70	763	CH ₃ NHCH ₂ CN
2.	6.419	4.4175	95	C ₁₃ H ₂₈	184	1185	Undecane, 4,7-dimethyl-
3.	7.162	2.4440	91	C ₁₂ H ₂₆	170	1150	Undecane, 2-methyl-
4.	9.926	6.7973	95	C ₁₂ H ₂₆	170	1086	Decane, 3,7-dimethyl-

5.	12.953	7.8962	95	C16H34	226	1612	Hexadecane
6.	13.550	4.4348	94	C14H30	198	1285	2,3-Dimethyldodecane
7.	16.365	4.2933	97	C18H38	254	1746	Heptadecane, 2-methyl-
8.	17.054	13.2647	94	C17H34O2	270	1814	Isopropyl myristate
9.	17.197	3.0552	90	C20H40O	296	2045	Phytol
10	18.447	2.3888	92	C18H38	254	1810	Octadecane
11	18.733	7.0855	92	C16H32O2	256	1968	n-Hexadecanoic acid
12	20.141	2.4387	92	C17H36	240	1711	Heptadecane
13	20.551	2.0476	92	C28H58	394	2804	Octacosane
14	20.904	2.2813	95	C20H42	284	1945	Nonadecane, 2-methyl-
15	23.569	5.1444	97	C19H40	268	1846	Octadecane, 2-methyl-
16	32.636	12.4925	91	C42H63O3P	646	0	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite

4. Discussion

4.1 Characterization, culture establishment and biomass preparation

The molecular identification of isolate C-16 as *Microcystis aeruginosa* based on 16S rRNA gene sequencing provided confirmation of its taxonomic identity beyond morphological observation. The establishment and maintenance of an axenic culture provided a controlled source of biomass for subsequent extraction and chemical analyses. This is particularly relevant for metabolite investigations because cyanobacterial cultures may be associated with other microorganisms that can contribute metabolites to crude extracts. The use of an axenic culture therefore helps reduce potential contributions from associated microorganisms and improves the attribution of the observed chemical profile to the investigated cyanobacterial isolate. The biomass obtained after controlled cultivation was subsequently dried and used for extraction. Since biomass composition can vary with culture conditions, growth phase and environmental factors, maintaining defined cultivation conditions is important when comparing metabolite profiles among cyanobacterial isolates or between studies.

4.2 Methanolic extraction of *M. aeruginosa*

The present investigation produced a 0.75% crude extract yield from dried *M. aeruginosa* biomass using 80% methanol. Extraction yield is influenced by several experimental variables, including solvent polarity, biomass condition, extraction time, solvent-to-biomass ratio and the chemical characteristics of the metabolites present [21], [22], [23]. Therefore, the yield obtained in the present study should primarily be regarded as a measure of recovery under the specific extraction conditions used rather than a general characteristic of the species. The use of aqueous methanol provides a relatively broad extraction range and

has been employed in previous investigations of *M. aeruginosa* secondary metabolites. Vasudevan et al. [12] for example, investigated methanolic extracts of *M. aeruginosa* biomass for secondary metabolite characterization and subsequent biological assessment. Differences in extraction yield between studies may arise from differences in strain, culture conditions, biomass age, drying procedure, solvent composition and extraction protocol. Consequently, direct comparison of extraction yields between unrelated *Microcystis* studies should be made cautiously.

4.3 Quantitative estimation of selected bioactive compound groups

The quantitative analysis indicated the presence of measurable polyphenolic, phenolic, flavonoid and alkaloid-equivalent constituents in the 80% methanolic extract. The total phenolic value of 0.24 µg/mg extract was obtained using the Folin–Ciocalteu-based approach. The method is widely used for estimating phenolic-reducing capacity, although the reagent can also respond to other reducing substances. Thus, the value should be interpreted as a colorimetric estimate of total phenolic equivalents rather than a measurement of only chemically confirmed phenolic molecules [15].

The flavonoid content was estimated as 9.8 µg/mg extract. Flavonoid colorimetric assays are useful for comparative screening, but the response depends on the structures of individual compounds and their ability to form complexes with the reagent [16], [17]. Consequently, the value represents total flavonoid-equivalent material under the selected assay conditions. The alkaloid-equivalent content was 110.52 µg AE/mg extract. This value represents a colorimetric estimate expressed relative to atropine and should not be interpreted as the actual concentration of chemically confirmed alkaloids in the extract.

These findings support the presence of several chemically reactive phytochemical groups in the methanolic extract and provide a preliminary basis for subsequent chromatographic and spectroscopic characterization.

4.4 Photosynthetic pigments

The pigment analysis confirmed the presence of chlorophyll and carotenoid fractions in the cultured *M. aeruginosa* C-16 biomass. Chlorophyll-a was the predominant chlorophyll detected, with a concentration of 2.30 ± 0.001 µg/mL, whereas chlorophyll-b was detected at 0.23 ± 0.006 µg/mL. The total carotenoid content was 1.50 ± 0.001 µg/mL. The higher chlorophyll-a content relative to chlorophyll-b is consistent with the central role of chlorophyll-a in oxygenic photosynthesis in cyanobacteria.

Carotenoids are also important components of photosynthetic systems and contribute to light harvesting and photoprotection. The detection of 1.50 ± 0.001 µg/mL total carotenoids therefore provides additional evidence of the pigment-rich nature of the cultured biomass. However, pigment concentrations are influenced by factors including light intensity, nutrient availability, growth phase and culture conditions. Thus, the values obtained in the present study represent the pigment composition of C-16 under the specific cultivation conditions used and should not be interpreted as fixed species-level values.

4.5 GC–MS characterization of the extract

GC–MS analysis revealed a chemically heterogeneous chromatographic profile. Among the representative assignments were phytol, n-hexadecanoic acid, octadecane, hexadecane, heptadecane and isopropyl myristate. Phytol is a diterpenoid alcohol associated with chlorophyll metabolism and has been reported from photosynthetic organisms. Its detection in the present cyanobacterial extract is therefore chemically plausible. However, because the present identification is based on library matching, the assignment should remain tentative until supported by additional analytical evidence.

n-Hexadecanoic acid, commonly known as palmitic acid, is a saturated fatty acid frequently reported in microbial and algal extracts. Similarly, other long-chain hydrocarbons detected in the chromatographic

profile may represent cellular lipid-associated or hydrocarbon-related constituents. The detection of isopropyl myristate indicates the presence of an ester-type compound in the chromatographic profile. Such assignments should nevertheless be interpreted cautiously because GC–MS library matching can sometimes produce closely related structural candidates with similar mass spectra.

A previous GC–MS investigation of *M. aeruginosa* using headspace-SPME coupled to GC–MS reported 210 volatile metabolites, demonstrating the extensive range of volatile compounds that may be detected from this cyanobacterium [11]. However, the analytical design of that study differed substantially from the present investigation. Headspace-SPME selectively samples volatile compounds from the headspace, whereas the present study involved extraction of dried biomass with 80% methanol. Therefore, the compound lists from the two studies should not be considered directly equivalent.

Another study of *M. aeruginosa* collected from the Muttukadu estuary employed methanolic extraction followed by isolation, purification, and structural elucidation of secondary metabolites, resulting in the identification of two compounds from the extract [12]. The findings from these studies collectively demonstrate that both culture conditions and extraction methodology can strongly influence the chemical profile obtained from *M. aeruginosa*.

4.6 Relationship of the present GC–MS findings to the broader metabolite diversity

The chemical composition observed in the present GC–MS analysis represents only one fraction of the metabolite space of *M. aeruginosa*. Many cyanobacterial secondary metabolites are peptides and other relatively non-volatile compounds that are more appropriately investigated using liquid chromatography coupled with mass spectrometry.

Recent LC–MS/MS metabolomics research has demonstrated extensive diversity of cyanopeptides in *Microcystis*. McDonald et al. [13] detected 82 cyanopeptides across five *Microcystis* strains, including cyanopeptolins, microviridins, microginins, cyanobactins, anabaenopeptins, aeruginosins and microcystins. Importantly, the profiles were strain-specific, indicating that different isolates can possess substantially different metabolite compositions.

Similarly, Stewart et al. [14] used mass-spectrometry-guided metabolomics of cultured *M. aeruginosa* UTEX LB2385 and reported five microginin peptides. Structural characterization involved additional spectroscopic and chemical approaches, demonstrating the importance of confirmatory techniques when reporting individual metabolites.

The broad chemical diversity of *Microcystis* has also been emphasized in recent reviews. Yancey et al. [6] noted that the genus contains numerous biosynthetic gene clusters and that many metabolites remain incompletely characterized. LC–MS and tandem mass spectrometry have become important tools for investigating this chemical diversity, particularly for compounds that are unsuitable for GC analysis. The continuing discovery of structurally diverse cyanobacterial metabolites is further reflected in recent literature covering metabolites reported between 2021 and 2024, including peptides, alkaloids, lipids, polyketides and terpenoid compounds [26].

4.7 Interpretation of GC–MS library matches

An important consideration in GC–MS metabolite profiling is the distinction between tentative identification and definitive structural characterization. A high-quality library match can provide strong evidence for a candidate compound, but mass spectral similarity alone does not necessarily prove molecular identity. The NIST mass spectral library is widely used for comparison of experimental spectra with reference spectra and also provides retention-index information [22], [23]. Where available,

agreement between mass spectrum, retention index and authentic standard provides stronger evidence than a library spectrum alone.

Accordingly, the compounds listed in the present study are referred to as tentatively identified compounds or putative assignments. Further confirmation using authentic standards, retention-index comparison and complementary analytical techniques would be required for definitive identification. This distinction is particularly important when interpreting biological significance. A tentative GC–MS assignment should not automatically be interpreted as proof that the biological activity associated with the reported compound is present in the crude extract.

4.8 Significance of the present study

The present study provides a preliminary chemical characterization of an axenically cultured *M. aeruginosa* isolate using an integrated extraction, quantitative phytochemical and GC–MS approach. The use of an axenic culture provides a controlled experimental source of biomass and reduces the possibility that metabolites detected in the extract originate from associated microorganisms. The detection of multiple phytochemical classes together with several GC–MS features demonstrate the chemical complexity of the extract. The results provide a foundation for subsequent investigations involving compound purification, structural confirmation and biological evaluation.

Further work should focus on confirmation of selected GC–MS compounds using authentic standards or complementary analytical methods. In addition, targeted investigation of individual metabolite classes may help determine whether the compounds detected in the crude extract have useful antioxidant, antimicrobial, nutraceutical or other biotechnological properties.

5. Conclusion

The present study provided a preliminary chemical characterization of an axenically cultured *Microcystis aeruginosa* isolate (C-16). Molecular identification based on 16S rRNA gene sequencing confirmed the isolate as *M. aeruginosa*, and the established axenic culture provided a controlled biomass source for subsequent extraction and analysis. Extraction of the dried biomass with 80% methanol yielded 0.75% crude extract.

Colorimetric analysis demonstrated measurable levels of selected bioactive compound groups, including phenolic, polyphenolic, flavonoid, alkaloid, terpenoid and saponin-equivalent constituents. Spectrophotometric pigment analysis further confirmed the presence of chlorophyll-a, chlorophyll-b and total carotenoids in the cultured biomass. GC–MS analysis revealed a chemically diverse chromatographic profile, with tentative library assignments including phytol, n-hexadecanoic acid, isopropyl myristate and several long-chain hydrocarbons.

Overall, the findings demonstrate that the axenically cultured *M. aeruginosa* C-16 isolate contains a range of chemically detectable metabolite and pigment constituents. The results provide a preliminary chemical basis for further investigation of this isolate. However, the colorimetric measurements represent class-level standard-equivalent estimates, while the GC–MS compound assignments are tentative and require confirmation using authentic standards and/or complementary analytical techniques. Further studies involving compound isolation, structural confirmation and biological evaluation are therefore warranted to establish the specific bioactive potential of the detected constituents.

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