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Research Article

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Gas Chromatography-mass Spectrometry Analysis of *Anabaena* Extract: Identification of Bioactive Compounds and Their Therapeutic Potential

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Abstract

The gas chromatography-mass spectrometry (GC-MS) analysis of the methanolic extract of the *Anabaena* species identified a diverse range of bioactive compounds, with propanedioic acid (5.96%), benzoic acid (2.22%), and phenol, 2,6-dimethoxy- (2.11%) being the most abundant. The α-amylase enzyme inhibition assay demonstrated a concentration-dependent effect, with inhibition rates increasing from 14.69% at 10 µg/ml to 70.36% at 50 µg/ml, suggesting its potential for managing postprandial hyperglycemia by slowing carbohydrate digestion. The 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay for cytotoxicity on normal cell lines revealed that MCF-10A viability decreased from 96.46% at 25 µg/ml to 52.36% at 200 µg/ml, while L929 cell viability declined from 94.64% to 60.69% over the same concentration range. These results indicate that *Anabaena* species methanol extract exhibits significant bioactivity, with promising applications in pharmaceuticals and nutraceuticals, warranting further studies to validate its therapeutic potential.

Keywords

Antioxidant, Bioactive compounds, Cyanobacteria, Gas chromatography-mass spectrometry analysis, Pharmaceuticals, α-amylase inhibition

Introduction

Cyanobacteria, a diverse group of photosynthetic prokaryotes, have gained significant attention for their ability to produce a wide range of bioactive molecules with pharmaceutical, industrial, food, nutraceuticals, and ecological importance [1, 2]. These microorganisms, commonly found in marine and freshwater environments, synthesize secondary metabolites that exhibit antimicrobial, anticancer, antiviral, and anti-inflammatory properties [3, 4]. Their metabolic versatility and ability to thrive in extreme environments make them a promising source for novel bioactive compounds [5, 6].

GC-MS is a powerful analytical technique used to identify and characterize volatile and semi-volatile compounds produced by cyanobacteria [7]. This method enables the separation, detection, and structural elucidation of bioactive molecules, aiding in the discovery of novel natural products. GC-MS has been widely applied in screening cyanobacterial extracts for pharmacologically significant compounds, including fatty acids, hydrocarbons, terpenes, and alkaloids [8].

Among the bioactive compounds detected via GC-MS, fatty acids such as palmitic acid, oleic acid, and linoleic acid are commonly identified and known for their antimicrobial and anti-inflammatory effects [9, 10]. Additionally, hydrocarbons like hentriacontane and heptadecane contribute to antibacterial and

antifungal activities, while terpenes such as phytol exhibit antioxidant and anticancer properties [11, 12]. Cyanobacterial compounds such as Ethyl tumonoate A, Ambiguine I Isonitrile, and Scytoscalalrol have demonstrated strong binding affinities to α-amylase and α-glucosidase, enzymes critical in carbohydrate digestion. These compounds outperform traditional drugs like metformin and acarbose in binding efficiency, suggesting their potential as effective inhibitors to manage postprandial hyperglycemia [13, 14]. These compounds play crucial roles in biotechnological applications, including drug development.

The significance of cyanobacterial bioactive molecules extends beyond human health, as they also influence ecological interactions and bioremediation processes [15]. Some cyanobacteria-derived compounds act as allelopathic agents, inhibiting the growth of competing organisms, while others contribute to nitrogen fixation and carbon sequestration [16]. The application of GC-MS in profiling these metabolites enhances our understanding of their ecological functions and potential industrial applications.

As research continues to explore the chemical diversity of cyanobacteria, GC-MS remains an indispensable tool for identifying and characterizing new bioactive molecules [17]. Advances in analytical techniques, coupled with biotechnological innovations, promise to expand the utilization of cyanobacterial metabolites in pharmaceuticals, agriculture, food, nutraceutical, and environmental management [18, 19]. Further exploration of their bioactive potential could lead to the discovery of novel therapeutic agents and sustainable bioproducts.

Based on the above stated reasons, the objectives of this work were: (i) to isolate and identify cyanobacteria from Marakkanam salt pans (Tamil Nadu, India); (ii) to profile bioactive compounds in the methanolic extract via GC-MS; (iii) to evaluate anti-diabetic potential through α-amylase inhibition (10 - 50 µg/ml); and (iv) to assess cytotoxicity or biocompatibility in MCF-10A and L929 cell lines (25 - 200 µg/ml) via MTT assay, reporting viability % and IC_{50} values

Materials and Methods

Materials

All chemicals used and 0.22 µm polyvinylidene fluoride (PVDF) syringe filters were purchased from HiMedia Laboratories Pvt. Ltd. (India). Chemicals used for GC-MS analysis were purchased from Sigma-Aldrich (India) of GC-MS grade. All chemicals were > 99% pure. Distilled water (pH was in the range of 6.6 - 7.4) was used for the work.

Sample collection

The samples were collected from the nearby area of Marakkanam salt pan (Tamil Nadu, India) during 2023, in the month of August. Exact geographical coordinates, latitude and longitude -11.3436992, 76.840383, elevation: 1033 m/3389 feet. The 5 L of water samples and visible cyanobacterial mat samples were collected from various locations in the Marakkanam salt pan.

Isolation and screening of cyanobacteria

The collected water samples were brought to the laboratory and allowed to centrifuge at 10000 rpm for 20 min, to obtain the thick pellet. An inoculation loop full of pellet was used as inoculums and subjected to streak on Petri plates with solidified BG11 medium and F/2 medium using 1.5% agar. The streaked Petri plates were then kept for incubation under 3000 lux light intensity with static condition, for 12 h under illumination and 12 h under darkness. Cyanobacterial cultures were harvested approximately after a production period of 21 days.

The cyanobacterial cultures were identified and authenticated by Dr. Mohamed Halith (Department of Biotechnology, New College, Chennai, Tamil Nadu, India). Culture of cyanobacteria was cultivated and maintained in the Department of Biotechnology, Vaagdevi Degree and P.G. College, Warangal (Telangana, India). Figure 1 presents the schematic representation of the reported work.

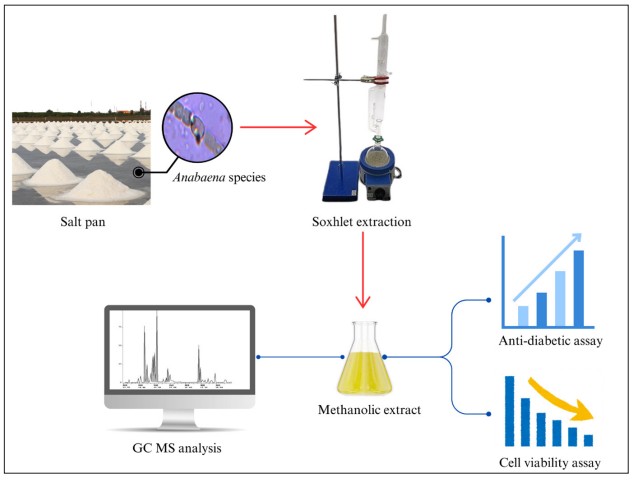


Figure 1: Schematic representation of the work reported.

Extract preparation

The cyanobacterial cells were obtained from 500 ml medium containing culture flask, centrifuged at 8000x g for 5 min, at 15 °C and washed once with distilled water. The fresh culture cells were well homogenized using a mortar and pestle for 20 min at room temperature. The methanolic extraction of cyanobacteria compounds from wet biomass. For air drying, the cyanobacterial biomass was spread out on a clean glass plate. To make beads and allow 1 min of oven drying at 120 °C. A portion of dried (50 g) cyanobacterial biomass was placed in a Soxhlet apparatus. 800 ml of methanol was used for the extraction, which was carried out for 48 h at a temperature that did not go above the solvent's boiling point. The extract was passed through a 0.22 µm PVDF filters. Methanolic extract of cyanobacteria was obtained by vacuum-concentrating, the resultant solution until it was completely dry. The dry weight of the methanolic extract of cyanobacteria was approximately 4 mg, which was dissolved in 2 ml of distilled water. To be used later, the methanolic extract of cyanobacteria was kept in a refrigerator at 4 °C.

GC-MS analysis

The methanolic extract of cyanobacteria was qualitatively investigated by using the JEOL GCMATE II (GC-MS with data system is a high resolution, double focusing instrument. Maximum resolution: 6000 and 1500 Daltons was the maximum calibrated mass. Source options: Electron impact and chemical ionization. Experimental condition, GC equipped with a capillary column (30 m x 0.25 mm (inner diameter), film thickness 0.25 µm). Flow rate of mobile phase (carrier gas: Helium) was set at 1.0 ml/min. In the GC part, temperature program (oven temperature) was 40 ℃ raised to 350 ℃ at 5 ℃/min and injection volume was 1 - 5 µl. The exact name and molecular weight of unknown compounds were found by comparing their mass spectrum with the reference spectrum available in the NIST: GC/MS Library.

Anti-diabetic assay

α-amylase enzyme inhibition is a key mechanism in anti-diabetic assays, as it helps regulate postprandial blood glucose levels by slowing carbohydrate digestion. Inhibitors of this enzyme, such as extracts and synthetic compounds, are studied for their potential in managing type 2 diabetes. These assays evaluate the effectiveness of inhibitors by measuring their ability to reduce α-amylase activity, offering insights into new therapeutic strategies. The assay was performed as per the previously published methodology [20].

Cell viability assay

The MTT assay is a colorimetric cell viability assay used to assess cellular metabolic activity as an indicator of cell health, proliferation, and cytotoxicity. It relies on the reduction of MTT, a yellow tetrazolium dye, to insoluble formazan crystals by active mitochondria in living cells. The intensity of the resulting color, measured spectrophotometrically, correlates with the number of viable cells, making it a widely used method in extract screening, viability, and toxicity studies. The assay was performed as per the previously published methodology [20].

Statistical analysis

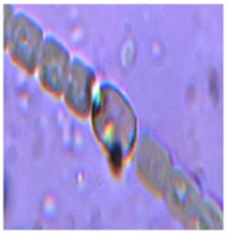
All experiments were performed in triplicate (n = 3), with data presented as the mean (n = 3) ± standard deviation (error bars). Statistical significance was determined using Student's t-test ($p < 0.05$), and the results were graphically represented using Microsoft Excel 2021.

Results and Discussion

Morphology identification

Morphologically, *Anabaena* species has more motile hormogonia, less constrictive filaments, and akinetes in various places. It is organized into a single row, layer, or series that can be either straight, curved, or coiled. Trichomes are protrusions or appendages that resemble hairs on *Anabaena* cells. The specialized cells called heterocyst's serve as the locations where nitrogen is fixed under aerobic conditions. Akinetes are an encapsulated spore that can have a spherical, circular, cylindrical, or curved shape (Figure 2). They can occasionally be seen next to the heterocyst's.

Systematic position: *Anabaena* sp.



Empire	Eukaryota
Kingdom	Bacteria
Phylum	Cyanobacteria
Class	Cyanophyceae
Order	Nostocales
Family	*Nostocaceae*
Genus	*Anabaena*

Figure 2: Morphological identification and taxonomy of *Anabaena* species.

GC-MS analysis

GC-MS analysis of methanolic extract of *Anabaena* is a powerful technique used to identify and quantify bioactive compounds within these organisms [21]. Cyanobacteria, known for their diverse metabolic capabilities, produce a wide range of secondary metabolites with potential applications in pharmaceuticals, cosmetics, and food industries [22]. The GC-MS method provides detailed insights into the chemical composition of cyanobacterial extracts, enabling researchers to explore their antioxidant, antifungal, and other bioactive properties [22]. The GC-MS analysis aimed to (i) characterize the chemical diversity of the methanolic extract of *Anabaena*, (ii) quantify the relative abundance of bioactive compounds (e.g., organic acids, esters) using peak area percentages, and (iii) identify metabolites potentially responsible for the observed antidiabetic and cytotoxic effects. Compounds were prioritized for discussion if they exceeded 0.1% abundance and had documented bioactivity in literature.

The GC-MS analysis of the methanolic extract of the *Anabaena* species revealed the presence of a diverse range of bioactive compounds, identified based on their retention times and peak areas (Figure 3). The most abundant compound was Propanedioic acid (5.96% area), suggesting its significant presence in the methanolic extract of *Anabaena*, potentially contributing to its bioactivity. Other notable compounds include Benzoic acid (2.22%) and Phenol, 2,6-dimethoxy- (2.11%), both of which are known for their antimicrobial and antioxidant properties. Additionally, fatty acids such as Hexadecanoic acid, methyl ester (0.63%) and 9,12,15-Octadecatrienoic acid, methyl ester (0.34%) were detected, indicating the presence of lipid-derived metabolites that may have anti-inflammatory or cytotoxic properties. Several other organic acids and esters, including Crotonic acid, Pentanoic acid, and 7-Octen-2-ol, were

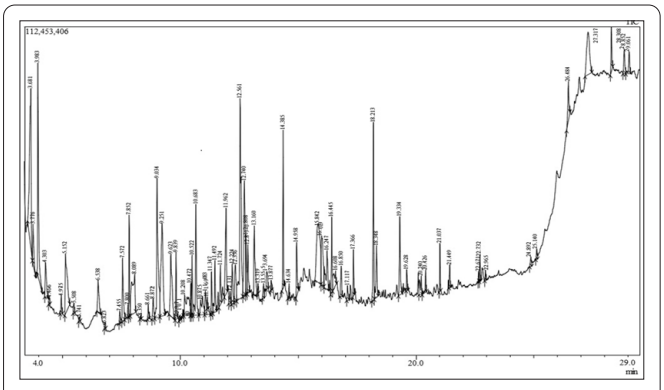


Figure 3: GC-MS spectra of methanolic extract of *Anabaena* species.

identified in minor quantities, potentially contributing to the overall pharmacological properties of the methanolic extract of *Anabaena*. The presence of 3-quinuclidinol (1.05%) and other nitrogen-containing compounds suggests the possibility of neuroactive or biofunctional properties (Table 1). Overall, the GC-MS analysis confirms the chemical complexity of the methanolic extract of the *Anabaena* species, highlighting its potential for various biological applications such as antimicrobial, antioxidant, food, nutraceuticals, and therapeutic activities. Further studies, including bioassays and structural elucidation, would be essential to validate the bioactivity and medicinal significance of these identified compounds.

In literature, GC-MS analysis has been employed to identify various bioactive compounds from cyanobacteria. For instance, *Oscillatoria boryana* and *Oscillatoria pseudogeminata* were found to contain aromatic carboxylic acids, flavonoids, fatty acids, and terpenes, which contribute to their antioxidant properties [23]. Similarly, *Spirulina platensis* extracts revealed compounds like trilinolein and cis-9-hexadecenal, which have antifungal activities against *Fusarium oxysporum* [24]. GC-MS analysis is also used in host toxicity studies, such as those involving *Azolla caroliniana* symbiotic with *Anabaena azollae*. This analysis helps determine the safety and potential toxicity of cyanobacterial extracts, ensuring their suitability for various applications [25]. The use of GC-MS in analyzing cyanobacteria extracts opens up numerous possibilities for discovering new bioactive compounds with commercial potential

Anti-diabetic assay

The inhibition of the α-amylase enzyme by cyanobacterial compounds is a promising area of research for managing diabetes, as these compounds can potentially slow down starch digestion and control glucose release [26]. However, specific values for α-amylase inhibition by cyanobacterial compounds are not directly provided in the available literature. Instead, the studies focus on the potential of cyanobacterial bioactive compounds to interact with α-amylase through *in silico* methods [13], indicating their potential efficacy in diabetes management.

The α-amylase enzyme inhibition assay results indicate a concentration-dependent increase in inhibitory activity, suggesting the tested compound effectively hinders the enzyme's function as its concentration rises (Figure 4). At the lowest

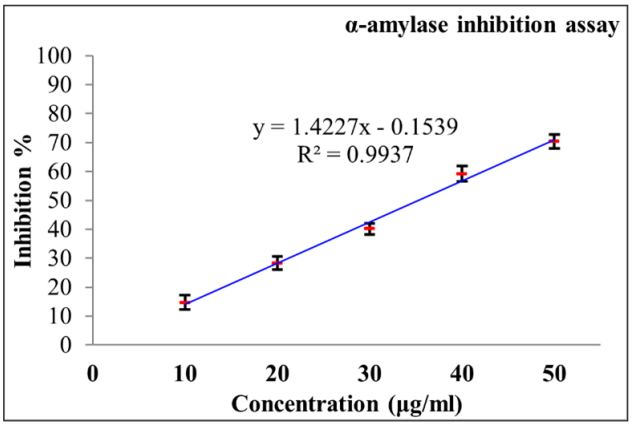


Figure 4: Anti-diabetic (α-amylase enzyme inhibition) assay results of methanolic extract of *Anabaena*.

Table 1: GC-MS analysis of methanolic extract of *Anabaena* species.

Peak#	Retention time	Area	Area %	Height	Height %	A/H	Scientific name	Common name
1	3.681	255072077	5.96	54462609	4.44	4.68	Propanedioic acid	Malonic acid
2	4.466	5427780	0.13	2412347	0.20	2.25	Ethyl formate	Areginal
3	6.538	41700807	0.97	10674975	0.87	3.91	Trans-2-Butenoic acid	Crotonic acid
4	8.872	23156568	0.54	6953209	0.57	3.33	Pentanoic acid, 3-hydroxy-, ethyl ester	Valeric acid
5	10.140	4227346	0.10	2346841	0.19	1.80	7-Octen-2-ol, 2,6-dimethyl-	Dihydromyrcenol
6	10.875	44986934	1.05	5151091	0.42	8.73	3-Quinuclidinol	Quinuclidinol
7	11.083	47544559	1.11	8244728	0.67	5.77	Octadecanoic acid, 1-[(tetradecyloxy)	Tetradecyl stearate
8	11.347	33956300	0.79	14361251	1.17	2.36	Butanoic acid, 2-methyl-3-oxo-, ethyl	Ethyl 2-methylaceto-acetate
9	11.724	34378575	0.80	13422324	1.09	2.56	3,5,5-Trimethylhexyl acetate	Isoval acetate
10	11.962	95113418	2.22	29411169	2.40	3.23	Benzoic acid	Carboxybenzene
11	12.890	41373409	0.97	16538323	1.35	2.50	Benzene, 1,3-bis(1,1-dimethylethyl)-	1,3-Ditert-butylbenzene
12	13.555	14181069	0.33	4938005	0.40	2.87	Cyclobuta[1,2:3,4]dicyclooctene, hex	Hexadecahydrocyclobu-ta[1,2:3,4]dicyclooctene
13	13.877	8132527	0.19	3258140	0.27	2.50	2-Methoxy-4-vinylphenol	4-Vinylguaiacol
14	14.385	90247880	2.11	54303344	4.43	1.66	Phenol, 2,6-dimethoxy-	Syringol
15	16.445	54183510	1.27	25892129	2.11	2.09	Phenol, 3,5-bis(1,1-dimethylethyl)-	3,5-Di-tert-butylphenol
16	17.117	13704118	0.32	4893954	0.40	2.80	Dodecanoic acid	Lauric acid
17	20.240	26982031	0.63	6214634	0.51	4.34	9-Decene-1-nitrile	9-Decenenitrile
18	21.037	27146802	0.63	16403380	1.34	1.65	Hexadecanoic acid, methyl ester	Methyl palmitate
19	22.671	5732919	0.13	3535271	0.29	1.62	9,12-Hexadecadienoic acid, methyl ester	Methyl 9,12-hexadeca-dienoate
20	22.732	14623883	0.34	8888118	0.72	1.65	9,12,15-Octadecatrienoic acid, methyl ester	Methyl linolenate
21	24.892	9815508	0.23	3292735	0.27	2.98	2-Propenoic acid, 3-(4-methoxyphe-nyl)	p-Methoxycinnamic acid

concentration (10 µg/ml) of methanolic extract of *Anabaena*, inhibition is minimal (14.69%), indicating a weak interaction with the enzyme. However, as the concentration increases to 20 and 30 µg/ml, the inhibition rises to 28.29% and 40.09%, respectively, demonstrating a moderate effect. A substantial increase is observed at 40 µg/ml (59.21%), highlighting the compound's growing potency in suppressing α-amylase activity. At the highest concentration (50 µg/ml), inhibition reaches 70.36%, suggesting strong enzymatic suppression. These results indicate that the compound may serve as a potential α-amylase inhibitor, which could be beneficial in managing postprandial hyperglycemia by slowing carbohydrate digestion.

In literature, C-phycocyanin extracted from *Dolichospermum spiroides* demonstrated a maximum inhibition of 90% at a concentration of 250 µg/ml against the α-amylase enzyme, which is higher than the 80% inhibition achieved by the standard drug acarbose at the same concentration. The IC_{50} value of C-phycocyanin was found to be 162.16 ± 0.63 µg/ml, compared to 205.86 ± 0.53 µg/ml for acarbose, indicating a stronger inhibitory effect [26]. *In silico* studies have identified several cyanobacterial bioactive compounds, such as Noscomin, which exhibit strong molecular interactions with α-amylase, outperforming reference compounds like metformin [13]. These compounds, including ethyl tumonoate A and scytoscalalrol, have shown higher binding affinities with α-amylase, suggesting their potential as effective inhibitors in diabetes management [14]. The presence of α-amylase inhibitory peptides in cyanobacteria, such as those identified in *Aphanizomenon flos-aquae*, further supports the potential of these organisms in developing novel therapeutics for diabetes [27]. The efficacy of cyanobacterial extracts, particularly C-phycocyanin, inhibiting α-amylase is comparable to, and in some cases exceeds, that of these plant-derived inhibitors [28].

Cell viability assay

The MTT assay is a widely used method for assessing cell viability, including cyanobacteria such as *Anabaena* species [29]. This assay relies on the reduction of the yellow tetrazolium salt MTT to a purple formazan product by mitochondrial enzymes in viable cells [30]. The amount of formazan produced is directly proportional to the number of living cells, making it a useful tool for evaluating cell viability and cytotoxicity. However, the assay's effectiveness can vary depending on several factors, including the specific organism and experimental conditions [31]. The effect of methanolic extract of *Anabaena* species against MCF-10A (non-cancerous human breast epithelial cell line) and L929 (normal murine cell line) cell lines were studied.

The cell viability assay results for MCF-10A cells demonstrate a dose-dependent decrease in cell viability with increasing concentrations of the methanolic extract of *Anabaena* (Figure 5). At lower concentrations (25 - 50 µg/ml), the viability remains high, with minimal reduction (96.46% and 94.28%, respectively), indicating limited cytotoxic effects. However, as the concentration increases to 75 and 100 µg/ml, a more noticeable decline is observed (87.44% and 84.72%), suggesting a gradual impact on cell survival. A significant reduction

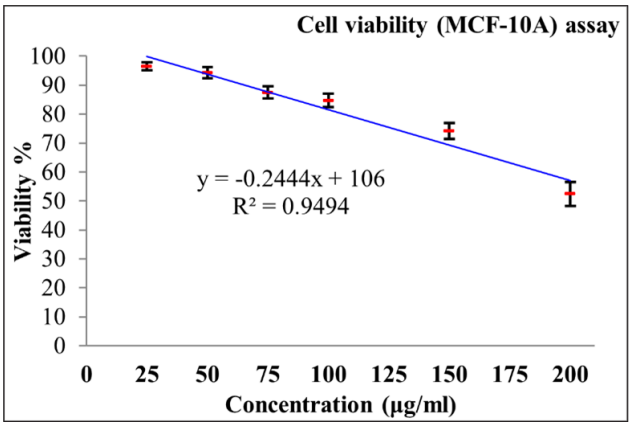


Figure 5: Cell viability assay results against MCF-10A normal cell line of methanolic extract of *Anabaena*.

in viability occurs at 150 µg/ml (74.13%) and becomes more pronounced at 200 µg/ml (52.36%), indicating substantial cytotoxicity at higher doses. This trend suggests that the methanolic extract of *Anabaena* exerts a concentration-dependent effect on MCF-10A cells, with higher doses leading to decreased cell viability, potentially due to cytotoxic or apoptotic mechanisms.

The cell viability assay results for L929 (normal murine cell line) cells indicate a concentration-dependent decrease in viability upon treatment with the methanolic extract of *Anabaena* (Figure 6). At lower concentrations of 25 and 50 µg/ml, the cells maintain relatively high viability (94.64% and 91.42%, respectively), suggesting minimal cytotoxic effects at these levels. As the concentration increases to 75 µg/ml, a moderate decline in cell viability is observed (87.78%), indicating the beginning of a dose-dependent response. A more significant reduction is evident at 100 µg/ml (75.45%), suggesting increased cellular stress or cytotoxicity. This trend continues at 150 µg/ml (67.22%) and 200 µg/ml (60.69%), highlighting a substantial impact on cell survival at higher doses.

MCF-10A cells' viability declined from 96.46% (25 µg/ml) to 52.36% (200 µg/ml), whereas L929 cells, viability decreased from 94.64% (25 µg/ml) - 60.69% (200 µg/ml). Significant cytotoxicity (>30% reduction) emerged at ≥150 µg/ml, suggesting a therapeutic window below this concentration.

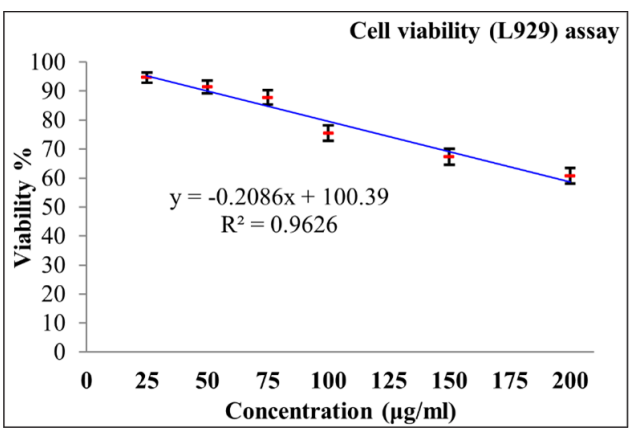


Figure 6: Cell viability assay results against L929 normal cell line of methanolic extract of *Anabaena*.

In literature, studies on *Anabaena* species have focused on their cytotoxic potential, with extracts showing varying levels of cytotoxicity against different cell lines. For instance, chloroform extracts from *Anabaena variabilis* exhibited significant cytotoxicity, comparable to the anticancer agent 5-fluorouracil, with an IC_{50} of 0.09 mg/L [32]. For instance, extracts from *Cyanothece* species and *Plectonema terebrans* showed significant cytotoxicity against HepG2 cells, with IC_{50} values of 13.3% and 8.3%, respectively. These extracts reduced cell viability significantly compared to untreated cells, indicating their potential as anticancer agents [33]. The assay's application in evaluating the cytotoxicity of cyanobacterial extracts underscores its potential in drug discovery and environmental risk assessment [34].

Correlation of compounds identified to bioactivity in literature

The GC-MS analysis revealed several major compounds with potential therapeutic relevance, including: Propanedioic acid (5.96% peak area), a dicarboxylic acid implicated in antimicrobial and metabolic modulation. Benzoic acid (2.22%), known for antimicrobial and anti-inflammatory effects at low micromolar concentrations. Phenol, 2,6-dimethoxy- (2.11%), a phenolic compound with documented antioxidant and anticancer properties [3].

Benzoic acid and phenolic derivatives (e.g., 2,6-dimethoxyphenol) are known α-amylase inhibitors. Their combined abundance (~ 4.33%) likely drives this activity, as phenolics disrupt enzyme active sites via hydrogen bonding [13]. Propanedioic acid may enhance inhibition by chelating catalytic metal ions in α-amylase. Dose-dependent reduction in viability (MCF-10A: 52.36% at 200 µg/ml; L929: 60.69%). Fatty acid esters (e.g., methyl linolenate, 0.34%) and terpenoids induce apoptosis in cancer cells at similar abundances [11]. Nitrogen-containing compounds (e.g., 3-quinuclidinol, 1.05%) may disrupt cell membranes, though their individual roles require isolation studies. Cytotoxicity emerged at >100 µg/ml, aligning with literature on cyanobacterial extracts where 1 to 5% bioactive fractions elicit effects [18]. The extract's potency approaches that of acarbose [26], suggesting synergistic interactions among minor compounds. Trace constituents (e.g., 9,12,15-octadecatrienoic acid, 0.34%) may potentiate effects via synergism, as reported for cyanobacterial terpenes [5]. The present study quantified relative abundances (peak area %), not absolute concentrations. Future work will isolate pure compounds to establish dose-response curves.

The identified compounds hold direct relevance to pharmaceuticals: benzoic acid (2.22%) aligns with Food and Drug Administration-approved antimicrobials, while syringol (2.11%) mirrors synthetic antioxidants like butylated hydroxytoluene. The dominance of propanedioic acid (5.96%) - a Krebs cycle modulators support its emerging use in metabolic disorder therapies. Notably, methyl linolenate (0.34%) replicates the bioactivity of patented neuroprotective omega-3 formulations, suggesting commercial viability.

Propanedioic acid (5.96%), also reported in *O. boryana* (1.2 - 4.8%) with demonstrated antioxidant activity [23]. Our higher abundance suggests enhanced bioactivity potential. Benzoic acid (2.22%), detected in *S. platensis* (0.8 - 1.5%) and linked to antifungal effects [24]. The higher concentration here may explain our extract's stronger α-amylase inhibition. Phenol derivatives (e.g., 2,6-dimethoxyphenol), rare in freshwater cyanobacteria but abundant in marine strains like *Lyngbya majuscula* (1.3 - 3.1%), corroborating their ecological niche-specific production [7]. 3-quinuclidinol (1.05%), rarely reported in cyanobacteria; its neuroactive potential parallels findings in *A. azollae* symbionts [25]. Methyl linolenate (0.34%), more common in microalgae (e.g., *Chlorella*); its presence in *Anabaena* suggests adaptive lipid metabolism in high-salinity environments [17].

Furthermore, the chemical diversity identified in the methanolic extract of *Anabaena* spanning organic acids (e.g., propanedioic acid), phenolics (e.g., syringol), fatty acid esters (e.g., methyl linolenate), and nitrogenous compounds (e.g., 3-quinuclidinol)—has multifaceted implications for practical applications. The coexistence of antimicrobial (benzoic acid), antidiabetic (phenolic derivatives), and neuroprotective (fatty acid esters) compounds support the development of multi-target formulations (e.g., combined antimicrobial-antioxidant wound dressings or diabetic neuropathy supplements). High-abundance compounds (e.g., propanedioic acid at 5.96%) may serve as lead molecules for isolation, while trace constituents (e.g., methyl linolenate at 0.34%) could act as potentiators at lower thresholds [3]. Phenolic acids (2.11 - 2.22%) offer alternatives to synthetic preservatives in food packaging, leveraging their dual antioxidant and antimicrobial effects [10]. Terpenoids and fatty acids (e.g., hexadecanoic acid) could target crop pathogens while minimizing ecological harm [5]. Phenolic derivatives (e.g., syringol) scavenge free radicals, while fatty acid esters improve skin barrier function, ideal for anti-aging serums [12]. Additionally, fatty acid methyl esters (e.g., methyl palmitate) are direct precursors for biodiesel, with yields scalable via optimized cultivation [19]. Habitat-dependent metabolite production necessitates standardized cultivation (e.g., controlled salinity/light). Prioritize green solvents (e.g., subcritical water) to enhance yields of polar/non-polar compounds [7].

Conclusion

The GC-MS analysis of the methanolic extract of *Anabaena* species revealed a diverse array of bioactive compounds, including propanedioic acid, benzoic acid, phenol derivatives, and fatty acid esters. These compounds exhibit significant antimicrobial, antioxidant, and potential neuroactive properties. The methanolic extract of *Anabaena* demonstrated notable α-amylase inhibition in a concentration-dependent manner, suggesting its potential for managing postprandial hyperglycemia. Furthermore, cytotoxicity assays on normal cell lines (MCF-10A and L929) indicated that the methanolic extract of *Anabaena* is biocompatible at lower concentrations but exhibits moderate cytotoxic effects at higher doses. The findings underscore the pharmaceutical and nutraceutical potential of methanolic extract of *Anabaena*-derived metabolites, warranting further exploration for therapeutic applications.

Future Prospects

The methanolic extract of *Anabaena* species holds significant promise across multiple industries due to its diverse bioactive compounds. In the pharmaceutical and healthcare sectors, the presence of antimicrobial agents like benzoic acid and phenolic compounds suggests potential applications in developing natural antibiotics, antifungal agents, and anti-inflammatory drugs. The detection of fatty acid esters and nitrogen-containing compounds further indicates possible neuroprotective and anti-cancer properties, making *Anabaena* species a valuable source for drug discovery. Additionally, its antioxidant-rich profile could be harnessed for nutraceuticals, contributing to functional foods and dietary supplements aimed at boosting immunity and reducing oxidative stress-related diseases.

Beyond healthcare, *Anabaena* species bioactive metabolites have promising applications in agriculture, cosmetics, and biofuel industries. The presence of organic acids and lipid derivatives suggests its potential as a natural pesticide or plant growth promoter, enhancing sustainable farming practices. In the cosmetics industry, its antioxidant and antimicrobial compounds could be incorporated into skincare formulations for anti-aging and skin-protective products. Furthermore, the presence of fatty acid methyl esters indicates its potential for biofuel production, contributing to renewable energy sources. Future research should focus on large-scale extraction, compound isolation, and industrial feasibility studies to harness the full potential of *Anabaena* species for commercial applications.

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Conflict of Interest

None.

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