



In Vitro and Analytical Study of *Spirulina Fusiformis* Extracts for Management of Diabetic Peripheral Neuropathy

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Abstract

Background/Aim: *Spirulina fusiformis* is a flexible spiral-covered cyanobacterium and potent nutraceutical. *Spirulina fusiformis* has been reported for its antioxidant, immune modulating or neuroprotective. The objective of the present study was to investigate the phytochemical profile, antioxidant potential and neuroprotective efficacy of aqueous and hydroethanolic extracts of *Spirulina fusiformis*.

Method: *Spirulina fusiformis* was extracted by maceration to obtain aqueous extract (AQE) and hydroethanolic extract (HEE) followed by its phytochemical screening. Antioxidant potential was assessed by DPPH assay. The analysis using GC-MS was carried out to determine bioactive metabolic compounds. *In vitro* neuroprotective effects were evaluated by MTT assay in RSC96 Schwann cell lines under hyperglycaemic conditions.

Results: Phytochemical analysis confirmed the presence of alkaloids, terpenoids, flavonoids, glycosides and amino acids. The antioxidant assay demonstrated that the hydroethanolic extract exhibited approximately twofold higher DPPH radical scavenging activity than the aqueous extract or the protective viability percentage at low concentrations. Gas chromatography-mass spectrometry (GC-MS) analysis revealed a total of 64 compounds-30 in the aqueous extract and 34 in the hydroethanolic extract. Major identified constituents included oxypoline, niacin, aucubin, N-acetyl-L-glutamic acid, leucine, niacinamide, various fatty acids and glutamic acid. These compounds are reported to possess neuroprotective and glucose-regulating properties. *In vitro* cytoprotective activity, assessed by MTT assay on RSC96 Schwann cells, showed improved cell viability at lower concentrations of both extracts, further supporting their neuroprotective potential.

Conclusion: *Spirulina fusiformis* extracts showed diverse phytochemicals with significant antioxidant and neuroprotective effects, particularly in the hydroethanolic extract. GC-MS identified metabolites linked to glucose regulation and neuroprotection, while *in vitro* studies confirmed protective effects on Schwann cells under hyperglycaemic stress.

Key words: *Spirulina fusiformis*; Neuroinflammatory diseases; Phytochemicals; Dietary supplements; Neuroprotection.

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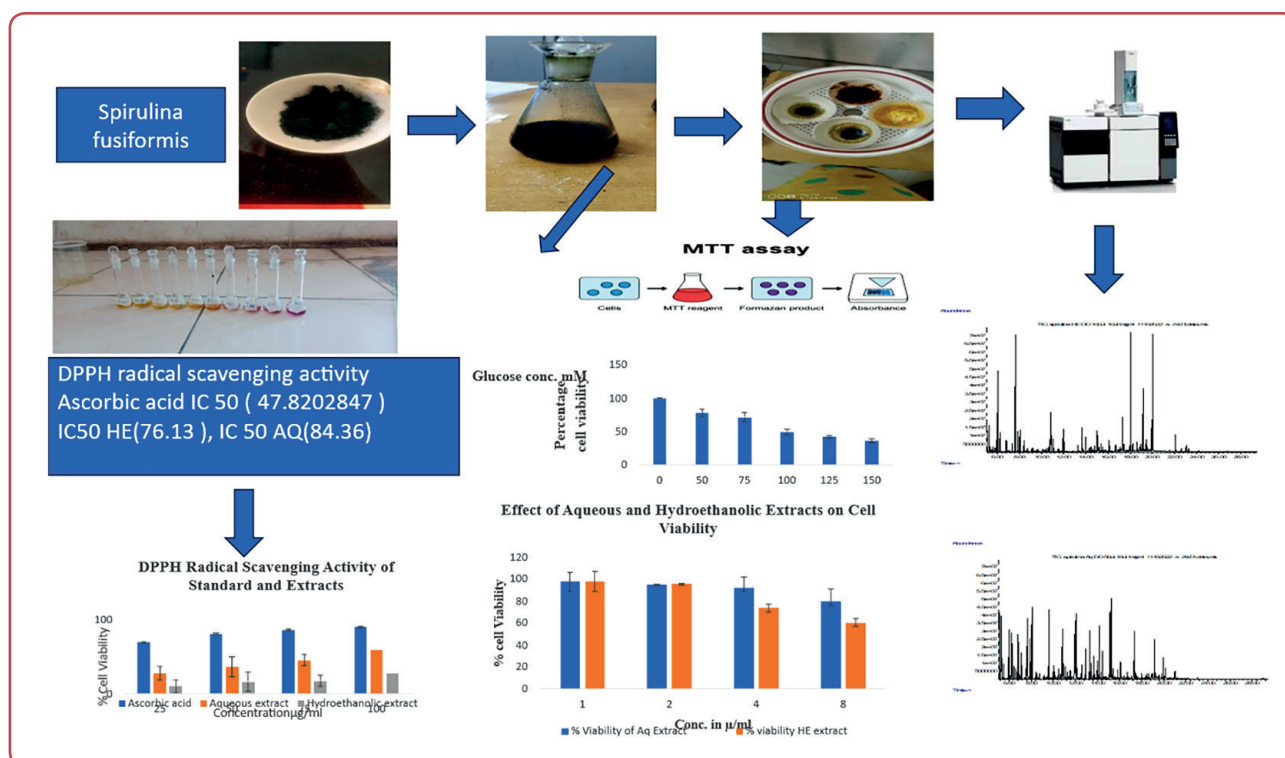
Citation:

Bajaj S, Garg K, Gupta S. *In vitro* and analytical study of *Spirulina fusiformis* extracts for management of diabetic peripheral neuropathy. Scr Med. 2026 Jul-Aug;57(4):733-44.

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Received: 3 October 2025
Revision received: 13 November 2025
Accepted: 13 November 2025



Graphical abstract

Introduction

Diabetes prevalence has considerably risen in the past few years, having a global estimation of 425 million persons in 2017 and growing 628 million of before 2045.^{1,2} Diabetic peripheral neuropathy (DPN) is most intricate complication of diabetes due to irregularity in metabolism which leads disparity which is non-physiological. Constitutes one among the main causes of mortality or affect as many as 50 % of people suffering with diabetes neuropathy.^{3,4} It is a worldwide health problem, associated with hyperglycaemia which leads to damage to the neurons. Some of the clinical manifestations of DPN are typical symptoms Includes discomfort, feeling numb, paraesthesia, problems in posture, feet and heel injuries, or sores.⁵⁻⁷ The pathophysiology of DPN is complex. Persistent hyperglycaemia in unification with oxidative stress leads to stimulate different pathways including advance glucose final products (AGEs), sorbitol pathway, hexosamine transport, oligo- (ADP ribose) polymerase, cyclooxygenase 2 activation, mitogen-activated protein kinases and changed function of Na⁺/K⁺-ATPase, increasing neural irregularities or nerve injury.⁸ Inflammation is one of the main culprits in causing DPN.^{9,10}

Management of DPN is difficult, effective medications are not accessible to many people; thus, enhanced pharmacology and suggestions are critical. The US FDA authorised only pregabalin and duloxetine as treatments for this disease. Conventional pharmacological agents mainly offer symptomatic relief of neuropathic pain but fail to halt the underlying emerging neurodegenerative processes. Therefore, using herbal remedies as sources of natural antioxidants being a suitable and complementary method to reduce the development of diabetic neuropathy.¹¹ The supplements may be provided as a follow-up treatment with drug for combined outcomes. Natural sources are more affordable and safer for the patients.

Microalgae and cyanobacteria belong to category of marine drugs are high within a variety of polyunsaturated fatty acids (PUFAs), such as 18-, 20- and 22-carbon fatty acids under a variety of phospholipids, neutral lipids, glycolipids, vitamin B12 sulpholipids and phenolics compound.^{12,13} *Spirulina fusiformis*, a cyanobacterium, has been employed as an amino acid-rich dietary supplement of ethnopharmacological importance in humans as well as animals. *Spirulina fusiformis*

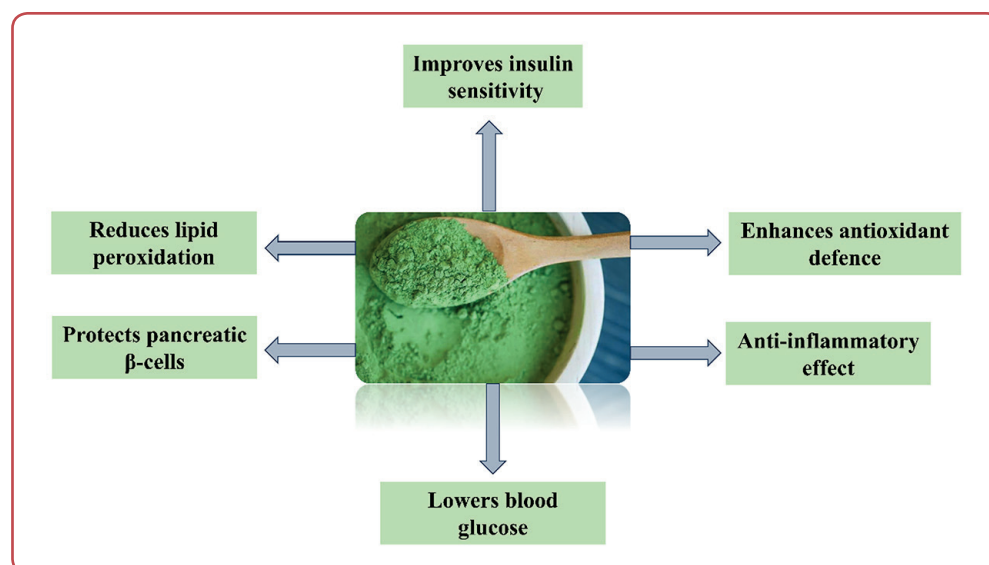


Figure 1: Pleiotropic effects of *Spirulina fusiformis* in diabetes management. *Spirulina* supplementation improves insulin sensitivity, reduces lipid peroxidation, protects pancreatic β -cells, lowers blood glucose, enhances antioxidant defence, or exerts anti-inflammatory effects.

is reported for various pharmacological property comprise anti-cancer immune-stimulating antigen-toxic, hepatotoxic, anti-diabetic, anti-inflammatory and antihypertensive (Figure 1).¹⁴

The current investigation aimed to evaluate the phytoconstituents of aqueous and hydroethanolic extracts of *Spirulina fusiformis* by gas chromatography-mass spectrometry (GC-MS). Antioxidant study of both extracts by DPPH assay and cell viability by MTT assay on RSC96 cell line.

Methods

Spirulina fusiformis powder was purchased from Recon (Bangalore, India) and cell lines were purchased from Pune (Pune, Maharashtra, India). Aqueous extract (AQE) and hydroethanolic extract (HEE) (80 % ethanol and 20 % water) of *Spirulina fusiformis* was prepared using 50 g of powder by maceration method. The extract was concentrated under reduced pressure using rotary evaporator until all the solvent has been removed. Percentage yield of both extracts of *Spirulina fusiform* was calculated in terms of w/w.¹⁵⁻¹⁷ Preliminary qualitative analysis of both extracts was done for identifying the presence of terpenoids, alkaloids, flavonoids, saponins, tannins, sterols, carbohydrates and glycosides by using different chemical tests (Table 1).^{18, 19}

Antioxidant study of *Spirulina fusiformis* extracts

DPPH radical scavenging assay: The antioxidant activity of the extract was evaluated using the DPPH free radical scavenging method. Stock solutions of the extract were prepared in ethanol and serially diluted to obtain working concentrations ranging from 0.01 to 0.5 mg/mL. An aliquot of 0.5 mL of each extract dilution was mixed with 1.0 mL of 125 μ M DPPH solution prepared in 95 % ethanol. The reaction mixture was vortexed gently and incubated in the dark at room temperature for 30 minutes to ensure complete interaction between the extract and the DPPH radical.

After incubation, the decrease in absorbance was measured at 515 nm using a UV-visible spectrophotometer.²⁰ A control was prepared by mixing 0.5 mL of ethanol with 1.0 mL of DPPH solution, while ascorbic acid served as the positive control. All experiments were performed in triplicate and the results were expressed as mean $\pm$ standard deviation (SD). The scavenging activity of both extracts and standard was calculated as:

$$\% \text{ DPPH radical scavenging activity} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

IC₅₀ was calculated from the graph plotted % inhibition versus concentration. The test was replicated thrice for each concentration. Based on IC₅₀, the antioxidant efficacy of the extracts can be determined.

GC-MS analysis *Spirulina fusiformis*

One microliter (1 μ L) of the derivatised extract was injected into an Agilent 7890/5975 GC-MS system (Agilent Technologies, USA) equipped with an HP-5MS capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 μ m). The oven temperature was programmed from 180 $^{\circ}$ C (held for 2 min) to 250 $^{\circ}$ C (held for 1 min) at a ramp rate of 5 $^{\circ}$ C/min. Helium was used as the carrier gas at a flow rate of 1 mL/min, with a split ratio of 1:100. The GC-MS interface (transfer line) temperature was maintained at 250 $^{\circ}$ C, while the electron ionisation (EI) source temperature was set at 230 $^{\circ}$ C. Ionisation was performed in EI mode at 70 eV and mass spectra were recorded in full-scan mode over an m/z range of 50–550 with a solvent delay of 3 min. Compound identification was performed by comparing the obtained mass spectra with reference spectra from the *National Institute of Standards and Technology (NIST)* library and the *Wiley Registry of Mass Spectral Data*. Metabolites were assigned based on the highest similarity match scores. Where applicable, an internal standard (4-phenylphenol, TMS derivative) was included to assist in the verification of retention time stability and for semi-quantitative comparison. This internal standard peak was not derived from the extract itself but was intentionally added during sample preparation.

In vitro study

The rat Schwann cell line (RSC96) was obtained from the National centre from cell science, Pune. The cells were cultured in Dulbeccos modified eagle medium (DMEM) media (4.5 g/L glucose) supplemented with 10 % fetal bovine serum (FBS), 1 % penicillin/streptomycin at 37 $^{\circ}$ C and 5 % CO₂. RSC96 cells were seeded at 1 $\times$ 10⁴ cells/well in a 96-well plate and were treated with various concentrations of glucose for 24 h. Then, the cells were incubated with 25 mM glucose (normal glucose, NG) or 100 mM glucose (high glucose, HG) with or without different concentrations of extracts for 24 h. Optimum concentration for RSC96 cell culture is 25 mM, we induce hyperglycaemia by increasing glucose concentration of medium 50, 75, 100, 125 and 150 mM for 24 h treated cell line with. RSC96 cells exposed to increasing levels of glucose at 50, 75, 100, 125 and 150 mM exhibited toxicity, reaching a maximal effect in 100 mM glucose, which resulted in 49.6 $\pm$ 12.82 % of relative cell viability. The glucose concentration of 100 mM was selected for further study as representative of hyperglycae-

mic conditions that can decrease the viability of RSC96 cells for assessing the protective effects of the *Spirulina Fusiformis* extracts.²¹

MTT assay

Cell viability was assessed using the MTT assay. This colorimetric method is based on the reduction of yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to insoluble purple formazan crystals by mitochondrial dehydrogenase enzymes in metabolically active cells. MTT solution (0.5 mg/mL) was added to the wells of a 96-well plate and the cells were incubated at 37 $^{\circ}$ C for 2 h. After incubation, the medium was removed and the formazan crystals were dissolved in 100 μ L of dimethyl sulfoxide (DMSO) per well. The absorbance was measured at 570 nm using an automated microplate reader (Elisa MAT 2000, DRG Instruments, GmbH).²²

Cell viability was expressed as a percentage relative to the control group, calculated using the formula: Viability percentage (%) = (Absorbance of treated cell - Absorbance of blank) / (Absorbance of untreated cell - Absorbance of blank) *100.

Results

Percentage yield (%w/w) of *Spirulina fusiformis* extracts was 46 % for HEE and 72 % for AQE. Preliminary phytochemical studies revealed the presence of alkaloids, terpenoids, steroids, flavonoids and glycosides etc (Table 1).

Table 1: Phytochemical screening of *Spirulina fusiformis* extracts

Test	HEE	AQE
Test for alkaloids		
Mayer's test	+	-
Hager's test	+	-
Wagner's test	+	-
Test for steroids and terpenoids		
Salkowski test	-	-
Liebermann Buchard test	-	-
Liebermann reaction	-	-
Test for flavonoids		
Lead acetate test	+	-
Shinoda test	+	-
Test for glycosides		
Born Trager's test	+	-

Test for proteins and amino acids		
Ninhydrin test	+	+
Biuret test	+	+
Test for saponins		
Foam test	+	+
Haemolytic test	+	+
Test for tannins		
Ferric chloride test	+	+
Test for carbohydrates		
Molisch test	+	+
Fehling test	+	+

(+) indicates presence of constituents; (-) indicates absence of constituents;
AQE: aqueous extract; HEE: hydroethanolic extract;

Antioxidant study

Hydroethanolic extract of *Spirulina fusiformis* showed better scavenging activity as compared to aqueous extract. With increase in extract concentration, the antioxidant activity also increased. The hydroethanolic extract showed maximum scavenging activity at concentration of 100 µg/mL as shown in Figure 2. The IC₅₀ of all samples was also calculated by plotting percentage inhibition against concentration (Table 2). The IC₅₀ value was found to be 27.36 µg·mL⁻¹ (standard) 83.24 µg·mL⁻¹ (hydroethanolic extract) 182.85 µg·mL⁻¹ (aqueous extract). Lower IC₅₀ value indicate stan-

dard (ascorbic acid) is the most potent free radical scavenger, while the hydroethanolic extract demonstrates substantially greater antioxidant potency than the aqueous extract. This pattern is consistent with differences in phytochemical composition and solvent extraction efficiency.

GC-MS analysis

GC-MS analysis was performed for both hydroethanolic and aqueous extracts, leading to the identification of a total of 34 compounds. Among these, 30 constituents were identified in the hydroethanolic extract (Table 3) and 34 in the aqueous extract (Table 4). The chromatograms of both extracts displayed distinct peak profiles (Figure 3 and Figure 4). Major compounds detected in the hydroethanolic extract included alanine, valine, isoleucine, niacin, niacinamide, glutamic acid, putrescine and gamma-linolenic acid. The aqueous extract was found to contain alanine, norvaline, niacin, aspartic acid, oxoproline, palmitic acid and a compound putatively identified as aucubin, among others. Compound identification was primarily based on spectral matching with the NIST library; no authentic standards were used for further confirmation. Should be considered tentative identifications pending further verification.

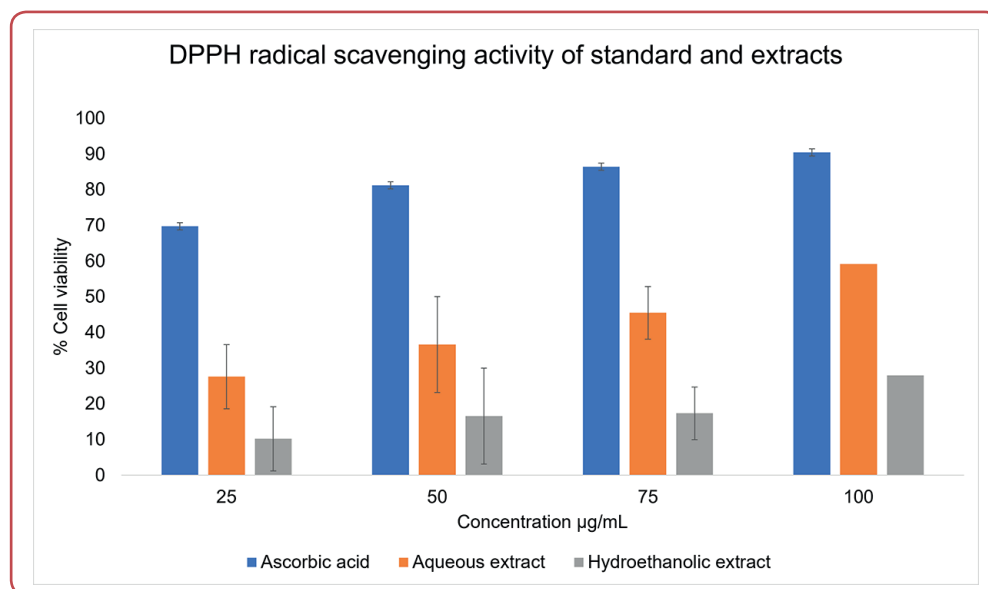


Figure 2: Graph showing the percentage inhibition of standard and the different plant extracts of *Spirulina fusiformis* at various concentrations by DPPH assay



Table 2: The percentage inhibition of standard and the different plant extracts of *Spirulina fusiformis* at various concentrations by DPPH assay

Concentration (µg/mL)	Ascorbic acid	HEE	AQE
25	69.72	27.62	10.22
50	81.24	36.62	16.55
75	86.40	45.49	17.35
100	90.43	59.17	27.99

AQE: aqueous extract; HEE: hydroethanolic extract;

Table 3: Phytoconstituents of hydroethanolic extract (HEE) of *Spirulina fusiformis*

SN	RT (min)	Area	Compound name	Biological role and pathological significance
1	5.276	4E+08	L-Alanine	Supports energy cycle, protein synthesis, mild hepatoprotective and antioxidant effects.
2	5.980	3E+08	L-Leucine	Stimulates mTOR, muscle protein synthesis; improves insulin signalling.
3	6.264	3E+08	L-Isoleucine	Supports muscle metabolism, blood sugar regulation, wound healing.
4	6.818	1E+08	L-Valine	Muscle growth, energy supply, nitrogen balance, neuroprotective.
5	7.361	8E+07	L-Serine	Neurotransmitter precursor; supports cognition and neuroprotection.
6	7.826	1E+07	Niacin	Lowers cholesterol, improves circulation, prevents pellagra.
7	7.877	2E+08	L-Threonine	Protein balance, collagen formation, gut and immune health.
8	7.924	2E+07	L-Proline	Collagen stability, wound healing, antioxidant.
9	8.058	2E+08	Glycine	Inhibitory neurotransmitter; neuroprotective, anti-inflammatory.
10	8.085	2E+08	Butane dioic acid	TCA intermediate; antioxidant, anti-hypoxic, cardioprotective.
11	9.522	2E+07	L-Aspartic acid	Urea cycle and neurotransmitter role; reduces fatigue.
12	9.596	2E+07	β-Alanine	Delay fatigue, enhances endurance.
13	9.683	2E+07	Butanoic acid, 3,4-bis[(trimethylsilyl)oxy]- trimethylsilyl ester	Neuroprotective, anticonvulsant, anti-inflammatory.
14	10.206	3E+07	Niacinamide	Anti-inflammatory, antioxidant, dermatological use.
15	10.620	2E+07	L-Threitol	Osmo-protectant, antioxidant.
16	10.816	5E+08	L-5-Oxoproline	Glutathione cycle intermediate; antioxidant defence.
17	10.860	3E+08	L-Glutamic acid, bis(trimethylsilyl) ester	Supports cognition, excess causes excitotoxicity.
18	13.283	1E+08	Putrescine, 4TMS derivative	Cell growth, wound healing, neuroprotection; excess linked to cancer.
19	13.653	3E+08	4-Phenylphenol (Internal standard)	Antimicrobial activity.
20	14.003	2E+08	9H-Purin-6-ol, 2TMS derivative	Antioxidant, neuroprotective.
21	15.373	8E+07	L-Tyrosine, 3TMS derivative	Precursor of catecholamines; cognitive and stress support.
22	15.518	5E+07	D-Mannitol, 6TMS derivative	Osmotic diuretic, antioxidant, neuroprotective.
23	16.116	1E+08	Palmitic acid, TMS derivative	Energy source, excess linked to inflammation and diabetes.
24	16.903	2E+07	D-Allose, pentakis (trimethylsilyl) ether, benzyl oxime (isomer 2)	Antioxidant, anticancer.

25	18.639	9E+07	Uridine	RNA synthesis; neuroprotective, cognitive benefits.
26	19.005	3E+07	2-Palmitoylglycerol, 2TMS derivative	Anti-inflammatory.
27	19.056	3E+07	2'-Deoxyinosine, tris(trimethylsilyl) derivative	DNA repair, antiviral.
28	19.162	5E+08	1-Monopalmitin	Antimicrobial; lipid metabolism role.
29	19.197	8E+07	Inosine	Neuroprotective, anti-inflammatory, immunomodulatory.
30	19.449	1E+08	Inosine	Adenosine

TCA: trichloroacetic acid;

Table 4: Showing phytoconstituents of aqueous extract (AQE) of *Spirulina fusiformis*

SN	RT (min)	Area	Compound name	Biological role and pathological significance
1	5.291	1.2E+09	Alanine	Helps regulate blood sugar.
2	5.370	1.7E+08	Norvaline	Nitric oxide (NO) regulation, vasodilation and potential neuroprotective effects.
3	5.996	6.5E+08	Leucine	Protein synthesis and nerve repair.
4	6.137	5.2E+07	2-Hydroxy-3-methylbutyric acid	Neuroprotective, antioxidant.
5	6.578	1E+08	Pentanoic acid	Sedative, neuroactive; potential anticonvulsant and mood stabiliser.
6	7.078	2.7E+08	2-Hydroxyisocaproic acid	Leucine metabolite; anti-catabolic, anti-inflammatory, antifungal.
7	7.361	2.4E+07	L-Serine	Crucial for neurotransmitter synthesis, nerve regeneration.
8	7.664	9.4E+08	Silanol	Antimicrobial and antioxidant; bioprotective in biological systems.
9	7.825	3.4E+07	Niacin	Lipid-lowering, vasodilatory, neuroprotective, anti-inflammatory.
10	7.892	7.1E+08	L-Isoleucine	Wound healing, blood sugar regulation.
11	8.081	1.2E+09	Glycine	Inhibitory neurotransmitter; neuroprotective, anti-inflammatory, collagen support.
12	9.136	3.4E+07	L-Threonine	Essential amino acid; supports protein balance, gut and immune health.
13	9.289	2.3E+07	Pyrimidine, 5-methyl-2,4-bis[(trimethylsilyl)oxy]-	DNA/RNA synthesis scaffold; antiviral, anticancer, anti-inflammatory base.
14	9.541	1.8E+08	L-aspartic acid	Supports urea cycle, gluconeogenesis, reduces fatigue.
15	9.624	9.3E+08	Gamma linolenic acid	Anti-inflammatory, neuroprotective, immunomodulatory, cardioprotective.
16	9.687	1.2E+07	Butanoic acid, 3,4-bis[(trimethylsilyl)oxy]-,	Neuroprotective, anticonvulsant, anti-inflammatory.
17	9.915	1.8E+07	Homoserine	Precursor for methionine, threonine, isoleucine; metabolic and antimicrobial roles.
18	10.029	1.6E+08	3-Aminoisobutyric acid, 3TMS derivative	Improves insulin sensitivity, cardioprotective.
19	10.840	9.4E+08	L-5-Oxoproline	Antiplatelets and antithrombotic activity.
20	11.981	7.9E+08	L-Glutamic acid	Major excitatory neurotransmitter, cognition, learning, excitotoxicity risk.
21	12.048	8.7E+08	5-Aminovaleric acid	Lysine metabolite, neuroactive, GABA-related.
22	13.303	5.7E+08	Putrescine	Cell growth, repair; excess linked with cancer.

23	15.231	1.1E+09	L-Lysine	Essential amino acid; protein synthesis, collagen, immunity, antiviral.
24	15.380	1.2E+08	L-Tyrosine	Precursor for dopamine and thyroid hormones; cognition, stress regulation.
25	16.120	1E+08	Palmitic acid	Saturated fat; energy storage, pro-inflammatory in excess.
26	17.273	1.9E+08	Spermine	Polyamine; DNA stabilisation, growth, neuroprotective, anti-aging.
27	17.332	4.5E+08	Phosphoric acid, bis[2,3-bis[(trimethylsilyl)oxy] propyl] trimethylsilyl ester	Energy metabolism, signalling, buffering, neuroprotection.
28	18.025	6.1E+07	Glyceryl-glycoside TMS ether	Osmo protectant, skin moisturiser, cytoprotective.
29	18.398	3.1E+07	D-(-)-Ribofuranose, tetrakis (trimethylsilyl) ether (isomer 2)	RNA sugar; nucleoside base for antiviral design.
30	18.638	6.5E+07	Uridine	Pyrimidine nucleoside; neuroprotective, antidepressant, RNA synthesis.
31	19.158	3E+08	1-Monopalmitin	Monoacylglycerol; lipid metabolism, anti-inflammatory.
32	19.193	5.9E+07	Inosine	Purine nucleoside; neuroprotective, immunomodulatory, cardioprotective.
33	19.945	2.1E+08	Glycerol monostearate	Emulsifier, energy source; anti-ulcer, moisturising.
34	20.047	5.5E+07	Aucubin, hexadic (trimethylsilyl) ether	Iridoid glycoside; hepatoprotective, antioxidant, antimicrobial, anticancer.

GABA: gamma-aminobutyric acid;

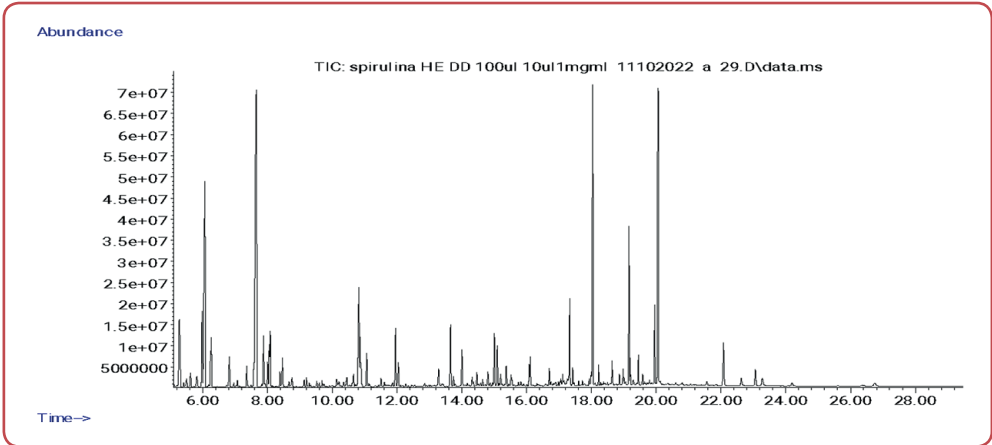


Figure 3: Gas chromatography-mass spectrometry (GC-MS) chromatograph of hydroethanolic extract (HEE) of *Spirulina fusiformis*

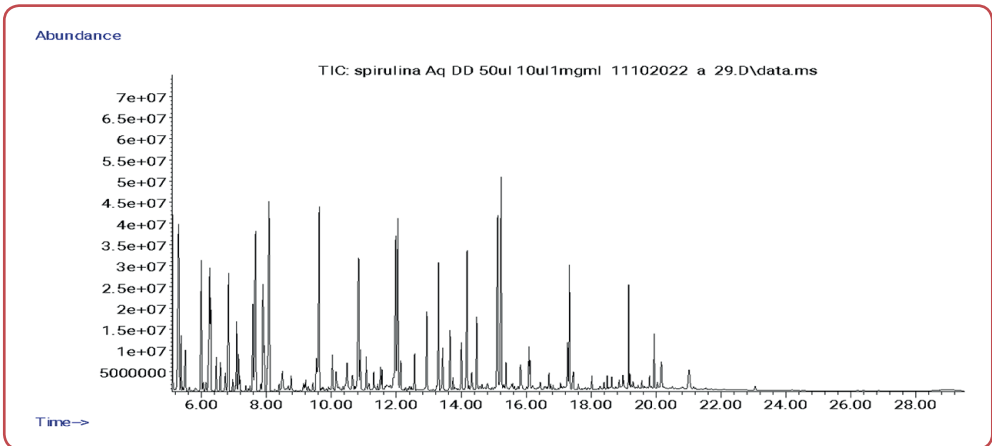


Figure 4: Gas chromatography-mass spectrometry (GC-MS) chromatograph of aqueous extract (AQE) of *Spirulina fusiformis*

MTT assay

Optimal level of glucose for RSC96 cell line is 25 mM, by increasing glucose concentration in medium will induce hyperglycaemic condition in cell line. Exposing RSC96 cells to rising doses of glucose at 50, 75, 100, 125 and 150 mM resulted in toxicity, with a maximum effect at 100 mM, resulting in 49.6 ± 12.82 % decrease cell viability as shown in Figure 5. The glucose concentration of 100 mM was chosen for further study as indicative of hyperglycaemic circumstances that can reduce the viability of RSC96 cells for testing the protective properties of *Spirulina fusiformis*. During this investigation, 24-hour therapy for culturing RSC96 Colonies at various dosages of *Spirulina fusiformis*, it shows with increase in dose of *Spirulina fusiformis* decrease cell viability. The cytotoxicity of AQE and HEE was assessed using the

MTT assay. At lower concentrations (1–2 $\mu\text{g/mL}$), AQE and HEE retained high cell viability (> 85 %), suggesting minimal cytotoxicity. But at greater doses (4–8 $\mu\text{g/mL}$), a significant reduction in viability was observed. At 8 $\mu\text{g/mL}$, AQE and HEE reduced cell viability to 63.27 ± 11.50 % and 55.10 ± 3.65 %, respectively ($p < 0.05$ vs control). These findings suggest that HEE exhibited slightly stronger cytotoxic potential than AQE at higher doses. Exposure to higher concentrations (4–8 $\mu\text{g/mL}$) of HEE could have triggered cellular stress responses leading to decreased mitochondrial enzyme activity, as reflected by reduced MTT reduction. In contrast, at lower concentrations (1–2 $\mu\text{g/mL}$), both extracts maintained high cell viability (> 85 %), indicating minimal cytotoxicity and suggesting that *Spirulina fusiformis* extracts are bio-compatible within a certain dose range (Figure 6).

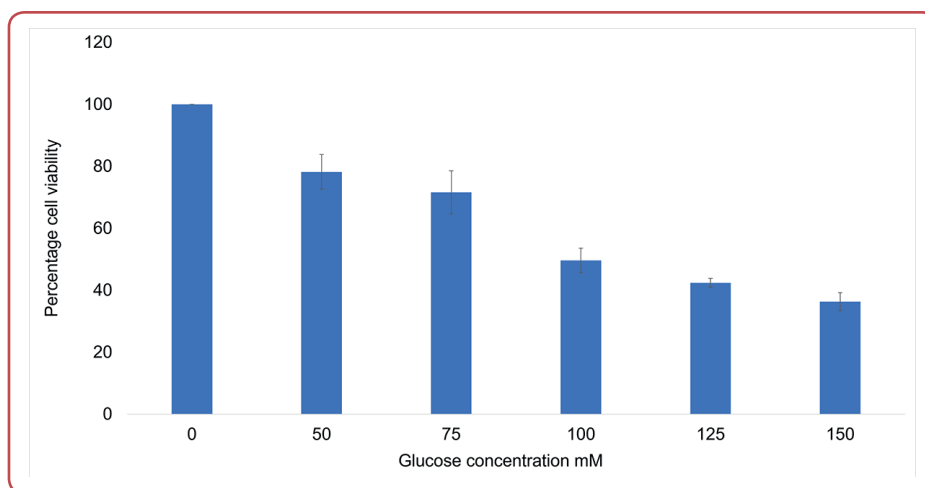


Figure 5: Cells were exposed to 0–150 mM glucose for 24 h and viability was measured by MTT assay

Data represent mean $\pm$ SD ($n = 3$). One-way ANOVA showed a significant, dose-dependent decrease in viability at higher glucose levels ($p < 0.05$).

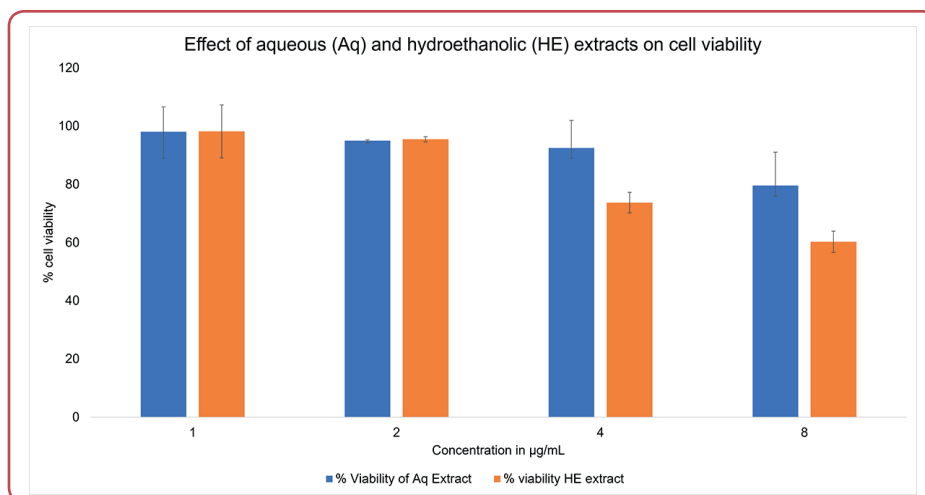


Figure 6: RSC96 cells were treated with varying concentrations (1–8 $\mu\text{g/mL}$) of aqueous (AQE) and hydroethanolic (HEE) extracts for 24 hours and cell viability was determined using the MTT assay

Data are presented as mean $\pm$ SD from three independent experiments ($n = 3$), each performed in triplicate wells. Statistical analysis was performed.

Discussion

DPN is a major complication of chronic hyperglycaemia, characterised by oxidative stress, inflammation and Schwann cell dysfunction. Nutraceuticals like *Spirulina fusiformis* have garnered attention for their potential neuroprotective and antioxidant properties, which may mitigate hyperglycaemia-induced neuronal damage. In this study, aqueous extract yield is higher than hydroethanolic extract because this plant contains highly polar, water-soluble compounds that are not efficiently extracted by mixed solvent. RSC6 Schwann cells exposed to hyperglycaemic conditions (100 mM glucose) showed a marked reduction in viability (~50 %), confirming glucose-induced cytotoxicity. Treatment with *Spirulina fusiformis* extracts demonstrated a dose-dependent effect on cell viability. At low concentrations (1–2 µg/mL), both AQE and HEE extracts maintained high cell viability (> 85 %), indicating minimal cytotoxicity. However, at higher concentrations (4–8 µg/mL), cell viability decreased, with HEE extracts exhibiting slightly higher cytotoxicity than AQE (55.1 % vs 63.3 % at 8 µg/mL), suggesting that lower doses are optimal for neuroprotection.

HEE extract had better bioactivity as the more phytochemicals like flavonoids; phenolics are present likely contributes to its stronger antioxidant effect. The antioxidant potential of *Spirulina fusiformis* extracts, assessed via DPPH experiment demonstrated dose-related free radical scavenge ability. HEE extracts exhibited slightly stronger antioxidant activity compared to AQE, likely due to higher levels of lipophilic antioxidants, due to which having higher scavenging activity and better neuroprotective action. This antioxidant property is particularly relevant in hyperglycaemia-induced oxidative stress, which contributes to Schwann cell dysfunction and neuronal injury. By mitigating reactive oxygen species (ROS), *Spirulina fusiformis* extracts can help maintain cellular integrity and viability, supporting their neuroprotective effect.

The GC–MS analysis of *Spirulina fusiformis* extracts revealed a complex profile of bioactive compounds, including amino acids, fatty acids, organic acids and other secondary metabolites. Amino acids such as L-alanine, L-leucine and L-valine are essential for neuronal metabolism, serving as precursors for neurotransmitter syn-

thesis and contributing to the maintenance of cellular homeostasis. These compounds can enhance neuroprotection by supporting neuronal energy metabolism, modulating excitotoxicity and stabilising protein synthesis under stress conditions. Fatty acids, including linoleic acid derivatives and palmitic acid, are critical for maintaining membrane fluidity, regulating signalling pathways and exerting anti-inflammatory effects, which can protect neurons from oxidative and inflammatory damage. Organic acids, particularly 2-hydroxybutyric acid, are known for their antioxidant properties, acting as free radical scavengers and supporting endogenous defence mechanisms against oxidative stress.

The HEE displayed a broader and more abundant metabolite spectrum compared to the AQE, which correlated with its higher antioxidant activity in the DPPH assay. This indicates that the extraction solvent significantly influences the yield and diversity of bioactive compounds, ultimately enhancing free radical scavenging potential. The antioxidant capacity aligns with the in vitro neuroprotective effects observed in RSC6 cell lines, where cells exposed to hyperglycaemic conditions demonstrated improved viability upon treatment with the HEE. This suggests that the identified metabolites can effectively reduce hyperglycaemia-induced oxidative stress, mitochondrial dysfunction and cytotoxicity in neuronal cells. Mechanistically, the bioactive compounds identified may exert neuroprotection through multiple pathways. Amino acids and fatty acids can modulate neuronal signalling and synaptic function, while organic acids can scavenge ROS and restore cellular redox balance. Fatty acids may also downregulate pro-inflammatory cytokines such as TNF- α and IL-6, reducing neuroinflammation, a key contributor to diabetic peripheral neuropathy. Moreover, some metabolites may activate endogenous antioxidant enzymes like superoxide dismutase (SOD) and catalase, enhancing cellular defences against oxidative damage. Together, these mechanisms support neuronal survival, reduce apoptosis and maintain functional integrity under stress conditions. Overall, the integration of GC–MS chemical profiling with functional antioxidant and cell-based assays establishes a strong link between the metabolite composition of *Spirulina fusiformis* extracts and their biological efficacy. The presence of amino acids, fatty acids and organic acids provides a chemical rationale for the observed antioxidant and neuroprotective effects and highlights the potential therapeutic

application of *Spirulina fusiformis* in managing oxidative stress-related neuropathies, particularly diabetic peripheral neuropathy. These findings also warrant further studies to isolate individual bioactive metabolites and elucidate their specific mechanisms of action in neuronal protection.

Conclusion

The GC-MS profile of the HEE and AQE of *Spirulina fusiformis* revealed the presence of numerous diverse metabolites including amino acids, fatty acids, organic acids, phenolic compounds and other secondary metabolites. However, one new iridoids namely aucubin was found in GC-MS but it is not naturally found in algae such as *Spirulina* or *Chlorella*. If detected in GC-MS data, it may be a database mis annotation or a structurally similar compound. *In vitro* study on cell line confirmed that the extract is non-toxic at low concentrations and supports cell viability under stress, but at higher concentrations it was somewhat cytotoxic. The observed cell-protective effect further supports its potential role in tissue regeneration and repair. The antioxidant potential assessed by the DPPH radical scavenging assay demonstrated that *Spirulina fusiformis* possesses significant free radical quenching activity in a concentration-dependent manner. This finding indicates its ability to mitigate oxidative stress, a major contributor to the pathogenesis of diabetic complications and neurodegeneration. In conclusion, *Spirulina fusiformis* exhibits a rich phytochemical composition, potent antioxidant activity and excellent cytocompatibility. Although the present study provides valuable insights into the antioxidant and cytoprotective potential of *Spirulina fusiformis* extracts, it is limited to *in vitro* evaluations. Therefore, these findings may not fully represent the *in vivo* pharmacological behaviour of the extracts. Furthermore, the underlying molecular mechanisms responsible for the observed effects, such as modulation of oxidative stress and inflammatory pathways, were not specifically examined in this study. Future research should aim to elucidate these mechanisms by assessing oxidative stress biomarkers and inflammatory mediators, as well as isolating and characterising the individual bioactive metabolites responsible for the observed activity.

Ethics

This study did not directly involve with human participants or experimental animals. Therefore, the ethics approval was not required in this paper.

Acknowledgement

The authors would like to express their sincere gratitude to all individuals and institutions who contributed to the successful completion of this research. We acknowledge the support of Global Research Institute of Pharmacy, Ch Devi Lal College of Pharmacy and Maharishi Markandeshwar College of Pharmacy for providing necessary facilities and resources.

Conflicts of interest

The authors declare that there is no conflict of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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