PLANT SCIENCE TODAY
Vol 12(sp1): 1-5
https://doi.org/10.14719/pst.9793

eISSN 2348-1900

RESEARCH ARTICLE

Evaluation of antioxidant properties of selected cyanobacterial strains for potential use as natural antioxidants

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Received: 04 June 2025; Accepted: 26 July 2025; Available online: Version 1.0: 21 August 2025; Version 2.0: 16 September 2025

Cite this article: Anjali S, Kamakshi, Devendra K. Evaluation of antioxidant properties of selected cyanobacterial strains for potential use as natural antioxidants. Plant Science Today. 2025; 12(sp1): 1-5. https://doi.org/10.14719/pst.9793

Abstract

Cyanobacteria are recognized for their antioxidant properties; however, comparative analyses of strain-specific antioxidant capacity are scarce, particularly among isolates from agricultural soils. In this study, seven cyanobacterial strains (*Nostoc* sp., *Anabaena* sp., *Westiellopsis* sp., *Oscillatoria* sp., *Tolypothrix* sp., *Calothrix* sp., *Phormidium* sp.), isolated from rice fields of Uttar Pradesh, India, were evaluated for their antioxidant capacity. Ferric reducing antioxidant power (FRAP) assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation decolorization assay were used to analyze antioxidant activity. Trolox (µmol trolox g^{-1}) was used to standardise the antioxidant capacity. *Anabaena* sp. showed the highest antioxidant activity among all the strains tested in all three assays, with values of 9.12 ± 0.05 µmol trolox g^{-1} ABTS assay, 8.96 ± 0.03 µmol trolox g^{-1} FRAP assay and 8.93 ± 0.03 µmol trolox g^{-1} DPPH assay. *Tolyprothrix* sp. showed the lowest antioxidant activity in all assays, with values of 5.02 ± 0.05 µmol trolox g^{-1} ABTS assay, 4.12 ± 0.03 µmol trolox g^{-1} FRAP assay and 4.01 ± 0.02 µmol trolox g^{-1} DPPH assay. These results imply that the species and assay technique significantly influence the antioxidant capacity of cyanobacterial strains. The study emphasises the importance of selecting appropriate assay techniques when assessing the antioxidant potential of cyanobacteria. The observed diversity underscores the necessity of using standardised protocols to evaluate and compare the antioxidant characteristics of various strains. Further research is recommended to explore potential applications in medicines and nutraceuticals and to investigate the underlying metabolic processes responsible for the observed antioxidant effects. This comparative investigation highlights the significance of selecting suitable cyanobacterial strains for the extraction of antioxidant compounds. By identifying high-activity strains, researchers can enhance the application of natural antioxidants in food preservation, cosmetic formulations and therapeutics.

Keywords: ABTS assay; antioxidant activity; cyanobacteria; DPPH assay; FRAP assay

Introduction

Paddy cultivation is a major agricultural activity in Uttar Pradesh. Traditional agricultural methods frequently rely on chemical fertilisers to meet the nutritional requirements of crops (1). Excessive use of synthetic fertilisers has resulted in environmental issues, including soil degradation, water contamination and greenhouse gas emissions (2). These challenges highlight the necessity for sustainable agriculture approaches to improve soil fertility and foster robust crop development (3).

Cyanobacteria are photosynthetic microorganisms that flourish in aquatic environments, such as paddy fields. They are integral to the nitrogen cycle by converting atmospheric nitrogen into forms available to plants (4). Cyanobacteria also synthesise several beneficial compounds, including antioxidants, which safeguard plants from oxidative stress and enhance overall plant health (5). The efficacy of cyanobacteria as biofertilizers is well-established, with research demonstrating that their use can augment yield, boost soil structure and reduce reliance on chemical fertilizers (6). However, the antioxidant properties of cyanobacterial strains extracted from paddy fields in specific regions remain inadequately investigated.

Moreover, there is an unmet need for thorough investigations comparing the antioxidant capabilities of various cyanobacterial strains through standardised assays such as 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH). This knowledge gap hinders understanding and obstructs the identification of superior strains for biofertilizer applications and their incorporation in sustainable agricultural practices (7).

This study seeks to address these gaps by investigating seven cyanobacterial strains procured from the Department of Microbiology, Chaudhary Charan Singh University (CCSU), Meerut. The strains isolated from regions

of Uttar Pradesh, were assessed for their antioxidant activity using ABTS, FRAP and DPPH assays. By comparing the antioxidant profiles of different strains, we aim to identify those with exceptional antioxidant potential for use as biofertilizers to improve rice agriculture. This research aspires to support eco-friendly agriculture practices by elucidating the antioxidant capabilities of native cyanobacterial strains. Harnessing the inherent potential of these microorganisms can reduce dependence on artificial fertilisers, foster sustainable agriculture and improve the overall health and yield of rice crops.

Materials and Methods

Selection of cyanobacterial strains

Seven cyanobacterial strains were isolated from paddy fields in the Meerut, Muzaffarnagar and Haridwar regions. These strains were obtained from the Algal Culture Collection of the Department of Microbiology, Chaudhary Charan Singh University (CCSU), Meerut, Uttar Pradesh, India. The selected strains are *Nostoc* sp., *Anabaena* sp., *Westiellopsis* sp., *Oscillatoria* sp., *Tolypothrix* sp., *Calothrix* sp. and *Phormidium* sp.

Identification of cyanobacterial strains

The identification was confirmed using the outlined techniques (8).

Total phenolic content (TPC)

A total of 100 µL of extract was combined with 500 µL of 10 % Folin-Ciocalteu reagent and incubated for 5 min. Subsequently, 400 µL of 7.5 % sodium carbonate (Na_2CO_3) was added and the mixture was incubated at room temperature for 30 min (9). The absorbance was quantified at 765 nm using a UV-Vis spectrophotometer.

Total flavonoid content (TFC)

A total of 500 µL of extract was added to 300 µL of 5 % sodium nitrate ($NaNO_2$) and incubated for 5 min. Subsequently, 300 µL of 10 % aluminium chloride ($AlCl_3$) was added, followed by the addition of 2 mL of 1 M sodium hydroxide (NaOH) after 6 min. The final volume was adjusted to 5 mL using distilled water (10). The absorbance was recorded at 510 nm.

Phycobiliprotein quantification

A total of 50 mg of dry biomass was suspended in 5 mL of 0.05 M phosphate buffer (pH 6.8) and subjected to repeated freezing and thawing cycles until the pigment was released in the supernatant and the pellet became colourless (11). After centrifugation, the absorbance of the supernatant was measured at 562, 615 and 652 nm.

Carotenoid content

To estimate carotenoids, 10 mL of homogenised algae was centrifuged at 4000 g for 10 min. After rinsing with distilled water to remove salts, the supernatant was removed. Then, 2.5 mL of 85 % acetone was added and the mixture was stored at 4 °C for one week. The freezing and thawing technique was repeated until the supernatant turned colourless (12). The absorbance was measured at 450 nm using 85 % acetone as a blank.

Antioxidant activity

Free radical scavenging activity using DPPH assay

A total of 10 µL of the extract was added to 90 µL of the DPPH solution (100 µM methanol) in a 96-well microplate and incubated at room temperature in the dark for 30 min. The absorbance was recorded at 517 nm using a microplate reader (13).

FRAP assay

FRAP was conducted following the outlined technique (14). The FRAP reagent was formulated by mixing 25 mL of acetate buffer, 25 mL of tripyridyltriazine (TPTZ) solution and 2.5 mL of ferric chloride hexahydrate ($FeCl_3.6H_2O$) solution. Then, the mixture was incubated at 37 °C prior to further use. A total of 100 µL of cyanobacterial extract was combined with 3 mL of the FRAP reagent and incubated at 37 °C for 30 min. Absorbance was measured at 593 nm with a spectrophotometer.

ABTS assay

ABTS was prepared by synthesizing equal volumes of ABTS stock solution (7 mM ABTS in distilled water) and 2.45 mM potassium persulfate solution (K_2S_2O) in distilled water, followed by incubation at room temperature in the dark for 12 hr to ensure complete production of the radical cation. Then, 3.9 mL of diluted ABTS radical cation solution was mixed with 100 µL of the extract. After 6 min of incubation, absorbance was measured at 734 nm using a spectrophotometer (15).

Statistical analysis

All experimental measurements were performed in triplicate and the data were expressed as mean ± standard deviation (SD) using SPSS 25.0. Duncan's multiple range test (DMRT) and least significant difference test (LSD) were used to compare the mean performance of strain-specific parameters.

Results

Antioxidant compounds

The seven cyanobacteria strains were selected for comparative analysis based on their potential antioxidant relevance (Table 1). These strains were preliminarily screened to assess the presence of antioxidant compounds (Table 2). The antioxidant activity evaluated using DPPH, ABTS and FRAP assays exhibited considerable variation among the examined cyanobacterial strains (Table 3). *Anabaena* sp. (AS2), *Phormidium* sp. (AS7) and *Calothrix* sp. (AS6) exhibited higher antioxidant activity, correlating with their increased concentrations of phenolics, flavonoids, phycocyanin, phycoerythrin and carotenoids (Table 2). *Tolypothrix* sp. (AS5) and *Oscillatoria* sp. (AS4) have low antioxidant activity, consistent with their relatively lower concentrations of

Table 1. Selection of cyanobacterial strains from the different regions of Uttar Pradesh and their label

S. No.	Label	Cyanobacterial species	Collection site
1	AS1	*Nostoc* sp.	Pabarsa, Meerut
2	AS2	*Anabaena* sp.	Jani Khurd, Meerut
3	AS3	*Westiellopsis* sp.	Mawana, Meerut
4	AS4	*Oscillatoria* sp.	Khatauli, Muzaffarnagar
5	AS5	*Tolypothrix* sp.	Budhana, Muzaffarnagar
6	AS6	*Calothrix* sp.	Laskar, Haridwar
7	AS7	*Phormidium* sp.	Khanpur, Haridwar

Table 2. Pre-screening of antioxidant compounds in selected cyanobacterial strains

Strains	Phenolics	Flavonoids	Phycocyanin	Phycoerythrin	Carotenoids
Anabaena sp.	+	+	+	+	+
Westiellopsis sp.	+	+	+	+	+
Nostoc sp.	+	+	+	±	+
Oscillatoria sp.	+	–	+	–	+
Phormidium sp.	±	±	+	–	+
Tolypothrix sp.	±	–	±	–	±
Calothrix sp.	–	–	±	–	–

± = trace or inconsistent reports, – = absent or undetected.

bioactive compounds. *Tolypothrix* sp. (AS5) and *Oscillatoria* sp. (AS4) lacked several essential categories, including flavonoids, carotenoids and vitamin C.

Antioxidant activity

In the DPPH assay, *Anabaena* sp. exhibited the highest antioxidant activity, with a mean value of 8.96 ± 0.01 µmol trolox g^{-1}, followed by *Westiellopsis* sp. at 7.01 ± 0.01 µmol trolox g^{-1}. The minimal DPPH activity was recorded in *Tolypothrix* sp. at 4.12 ± 0.01 µmol trolox g^{-1} (Table 3). In the ABTS assay, *Phormidium* sp. demonstrated the highest scavenging capacity (9.12 ± 0.02 µmol trolox g^{-1}), followed by *Calothrix* sp. (7.12 ± 0.01 µmol trolox g^{-1}) and *Nostoc* sp. (6.34 ± 0.02 µmol trolox g^{-1}). The minimal ABTS activity was observed in *Oscillatoria* sp. at 5.02 ± 0.01 µmol trolox g^{-1} and *Tolypothrix* sp. at 5.22 ± 0.01 µmol Trolox g^{-1} (Table 3).

The FRAP assay, *Phormidium* sp. exhibited the maximum ferric reducing power at 8.96 ± 0.01 µmol trolox g^{-1}, followed by *Calothrix* sp. at 7.01 ± 0.01 µmol trolox g^{-1} and *Nostoc* sp. at 5.78 ± 0.02 µmol trolox g^{-1}. The minimal FRAP activity was seen in *Oscillatoria* sp. at 4.12 ± 0.01 µmol trolox g^{-1} (Table 3).

Duncan's multiple range test (DMRT) was utilised to statistically categorise the strains according to their antioxidant efficacy, based on the mean values obtained from the ABTS, FRAP and DPPH assays (Table 3). In the ABTS assay, *Phormidium* sp. and *Calothrix* sp. demonstrated statistical superiority, exhibiting substantial group differentiation at both the 5 % and 1 % LSD levels. Similar trends were observed in the FRAP assay, where *Phormidium* sp. and *Calothrix* sp. displayed markedly enhanced reducing power. In the DPPH assay, *Anabaena* sp. exhibited the highest radical scavenging capacity, distinctly separating itself from other strains according to DMRT grouping.

These results affirm the strain-specific antioxidant activity of cyanobacterial isolates and support the selection of effective strains for prospective biotechnological, nutraceutical and medical applications.

Discussion

The comparative examination of antioxidant activity across seven cyanobacterial strains indicated significant interspecies variations, with *Anabaena* sp., *Westiellopsis* sp. and *Nostoc* sp. exhibiting the best radical scavenging ability in DPPH, ABTS and FRAP assays. The result provides a comprehensive comparison including LSD and DMRT tests. *Anabaena* sp. exhibited the highest antioxidant activity in the DPPH assay. The antioxidant activity of *Tolypothrix* sp. was comparatively significantly lower antioxidant activity of other species ($p < 0.05$).

The reduced radical scavenging capacity of *Tolypothrix* sp. may be related to strain-specific differences in metabolite profiles, notably a reduced concentration of phenolics and phycobiliproteins, antioxidant components. The reduced radical scavenging capacity of *Tolypothrix* sp. may be related to strain-specific differences in metabolite profiles, notably a reduced concentration of phenolics, phycobiliproteins and antioxidant components.

In the ABTS assay, *Phormidium* sp. has superior antioxidant activity (9.12 ± 0.02) µmol trolox g^{-1}, significantly higher than all other strains. In the FRAP assay, which quantifies ferric reducing antioxidant capacity, *Phormidium* sp. exhibited the highest activity (8.96 ± 0.01) µmol trolox g^{-1}, followed by *Calothrix* sp. (7.01 ± 0.01 µmol trolox g^{-1}). *Oscillatoria* sp. exhibited the lowest FRAP activity (4.12 ± 0.01 µmol trolox g^{-1}), indicating low reduction power. *Westiellopsis* sp. also displayed an excessive amount of antioxidant activity and it was statistically categorised as the second most influential strain in the DPPH assay. This is an interesting finding because there isn't much research on the antioxidant qualities of *Westiellopsis*. This suggests that it could be a good source of natural antioxidants for biotechnological uses. *Calothrix* sp. and *Nostoc* sp. demonstrated statistically comparable antioxidant capabilities in the DPPH assays ($p > 0.05$), as seen by DMRT groupings.

Table 3. Comparative antioxidant activity (µmol trolox g^{-1}) through ABTS, FRAP and DPPH assay with DMRT grouping.

Cyanobacterial strains	DPPH (Mean ± SD)	ABTS (Mean ± SD)	FRAP (Mean ± SD)
Anabaena sp.	8.96 ± 0.01^{f}	6.12 ± 0.01^{c}	5.01 ± 0.01^{b}
Calothrix sp.	5.12 ± 0.01^{c}	7.12 ± 0.01^{e}	7.01 ± 0.01^{e}
Nostoc sp.	5.12 ± 0.01^{c}	6.34 ± 0.02^{d}	5.78 ± 0.02^{d}
Oscillatoria sp.	5.78 ± 0.01^{d}	5.02 ± 0.01^{a}	4.12 ± 0.01^{a}
Phormidium sp.	5.01 ± 0.01^{b}	9.12 ± 0.02^{f}	8.96 ± 0.01^{f}
Tolypothrix sp.	4.12 ± 0.01^{a}	5.22 ± 0.01^{b}	5.12 ± 0.01^{b}
Westiellopsis sp.	7.01 ± 0.01^{e}	6.14 ± 0.01^{c}	5.12 ± 0.01^{c}

Significant differences among strains at the 5 % level are indicated by different letters in the mean values (± SD) for DMRT. Different letters indicate significant differences between groups as per DMRT at 5 % and 1 % level. n = 3 is the number of replicates.

The compound screening data confirmed these tendencies, revealing that the more active strains exhibited higher levels of phenolics, flavonoids, carotenoids, phycobiliproteins, ascorbic acid and tocopherols. The strong correlation between bioactive content and antioxidant potential aligns with recent studies, which indicate those cyanobacteria with increased phenolic and flavonoid content exhibit enhanced oxidative stress-mitigating abilities (16).

The primary aim of this study was to assess the biochemical potential of cyanobacterial strains for application as antioxidant-enriched biofertilizers, particularly in rice agroecosystems. The results not only corroborate this idea but also furnish a biochemical foundation for strain selection. Antioxidant-rich cyanobacteria, such as *Anabaena* sp. and *Nostoc* sp., enhanced the vigour and salinity tolerance of rice seedlings by improving the scavenging mechanisms of reactive oxygen species in plant tissues (17, 18).

The existence of phycobiliproteins (phycocyanin, allophycocyanin and phycoerythrin) and carotenoids significantly enhances photosynthetic efficiency and stress resilience, rendering these strains resilient in field circumstances (19). Ascorbic acid, found in the stronger strains, is a recognised antioxidant that enhances cellular protection in microorganisms (20).

These characteristics correspond with the prevailing trend in sustainable agriculture to incorporate multifunctional bioinoculants that merge fertilisation capabilities with the development of plant health (21). In rice cultivation, where crops frequently experience oxidative stress from waterlogging, salt, or nutrient deficiencies, the combined effects of nitrogen fixation and antioxidant provision by cyanobacteria present an innovative and environmentally sustainable approach (22). By augmenting the rhizosphere with necessary nutrients and antioxidative compounds, these strains can enhance rice plant resistance, diminish reliance on chemical fertilisers and promote overall soil health.

This study contributes to the expanding evidence supporting cyanobacterial biofertilizers as not only a nutritional source but also as biochemical enhancers of plant stress resilience. The results endorse forthcoming field trials and the construction of antioxidant-rich cyanobacterial consortia designed for rice-based agroecosystems.

Conclusion

The research indicates the substantial antioxidant capacity of several cyanobacterial strains through a thorough assessment utilising DPPH, ABTS and FRAP assays, evaluated against trolox equivalents. The findings emphasise the biochemical compounds of cyanobacteria and their potential as natural sources of antioxidants for nutraceutical, medicinal and biotechnological uses. The research presents a core framework for selecting effective strains with high antioxidant capacity. These strains can be further explored for the isolation of bioactive compounds, the production of functional foods and the production of natural antioxidants for industrial applications.

Acknowledgements

Authors are thankful to SRM Institute of Science and Technology, Delhi-NCR Campus, Modi Nagar, Ghaziabad, UP, India and CCS University Campus, Meerut, UP, India, for providing the research platform and availability, which was helpful during the research work.

Authors' contributions

AS participated in data collection and carried out the statistical analysis. K and DK participated in designing and manuscript correction. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors do not have any conflict of interest to declare.

Ethical issues: None

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Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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