

Bioactive potential of freshwater *Spirogyra* species: Comparative antioxidant and antifungal activity

Tatlı su *Spirogyra* türlerinin biyoaktif potansiyeli: Karşılaştırmalı antioksidan ve antifungal aktivite

Hamna Sohail^{1*} • Ghazala Yasmeen Butt²

¹Department of Botany, Faculty of Chemistry and Life Sciences, Government College University, Lahore, Pakistan

²Institute of Botany, University of the Punjab, Quaid-i-Azam Campus, Lahore, Pakistan

*Corresponding author: hamnasohail60@gmail.com

Received date: 27.01.2025

Accepted date: 24.03.2026

How to cite this paper:

Sohail, H., & Butt, G.Y. (2026). Bioactive potential of freshwater *Spirogyra* species: Comparative antioxidant and antifungal activity. *Ege Journal of Fisheries and Aquatic Sciences*, 43(2), 145-151. <https://doi.org/10.12714/egejfas.43.2.07>

Abstract: Freshwater green algae, particularly *Spirogyra* sp., is a potential source of bioactive compounds with pharmacological relevance. This study evaluated the antioxidant and antifungal activities of *Spirogyra* sp. collected from Jilani Park Racecourse, Lahore, Pakistan. Methanolic and chloroform extracts were analysed using total antioxidant activity (TAA), ferric reducing antioxidant power (FRAP), total phenolic content (TPC), and metal chelating (MC) assays. Antifungal activity was assessed against *Aspergillus niger* Tiegh and *Rhizopus stolonifer* (Ehrenb.) Vuill., using fluconazole as a positive control. Methanolic extracts exhibited significantly higher TAA, TPC and MC activities compared to chloroform extracts, whereas chloroform extracts showed markedly higher FRAP values. Differences between methanolic and chloroform extracts were statistically significant ($p < 0.0001$). Both extracts demonstrated antifungal activity; methanolic extracts showed stronger initial inhibition, whereas chloroform extracts maintained sustained inhibitory effects over 48 h. Overall, *Spirogyra* sp. exhibited notable antioxidant and antifungal potential. These findings provide evidence of its solvent-dependent bioactive properties and support further in-depth investigations to explore its potential applications in pharmaceutical, nutraceutical, and biotechnological formulations.

Keywords: *Spirogyra*, antioxidant assays, antifungal activity, methanol and chloroform extracts

Öz: Tatlısu yeşil algleri, özellikle *Spirogyra* sp., farmakolojik öneme sahip biyoaktif bileşiklerin potansiyel bir kaynağıdır. Bu çalışma, Pakistan'ın Lahor şehrindeki Jilani Park Hipodromu'ndan toplanan *Spirogyra* sp.'nin antioksidan ve antifungal aktivitelerini değerlendirmiştir. Metanolik ve kloroform özütleri, toplam antioksidan aktivite (TAA), demir indirgeyici antioksidan güç (FRAP), toplam fenolik içerik (TPC) ve metal şelatlama (MC) testleri kullanılarak analiz edilmiştir. Antifungal aktivite, pozitif kontrol olarak flukonazol kullanılarak *Aspergillus niger* Tiegh ve *Rhizopus stolonifer* (Ehrenb.) Vuill.'e karşı değerlendirilmiştir. Metanolik özütlere kıyasla önemli ölçüde daha yüksek TAA, TPC ve MC aktiviteleri sergilerken, kloroform özütleri belirgin şekilde daha yüksek FRAP değerleri göstermiştir. Metanolik ve kloroform özütleri arasındaki farklılıklar istatistiksel olarak anlamlıdır ($p < 0.0001$). Her iki özütle de antifungal aktivite göstermiştir; metanolik özütlere daha güçlü başlangıç inhibisyonu gösterirken, kloroform özütleri 48 saat boyunca sürekli inhibitör etkilerini korumuştur. Genel olarak, *Spirogyra* sp. önemli antioksidan ve antifungal potansiyel sergilemiştir. Bu bulgular, çözücüye bağlı biyoaktif özelliklerine dair kanıt sağlamakta ve farmasötik, nutrasötik ve biyoteknolojik formülasyonlardaki potansiyel uygulamalarını araştırmak için daha derinlemesine araştırmaları desteklemektedir.

Anahtar kelimeler: *Spirogyra*, antioksidan testleri, antifungal aktivite, metanol ve kloroform özütleri

INTRODUCTION

Algae are globally recognized as a rich source of bioactive compounds with diverse pharmacological properties, including antioxidant, antimicrobial, antiviral, anti-inflammatory, and cytotoxic activities (Bhattacharya, 2011; Lee et al., 2013; Lordan et al., 2011; Muthuirulappan and Francis, 2013; Nobili et al., 2009). Synthetic antioxidants, commonly used in foods and pharmaceuticals, are often linked to adverse effects such as carcinogenesis and liver toxicity, which has intensified interest in natural alternatives. Freshwater algae, particularly *Spirogyra*, are promising due to their high content of phenolics, flavonoids, terpenoids, and other secondary metabolites capable of mitigating oxidative stress and inhibiting pathogenic microorganisms. *Spirogyra*, a filamentous green alga of the order Zygnematales (class Chlorophyta), forms free-floating mats in shallow, slow-moving waters such as ponds, canals, and littoral zones of lakes (Hainz et al., 2009; Naik et al., 2012; Thiamdao and Peerapornpisal, 2011). With over 400 species

reported worldwide, its characteristic spirally arranged chloroplasts facilitate easy identification. Beyond ecological significance, *Spirogyra* has gained attention for industrial applications, including biofuel production, sorption technologies, cosmetics, and as a natural antioxidant in foods and pharmaceuticals (Lee, 2008; Punyoyai, 2008; Taş et al., 2015).

Recent research has highlighted the significant bioactive potential of *Spirogyra*. Samples from the Algerian Sahara demonstrated antioxidant, antibacterial, and cytotoxic activities, with compounds such as gallotannins and monolinolenin identified as key metabolites (Belyagoubia et al., 2025). Similarly, a study reported diverse phytochemical constituents and strong antimicrobial effects of *Spirogyra* extracts against several pathogenic bacteria (Belyagoubi et al., 2023). These findings emphasize the growing pharmacological relevance of *Spirogyra* and support the need for systematic, location-specific

studies to fully explore its bioactive properties. Despite its recognized industrial potential, systematic biochemical and pharmacological investigations at the genus level remain scarce. Most prior studies focus on ecological or industrial aspects, with limited evaluation of antioxidant and antifungal activities, often restricted to one or two assays or species-level observations. Consequently, temporal dynamics and solvent-dependent bioactivities of *Spirogyra* remain largely unexplored.

To bridge this gap, the present study delivers the first comprehensive, location-specific assessment of antioxidant and antifungal properties of *Spirogyra* sp. from Jilani Park Racecourse, Lahore, Pakistan. This research integrates four antioxidant assays (TAA, FRAP, TPC, and MC) with a time-based antifungal evaluation against *Aspergillus niger* Tiegh and *Rhizopus stolonifer* (Ehrenb.) Vuill. ([Index Fungorum, 2025](#)) comparing methanolic and chloroform extracts. A key novel finding is the contrasting durability of antifungal activity: methanolic extracts exhibit strong but transient inhibition, whereas chloroform extracts maintain sustained antifungal effects over 48 h. By combining solvent comparison with temporal bioactivity profiling, this study provides preliminary data on antioxidant and antifungal activities, forming a basis for future detailed investigations on *Spirogyra* sp. as well as new and significant insights into pharmacological potential of freshwater *Spirogyra*, highlighting its promise for natural product development in pharmaceutical and biotechnological applications. The present study meant to investigate the antioxidant and antifungal potential of freshwater green algae *Spirogyra* sp. Antioxidant activity was evaluated using multiple assays, whereas antifungal efficacy was assessed

against *A. niger* and *R. stolonifer* using fluconazole as a standard antifungal control. Furthermore, this study provides baseline data to support future pharmacological and biotechnological research on *Spirogyra*-derived bioactive compounds.

MATERIALS AND METHODS

Algal sample collection and preparation

Freshwater *Spirogyra* sp. samples were collected in bulk from Jilani Park Racecourse, Lahore, Pakistan, during February-March 2017, when the ambient temperature ranged between 24-32 °C. Samples were examined under a compound light microscope and identified based on morphological characteristics with reference to standard taxonomic literature ([Shameel, 2001](#)). Collected samples ([Figure 1](#)) were thoroughly washed with distilled water to remove debris, air-dried and were manually ground into fine powder (200 g dry weight). The powdered sample was divided into two equal portions (100 g each) for extraction with methanol and chloroform.

Extraction of bioactive compounds

Extraction of bioactive compounds was carried out using solvent maceration method as described by [Harborne \(1973\)](#), with slight modifications. Each portion of algal powder was soaked in the respective solvent (methanol or chloroform) for approximately 7 days to ensure complete extraction of bioactive compounds. After soaking, extracts were concentrated using a rotary evaporator for 2 h at 35 °C. The resulting crude extracts were collected and stored at 4 °C until further analysis ([Figure 2](#)).

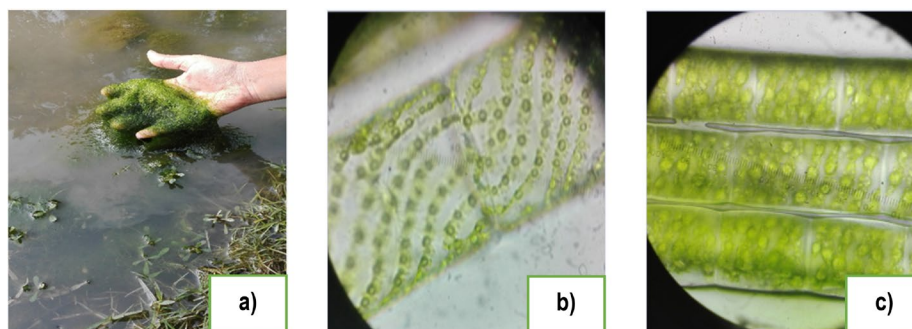


Figure 1. a) Fresh water algae *Spirogyra* sp., growing in bulk quantity, collected from Jilani Park Racecourse, Lahore, Pakistan. b) Surface view of *Spirogyra* sp., pyrenoids are present, c) Surface view of *Spirogyra* sp., chloroplasts present

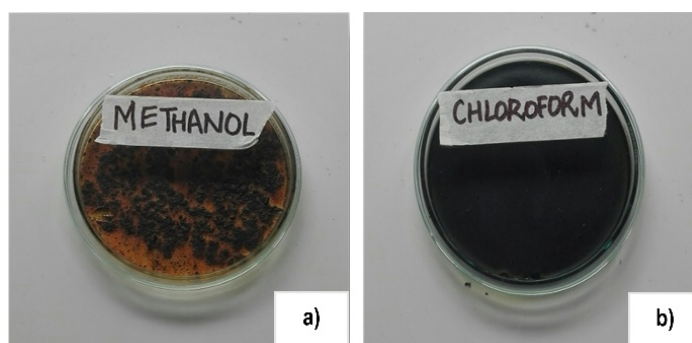


Figure 2. a) Crude extract of freshwater algae *Spirogyra* sp., in methanol solvent, b) Crude extract of freshwater algae *Spirogyra* sp., in chloroform solvent

Antioxidant activity

Antioxidant activity was evaluated by Total Antioxidant Activity (TAA), Ferric Reducing Antioxidant Activity (FRAP) Assay, Quantification of Total Phenolic Contents (TPC) and Metal Chelating activity (MC).

Total antioxidant activity (TAA)

Total antioxidant activity was determined by the phosphomolybdenum complex formation method described by Prieto et al. (1999). The reagent solution was prepared by mixing 5.32 g sodium phosphate, 2.47 g ammonium molybdate, and 16.7 mL sulfuric acid in 500 mL distilled water. Algal extract (sample) was mixed with 4 mL of reagent solution. The blank contained 4 mL reagent solution. Test tubes were cotton-plugged and incubated in a water bath for 90 min at 95°C. After cooling to room temperature, absorbance was measured at 695 nm. Results were expressed as milligrams, per gram of ascorbic acid, calculated by following equation derived from calibration curve of ascorbic acid at different concentrations (Annexure I, 1.1).

$$X = \frac{Y + 0.0328}{0.0112}$$

Y= Sample absorbance

X= mg / mL of ascorbic acid

Ferric reducing antioxidant activity (FRAP)

FRAP was determined according to the method of Benzie and Strain (1996). The FRAP reagent was prepared by mixing 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of 10 mM TPTZ solution (in 40 mM HCl), and 2.5 mL of 20 mM ferric chloride (FeCl₃). 2990 µL of FRAP reagent was mixed with 10 µL of algal extract and incubated in the dark for 30 min. Absorbance was measured at 593 nm. Results were expressed in Trolox equivalents (TE µM / mL) using standard calibration curve. (Annexure I, 1.2).

$$X = \frac{Y - 0.069}{0.002}$$

Y= Sample absorbance

X= TE (µM / mL)

Total phenolic content (TPC)

Total phenolic content was determined by the method of Makkar et al. (1993). The algal sample was mixed with 2.8 mL of 10% Na₂CO₃ and 0.1 mL of 2N Folin-Ciocalteu reagent. Absorbance was measured at 725 nm after 40 min. TPC values were expressed in GAE µg / mL, following equation, derived from standard calibration curve (Annexure I, 1.3).

$$Y = 0.006X + 0.139$$

Y= Sample absorbance

X= TPC value

Metal chelating activity (MC)

Metal chelating activity was evaluated using the method of Dinis et al. (1994). 50 µL of 2 mM ferrous sulfate and 200 µL of ferrozine were added to 100 µL of algal extract, and the volume was adjusted to 4 mL with methanol. The solution was incubated at room temperature for 15 min, and absorbance was measured at 562 nm. Percentage inhibition of ferrozine ferrous complex formation was determined by following formula:

$$\% \text{ Inhibition} = [(Ab-As)/Ab] \times 100$$

Ab = absorbance of blank solution

As = absorbance of the algal extract

Antifungal activity

Antifungal activity was carried out using the agar well diffusion method as described by Perez et al. (1990), with slight modifications. Fungal strains *A. niger* and *R. stolonifer* used for the evaluation of antifungal activity were obtained from Botany Department. Investigation was accomplished by extracting raw algae in solvents methanol and chloroform by rotary evaporator and assessment of antifungal activity by zone of inhibition method.

Preparation of algal extract dilutions

Algal extracts were prepared from the rotary evaporator and three dilutions were made from it. In 0.5 mL of each of the solvents (methanol and chloroform) 0.5 g of *Spirogyra* sp. extract was dissolved to make final concentration of 1 /1 g/ mL. Likewise, second and third dilutions were made by dissolving 0.5 g of each extract in 5 mL and 50 mL of each of the two solvents to make final concentration of 1 /10 g/ mL and 1 /100 g/ mL respectively. Later, these concentrations were transferred to glass test tubes with tagging and were stored at 20 °C until use.

Preparation of fungal cultures

Fungal specimens (*A. niger* and *R. stolonifer*) were cultured on Potato Dextrose Agar (PDA) medium. PDA medium was prepared by dissolving 9.75 g in 250 mL distilled water, adjusting pH to 5.6 ± 0.2, and sterilizing by autoclaving at 121 °C and 15 psi for 15 min. Following autoclaving, 15 mL of medium was poured into each sterile petri plate, which was then sealed with cling film and stored at room temperature until use. After solidification, four wells were aseptically made in each plate using a sterile cork borer (No. 4) and labelled according to extract concentration: 1/ 1, 1/ 10, 1/ 100 and crude.

Agar well diffusion assay

Each well was loaded with 10 µL of the corresponding extract, corresponding to final extract doses of 10 mg/well (1/1 g/ mL), 1 mg/well (1/10 g/ mL), 100 µg/well (1/100 g/ mL), and 10 mg/well (crude extract). Petri plates were further labeled with the test organism and solvent used. For inoculum preparation, fungal strains were aseptically transferred to sterile glass vials containing 20 mL of autoclaved distilled water using cotton swabs. Inoculum was subsequently applied to

labeled petri plates. The same technique was repeated for the control group as well. Fluconazole (25 µg/ well) was used as a positive control. Plates were incubated at 25 ± 2 °C for 48 h, antifungal activity was assessed by measuring diameter of inhibition zones (mm) encircling each well. The diameter of zone was measured in cm using ruler and later converted into mm.

Statistical analysis

All experiments were performed in triplicate, and results are expressed as mean \pm SE. Data were analyzed using one-way ANOVA to evaluate differences between methanol and chloroform extracts. Post hoc Tukey's test was used for pairwise comparisons. Differences were considered statistically significant at $p < 0.0001$.

RESULTS

Extraction yield

The extraction of *Spirogyra* sp. was performed using 100 g of dry algal biomass for each solvent. Methanol extraction yielded 2.86 g (2.86 %), while chloroform extraction yielded 2.37 g (2.37 %). Post-extraction biomass was 89.80 g for methanol and 90.46 g for chloroform, indicating minimal loss during the process and consistent reproducibility across solvents.

Antioxidant activity

The antioxidant potential of *Spirogyra* sp. extracts was assessed through total antioxidant activity (TAA), ferric reducing antioxidant power (FRAP), total phenolic content (TPC), and metal chelating (MC) assays. The combined results of these are presented in Table 1.

Table 1. Antioxidant potential of *Spirogyra* sp. extracts in methanol and chloroform solvents

Test	Extract	Mean \pm SD	ANOVA p-value	Tukey Comparison
TAA	Methanol	111.33 \pm 0.72	<0.0001	Methanol vs Chloroform $p < 0.0001$
	Chloroform	24.98 \pm 0.92		
FRAP	Methanol	78.00 \pm 0.33	<0.0001	Methanol vs Chloroform $p < 0.0001$
	Chloroform	840.50 \pm 1.20		
TPC	Methanol	90.666 \pm 0.12	<0.0001	Methanol vs Chloroform $p < 0.0001$
	Chloroform	57.1666 \pm 0.11		
MC	Methanol	9.782 \pm 0.17	<0.0001	Methanol vs Chloroform $p < 0.0001$
	Chloroform	4.42 \pm 1.15		

*Values represent mean \pm SE of three independent replicates (n = 3).

Methanolic extracts showed significantly higher TAA (111.33 \pm 0.72 mg/ g) and TPC (90.666 \pm 0.12 µg/ g) compared to chloroform extracts (24.982 \pm 0.92 mg/ g and 57.1666 \pm 0.11 µg/ g, respectively; $p < 0.0001$). Metal chelating activity was also greater in methanol (9.782 \pm 0.17%) than in chloroform (4.42 \pm 1.15%; $p < 0.0001$). Conversely, FRAP values were higher in chloroform extract (840.5 \pm 1.20 µM/ mL) than in methanol (78.00 \pm 0.33 µM/ mL; $p < 0.0001$). These findings indicate that solvent choice influences the extraction of bioactive compounds, with methanol enriching phenolics contributing to TAA and MC, while chloroform favors compounds responsible for ferric reducing activity.

Antifungal activity

The antifungal effects of methanol and chloroform extracts were evaluated against *A. niger* and *R. stolonifer* at four concentrations (crude, 1/1, 1/10, 1/100 g/ mL). Methanolic extract showed initial antifungal activity at 24 h, with inhibition zones ranging from 10-31 mm for *A. niger* and 6-18 mm for *R. stolonifer*. (Figure 3).

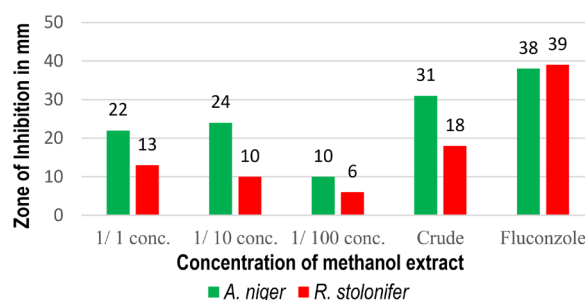


Figure 3. Zone of inhibition of methanolic extract against fungal species at different concentrations after 24 h

However, after 48 h (Figure 4), all methanol concentrations lost activity, showing no inhibition against either fungus, while fluconazole consistently exhibited strong inhibition (39 mm for both fungi).

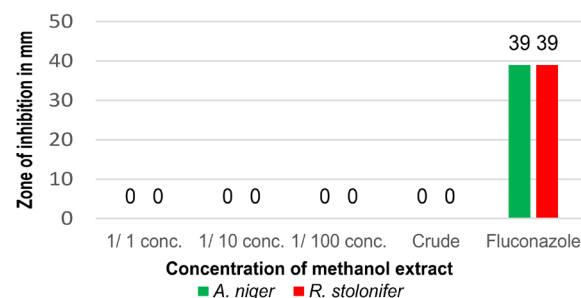


Figure 4. Zone of inhibition of methanolic extract against fungal species at different concentrations after 48 h

Chloroform extract exhibited strong and sustained antifungal activity. After 24 h, the crude extract inhibited both *A. niger* and *R. stolonifer* by 34 mm, and diluted forms maintained moderate activity. (Figure 5).

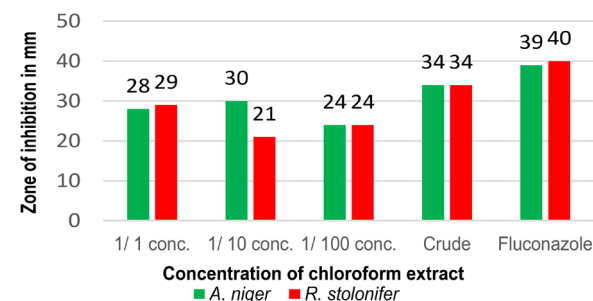


Figure 5. Zone of inhibition of chloroform extract against fungal species at different concentrations after 24 h

After 48 h (Figure 6), crude extract continued to inhibit *A. niger* and *R. stolonifer* at 34 mm and 33 mm, respectively, while

diluted forms showed partial activity (6-25 mm). Fluconazole sustained strong inhibition against both fungi.

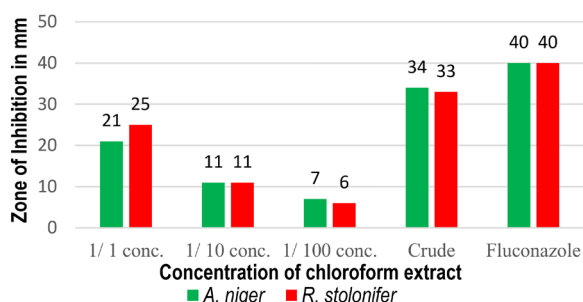


Figure 6. Zone of inhibition of chloroform extract against fungal species at different concentrations after 48 h

Based on these results, antifungal activity can be interpreted using standard sensitivity terms. Methanolic extract demonstrated rapid loss of activity, with crude initially classified as Sensitive (S) for *A. niger* and Intermediate/ Resistant (I/ R) for diluted forms, becoming Resistant (R) after 48 h. *R. stolonifer* showed Intermediate at crude and Resistant at all dilutions. Chloroform extract largely retained activity, with crude remaining Sensitive even after 48 h, and diluted forms classified as Intermediate or Resistant. Fluconazole remained Sensitive for both strains throughout the experiment. These observations highlight the solvent-dependent stability of antifungal compounds, with chloroform providing prolonged inhibition compared to the rapid decline observed in methanol extracts.

DISCUSSION

The present study demonstrates that the bioactive potential of *Spirogyra* sp. is strongly influenced by the choice of extraction solvent. The antioxidant assays showed that methanolic extracts had significantly higher total antioxidant activity (TAA, 111.33 ± 0.72 mg/ g, $p < 0.0001$), total phenolic content (TPC, 90.666 ± 0.12 μ g/ g, $p < 0.0001$), and metal chelating activity (MC, $9.782 \pm 0.17\%$, $p < 0.0001$) compared to chloroform, while ferric reducing antioxidant power (FRAP) was substantially higher in chloroform extracts (840.50 ± 1.20 μ M/ mL, $p < 0.0001$). These findings indicate that polar solvents preferentially extract phenolic and other hydrophilic compounds, which play a major role in neutralizing free radicals, whereas non-polar or moderately polar compounds contribute to ferric reducing capacity. Such solvent-dependent differences are consistent with the chemical diversity of algal metabolites, highlighting that a single solvent cannot comprehensively extract all bioactive constituents. These observations align with previous reports on *Spirogyra* and other freshwater algae (Rutikanga et al., 2011; Thitiphan and Waranya, 2015).

Similarly, earlier studies have reported higher phenolic recovery and antioxidant activity in methanolic fractions compared to less polar solvents, supporting the present findings. However, some studies have documented uniformly higher overall antioxidant performance in methanol extracts

without observing elevated FRAP values in non-polar fractions. This difference may be attributed to species-specific metabolite composition, ecological variation, or differences in extraction protocols and solvent purity. The metal chelating activity suggests that *Spirogyra* may mitigate oxidative stress by binding pro-oxidant metal ions, a trait often associated with mineral-rich algal matrices and certain secondary metabolites. Compared to reports on certain marine macroalgae showing higher chelation percentages, the moderate activity observed in the present study may reflect freshwater habitat differences, nutrient availability, or lower abundance of strong chelator compounds. This finding emphasizes the potential role of *Spirogyra* in applications targeting oxidative stress-related disorders.

The antifungal assays further confirmed the solvent-dependent bioactivity of *Spirogyra* extracts. Methanolic extracts initially inhibited *A. niger* and *R. stolonifer* (e.g., crude inhibition zones 31 mm and 18 mm, respectively), but activity declined rapidly over 48 h, indicating possible instability or degradation of polar antifungal compounds. While some previous investigations reported sustained antifungal activity in methanolic algal extracts, the rapid decline observed here suggests possible volatility, oxidation, or thermal instability of polar bioactive constituents under incubation conditions. In contrast, chloroform extracts maintained strong and prolonged antifungal activity, with crude inhibition zones remaining at 34 mm (*A. niger*) and 33 mm (*R. stolonifer*) after 48 h. Diluted concentrations of chloroform extract retained partial activity, demonstrating solvent-dependent stability. This sustained inhibition is consistent with studies indicating that organic solvents often extract more stable lipophilic antimicrobial compounds such as terpenoids and steroids.

Based on standard inhibition zone thresholds, methanolic extracts were initially Sensitive (S) against *A. niger* at crude concentration, Intermediate (I) at 1/1 and 1/10, and Resistant (R) at 1/100; after 48 h, all methanol concentrations became Resistant (R). For *R. stolonifer*, methanol extract showed Intermediate (I) at crude and Resistant (R) at all diluted concentrations. Chloroform extracts largely remained Sensitive (S) at crude for both fungi, while diluted forms were Intermediate (I) or Resistant (R), even after 48 h. Fluconazole remained Sensitive (S) throughout. These interpretations emphasize the greater stability and long-lasting efficacy of non-polar antifungal constituents in chloroform extracts, while polar methanol extracts exhibited rapid decline. Similar solvent-specific antifungal patterns have been reported in freshwater and marine algae, where organic solvents preferentially extract stable antimicrobial agents (Khalid and Shameel, 2012; Okunowo et al., 2018; Sitthiwong, 2019; Wanlambok et al., 2021; Mohammed and Al-Katib, 2023; Sitthiwong et al., 2024; Soiklom et al., 2025). However, variations in inhibition zone diameters between studies may result from differences in fungal strains, inoculum density, incubation time, algal species, and extraction yield. The inclusion of fluconazole as a positive

control validated the experimental approach and confirmed the sensitivity of the tested fungal strains. While algal extracts did not exceed the efficacy of fluconazole, their inhibitory effects demonstrate promising natural antifungal potential as alternative or complementary bioactive sources. Unlike the synthetic antifungal agent fluconazole, which acts through a well-defined mechanism targeting ergosterol biosynthesis, algal extracts likely exert antifungal effects through multiple bioactive constituents acting synergistically. This multi-component mode of action may reduce the likelihood of rapid resistance development, although further mechanistic studies are required to substantiate this possibility.

Overall, the findings support the view that *Spirogyra* sp. represents a valuable reservoir of biologically active compounds. The observed antioxidant and antifungal properties can be attributed to a diverse range of secondary metabolites, including alkaloids, steroids, terpenoids, tannins, and flavonoids, which have been documented across algal taxa. These results reinforce the potential of *Spirogyra* for future applications in pharmaceutical, nutraceutical, and functional food industries. Although the present study demonstrates significant bioactivities, it represents a preliminary investigation. Future research should focus on isolation and characterization of specific bioactive compounds, detailed mechanistic studies, and in vivo assessments to validate and expand upon these findings. Incorporating p-values and statistical analysis strengthens the reliability of the results and provides a quantitative measure of solvent-dependent bioactivity. Furthermore, recent studies on *Spirogyra* (Belyagoubi et al., 2023; Belyagoubi et al., 2025) continues to highlight its versatile bioactive potential, supporting the relevance of the present findings.

CONCLUSION

This study provides the first comprehensive, location-specific assessment of the antioxidant and antifungal potential of *Spirogyra* sp. from Jilani Park Racecourse, Lahore, Pakistan. Methanol efficiently extracted polar antioxidant compounds, while chloroform yielded stable antifungal metabolites, highlighting the genus' solvent-dependent bioactivity. These findings demonstrate that *Spirogyra* is a

REFERENCES

- Belyagoubi, L., Chaibi, R., Gouzi, H., Aissaoui, F.Z., Benamar, Z.E.O., & Benahmmou, B.N. (2023). Phytochemistry study and antimicrobial activity of *Spirogyra* freshwater green microalgae from Algeria. *Journal of Natural Product Research and Applications*, 3(2), 47-60. <https://doi.org/10.46325/jnpra.v3i02.65>
- Belyagoubi, L., Belyagoubi-Benahmmou, N., Mokhtari-Soulimane, N.A., Abdelmoumene, W., Dicha, A., Benlarbi, L., Ceglowska, M., Konke, R., Surosz, W., Toruńska-Sitarz, A., Zloch, I., & Mazur-Marzec, H. (2025). Biological activity of the freshwater alga *Spirogyra* sp. from the Algerian desert. *Algal Research*, 104292. <https://doi.org/10.1016/j.algal.2025.104292>
- Benzie, I.F.F., & Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry*, 239, 70-76. <https://doi.org/10.1006/abio.1996>
- Bhattacharya, S. (2011). Natural antimutagens: A review. *Research Journal of Medicinal Plant*, 5(2), 116-126. <https://doi.org/10.3923/rjmp.2011.116.126>
- Dinis, T.C.P., Madeira, V.M.C., & Almeida, L.M. (1994). Action of phenolic derivatives (acetoaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Archives of Biochemistry and Biophysics*, 315, 161-169. <https://doi.org/10.1006/abbi.1994.1485>
- Hainz, R., Wobber, C., & Schagerl, M. (2009). The relationship between *Spirogyra* (Zygnematophyceae, Streptophyta) filament type groups and environmental conditions in Central Europe. *Aquatic Botany*, 91, 173-180.
- Harborne, J.B. (1973). *Phytochemical methods: A guide to modern techniques of plant analysis*. Chapman and Hall.

promising natural source of bioactive compounds with potential applications in functional foods, nutraceuticals, and eco-friendly antifungal formulations. Further research should focus on isolating and characterizing specific bioactive compounds, evaluating their stability, and validating their efficacy in biological systems. This work emphasizes the untapped potential of freshwater algae as sustainable and multifunctional resources for human health, biotechnology, and environmental applications.

ACKNOWLEDGEMENTS AND FUNDINGS

The authors would like to thank Botany Department, Government College University, Lahore, for providing laboratory facilities. This research is self-funded by the corresponding author and has not received a specific grant, fund or other support from any funding agency in the public, commercial, or not-for-profit sectors.

AUTHORSHIP CONTRIBUTIONS

All authors contributed to the idea and design of the study. Hamna Sohail: Conceptualization, methodology, investigation, resources, funding acquisition, data curation, writing-original draft preparation, formal analysis, visualization. Ghazala Yasmeen Butt: Supervision, project administration, validation, review and editing.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest or competing interests.

ETHICS APPROVAL

This article does not contain any human or animal studies performed by any authors. No specific ethical approval was necessary for this study.

DECLARATION OF AI USE

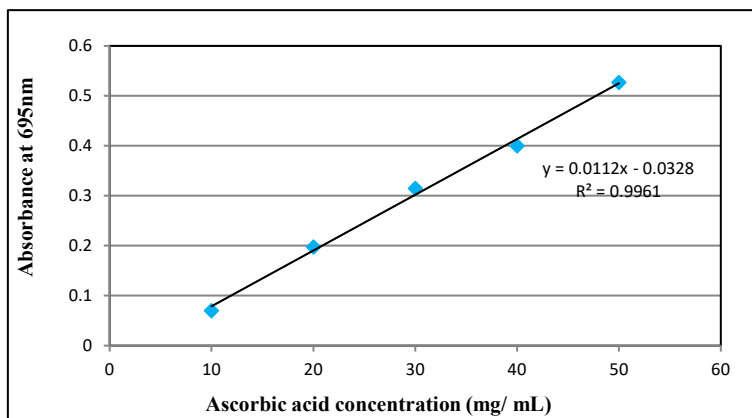
The authors declare that no generative AI model was used in any stage of this research or manuscript preparation.

DATA AVAILABILITY STATEMENT

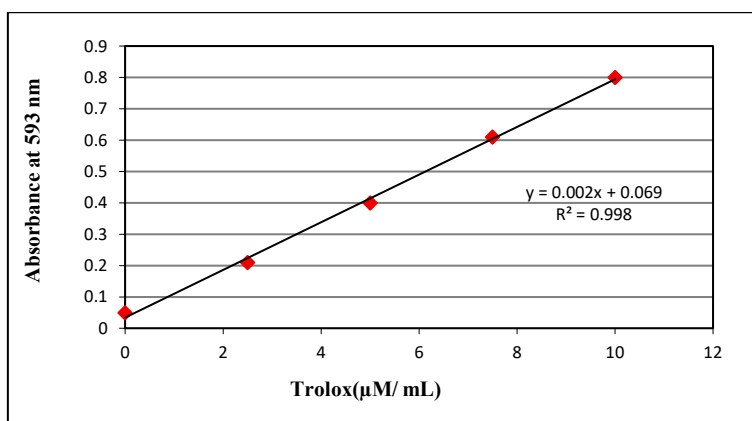
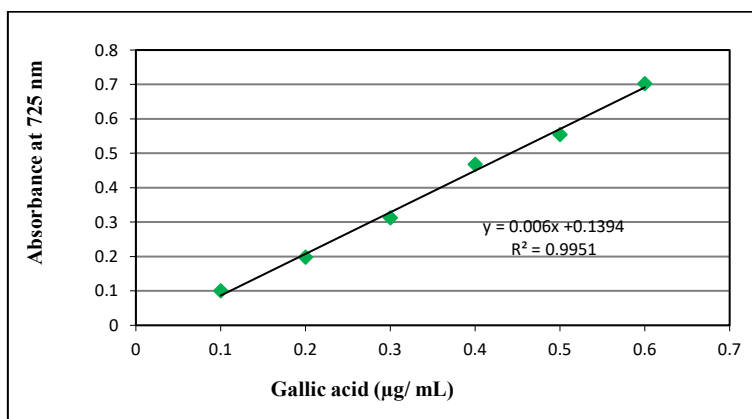
All relevant data is in the article.

- Index Fungorum Partnership. (2025). *Index Fungorum*. <https://www.indexfungorum.org/> (Accessed 18 Dec 2025)
- Khalid, M.N., & Shameel, M. (2012). Studies on the phycochemistry and biological activity of *Spirogyra rhizoides* (Chlorophyta). *Pakistan Journal of Botany*, 44(5), 1815–1820.
- Lee, J.C., Hou, M.F., Huang, H.W., Chang, F.R., Yeh, C.C., Tang, J.Y., & Chang, H.W. (2013). Marine algal natural products with anti-oxidative, anti-inflammatory, and anticancer properties. *Cancer Cell International*, 13, 55. <https://doi.org/10.1186/1475-2867-13-55>
- Lee, R.E. (2008). *Phycology* (4th ed.). Cambridge University Press.
- Lordan, S., Ross, R.P., & Stanton, C. (2011). Marine bioactives as functional food ingredients: Potential to reduce the incidence of chronic diseases. *Marine Drugs*, 9(6), 1056–1100. <https://doi.org/10.3390/md9061056>
- Makkar, H.P.S., Bluemmel, M., Borowy, N.K., & Becker, K. (1993). Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *Journal of the Science of Food and Agriculture*, 61, 161–165. <https://doi.org/10.1002/jsfa.2740610205>
- Mohammed, D.H., & Al-Katib, M.A. (2023). Active and phenolic compounds in *Spirogyra* sp. PDNA1 is an antibiotic for some bacteria and fungi. *Al-Kitab Journal of Pure Science*, 7(1), 100–113. <https://doi.org/10.32441/kjps.07.01.p9>
- Muthuruppan, S., & Francis, S.P. (2013). Anti-cancer mechanism and possibility of nano-suspension formulation for a marine algae product fucoxanthin. *Asian Pacific Journal of Cancer Prevention*, 14(4), 2213–2216. <https://doi.org/10.7314/apjcp.2013.14.4.2213>
- Naik, A.A., Hemavani, C., & Thippeswamy, B. (2012). Evaluation of antimicrobial property of *Spirogyra* species. *International Multidisciplinary Research Journal*, 2(2), 13–15.
- Nobili, S., Lippi, D., Witort, E., Donnini, M., Bausi, L., Mini, E., & Capaccioli, S. (2009). Natural compounds for cancer treatment and prevention. *Pharmacological Research*, 59(6), 365–378. <https://doi.org/10.1016/j.phrs.2009.01.017>
- Perez, C., Pauli, M., & Bazerque, P. (1990). An antibiotic assay by the agar well diffusion method. *Acta Biologica et Medicinæ Experimentalis*, 15, 113–115.
- Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemistry*, 269(2), 337–341. <https://doi.org/10.1006/abio.1999.4019>
- Punyoyai, T. (2008). *Antioxidant activity of Tao, Spirogyra neglecta (Hassall) Kützting* (M.S. thesis). Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.
- Rutikanga, A., Gitu, L., Oyaro, N., & Chacha, S. (2011). Mineral composition, antioxidant and antimicrobial activities of freshwater algae (*Spirogyra* genus), Kenya. *World Rural Observations*, 3(2), 64–71.
- Shameel, M. (2001). An approach to the classification of algae in the new millennium. *Pakistan Journal of Marine Biology*, 7(1–2), 233–250.
- Sitthiwong, N. (2019). Pigment and nutritional value of *Spirogyra* spp. in Sakon Nakhon, Nakhon Phanom and Mukdahan provinces. *Progress in Applied Science and Technology*, 9(1), 10–21.
- Sitthiwong, N., Sumangka, J., Michaitrakun, S., & Sotthisawad, K. (2024). Total phenolic compound and antimicrobial activity of *Spirogyra* spp. *Progress in Applied Science and Technology*, 14(1), 65–72. <https://doi.org/10.60101/past.2024.252833>
- Soiklom, S., Siri-anusornsak, W., Petchpoung, K., Soiklom, S., & Maneeboon, T. (2025). Development of bioactive edible film and coating obtained from *Spirogyra* sp. extract applied for enhancing shelf life of fresh products. *Foods*, 14(5), 804. <https://doi.org/10.3390/foods14050804>
- Taş, B., Ertürk, Ö., Yılmaz, Ö., Çol Ayvaz, M., & Ertürk, E. (2015). Chemical components and biological activities of two freshwater green algae from Ordu, Turkey / Türkiye'nin Ordu ilinden iki tatlı su yeşil alglerinin kimyasal bileşenleri ve biyolojik aktiviteleri. *Turkish Journal of Biochemistry*, 40(6), 508–517. <https://doi.org/10.1515/tjb-2015-0042>
- Thiamdao, S., & Peerapornpisal, Y. (2011). Morphological observation of *Spirogyra ellipsospora*, an edible freshwater macro-algae. *Journal of the Microscopy Society of Thailand*, 4(2), 94–97.
- Thitiphan, C., & Waranya, W. (2015). Effect of ultrasonic-assisted extraction on phenolic content of freshwater macroalgae in Northern Thailand. *MATEC Web of Conferences*, 35, 04002. <https://doi.org/10.1051/mateconf/20153504002>
- Wanlambok, S.M., Carefulness, D., & Papiya, R. (2021). Determination of antioxidant activities of algae from lentic ecosystems under anthropogenic stress: A comparative study. *International Journal of Life Sciences*, 9(1), 62–70.
- Okunowo, W.O., Oyediji, A.O., Ilesanmi, J.A., Afolabi, L.O., & Umunnakwe, I.E. (2018). Antimicrobial, antioxidant potential and chemical composition of the methanolic extracts of *Spirogyra setiformis* and *Navicula* species. *Journal of Scientific Research and Development*, 17(1), 15–20. <https://jsrd.unilag.edu.ng/article/view/32>

ANNEXURE-I



1.1. Standard curve of ascorbic acid in mg/ mL versus absorbance at 695 nm plotted through TAA method

1.2. Standard curve of trolox in $\mu\text{M/ mL}$ versus absorbance at 593 nm plotted through FRAP method1.3. Standard curve of gallic acid equivalent (GAE) in $\mu\text{g/ mL}$ versus absorbance at 725 nm plotted through TPC method