



Agronomic potential of ethanolic extracts from the microalgae *Limnospira platensis* and *Porphyridium cruentum* on common bean (*Phaseolus vulgaris*) crops

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Abstract

In recent years algae extracts have gained recognition in global agriculture for their capacity to enhance the growth and yield of leguminous plants like the common bean (*Phaseolus vulgaris*), which is vital in many countries and plays a key role in ensuring global food security. This study evaluated the biostimulant and biopesticide potential of ethanolic extracts from *Limnospira platensis* and *Porphyridium cruentum* on common bean, using seed priming and soil application methods. Extracts were tested at 0.1, 0.5, and 1.0 mg mL⁻¹. Seed treatment with *L. platensis* at 0.1 mg mL⁻¹ increased shoot length by 59% and total seedling length by 32%. At 1.0 mg mL⁻¹, shoot dry weight rose by 21%, and total dry weight by 20%. In soil application, *L. platensis* extract at 1.0 mg mL⁻¹ increased root fresh weight by 28.8% and shoot dry weight by 25.9%. *Porphyridium cruentum* extract promoted seedling elongation (up to 42%) and fresh biomass accumulation (17.6%) at 1.0 mg mL⁻¹. Photosynthetic pigment levels were enhanced in *L. platensis* treatment, with chlorophyll *a* and carotenoids increasing by 17% and 31%, respectively. The chemical characterization revealed the presence of key bioactive compounds, such as peptides, sphingolipids, fatty acids, and phospholipids, commonly associated with plant growth promotion and physiological responses. The extracts enhanced growth, biomass accumulation, and pigment levels, suggesting their potential as eco-friendly agricultural inputs. Further research is needed to explore their application in other crops.

Keywords Agroecology · Biostimulant · Bioproduct · Eco-friendly agricultural inputs · Sustainability

Introduction

In recent years, algae extracts sourced from both seaweeds and microalgae have gained recognition in global agriculture (Khan et al. 2009; Garcia-Gonzalez and Sommerfeld 2016; Martins et al. 2024; Pereira et al. 2025; Rantanen et al. 2025; Moraes et al. 2026) for their capacity to enhance the growth and yield of leguminous plants like the common bean (*Phaseolus vulgaris*), which is vital in many countries and plays a key role in ensuring global food security (Bhupenchandra et al. 2022; González-Pérez et al. 2022; Ferreira et al. 2023; Parmar et al. 2023; Moraes et al. 2025a). However, common bean cultivation faces challenges from various biotic and abiotic factors, leading to inconsistent productivity and losses in production (Wani et al. 2023). One of the major biotic threats is anthracnose, caused by the hemibiotrophic fungus *Colletotrichum lindemuthianum*. This disease results

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in necrotic lesions on leaves, stems, and pods, leading to decreased photosynthetic capacity and grain quality.

Traditional methods of managing anthracnose involve the use of chemical fungicides and resistant cultivars, but the environmental impact of chemical control and the variability of the pathogen often outweigh the benefits of genetic resistance (Paulino et al. 2022; Shafi et al. 2023; Santos et al. 2025). It is estimated that billions of kilograms of active ingredients are used each year in agricultural settings globally, playing a crucial role in pest control and crop yield stability. Nevertheless, this approach has come under scrutiny due to its environmental and health impacts (Shattuck et al. 2023). Scientific evidence accumulated in recent decades indicates that the intensive and often continuous use of these chemicals has resulted in various adverse effects on the environment and public health, including soil degradation, contamination of aquatic ecosystems, eutrophication, greenhouse gas emissions, and the loss of biodiversity (Bhupenchandra et al. 2022; Ferreira et al. 2023; Parmar et al. 2023).

In light of these challenges, it is evident that there is a requirement for innovative approaches that enhance plant growth and provide defense against pathogens like *C. lindemuthianum*. Natural biostimulants, particularly those derived from algae, may present a sustainable solution to reduce reliance on chemical fertilizers and synthetic pesticides by promoting growth and enhancing pest resistance.

The use of microalgae extracts has become a promising approach to enhance plant growth, stress tolerance, and disease resistance in an environmentally friendly and sustainable manner (Chabili et al. 2024; Moraes et al. 2025a). Microalgae-based biostimulants are particularly noteworthy for containing a diverse range of bioactive compounds that have been shown to positively impact plant physiology, development, and defense mechanisms. Microalgae and cyanobacteria are highly adaptable and efficient biological platforms for generating valuable compounds. When extracted and applied correctly, these compounds can serve as versatile bioinputs that not only boost plant growth but also safeguard crops against various biotic stressors (Gitau et al. 2022; Mahapatra et al. 2022; Braun and Colla 2023; Mutale-Joan et al. 2023; Chabili et al. 2024).

Biostimulants derived from microalgae act primarily by modulating plant metabolism rather than directly supplying nutrients (Colla and Rouphael 2020). Their effectiveness is due to their diverse biochemical profiles, which include phytohormones (such as auxins, cytokinins, and gibberellins), amino acids, vitamins, polyphenols, pigments, and sulfated polysaccharides (Arenas Colarte et al. 2025). These components can impact seed germination, promote shoot and root growth, enhance photosynthesis, and improve nutrient absorption (Kapoor et al. 2021). Additionally, microalgal extracts can boost resilience to environmental stresses like drought, salinity, and temperature changes by activating

antioxidant defense mechanisms and stress-responsive genes. Some metabolites, especially phenolic compounds and polysaccharides can also offer protection against pathogens by directly affecting microbial structures or priming plant immune responses (Colla and Rouphael 2020; Kapoor et al. 2021).

The application of microalgal extracts to seeds or growing plants has demonstrated consistent improvements in plant vigor and resilience across a range of crops (Chabili et al. 2024). Seed treatments, in particular, offer a cost-effective strategy to boost early germination and seedling establishment, setting the foundation for better yields under field conditions. Foliar sprays and soil drenches are also effective in boosting plant metabolism and reducing the impact of environmental stress. As a result, microalgae extracts are increasingly being recognized as versatile resources that can promote plant growth throughout all growth stages, making them beneficial in agricultural practices (Garcia-Gonzalez and Sommerfeld 2016; Supraja et al. 2020; Youssef et al. 2022). However, there are numerous gaps that need to be elucidated in the realm of microalgae-based biostimulants. It should be emphasized that the efficacy of extracts varies. The quality and impact are influenced by factors such as the species of microalgae, extraction technique, chemical makeup, and application approach (Colla and Rouphael 2020; Kapoor et al. 2021).

Among the wide variety of microalgae, our research group has identified two species, *Limnospira platensis* and *Porphyridium cruentum*, as particularly noteworthy. *Limnospira platensis* is a filamentous cyanobacterium with a reputation for its high protein content and abundance of phycocyanin, essential fatty acids, and vitamins. Extensively researched for agricultural applications, *L. platensis* has shown promise in promoting plant growth, enhancing chlorophyll levels, and boosting resilience to environmental stress (Salvi et al. 2020; Fadia et al. 2023; Rady et al. 2023). In contrast, *P. cruentum* is a unicellular red microalga that has received less attention in agricultural research. Known for its production of phycoerythrin, sulfated polysaccharides, and antioxidant compounds like gallic acid, ferulic acid, and flavonoids, *P. cruentum* offers potential benefits such as antimicrobial and immunomodulatory effects in plants. Despite its biochemical richness, the biostimulant and biopesticide properties of *P. cruentum* have not been thoroughly assessed, especially in crops like common beans (Casas-Arrojo et al. 2023).

While most studies exploring algal biostimulants rely on whole-cell extracts obtained through mechanical disruption or aqueous extraction, the use of ethanolic extracts offers advantages in selectively concentrating lipophilic and bioactive secondary metabolites, such as phenolic compounds, flavonoids, and certain fatty acids, which are often associated with plant growth promotion and defense responses.

Therefore, ethanol emerges as a valuable solvent for obtaining enriched extracts with enhanced biostimulant potential, making it a promising approach for the development of more effective algal-based bioinputs (Alassali et al. 2016).

In addition to bioassays and phenotypic assessments in plant systems, the chemical characterization of microalgal extracts represents a crucial step toward elucidating their modes of action. Analytical tools such as ultra-performance liquid chromatography (UPLC) allow the identification of diverse metabolites potentially involved in plant growth promotion or stress mitigation. This molecular profiling provides a comprehensive understanding of the biochemical complexity of the extracts and supports the identification of correlations between specific compounds and observed bioactivities. Such insights are essential for the rational development of biostimulant and biopesticide formulations with enhanced efficacy and specificity (Yakhin et al. 2017).

In this study, ethanolic extraction was used to obtain extracts from *L. platensis* and *P. cruentum*. The goal was to assess the biochemical composition of the ethanolic extracts and their potential as biostimulants for promoting early establishment and sustained vegetative growth of common beans, in addition to evaluating the biopesticidal properties of the extracts against *C. lindemuthianum*, the etiological agent of anthracnose.

Material and methods

Microalgae strains and biomass processing

Limnospira platensis and *Porphyridium cruentum* were cultivated at the Laboratory of Algae Cultivation (LCA) at the Federal University of Santa Catarina – Brazil. *Limnospira platensis* was grown in 2.0-L borosilicate Schott flasks containing LCA-AD (freshwater) medium, consisting of 1.05 g L⁻¹ NaNO₃, 0.075 g L⁻¹ MgSO₄·7H₂O, 0.025 g L⁻¹ K₂HPO₄, 0.06 g L⁻¹ KH₂PO₄, 0.025 g L⁻¹ NaCl, 0.025 g L⁻¹ CaCl₂·2H₂O, 0.50 g L⁻¹ C₁₀H₁₆N₂O₈-Na₂ (EDTA-Na₂), 0.031 g L⁻¹ KOH, 0.005 g L⁻¹ FeSO₄·7H₂O, 0.01142 g L⁻¹ H₃BO₃, 0.0014 g L⁻¹ MnCl₂·4H₂O, 0.000071 g L⁻¹ MoO₃, 0.00157 g L⁻¹ CuSO₄·5H₂O, and 0.000049 g L⁻¹ Co(NO₃)₂·6H₂O (Oliveira et al. 2020) with the addition of sodium bicarbonate (10 g L⁻¹) and sodium carbonate (5 g L⁻¹). *Porphyridium cruentum* was grown in LCA-AM (seawater) medium, consisting of nitrogen 255 mg L⁻¹, phosphorus 27 mg L⁻¹, nitrate 2 mL of stock solution (SS) (SS = 510.0 g L⁻¹ NaNO₃, 8.0 g L⁻¹ FeCl₃·6H₂O, 10.0 g L⁻¹ EDTA Na₂) phosphate 2 mL of stock solution (SS = 48.0 g L⁻¹ NaH₂PO₄), trace metals 1 mL of stock solution (SS = 8.82 g L⁻¹ ZnSO₄·7H₂O, 1.44 MnCl₂·4H₂O, 6.61 g L⁻¹ (NH₄)₆Mo₇O₂₄·4H₂O, 1.57 g L⁻¹ CuSO₄·5H₂O, 0.40 g L⁻¹ CoCl₂·6H₂O), and 1.0 mL vitamins (Citoneurin 5,000 mg

(Sales et al. 2019). The cultures were maintained at 22 °C, with constant agitation by bubbling air enriched with 0.2% CO₂ (v/v), irradiance of 150 and 250 μmol photons m⁻² s⁻¹ for *L. platensis* and *P. cruentum*, respectively, and a continuous 24 h photoperiod. The pH of the cultures was kept between 9.6 and 9.8 for *L. platensis* and 7.5 and 8.5 for *P. cruentum* using an automated system — APEX AQUACONTROLLER — from Neptune System. After reaching the stationary phase, i.e., three consecutive days without significant growth (9th–11th day for *L. platensis* and 7th–9th day for *P. cruentum*), cultures were separated by centrifugation at 6000 × g, -4 °C, for 5 min. The sedimented biomass was collected, frozen, and kept at -80 °C, and then lyophilized.

Extracts preparation

The extracts were prepared according to Abreu et al. (2008), with some modifications. Briefly, 3.5 g of freeze-dried algal biomass was added to 100 mL of ethanol (70° GL), and maintained for 24 h on an orbital shaker at 100 rpm and 25 °C. The ethanolic extracts were filtered using Qualy filter paper (n° 9) in a Buchner funnel and concentrated in a rotary evaporator (Quimis, Brazil) until complete ethanol removal. After quantification, the crude extract was diluted in water to prepare concentrations for the development of experiments, which were 0.1, 0.5 and 1.0 mg mL⁻¹.

Experimental conditions

In vitro and *in vivo* experiments were conducted in a completely randomized design with four replications. Each replication for the *in vitro* tests consisted of one Petri dish to assess the impact of the extracts on mycelial growth. For the *in vivo* experiments, each replication included three tubes with one plant in each to evaluate the effects of the extracts on common bean growth parameters and pigment content.

In vitro effects on *Colletotrichum lindemuthianum* – Biopesticide trial

The experiment used the MANE 003 isolate of race 73 of *C. lindemuthianum*, provided by the Laboratory of Plant Pathology at the Federal University of Santa Catarina. To assess the impact of *L. platensis* and *P. cruentum* extracts on the mycelial growth of *C. lindemuthianum*, *in vitro* trials were conducted following the methodology outlined by Paulert et al. (2009). In a sterile laminar flow chamber, 10 mL of each microalgal extract was added to 15 mL of potato dextrose agar (PDA) medium at 70 °C, resulting in a final volume of 25 mL at concentrations of 0.1, 0.5, and 1.0 mg mL⁻¹. Subsequently, 20 mL of culture medium (pH 6.0) was poured into 90 mm Petri dishes. After solidification of the medium, an 8.0 mm disc of the fungal culture (15 days

incubation) was transferred to the center of the plates and these were incubated in a growth chamber at $21 \pm 2^\circ\text{C}$ and a photoperiod of 12 h for 14 days. Petri dishes containing 15 mL of PDA medium supplemented with 10 mL of sterile distilled water were utilized as the control group. Mycelial growth was evaluated by measuring the colony diameter along two perpendicular axes using a digital caliper (Mitutoyo, Suzano, Brazil) 14 days after the transfer of the pathogen culture discs. The mean diameter between the axes was recorded twice for each repetition and expressed in mm. Each Petri dish was considered as an individual experimental unit for the mycelial growth assessments.

Effects on the initial development of bean seedlings – Seed priming

The effects of the ethanolic extracts on seedlings length and weight were conducted according to Paulert et al. (2009) and the standardized procedures of the Association of Official Seed Analysts (AOSA 2005) with some modifications. Briefly, seeds of common bean (*P. vulgaris* L. cv. Uirapuru) were rinsed with sterile water for 5 min and then soaked (0.33 mL per seed) in the different concentrations of the ethanolic extract and in water (control) for 4 h at 25°C , in order to absorb the solutions. Thirty treated seeds were placed equidistantly between three sheets of germitest paper moistened with distilled water in a ratio of 2.5:1 (distilled water:dry paper mass) and subsequently made into rolls and placed in plastic bags. These were kept in a germination chamber at a temperature of $25.0 \pm 1.0^\circ\text{C}$, with a humidity level of at least 95%, for 9 days. Four replications were performed for each treatment (120 seeds per replication), and each moistened roll (with 30 seeds) was considered an experimental unit. After 9 days, 5 normal seedlings from each replicate were randomly chosen by treatment, for measuring shoot and root length with the aid of a graduated ruler. To determine shoot and root dry masses, the same seedlings had their parts separated and placed in paper bags to dry in an oven at 60°C for 72 h.

Effects on the initial development of bean seedlings – Soil application

Four replications were performed for each treatment (12 seeds per replication). Bean seeds were sown in 75 cm^3 non-toxic polypropylene conic tubes (Bom Cultivo, Rio do Sul, Brazil) filled with medium-grade vermiculite (pH 6–9; maximum humidity 8.0% w/w, Agrinobre, Brazil) and maintained in a controlled growth chamber set to 22.5°C , 80% relative humidity, and a 12-h photoperiod for 11 days. Irrigation was performed daily with 3.0 mL of sterile distilled water, except on treatment days. Treatments were applied by irrigation on the day of sowing and on the 3rd, 6th, and

9th day. Each tube received 3.0 mL of either sterile distilled water (control) or microalgae extract at concentrations of 0.1, 0.5, or 1.0 mg mL^{-1} .

On the 11th day after sowing, plants were carefully removed from the tubes and their roots were thoroughly washed under running water to eliminate residual vermiculite. Shoot, root and total lengths were measured using a ruler. Shoots and roots were separated, and fresh weights of both parts were recorded. After that, seedlings were packed in paper bags and placed in a laboratory oven with mechanical air circulation for drying at 65°C for 72 h to determine shoot and root dry weight.

Quantification of photosynthetic pigments in leaves of bean seedlings

The content of chlorophyll *a* (Ca), chlorophyll *b* (Cb), total chlorophyll (Total C) and carotenoids (Ct) of common bean leaves after soil application were determined according to Hiscox and Israelstam (1979), with adaptations. On the 11th day after sowing, (8–10) 8.0 mm discs equidistant from the leaf blade (around 100 mg) were placed in Falcon tubes with 7.0 mL of dimethylsulfoxide (DMSO) and heated to 65°C in a water bath for 2 h. Subsequently, the Falcon volumes were completed with DMSO to 10 mL and 250 μL aliquots of each sample were transferred to microplates. Absorbance was measured using a microplate reader (SpectraMax Paradigm, Molecular Devices, USA) at 480, 649 and 665 nm. The equipment was calibrated for zero absorbance using pure DMSO and the absorbance values of the samples were then subtracted from the value obtained for the blank. Pigment concentration was calculated from Eqs. 1, 2, 3 and 4 according to Wellburn (1994):

$$Ca = 12.19 \times A_{665} - 3.45 \times A_{649} \quad (1)$$

where, *Ca* represents the concentration of chlorophyll *a* (mg g^{-1}), A_{665} and A_{649} the absorbances of 665 and 649 nm, respectively.

$$Cb = 21.99 \times A_{649} - 5.32 \times A_{665} \quad (2)$$

where, *Cb* represents the concentration of chlorophyll *b* (mg L^{-1}), A_{649} and A_{665} the absorbances of 649 and 665 nm, respectively.

$$\text{Total } C = Ca + Cb \quad (3)$$

where, total *C* represents the concentration of total chlorophyll (mg L^{-1}).

$$Ct = (1000 \times A_{480} - 2.14 \times Ca - 70.16 \times Cb) / 220 \quad (4)$$

where, *Ct* represents the concentration of total carotenoids (mg L^{-1}) and A_{480} the absorbance of 480 nm.

Extracts characterization—Ultra Efficiency Liquid Chromatography coupled to Mass Spectrometry Analysis (CLUE-ESI-MS)

The ethanolic extracts of *L. platensis* and *P. cruentum* were subjected to CLUE-ESI-MS analysis to identify the compounds present within the extract. Samples of 10 µL of the extracts were diluted in 2.0 mL of MS grade methanol. The samples were filtered in a vial using a 0.22 µm pore size filter in order to prevent particles from passing into the final solution.

An Ultra Performance Liquid Chromatography system (Waters, USA) equipped with a photodiode detector, a sample manager and a quaternary solvent manager, as well as a reversed-phase column (BEH, 5 × 1 C18 cm and 1.7 µm particles) was used for separation. The column and sample tray were maintained at temperatures of 40 °C and 20 °C, respectively. A sample volume of 2.0 µL was subjected to a mobile phase gradient condition containing water with 0.1% formic acid (A) and acetonitrile (B) at a constant flow rate of 0.3 mL min⁻¹: 0–1 min, 80% A; 1–12 min, 10% A; 12–14 min, 10% A; 14–15 min 80% A; 15–20 min, 80% A (Pradi et al. 2023).

An orthogonal quadrupole time-of-flight mass spectrometer (Xevo G2-S Qtof, Waters) with an electrospray ionization (ESI) probe operating in positive and negative ionization modes was coupled to the CLUE system and used to detect the components chemicals from each fraction. Mass acquisition parameters were set to centroid mode and monitored for a scan time of 1.0 s, in a mass range of 50–1500 Da. The collision energy was 6 eV in function 1, rising from 20 to 35 eV in function 2, in fast data dependent acquisition mode (Fast DDA – Fast Data Dependent Acquisition). Each data acquisition cycle included an m/z referring to MS¹ above a threshold of 30,000, these being the peaks selected as precursors for subsequent fragmentation. MS/MS spectra were recorded while the threshold remained above 20,000. Accuracy of mass was ensured during acquisition using infusion correction of an external reference standard (LockSpray™), in constant infusion of a leucine-enkephalin solution (1.0 ng mL⁻¹ at 20 µL min⁻¹) (Gao et al. 2020).

The positive ionization mode used a capillary voltage of 3 kV, sample cone voltage of 40 V, offset voltage of 80 V, desolvation temperature of 350 °C, source temperature of 120 °C, gas flow in the cone 100 L h⁻¹ and desolvation gas flow of 800 L h⁻¹. The negative ionization mode used a capillary voltage of 3 kV, sample cone voltage of 40 V, offset voltage of 80 V, desolvation temperature of 300 °C, source temperature of 90 °C, gas flow in the cone of 80 L h⁻¹ and desolvation gas flow of 500 L h⁻¹.

Extract characterization—Processing of chromatographic and spectrometric data for metabolite identification

A combination of MS-DIAL (ver. 4.92) and MS-FINDER (ver. 3.52) software was used to assist in the identification of metabolites from microalgal extracts. The raw data from the FastDDA analyses, converted to the corresponding format (mzML), were imported into MS-DIAL, where information was selected according to the analyses carried out by CLUE-MS, and parameters for the processing and alignment of the data were selected.

Data collection was carried out with a tolerance of 0.02 Da and 0.06 Da for MS1 and MS2, respectively. Peak detection was applied with an amplitude of 10,000 for the minimum peak height and 0.1 Da for the mass cutoff width. For deconvulsion parameters, a sigma window value of 0.5 and an MS/MS abundance cutoff of 30 amplitudes were used. For alignment, the solvent blank file was used as a reference. Retention time tolerance set to 0.5 min and 0.2 Da for MS1 tolerance. The option to remove features based on solvent blank data was also applied and thus obtain annotations referring only to the samples. After finishing processing, precursor ion peaks were detected exploring two continuous data axes: retention time (tR) and charge mass ratio (m/z), where each point represents a detected peak. In addition, a series of information was obtained, including a table containing m/z values, retention time, type of adduct, statistical values and a bar graph indicating the abundance of each ion in the samples analyzed (Tsugawa et al. 2015).

The data obtained in MS-DIAL were exported to MS-FINDER, where metabolites were identified by comparing MS/MS fragmentation spectra with several databases (COCONUT, PubChem, UNPD, ChEBI, NNPDB, KNApSAcK, NPA). To select the most possible structure among the candidates, the score value (greater than or equal to 5), fragments obtained and knowledge of the possible classes of compounds that the type of sample is capable of producing were considered (Wang et al. 2022).

Statistical analysis

The data underwent Shapiro–Wilk and Levene tests to evaluate homoscedasticity and normality, respectively. One-way analysis of variance (ANOVA), followed by the Tukey's test, when necessary, was applied, using a significance level of 5%. Statistical analyses were conducted using Statistica software (version 7.0).

Results

In vitro effects on *C. lindemuthianum* – Biopesticide trial

The ethanolic extracts of *L. platensis* and *P. cruentum*, tested at concentrations of 0.1, 0.5, and 1.0 mg mL⁻¹ did

not significantly affect ($p=0.64$) the mycelial growth of *C. lindemuthianum* after 14 days of incubation on PDA medium, suggesting that the extracts did not inhibit fungal development under the tested conditions. Mycelial diameters ranged from 48.14 ± 0.61 to 49.18 ± 0.71 mm for *L. platensis* and from 47.73 ± 0.94 to 48.14 ± 0.61 mm for *P. cruentum*, values comparable to the control treatment (47.67 ± 0.77 mm) (Table 1).

Effects on the initial development of bean seedlings – Seed priming

Seeds treated with *L. platensis* extract showed significant ($p=0.0002$) improvements in shoot and total seedling length, indicating a potent growth-promoting impact at varying concentrations of the extract (Fig. 1a). Shoot length increased at all extract concentrations, with the highest value observed at 0.1 mg mL^{-1} (14.92 cm), representing a 59% increase over the control and significantly exceeding the value at 1.0 mg mL^{-1} . Treatments with 0.5

and 1.0 mg mL^{-1} also led to increases of 45.2% and 33.6%, respectively. Total seedling length similarly increased by 32, 25, and 19% at 0.1 , 0.5 , and 1.0 mg mL^{-1} , respectively, with 0.1 mg mL^{-1} showing the most pronounced effect. Application of *P. cruentum* extract also significantly enhanced ($p=0.039$) seedling elongation (Fig. 1b). At 0.1 mg mL^{-1} , shoot length rose by 33%, root length by 51%, and total seedling length by 42% compared to the control. At 1.0 mg mL^{-1} , shoot and root lengths each increased by 42%, and total seedling length by 35%.

In terms of biomass accumulation, the extract of *L. platensis* significantly improved ($p=0.0048$) dry weight (Fig. 2a and b). At 1.0 mg mL^{-1} , shoot dry weight was 24% higher than at 0.5 mg mL^{-1} and 21% greater than the control, while total dry weight increased by 20%.

Porphyridium cruentum extract at 1.0 mg mL^{-1} significantly increased ($p=0.031$) shoot fresh weight by 18.1% and total fresh weight by 17.6% compared to the control (Fig. 2c). However, shoot dry weight decreased by 18.1% at this same concentration, indicating that while the extract boosts fresh weight, it could potentially limit dry matter accumulation in shoot tissues (Fig. 2d).

Table 1 *In vitro* effect of *Limnospira platensis* and *Porphyridium cruentum* extracts on mycelial growth of *Colletotrichum lindemuthianum*

Treatments	Concentration (mg mL ⁻¹)	Mycelial growth (mm)*
<i>L. platensis</i> extract	0.1	48.40 ± 0.80
	0.5	48.35 ± 0.80
	1.0	49.18 ± 0.71
<i>P. cruentum</i> extract	0.1	48.14 ± 0.61
	0.5	48.13 ± 0.39
	1.0	47.73 ± 0.94
Control (water)	-	47.67 ± 0.77

*Mycelial growth after 14 days

Effects on the initial development of bean seedlings – Soil application

Changes in seedling growth parameters (shoot length, root length, and total length) of *P. vulgaris* in response to different concentrations of *L. platensis* and *P. cruentum* extracts are presented in Fig. 3. For shoot length, treatments with *L. platensis* extract (Fig. 3a) resulted in enhancements of 15 and 9% at 0.5 and 1.0 mg mL^{-1} , respectively. Total seedling length was significantly impacted ($p=0.016$), with the 0.5 mg mL^{-1} treatment resulting in the highest value ($30.98 \pm 1.01 \text{ cm}$), showing an increase of around 8%

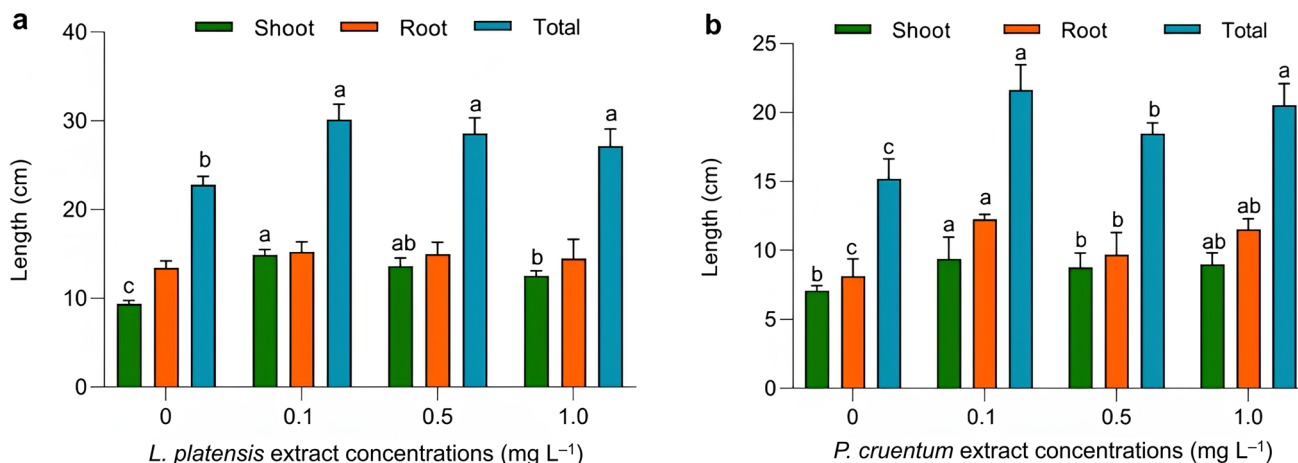


Fig. 1 Effect of *Limnospira platensis* (a) and *Porphyridium cruentum* (b) extracts on shoot, root and total length of *Phaseolus vulgaris* after nine days. Data are presented as mean ($n=4$) $\pm$ standard deviation ($p < 0.05$). Different letters indicate significant differences between treatments

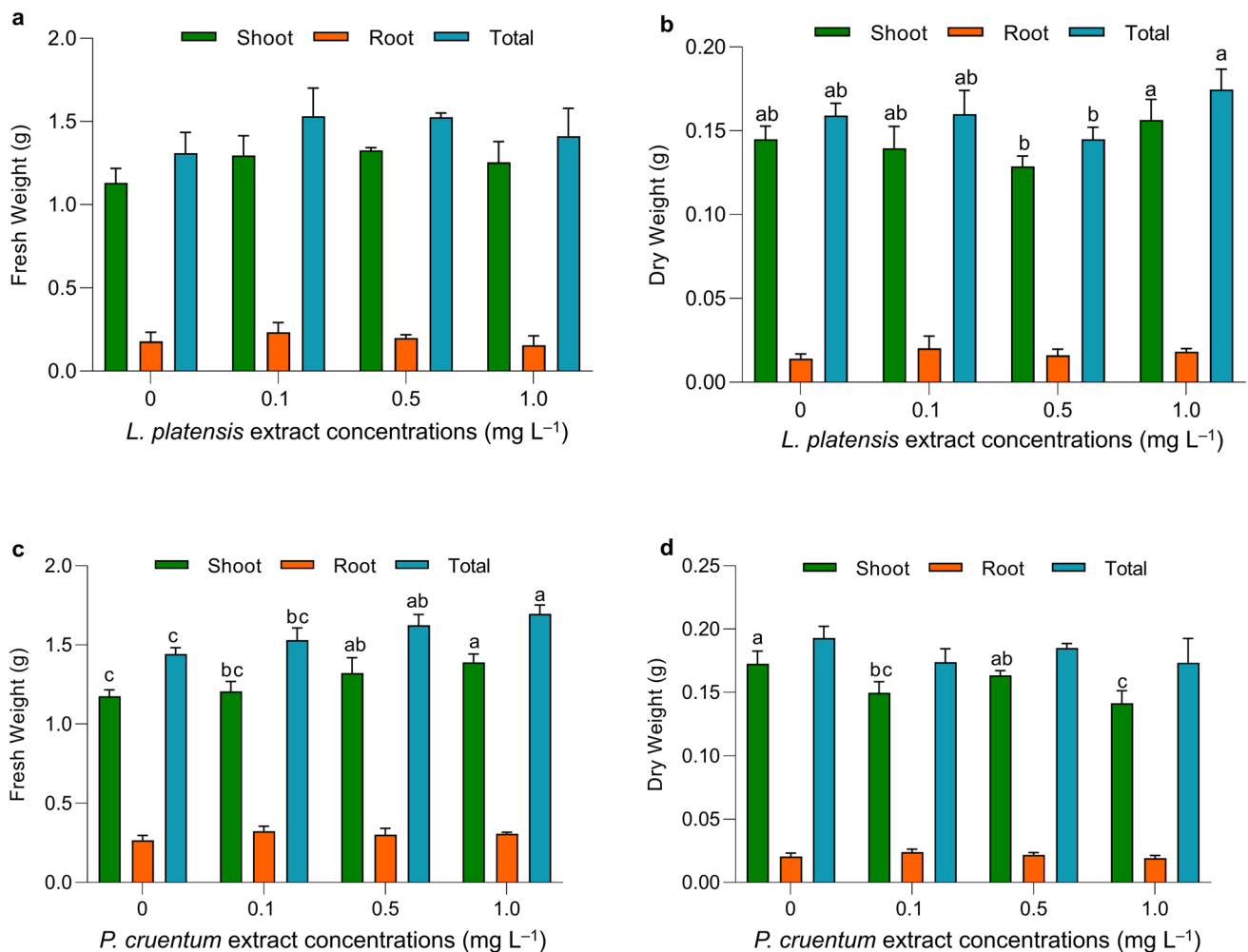


Fig. 2 Effect of *Limnospira platensis* (**a**, **b**) and *Porphyridium cruentum* (**c**, **d**) extracts on fresh and dry shoot, root and total weight of *Phaseolus vulgaris* after nine days. Data are presented as mean

($n=4$) $\pm$ standard deviation ($p < 0.05$). Different letters indicate significant differences between treatments

compared to the control. No significant ($p=0.362$) changes in growth parameters were observed with *P. cruentum* treatments (Fig. 3b).

Statistically significant enhancements ($p=0.0009$) were noted in biomass parameters in plants exposed to *L. platensis* extract at a concentration of 1.0 mg mL^{-1} . Root fresh weight increased by 28.8% compared to the control, with no significant changes ($p=0.477$) at lower concentrations (Fig. 4a). Shoot dry weight also increased by 25.9% at 1.0 mg mL^{-1} and by 19.5% at 0.5 mg mL^{-1} compared to the control. Total dry weight showed similar increases, with gains of 25% and 16.3% at 1.0 and 0.5 mg mL^{-1} , respectively (Fig. 4b).

In seeds treated with *P. cruentum* extract, significant differences ($p=0.0007$) were observed in root and total fresh weights (Fig. 4c). The 1.0 mg mL^{-1} treatment produced the highest values ($0.74 \pm 0.09 \text{ g}$ and $1.90 \pm 0.19 \text{ g}$), reflecting improvements of approximately 32% and 20%

over the control, suggesting that the highest concentration of *P. cruentum* extract was the most effective in improving fresh biomass, especially in root tissues.

Quantification of photosynthetic pigments

Treatment with *L. platensis* extract at 1.0 mg mL^{-1} significantly increased ($p=0.021$) chlorophyll *a* content by 17%, while chlorophyll *b* showed no significant ($p=0.090$) changes. Total chlorophyll was highest at 1.0 mg mL^{-1} , increasing by 16%. Carotenoid content increased significantly ($p=0.0009$) across all extract concentrations, with rises of 18%, 22%, and 31% compared to the control (Fig. 5a).

Treatment with *P. cruentum* extract at 0.1 mg mL^{-1} significantly decreased ($p < 0.0001$) chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid contents compared to 0.5 and 1.0 mg mL^{-1} treatments. There was no

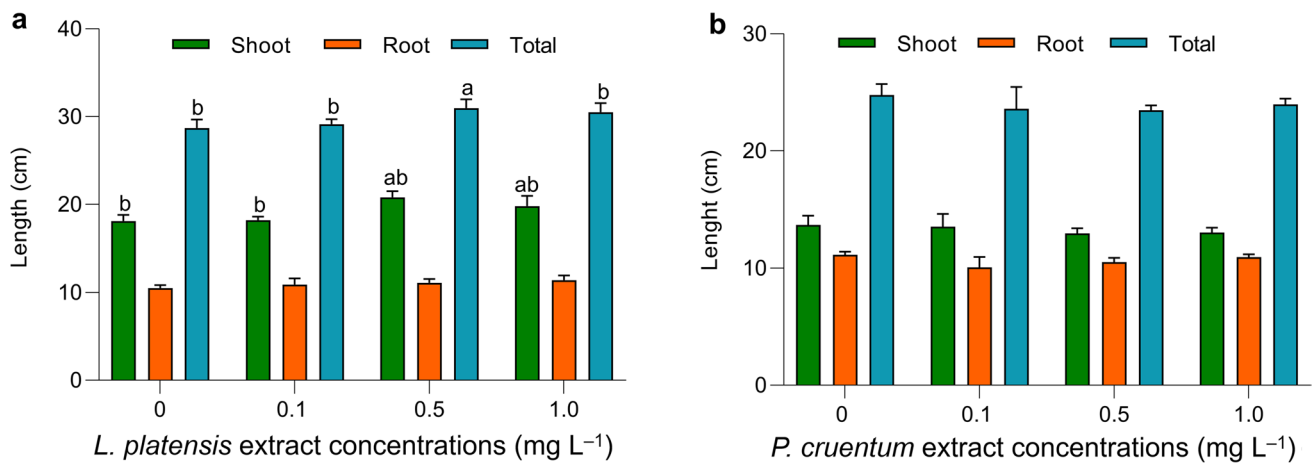


Fig. 3 Effect of *Limnospira platensis* (a) and *Porphyridium cruentum* (b) extracts on shoot, root and total length of *Phaseolus vulgaris* after 11 days. Data are presented as mean ($n=4$) $\pm$ standard deviation ($p < 0.05$). Different letters indicate significant differences between treatments

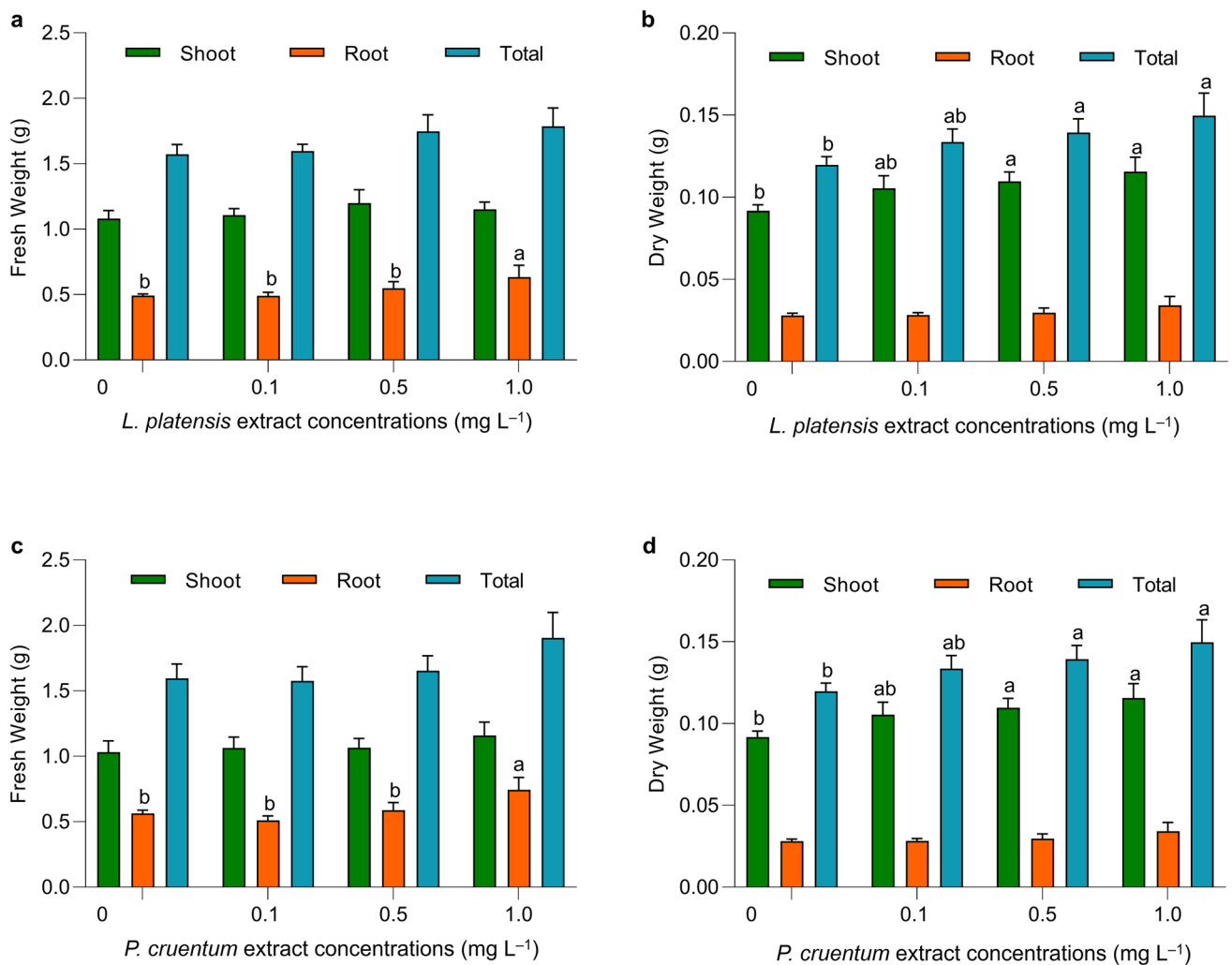


Fig. 4 Effect of *Limnospira platensis* (a, b) and *Porphyridium cruentum* (c, d) extracts on fresh and dry shoot, root and total weight of *Phaseolus vulgaris* after 11 days. Data are presented as mean

($n=4$) $\pm$ standard deviation ($p < 0.05$). Different letters indicate significant differences between treatments

statistical difference ($p=0.919$) for Chlorophyll *b* among the 0.1 mg mL^{-1} treatment and the control group. No significant differences ($p=0.778$) were observed among the control, 0.5 and 1.0 mg mL^{-1} treatments for any pigment (Fig. 5b).

Extracts characterization

The UPLC analysis of *L. platensis* (Table 2) and *P. cruentum* (Table 3) extracts revealed the presence of various compounds with distinct chemical structures and functional groups, indicating the intricate and diverse chemical composition of the extracts.

The identified compounds in *L. platensis* included sphingolipids (sphinganine), phospholipids (lysophosphatidylglycerols, phosphatidylcholines), peptides (l-valyl-L-tyrosine), long-chain amino alcohols (2-amino-tetradecane-1,3-diol, 2-amino-hexadecane-1,3,4-triol, 2-amino-hexadecane-1,3-diol), ardenolide glycosides and derivatives, sesquiterpenoids, sterols (a diverse class of lipids), polyunsaturated fatty acids (linolenic acid and linoleic acid) and oxygen-containing carotenoid pigments (xanthophylls). The *P. cruentum* extract contained an amino alcohol (2-aminooctadecane-1,3-diol) and phosphatidylcholines, similar to those found in *L. platensis*. Additionally, a steroid, a glycolipid (gingerglycolipid A), and a triterpenoid were identified.

Discussion

The present study explored the use of ethanolic extracts from the microalgae *L. platensis* and *P. cruentum* as eco-friendly alternatives for growing common beans (*P. vulgaris*). The findings support the development of more efficient and environmentally friendly bioinputs, highlighting the importance

of microalgal secondary metabolites in promoting plant growth. Although the extract had a complex chemical composition, it did not show any *in vitro* antifungal activity against *C. lindemuthianum*. This outcome could be attributed to factors such as the concentration utilized, the polarity of the solvent, or the mechanism of action of the compounds, which might act indirectly enhancing plant defenses rather than directly inhibiting the pathogen. This is consistent with the findings of Paulert et al. (2007), who reported no inhibition of mycelial growth using alcoholic extracts of *Ulva fasciata* up to 2 mg mL^{-1} , and Dmytryk et al. (2022), who noted antifungal activity with *L. platensis* extracts only when obtained via supercritical CO_2 . In this study, while no direct antifungal activity was detected, the compounds in the extracts may have indirectly influenced plant growth by stimulating biochemical and structural mechanisms. This led to significant improvements in bean growth.

Seed priming with both *L. platensis* and *P. cruentum* extracts significantly enhanced early seedling development, but with species-specific patterns. The extract from *L. platensis* notably boosted total shoot and seedling length, especially at a concentration of 0.1 mg mL^{-1} . This enhancement could be attributed to the presence of sphinganine derivatives such as 2-amino-hexadecane-1,3-diol and 2-amino-hexadecane-1,3,4-triol, which function as signaling molecules in cell expansion and stress responses (Huby et al. 2020). The presence of L-valyl-L-tyrosine may further support these responses by modulating nitrogen metabolism and the biosynthesis of hormones and pigments (El-Sherbeny and Silva 2014). Refaat and Naguib (1998) found that tyrosine application increased carbohydrate content in peppermint (*Mentha piperita*) leaves, while Wahba et al. (2007) observed positive growth effects in *Urtica pilulifera* after tyrosine application. Essential fatty acids (linoleic

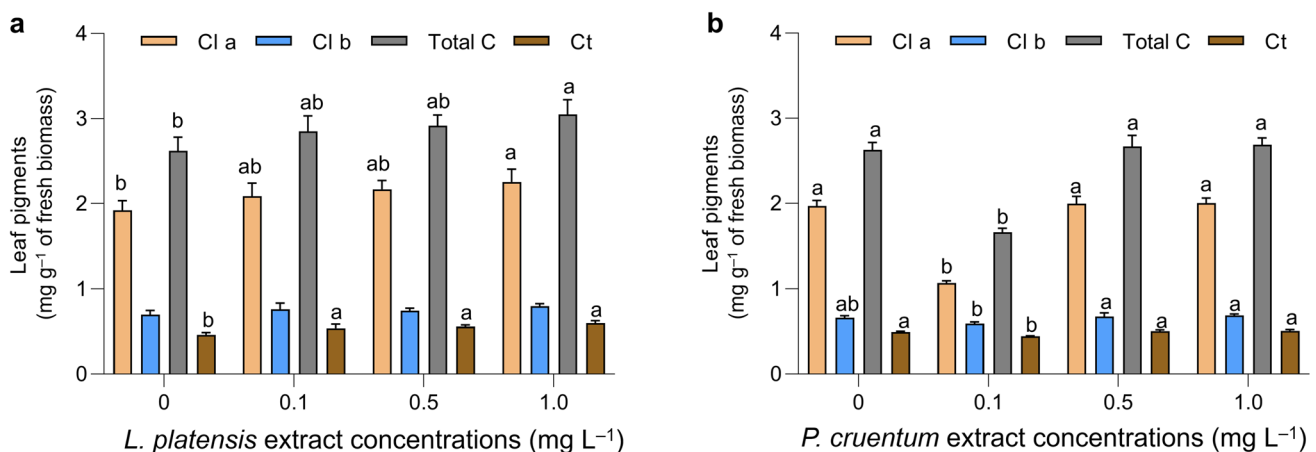


Fig. 5 Effect of *Limnospira platensis* (a) and *Porphyridium cruentum* (b) extracts on leaf pigment composition (chlorophyll a (Ca), chlorophyll b (Cb), total chlorophylls (Total C) and total carotenoids (Ct)

(mg g^{-1}) in *Phaseolus vulgaris* after 11 days. Data are presented as mean ($n=4$) $\pm$ standard deviation ($p<0.05$). Different letters indicate significant differences between treatments

Table 2 Chemical profile obtained from UPLC-MS data for *Limnospira platensis* ethanolic extract sample. NI: not identified

Peak n°	t _R (min)	Molecular formula	m/z Precursor error	Proposed compound
1	2.99	[C ₁₂ H ₂₇ NO ₂ + H] ⁺	218.2117	Class of sphinganine
2	3.94	[C ₁₄ H ₂₀ N ₂ O ₄ + Na] ⁺	303.1334 (1.7)	L-valyl-L-tyrosine
3	5.11	[C ₁₄ H ₃₁ NO ₂ + H] ⁺	246.2430	2-amino-tetradecane-1,3-diol
4	5.27	[C ₁₆ H ₃₅ NO ₃ + H] ⁺	290.2692	2-amino-hexadecane-1,3,4-triol
5	5.44	[C ₄₂ H ₆₄ O ₂₀ + H] ⁺	889.4088	Ardenolide glycosides and derivatives
6	5.81	[C ₃₇ H ₅₈ N ₁₂ O ₁₈ + H] ⁺	959.4083	Peptide
7	6.60	[C ₁₆ H ₃₅ NO ₂ + H] ⁺	274.2747	2-amino-hexadecane-1,3,-diol
8	8.21	[C ₃₅ H ₅₄ O ₁₄ + H] ⁺	699.3572	Glycosylated steroid
9	8.33	[C ₄₀ H ₅₀ O ₉ + H] ⁺	675.3550	NI
10	9.12	[C ₁₈ H ₂₈ O + H] ⁺	261.2216	Sesquiterpenoid
11	9.12	[C ₁₁ H ₂₀ N ₂ O ₆ + H] ⁺ [2 M + H] ⁺	277.1382 553.2776	Peptide
12	9.12	[C ₂₉ H ₅₅ O ₉ + H] ⁺	537.3041	Class sterol
13	9.86	[C ₃₄ H ₄₀ N ₂ O ₅ + H] ⁺ [M + Na] ⁺	557.2994 579.2829	NI
14	10.13	[C ₂₂ H ₄₅ O ₉ P - H] ⁻	483.2722	Lysophosphatidylglycerols
15	11.64	[C ₁₈ H ₃₀ O ₂ - H] ⁻	277.2166	Linolenic acid
16	12.23	[C ₄₄ H ₈₄ NO ₈ P + H] ⁺	786.5999 (1.0)	Phosphatidylcholines
17	12.37	[C ₁₈ H ₃₂ O ₂ - H] ⁻	279.2326	Linoleic acid
18	15.47	[C ₄₀ H ₅₄ O ₂ + H] ⁺	567.4196	Xanthophyll
19	15.47	[C ₄₂ H ₈₂ NO ₈ P + H] ⁺	760.5863	Class phosphatidylcholines

Table 3 Chemical profile obtained from UPLC-MS data for *Porphyridium cruentum* ethanolic extract sample. NI: not identified

Peak n°	t _R (min)	Molecular formula	m/z Precursor	Proposed compound
1	4.27	[C ₁₄ H ₂₀ N ₂ O + H] ⁺	233.1652	NI
2	7.91	[C ₁₈ H ₃₉ NO ₂ + H] ⁺	302.3058 (-0.5)	2-amino-octadecane-1,3-diol
3	8.95	[C ₂₈ H ₅₀ NO ₇ P + H] ⁺	544.3406	Class phosphatidylcholines
4	9.03	[C ₃₁ H ₄₄ O ₉ + H] ⁺	561.3041	Steroid
5	9.32	[C ₃₃ H ₅₆ O ₁₄ + H] ⁺	677.3726	Gingerglycolipid A
6	12.12	[C ₂₈ H ₄₀ + H] ⁺	377.3205	Class triterpenoids
7	15.47	[C ₄₄ H ₈₄ NO ₈ P + H] ⁺	786.6013	Class Phosphatidylcholines

and linolenic acids), known precursors of oxylipins and jasmonates, may also contribute to these growth-promoting effects by stimulating endogenous defense and growth pathways (Mata-Pérez et al. 2015; Saffaryazdi et al. 2020). Seed priming improved early seedling growth, potentially by activating physiological and hormonal mechanisms through bioactive compounds. This suggests potential use of these extracts as organic biostimulants in agriculture.

At increased extract concentrations, seed treatments still enhanced shoot elongation and biomass accumulation, albeit to a lesser extent. The rise in dry biomass at higher doses indicates a transition towards biomass deposition, possibly influenced by phosphatidylcholines (PC), phosphatidylglycerols, and sterols. These compounds play a role in membrane formation, nutrient transport, and hormone signaling, regulating important processes like stress responses, elongation, root growth, and flowering (Furse 2017; Rogowska and

Szakiel 2020; Nakamura 2021). Sun et al. (2022) conducted a study showing that exogenous PC enhanced salt stress tolerance in peach trees (*Prunus persica*) by promoting root growth and increasing plant biomass. The extract contains peptides and sesquiterpenoids that may serve as signaling molecules to support developmental and stress-related responses (Guedes et al. 2018), contributing to the increase in dry matter observed in the present study.

Likewise, the significant enhancements in stem, root, and total seedling length of common bean following treatment with *P. cruentum* extract at doses of 0.1 and 1.0 mg mL⁻¹ may be due to the presence of 2-amino-octadecane-1,3-diol, a sphingolipid structurally similar to sphingosine, which is known to impact membrane integrity and hormonal signaling. Studies have shown that sphingolipids play a pivotal role in plant vegetative growth and pollen development (Chen et al. 2008; Dietrich et al. 2008; Rennie et al. 2014), and

their role in enhancing early-stage elongation is consistent with the pronounced increases in total seedling length (up to 42%) observed in this study. Triterpenoids and steroids in the extract may act as analogs of brassinosteroids which are known to regulate growth and stress responses and are able to promote cell elongation (Noushahi et al. 2022). This highlights the potential of *P. cruentum* extracts as effective agricultural bioinputs.

Interestingly, while *L. platensis* stimulated dry biomass accumulation, *P. cruentum* extract led to increased fresh weight but reduced shoot dry weight at 1.0 mg mL^{-1} , suggesting a stimulation of tissue hydration without proportional structural development. This can relate to the presence of phosphatidylcholines, key components of biological membranes that are able to enhance membrane fluidity and facilitate vesicle trafficking and auxin transport, contributing to improved water and nutrient uptake (Furse 2017; Nakamura 2021). Moreover, phospholipids play crucial roles in plants, extending beyond cellular structure to serve as signaling molecules during abiotic stress, facilitate lipid transport, and act as precursors for bioactive molecules (Nakamura 2021). These functions are essential for the survival and optimal functioning of bean plants.

Our findings are consistent with previous studies showing the biostimulant potential of microalgal extracts in seed treatments. For example, Shedeed et al. (2022) found that *L. platensis* extract at 0.25% significantly enhanced seedling shoot and root length as well as fresh and dry weight in yellow lupine (*Lupinus luteus*). Similarly, Bumandalai and Tserennadmid (2019) reported optimal cucumber (*Cucumis* sp.) seedling growth at 0.25 mg mL^{-1} , supporting the effectiveness of lower doses, which aligns with our observations for both species. El-Naggar et al. (2005) demonstrated that aqueous extract from *Chlorella kessleri* improved the fresh and dry weight of *Vicia faba* seedlings, while Garcia-Gonzalez and Sommerfeld (2016) observed that *Acutodesmus dimorphus* enhanced root development in tomato seedlings. Guedes et al. (2018) examined the biostimulant effects of *L. platensis* on papaya (*Carica papaya*) seedlings, finding that a 1.08% root application led to optimal growth and biomass yield. In our study, lower extract concentrations showed positive results in soil applications, suggesting effectiveness dependent on cultivation conditions or preparation methods.

When applied to the soil, *L. platensis* extract continued to support seedling development, with moderate improvements in shoot and total length, and more pronounced increases in biomass (especially root fresh weight), and in shoot and total dry weight (at 1.0 mg mL^{-1}). These effects are likely linked to lipophilic molecules such as phospholipids and sterols, which enhance membrane functionality and nutrient transport. Ardenolide glycoside derivatives, though not fully characterized, can stimulate root growth through auxin response pathways, potentially explaining the increased root

biomass observed in treated bean seedlings (Mazepa et al. 2021).

Porphyridium cruentum extract, in contrast, did not significantly alter shoot or root elongation when applied to the soil, but significantly increased root fresh weight. This may be partially attributed to the presence of glycolipids such as gingerglycolipid A, known for their antioxidant and membrane-stabilizing properties. These effects could enhance water uptake and retention in root tissues, contributing to biomass accumulation (Khan et al. 2009). This compound is also observed in the leaves of *Sideroxylon obtusifolium*, but its role in microalgae extracts is still under investigation. This discovery may represent the first documented occurrence of gingerglycolipid A in a microalga.

Previous studies have demonstrated the growth-promoting potential of microalgal extracts, though responses often vary depending on species, concentration, and application method. Chabili et al. (2025) suggested screening soil cyanobacteria and microalgae extracts to explore their biostimulant effects on wheat seed emergence and vigor. They found that lower doses (0.1 g L^{-1}) were associated with higher seed germination rates, leading to improvements in germination, vigor, and biometric parameters. In contrast, Moraes et al. (2025b) observed that foliar application of microalgae extracts from *L. platensis*, *Nannochloropsis oculata*, *Tetrademus obliquus*, and *Chaetoceros muelleri* affected the seed yield of white lupine (*L. albus*). A single application of microalgae extract led to a significant increase (13.12%) in the number of seeds per pod, regardless of the specific extract used. This highlights that the differences in outcomes may be linked to the species investigated and the application techniques utilized.

Mutale-Joan et al. (2020) reported significant increases in root and shoot growth in tomato plants treated with acid-hydrolyzed extracts of *L. platensis* and *Porphyridium* sp., highlighting species-specific effects. Barone et al. (2018) and Puglisi et al. (2020) observed enhanced root architecture and biomass in hydroponically grown plants treated with green microalgae extracts, likely due to improved nutrient availability under controlled conditions. In the present study, both *L. platensis* and *P. cruentum* extracts promoted fresh biomass accumulation under soil conditions, with *L. platensis* additionally increasing shoot and total dry weights and pigment content. These effects may be attributed to soluble compounds such as proteins, pigments, and polysaccharides. Moreover, previous reports on *L. platensis* enhancing shoot growth in crops like rice suggest the involvement of phytohormones and chelating polysaccharides, which improve nutrient uptake and mobilization (particularly phosphorus) through increased root-associated enzyme activity (Bauenova et al. 2024).

The extracts also positively affected pigment content, only in *L. platensis*-treated plants. The found compounds,

xanthophylls, linolenic acid, and phospholipids, may contribute to photoprotection, photosynthetic efficiency and oxidative stress mitigation, reinforcing the results (Zhu et al. 2011; Barone et al. 2018). Comparable effects were also reported by Mala et al. (2017) in *Amaranthus dubius* treated with *L. platensis*, likely due to increased availability of certain nutrients.

Microalgae are becoming increasingly important in agriculture as valuable bioinputs. However, further research is needed to identify suitable and promising strains (cyanobacteria or microalgae) that are easy to cultivate and can produce sufficient biomass. It is also worth noting that environmental factors such as temperature, light, and nutritional mode (photoautotrophic, heterotrophic, or mixotrophic) can influence the production of desired metabolites for agricultural purposes (Chabili et al. 2024).

In the present study, notable differences were observed between the effects of *L. platensis* and *P. cruentum* extracts on *P. vulgaris* development, which may be attributed to their distinct phylogenetic origins and chemical profiles. These microorganisms differ not only in cell structure and metabolism but also in the composition of their bioactive compounds, which likely underlies their divergent bioactivity profiles.

Conclusion

This study showcased the biostimulant properties of *L. platensis* and *P. cruentum* extracts, emphasizing the unique impacts of each species on the growth of common beans. The enhancements in growth, biomass accumulation, and pigment levels provide evidence for the potential use of these microalgae as eco-friendly inputs in agriculture. The observed effects are believed to be associated with the distinct chemical compositions and evolutionary backgrounds of the microalgae, underscoring the significance of the application method and extract characteristics. While the extracts positively influenced seedling growth, no antifungal activity was detected in the tested conditions, indicating that both extracts are more effective as growth enhancers. Future research should investigate the potential use of these extracts in other crop species.

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Writing – Original draft. Herculano Cella: Data curation, Writing—review and editing. Alessandro Antônio dos Santos: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation. Marciel João Stadnik: Resources, Supervision, Project administration. Mateus Brusco de Freitas: Resources, Supervision, Project administration. Carlos Yure Barbosa de Oliveira: Data curation, Writing—review and editing. Rafael Garcia Lopes: Conceptualization, Methodology, Investigation. Marco Shizuo Owatari: Writing – Original draft, Writing—review and editing. Jhuly Wellen Ferreira Lacerda: Formal analysis, Data curation. Louis Pergaud Sandjo: Formal analysis, Data curation. Silvana Ohse: Supervision, Conceptualization, Methodology. Roberto Bianchini Derner: Resources, Supervision, Conceptualization, Methodology, Validation, Investigation, Project administration.

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Data availability Data will be made available on request.

Declarations

Competing interest The authors declare no competing interests.

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