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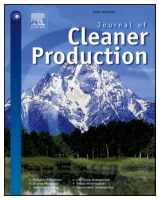

Exploring the biofertilizer potential of *Gracilaria birdiae* aqueous extract for sustainable agriculture

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ABSTRACT

Macroalgal generates valuable bioproducts, primarily extracted using inorganic solvents. However, water can dissolve nutrients, offering an alternative for clean agriculture from a renewable inexpensive source. Therefore, the main objective of this study was to produce and characterize the *aqueous* extract of *Gracilaria birdiae* and evaluate its potential for enhancing plant growth. The first and second extraction stages presented 5.3 % and 11.5 % solid biomass, yielding 88.5 % of the final aqueous extract. Lyophilization preserved 0.3 % of initial weight and was the most effective storage condition for maintaining extract's compounds. Gas chromatography-mass spectrometry identified 54 metabolites in the algae extract, including organic acids (63.5 %), sugars and derivates (17.2 %), amino acids (14.5 %), and other compounds (3.9 %). The extract also retained high levels of macronutrients, including nitrogen (N), phosphorus (P), and potassium (K), 14.8, 16.0, and 276.7 g/kg, respectively along with macro and micronutrients. Rice plants sprayed with the aqueous extract showed a tendency to increase tiller number, carotenoids, carboxylation efficiency, and biomass. It was followed by a negative correlation with electrolyte leakage. The principal component analysis (PCA) showed the divergence of the control and treated groups, with axes representing 93.2 % of the variations. Overall, a water-based product can be considered for use as a potential biofertilizer or biostimulant due to its high nutrient content and abundance of organic molecules. The reduced electrolyte leakage may also indicate physiological conditioning or an antioxidant role. Nevertheless, future research should investigate the optimal percentages, frequencies, and methods for applying the extract.

1. Introduction

Seaweeds are photosynthetic organisms belonging to the Ochrophytes (brown), Chlorophytes (green), and Rhodophytes (red) phylum (Badmus et al., 2024). The positive impacts of seaweed cultivation on ecosystem services include the enhancement and management of biodiversity, restoration of aquatic ecosystems, adaptation and mitigation of climate change, and bioremediation (Elouardi et al., 2023; Pessarrodona et al., 2023). In the aquaculture systems, these species act as a biofilter, removing nutrients, such as phosphate, ammonium, and nitrate, from wastewater and gradually increasing its biomass (Estevam et al., 2017). Seaweed has a high potential for CO_2 sequestration and requires minimal nutritional inputs for vegetative propagation and farm production, contributing to natural stock reestablishment (Peixoto and

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Chow, 2025). Additionally, it contributes to food security and green circular bioeconomy (Rajkumari et al., 2023). This practice provides an additional source of income through the production of low-cost, easily manufactured products.

The importance of seaweed farming is globally acknowledged for creating sustainable new by-products. They are known to be rich sources of polysaccharides, minerals, and vitamins. Although the protein and lipid content are relatively low in brown seaweed, it is higher in most red seaweeds (Fleurence, 1999). Recently, seven species demonstrated the profile of inorganic elements, and the most abundant elements found were calcium (Ca), potassium (K), sodium (Na), magnesium (Mg), and phosphorus (P) potential to be used for different purposes (Ribeiro et al., 2024).

Seaweed-based biofertilizers for agriculture are developing rapidly through the innovation and improvement of used raw materials, formulations, methods, and processes. There were 1731 patent documented in the last 20 years. Among them, brown seaweeds such as *Sargassum* and *Ascophyllum* genera are most employed to produce the liquid extracts, and the red seaweeds are still few studied, as well as the cultivation methodologies in the sea to avoid the depredation of natural stocks and morphophysiological and biochemical traits of treated plants (Fatimi, 2022). Foliar treatment with algal extract significantly enhanced biomass and biochemical characteristics in maize (Zekry et al., 2022). It improves the efficiency of nitrate uptake in leaves, ammonium assimilation, and potassium uptake in rice (Castro et al., 2024). These benefits make it a valuable resource for promoting plant growth and improving crop yield. It suggests that these seaweeds could serve as clean sources of essential eco-friendly mineral supplements for fertilizing plants in a scenario comprising the world's population growth in an increasing demand for food production, which may be insufficient to reach the food needs by 2030 (De Wrachien et al., 2021; Ray et al., 2022). On this hand, biofertilizers are feasible alternatives from an ecological and economic point of view and are used in organic agriculture. It may enhances soil quality and can boost crop yield by up to 40 %, while also promoting the accumulation of amino acids in plants (Rai and Shukla, 2020). The alternative foliar spray is gaining attention for its ability to stimulate plants to accumulate biomass (Zafar et al., 2022). Although there are commercial seaweed products used in agriculture as plant growth stimulants and biofertilizers, they do not guarantee the organic molecules and mineral constituents that need to be explored especially in algal extracts, as well as there is a need to investigate the ways of applying and how it works (Nabti et al., 2017).

The socioeconomic benefits of red seaweed cultivation have supported the maintenance and development of fishing and algaculture communities, particularly in the coastal region of Northeast Brazil, where species are present (Holanda et al., 2025; Marinho-Soriano, 2017). Among seaweed species, the Rhodophyta phylum is abundant and diverse, accounting for 49.3 % of global production, making *Gracilaria* the third most cultivated genus in the world (FAO, 2022). In general, the *Gracillaria* has an elevated nutritional content, such as potassium and manganese (Rohani-Ghadikolaei et al., 2012), magnesium, iron, zinc, and copper (Rosemary et al., 2019), and high levels of total nitrogen and trace elements (Premarathna et al., 2022). Acid hydrolysis of the biomass release phenolic compounds, fibers, minerals, and agar, a sulfated polysaccharide used in the production of food, cosmetics, prebiotics, and food additive (Aguiar et al., 2023; Albuquerque et al., 2021; Torres et al., 2019). Indeed, G. *birdiae* is an outstanding renewable source of compounds such as R-Phycoerythrin, fine chemicals (glucose, galactose, cellobiose, and acid formic, among others). The proximate composition of the dry *G. birdiae* showed high contents of ash (9.5 %), protein (12.2 %), and carbohydrate (67.3 %) and low lipid (0.54 %), being an interesting source of macronutrients and antioxidant compounds aqueous soluble (Guaratini et al., 2012). However, we don't know the nutritional content of a water-based extract and if it could benefit plant growth.

Accordingly, this work aimed to produce and characterize the physical-chemical and biochemical properties of the aqueous extract of *Gracilaria birdiae* and evaluate its potential in plant growth improvement as an eco-friendly alternative for agriculture, focusing on its organic molecules and mineral contents, from easy and cheap obtaining. We hope that our results will contribute to global food security by providing incentives for sustainable strategies to reduce the risks of climate change and by offering new strategies to improve communities' use of algae-derived products.

2. Material and methods

2.1. Red seaweed Gracilaria birdiae

The red seaweed was provided by Associação de Produtores de Algas de Flecheiras e Guajiru (APAFG) located in Trairi, Ceará State, Brazil. The red seaweed species was confirmed by specialists from the Prisco Bezerra herbarium, located at the Federal University of Ceará, and deposited with the identification EAC 66166 - *Gracilaria birdiae* E.M. Plastic & E.C. Oliveira. The algal biomass had its specific weight determined using the biomass weight: volume ratio. The solid biomass per liquid biomass (weight: volume ratio) was measured before starting the extraction stages. The fresh material was weighed and dried in an oven at 60 °C to constant weight to obtain the moisture content.

2.2. Chemicals

GC and LC grade N-Hexane, chloroform and methanol (Merck, Germany), as well as ultrapure water (Millipore) were used to extract biomolecules. Methoxyamine hydrochloride, pyridine and N-methyl-N-(trimethylsilyl) trifluoro-acetamide for gas chromatography (SigmaAldrich) were used in the derivatization step. Standards (ribitol and organic acids) were purchased from Sigma-Aldrich (99 % purity). Other inputs had a high degree of analytical purity.

2.3. Aqueous extract from seaweed and process yields

The density of the red seaweed *G. birdiae* was obtained by checking the volume of distilled water occupied by 20 g of seaweed and expressed in $g.cm^{-3}$. The seaweed was dried in an oven at 60 °C until it reached constant weight, and the moisture was calculated by gravimetry. To aqueous extraction, the wet biomass was weighed and crushed in a food processor for 3 min. The distilled water was added (3:1, water:biomass) and stirred with an mechanical homogenizer at 750 rpm for 3 h at 25 °C. The gross extract (GE) was filtered through nylon fabric and centrifuged at 2000×*g* under refrigeration (4 °C).

During the extraction steps, the GE volumes, the seaweed residue (SR), the centrifuged extract (CE) volume, the centrifugation residue (CR) weight, and the lyophilized extract (LE) weight were recorded. The volumetric yields of the extracts (Y_V) and the losses of solid residues (L_{SR}) during the process steps were calculated second Equations (1) and (2) and were expressed in percentage. The pH of CE was checked.

$$Y_V(\%) = \frac{V_e}{V_i} \cdot 100 \quad \text{Eq. 1}$$

Where V_e is the extract volume recovered (mL) after such process' step and V_i is the homogenate (seaweed plus distilled water).

$$L_{SR}(\%) = \frac{B_i - B_g}{B_i} \cdot 100 \quad \text{Eq. 2}$$

Where B_i is the initial weight (g) of wet seaweed and B_g is the weight (g) of solid residues of such process' step.

2.4. Effect of storage method on the physicochemical properties of the GEB

The thermogravimetric analysis (TGA) of lyophilized extract was performed in a thermobalance (Shimadzu TGA-60) at a temperature range of 25–900 °C (rate 10 °C.min^{-1}) under synthetic air flow (40.0 mL min^{-1}) represented by the percentage of weight obtained in the temperature range divided by the initial weight (M/Mo) until 900 °C.

The ultraviolet–visible (UV–Vis) profile scanning range of 190–1000 nm in spectrophotometer (Cary 60 UV–Vis, Agilent) was performed to compare aqueous frozen stored (−20 °C), aqueous refrigerated (4 °C) or lyophilized extract to evaluate the most efficient form of storage for 90 days of the CE.

Fourier Transform-Infrared spectra (FT-IR) in an interval between 400 and 4000 cm^{-1} with a resolution of 4 cm^{-1} (model ABB-FTIR- FTLA, 2000) were used. The samples were analyzed as KBr pellets.

2.4.1. Biochemical composition

Total soluble proteins (Bradford, 1976), total carbohydrates (Albalasmeh et al., 2013; DuBois et al., 1956), and total lipids (Lutz, 1985); were quantified in aqueous extract.

Organic acids were quantified by solubilizing the LE in ultrapure water (1:10, m/v) (Nascimento et al., 2023). The samples were analyzed using High-Performance Liquid Chromatography (HPLC model SPD-10A VP, Shimadzu, Japan) using the isocratic method, with mobile phase 0.005 M sulfuric acid, flow rate 0.6 mL min^{-1}, on HPX-87H BIORAD column (30 cm × 4.5 mm) and pressure 48 kgf. The reading was performed by a detector coupled to a UV–vis detector at wavelength 210 nm. The quantification occurred by a standard curve and expressed as (mM.g^{-1}).

The other polar biomolecules were extracted using methanol, chloroform, and ultrapure water (Lisec et al., 2006) and analyzed by gas chromatography coupled to mass spectrometry (GC-MS model QP-PLUS 2010; Shimadzu, Japan) using split mode (1:5) with helium gas as a carrier (flow rate 1.2 mL min^{-1}) in a capillary column RTX-5MS (30 m × 0.25 mm × 0.25 μm). The data obtained were analyzed by the Xcalibur™ 2.1 software, the retention rates and mass percentages were determined according to the fragmentation pattern (Hummel et al., 2007). Relative quantification was performed using ribitol as a standard.

2.5. Chemical composition of aqueous extract

Quantification of the total nitrogen content was carried out on the samples previously digested in nitric acid (10:1; m/v) by titration using the Kjeldahl method (Bremner, 1996).

The quantification of elements was performed by inductively coupled plasma-optical emission spectrometer (ICP OES), (iCap 6000 Model, Thermo Scientific, 7000DV, USA), the specific wavelength was set for each element such as Al (167.0 nm), As (193.7 nm), Zn (206.200 nm), P (213.617 nm), Fe (239.562 nm), B (249.677 nm), Mn (257.610 nm), Mg (285.213 nm), Ca (317.933 nm), Cu (327.393 nm) Na (589.592 nm) and K (766.940 nm).

2.6. Test of extract as a foliar biofertilizer spray for rice plants

The experiment was carried out in the greenhouse of the Laboratory of Plant Physiology of the Federal University of Ceará (3.7448° S, 38.5744°W) Aw' climate (Alvares et al., 2014; Köppen and Geiger, 1928). Rice seeds were sown in vermiculite and regularly moistened with desalinated water during the first 15 days. After the appearance of three fully expanded leaves (V3 stage), the plants were transferred to a half-strength nutrient solution (Hoagland and Arnon, 1950) and acclimatized for seven days. It was divided into 2 groups, one group sprayed with 20 mL distilled water (Control) and other group was sprayed with 20 mL per plant of the no diluted aqueous extract once a week for four weeks directly on leaves (every seven days for 28 days). Each group was comprised of 4 repetitions, each repetition composed of two plants.

Plant fresh weight, dry weight, number of tillers, and photosynthetic pigments (Wellburn, 1994) were quantified at the end of the experiment. Electrolyte leakage (EL) was determined in shoots. Samples were incubated in deionized water and the electrical conductivity 1 (EC1) using an electrical conductivity meter (Micronal®). Then samples were incubated in water bath at 95 °C, for 30 min and membrane electrical conductivity 2 (EC2) was measured (Dionisio-Sese and Tobita, 1998). The percentage of EL was determined using the formula:

$$EL\ (\%) = 100 \times (EC1\ /\ EC2) \quad \text{Eq. 3}$$

Gas exchanges were monitored with an infrared gas analyzer (IRGA model LI6400XT, LI-COR Biosciences Inc) on the first fully expanded leave under a constant concentration of 400 μmol mol^{-1} CO_2 and photosynthetic photon flux density of 1200 μmol photon m^{-2} s^{-1} (Gadelha et al., 2021).

2.7. Statistics analysis

The analysis of rice plants was carried out from four repetitions, with each experimental unit consisting of two plants. The Multivariate analysis was processed on a R web-based interface. The data were normalized by an automatic scaling (mean-centered and divided by the standard deviation of each variable) that fitted a better statistical normal distribution.

3. Results

3.1. Obtaining aqueous extract from seaweed

The density of the *G. birdiae* red seaweed was 1.03 ± 0.02 g cm^{-3}, and the moisture content of the seaweed biomass was approximately 85.99 ± 0.66 %.

The process used to obtain the extract of *G. birdiae* was shown (Fig. 1). Algae tissues were crushed, mixed, and filtered (first stage). From the total volume yield, 94.7 ± 5.7 % was gross extract (GE), and 5.3 % corresponded to losses during processing. The solid seaweed residue (SR) accounted for 92.2 % ± 6.2 %, with 7.8 % attributed to losses. In the second stage, the GE was processed to obtain centrifuged extract (CE), yielding 88.5 % ± 1.6 % of supernatant and 10.6 % residual volume, with 0.9 % losses. This resulted in a total loss of 11.5 %, which was discarded. From the supernatant, only 0.3 % ± 0.03 of lyophilized extract (LE) was obtained, while 99.7 % corresponded to the eliminated water.

The consistent values among independent extraction rounds demonstrated the easy reproducibility and efficiency of the established procedure with low standard errors (Table S1). There was a slight difference between the density of the GE and the CE from G. *birdiae*, with mean values of 0.97 ± 0.001 and 0.98 ± 0.002 g cm^{-3}, respectively. Besides, the average volumetric yield of the CE was 88.5 %, with an average pH of 7.56, ranging from 7.16 to 7.71, and an average humidity of 99.7 %.

3.2. Storage methods and physicochemical properties of extract

The breakdown kinetics for the degradation of LE showed three predominant M/Mo declines. First, from the initial temperatures until 150 °C, the weight loss was due to volatile compounds and water loss (Fig. 2A). It accounted for approximately 70 % of the total sample mass represented mainly by organic compounds and water. The second decrease in weight loss ranged from 160 °C to 320 °C. It represents approximately 35 % of the total sample mass where carbohydrate decomposition occurs. The third drop occurred from 320 °C to 450 °C, corresponding to the degradation of protein structures, reaching 25 % of total mass. The weight became constant from 525 °C, where only mineral residues are present, such as metals, salts, and compounds

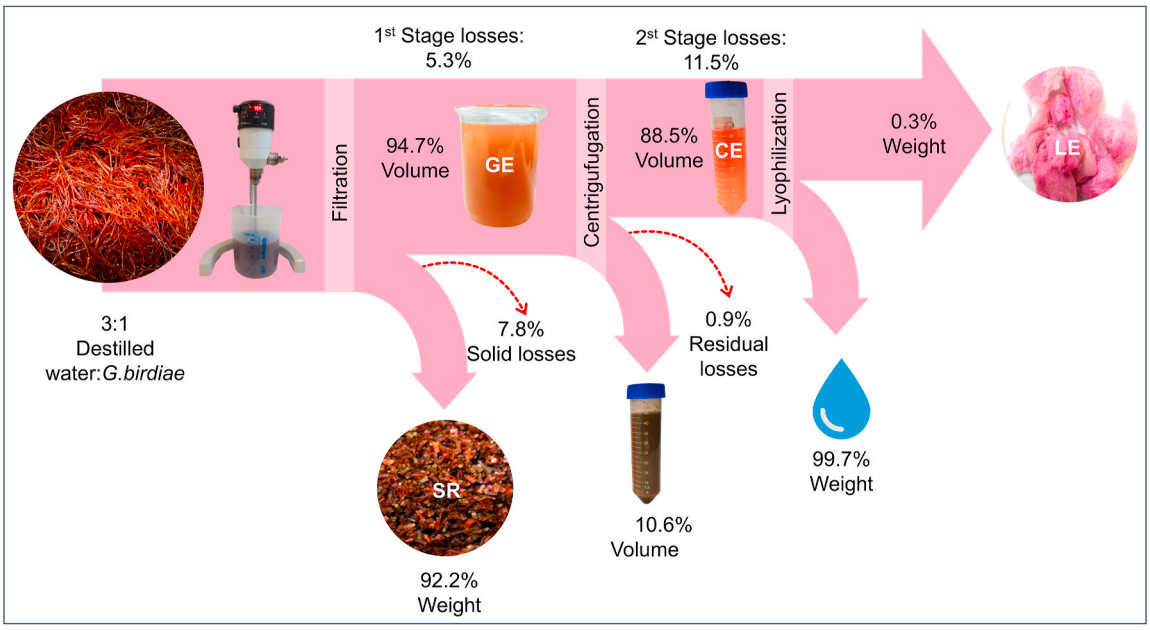

Fig. 1. Process for obtaining *Gracilaria birdiae* aqueous extract. The first stage involves crushing, mixing, and filtering through nylon fabric to produce a gross extract (GE). The second stage involves centrifugation to yield the final aqueous extract (CE). Optionally, the third stage involves lyophilization to obtain a lyophilized extract (LE). The values presented are the means of six independent extractions.

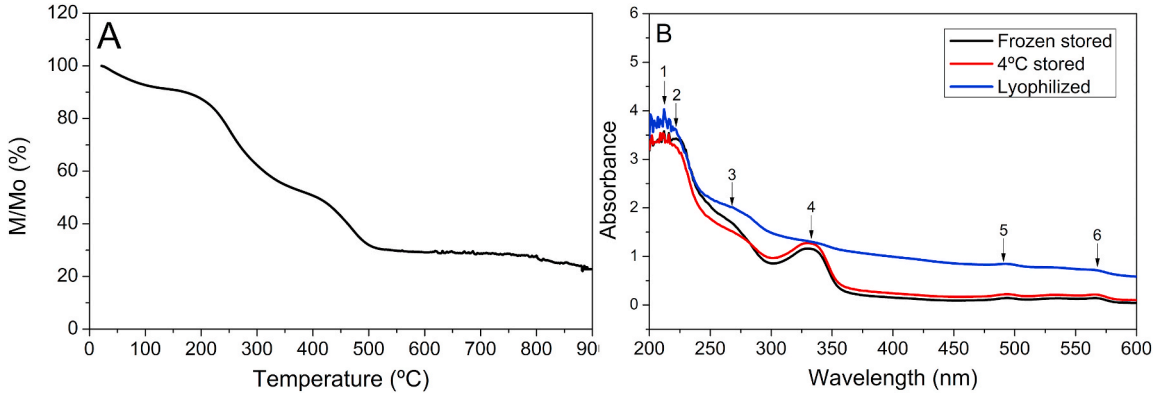

Fig. 2. Physicochemical characterization of aqueous extract. A) Thermogravimetric analysis of the lyophilized extract. B) Spectrophotometric profiles of the extract after 90 days of storage under different conditions: centrifuged extract stored at 4 °C and −20 °C, or lyophilized extract stored at −20 °C, then resolubilized to the original volume in distilled water for analysis.

containing C, H, and O.

The spectrophotometric absorbance profiles of CE stored at 4 °C (red line) and at −20 °C (black line), and LE stored at −20 °C (blue line) were evaluated after 90 days of storage. In general, the lyophilized storage showed the highest optical densities at all wavelengths compared to 4 °C and −20 °C storage (Fig. 2B). The highest optical density value was 4.029, which was obtained at wavelength 212 nm, while there was a reduction of 12.8 % and 11.2 % for the 4 °C and −20 °C stored extracts, respectively. There was a very close absorbance profile between 4 °C and −20 °C storage conditions, except for the wavelength around 265 nm in which −20 °C storage presented higher optical density. It was the only one in the 4 °C storage that retained more organic components than the frozen extract, with a decrease of 11.02 %. The six accentuated peaks in the extract absorbance profiles showing greater prominence were compared by Tukey's mean (Table S2). The comparison test showed that lyophilization was more efficient for the storage of organic components due to higher absorbance at 212 nm (peak 1), 494.99 nm (peak 5), and 565 nm (peak 6). Conversely, there was no statistical difference between 4 °C stored and Frozen stored. Stability in readings occurred from the wavelength from 600 nm until readings at 1200 nm.

The infrared spectrum of the lyophilized extract (Fig. 3) revealed seven prominent bands with minimum transmittance at wavenumbers 3390, 2929, 2148, 1652, 1417, 1110, and 617 cm^{-1}. These bands were analyzed for their spectroscopic assignments and corresponding functional groups based on relevant literature (Table S3). The alcohol clusters were identified at higher intensity peaks 3390 representing H-bonded and O–H stretching, also observed at 1417 cm^{-1} and 1110 cm^{-1}, along with carbonyls arising from carboxylic acids. Nitrogen-containing groups were identified at 2929 cm^{-1}, 1652 cm^{-1}, and 1417 cm^{-1}. In addition, phenols (including polyphenolic carbonyls) were identified at 3390 cm^{-1}, 1652 cm^{-1}, and 1110 cm^{-1}; aliphatic hydrocarbons, such as alkenes and alkynes, were observed at 2929 cm^{-1} and 2148 cm^{-1}, respectively. Aromatic rings appeared at 1652 cm^{-1}, while esters and ethers were detected at 1110 cm^{-1}, including thioethers at 617 cm^{-1}. Amines, including aliphatic amines, were present at 3390 cm^{-1} and 1110 cm^{-1}. Additionally, bands between 1258 and 933 cm^{-1} were attributed to sulfated polysaccharides (S=O), such as the C–O–C group of 3,6-anhydro-α-L-galactopyranose, a signature compound for

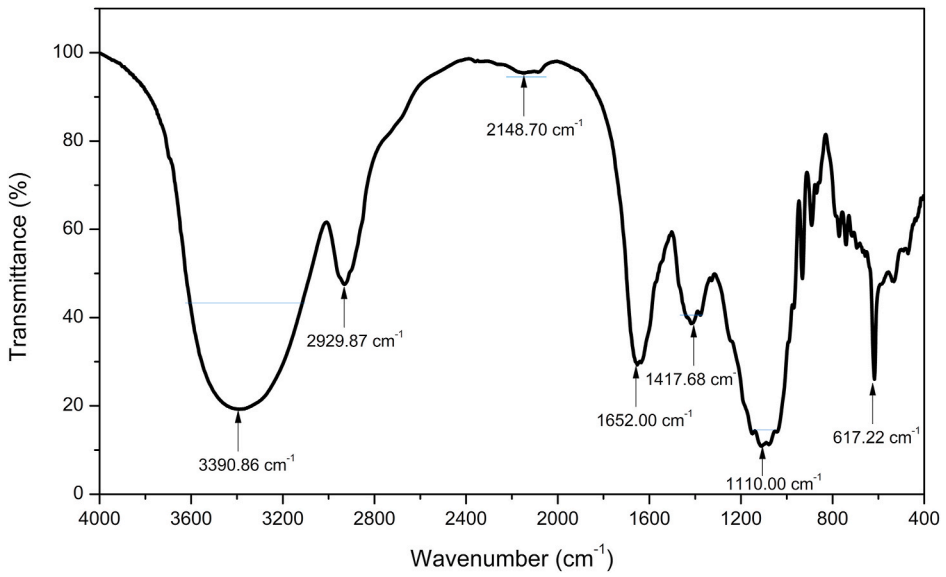

Fig. 3. Fourier Transform Infrared (FT-IR) spectrum of *Gracilaria birdiae* lyophilized extract, ranging from 400 cm^{-1} to 4000 cm^{-1} wavenumbers. Arrows indicated the prominent bands with minimum transmittance.

G. birdiae.

3.3. Characterization of lyophilized algal aqueous extract

3.3.1. Biochemical composition

There were 1.67 μg mL^{-1} of total protein, 1330 μg mL^{-1} of total carbohydrates, and 88.67 μg mL^{-1} of total lipids, as well as 41.15 μg mL^{-1} of mineral residues in the resolubilized lyophilized extract, after incineration at 500 °C. The organic acids identified by liquid chromatography in greater quantity were butyric acid, propionic acid, and acetic acid, with a relative abundance of 36.92 %, 27.24 %, and 23.07 %, respectively (Table 1).

GC-MS revealed the main primary metabolites in the lyophilized extract (Table 2). A total of 54 metabolites were identified, including 63.95 % organic acids, 17.22 % sugars and derivatives, 14.95 % amino acids, and 3.88 % nitrogenous compounds. Metabolites found in higher amounts were phosphoric, lactic, phthalic, and citric acids, all included as organic acids, as well as glycerol in the sugars and derivatives group.

3.3.2. Mineral composition of extract

The highest mineral content was potassium, which reached 276.7 g/kg of biomass. It was followed by sodium (58.4 g/kg), calcium (17.3 g/kg), phosphorus (16 g/kg), nitrogen (14.8 g/kg), and magnesium (14.4 g/kg). Trace elements were also detected, including aluminum (476 mg/kg), boron (259 mg/kg), iron (223 mg/kg), manganese (29.3 mg/kg), zinc (20 mg/kg), and copper (3.7 mg/kg) (Table 3).

Table 1
Organic acids content of the aqueous extract from *Gracilaria birdiae*. The amount corresponds to mean (n = 3) ± standard errors.

Compound	Retention Time (RT)	Amount (mM.g^{-1})	
Oxalic acid	6.5	8.15	±0.43
Citric acid	8.2	3.10	±0.12
Malic acid	9.8	0.95	±0.06
Succinic acid	12.1	1.15	±0.07
Acetic acid	15.3	38.60	±1.65
Propionic acid	18.0	28.48	±1.39
Butyric acid	22.8	24.12	±0.83

3.4. Morpho-physiological analysis of rice plants sprayed with algal aqueous extract

The lyophilized extract was prepared as previously described and sprayed on rice leaves once a week. After four weeks, the plants were harvested, and the morpho-physiological variables were compared with control plants (Fig. 4A). There was a tendency to decrease the content of chlorophyll *b*, the leakage of electrolyte, and the internal carbon (C*i*). On the other hand, there was a tendency to increase the fresh and dry weight of shoots and roots, tiller number, water use efficiency, chlorophyll *a* content, carotenoids, carboxylation efficiency, and assimilation of Carbon (A) colored in the heatmap (Fig. 4B). Additionally, there was maintenance of transpiration, and stomatal conductance (*Gs*).

The three-dimensional model showed a complete separation of treatments, accounting for 93.2 % of the variation in morpho-physiological data, 50.6 %, 31.3 %, and 11.3 % of the variance for PC1, PC2, and PC3, respectively (Fig. 5A). Three main groups of variables had positive correlations (Fig. 5B). The first group comprised carboxylation and water use efficiencies (A/Ci and A/G*s*) and carotenoids that present strong networks (Fig. 5C). The second group included the number of tillers, dry and fresh weight of roots, and shoots that also present strong networks (Fig. 5C). The third group comprised internal carbon, chlorophyll *b*, and stomatal conductance variables, exhibiting strong networks (Fig. 5C). On the other hand, it was possible to observe a negative relationship of electrolyte leakage with the largest of the analyzed parameters, except for the internal carbon. In addition, it is also possible to infer a negative correlation between carboxylation and water use efficiencies, carotenoids, and tiller number with internal carbon content, chlorophyll *b*, stomatal conductance, and transpiration. Orthogonal PLS-DA further provided a list of traits, based on crescent scores of variable importance in projection (VIP), which mostly contributed to the discrimination observed in the PLS-DA model (Fig. S1). The most discriminant for the extract sprayed treatment was chlorophyll *A* content, followed by carotenoids content, carboxylation efficiency and assimilation of Carbon.

4. Discussion

4.1. Gracilaria birdiae provides a low-cost water-based product

The macroalgae *Gracilaria birdiae* is a renewable source of nutrients

Table 2
Biochemical composition of the aqueous extract from *Gracilaria birdiae*. The values correspond to means of relative amount (metabolite/ribitol) of five independent extractions ± Standard Errors.

Compound	Relative amount	SE	Function
Alanine	0.3529	±0.0389	Amino acids
Glycine	0.1931	±0.0627	
Isoleucina	0.7237	±0.2849	
Leucine	0.8972	±0.3707	
Proline	0.3148	±0.0689	
Serine	0.1819	±0.0919	
Threonine	0.0348	±0.0122	
Tyrosine	0.0915	±0.0299	
Valine	1.0085	±0.4581	
Hydroxylamine	0.1005	±0.0166	Other compounds
Putrescin	0.1628	±0.0865	
Spermidine	0.0777	±0.0093	
Uracil	0.2043	±0.0551	
Urea	0.4402	±0.1949	
Adipic acid	0.1255	±0.0148	Organic acids
Butyric acid	1.0173	±0.2244	
Caffeic acid	0.1610	±0.0140	
Citric acid	2.1454	±0.4868	
Erythronic acid	0.6285	±0.0792	
Galactonic acid	0.0779	±0.0164	
Glucaric acid	0.0193	±0.0057	
Gluconic acid	0.5450	±0.0689	
Glutaric acid	0.0631	±0.0077	
Glyceric acid	0.3296	±0.0761	
Glycolic acid	0.1644	±0.0289	
Gulonic acid	0.1414	±0.0316	
Hexadecanoic acid	1.0415	±0.0817	
Isocitric acid	0.1699	±0.0343	
Lactic acid	3.1699	±0.6389	
Phosphoric acid	3.3559	±1.4175	
Phthalic acid	2.4511	±0.2463	
Pyroglutamic acid	0.4399	±0.1227	
Pyruvic acid	0.0703	±0.0174	
Quinic acid	0.1057	±0.0158	
Shikimic acid	0.0063	±0.0021	
Threonic acid	0.0177	±0.0043	
Cellobiose	0.1862	±0.0312	Sugar and derivates
Erythritol	0.0200	±0.0041	
Fructose	0.2132	±0.0173	
Fucose	0.0174	±0.0025	
Galactose	0.0337	±0.0068	
Glucoheptose	0.0188	±0.0047	
Glucopyranoside	0.1315	±0.0172	
Glucose	0.1859	±0.0315	
Glycerol	1.7842	±0.5726	
Inositol (Myo)	0.2646	±0.0741	
Lactulose	0.1862	±0.0312	
Maltotriose	0.7808	±0.1486	
Mannitol	0.0677	±0.0269	
Ribose	0.1212	±0.0127	
Sorbitol	0.0257	±0.0025	
Sorbose	0.2158	±0.0720	
Tagatose	0.0893	±0.0144	
Trehalose	0.0326	±0.0047	

able to resist to climate variations (Borburema et al., 2025). It has been cultivated in the sea of the Brazilian northeast coast by local communities, particularly for fresh food preparations and handmade cosmetics and agar extraction (Holanda et al., 2025; Marinho-Soriano, 2017). However, an additional extract can be generated. The biomass produced by sustainable methods has nutrients that can improve the commercial values of algal-based products and may find additional benefits in agriculture (Glauco et al., 2024).

Usually, methanol, ethanol, and other organic solvents are highly efficient in extracting compounds (Chan et al., 2015). Otherwise, water can dilute a wide range of organic carbon content, including proteins, water-soluble polysaccharides, plant regulators, antioxidant molecules, and micro and macronutrients without organic solvents (Benítez-García et al., 2020; Wahlström et al., 2018). Here, the extract from *G. birdiae* employed a simple process, using cheaper equipment and utensils, water, and only two steps to obtain a nutritive liquid to apply in the plants with a high yield and a viable nutritional solid residue that still needs to be explored. It is a low-cost water-based product obtained with high yield in the laboratory. Therefore, this process has a high potential to be scaled up and used by coastal communities. The aqueous extract may provide potential environmentally friendly organic molecules for biofertilizers. It has great potential for industrial scale-up, with reduced losses and lower energy requirements (Fig. 1), and the residue could still be used for agar extraction, for example.

Table 3
Mineral composition of the aqueous extract from *Gracilaria birdiae*. The values correspond to means of 4 independent extractions ± SE are standard errors. Plant metabolic function was based on literature (Taiz and Zeiger, 2013).

Macroelements	Means (g/Kg)	SE	Plant metabolic function
N	14.8	±0.9	Protein and nucleic acid constituent; Energy metabolism; Photosynthesis
P	16.0	±0.1	Energy metabolism; Photosynthesis
Mg	14.4	±0.1	Enzyme activator; Photosynthesis; Respiration; DNA and RNA synthesis
Ca	17.3	±0.2	Structural; Signaling role
Na	58.4	±0.6	Cell expansion; Osmotic regulation
K	276.7	±2.7	Osmotic regulation; Enzyme activator; Photosynthesis; Respiration
Microelements	**Means (mg/Kg)**	**SE**	**Plant metabolic function**
Zn	20.0	±1.9	Cofactor enzyme;
Fe	223.0	±13.0	Redox metabolism
B	259.0	±25.0	Cell elongation; Nucleic acid synthesis; Hormonal responses
Mn	29.3	±2.5	Enzyme activator; Energy metabolism
Al	476.0	±49.0	Toxic
Cu	3.7	±0.4	Redox metabolism

4.2. *The aqueous extract presents stable physicochemical properties*

The extract demonstrated high thermal stability decomposition through different rates. The initial weight loss was due to water dehydration and structural decomposition of carbohydrates starting at 160 °C, protein at 320 °C, and mineral content from 900 °C (Fig. 2A), which is corroborated by literature (Roslee and Munajat, 2017). Similar pattern properties were observed in the *G. birdiae* biomass, it presents a higher thermal decomposition compared to other species indicating higher levels of components such as lipids, proteins, and carbohydrates (Khan et al., 2022; Mohammed et al., 2021). A pattern that was followed by the agar thermal behavior comprising at least 4 states of degradation (Ferreira et al., 2023). This high thermal stability indicates a broad biotechnological prospect for the extract for food products, and agricultural inputs (Al-Mamary and Moussa, 2021). Overall, due to the high complexity of the extract composition, the UV–vis analysis had a qualitative purpose to verify the best way to pack a prospective product. The best method for storing the extract was lyophilization and solubilization of the material in water (Fig. 2B). This process is widely used for the preservation and storage of products of industrial interest, mainly in the pharmaceutical and food industries (Pisano et al., 2019).

The bands observed in the FTIR confirmed the functional groups that comprise biomolecules, such as proteins, polysaccharides, and amino acids, which retained their structure after lyophilization. A comparison with the spectra of several studies recognized the main groups (Table S4). The presence of alcohols, phenols, amines, alkanes, carboxylic acids, esters, ethers, and thioether in algae is strong evidence of bioactive compounds (Sumayya et al., 2020). For instance, the functional groups belonging to amino acids and polysaccharides in the band 3390.86 cm^{-1} corroborated the TG results. The FTIR of *G birdiae* agar

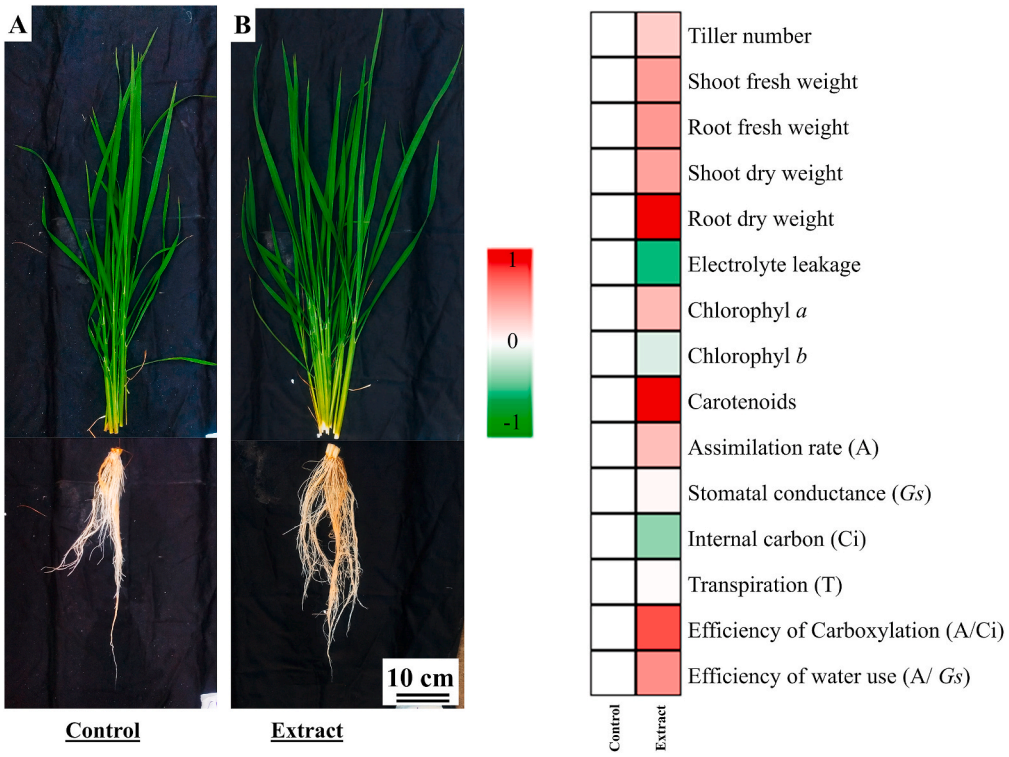

Fig. 4. Morpho-physiological analysis of rice plants submitted to algal extract foliar spraying once a week for four weeks. (A) Phenotypic apparency of the control and algal extract treated plants, and (B) color map shows an increasing (red scale) or decreasing (green scale) of morphological, biochemical, and variable evaluated.

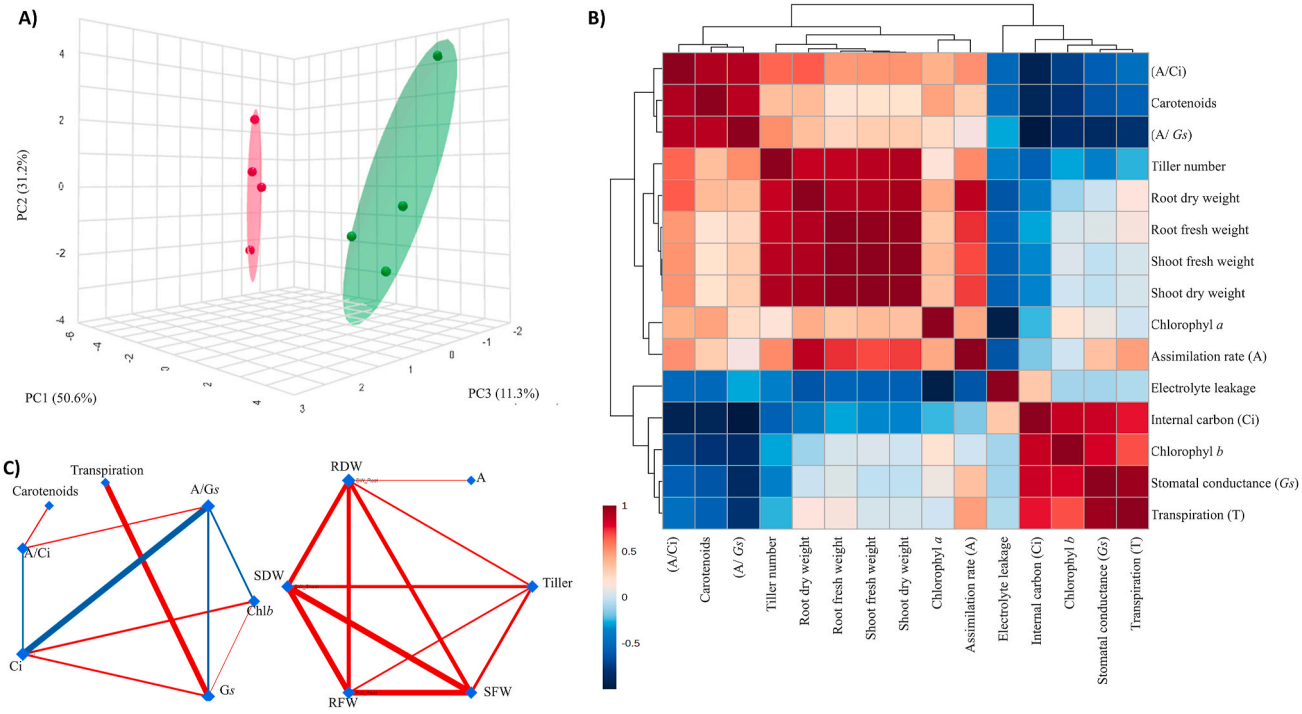

Fig. 5. Multivariate analysis of morphophysiological and biochemical traits from rice plants. A) Spatial distribution of treatments by three principal component (PC) analysis, in which the circles represent a mean of four plants and ellipsis correspond to standard deviations, B) Global correlations among traits, and C) Main networks of major correlations. The color indicated an increase (red scale) or decrease (blue scale) of correlation between the observed morphological characteristics.

shows the presence of polysaccharides by hydroxyl stretching vibration in the 3444 band, and galactopyranose and sulfate groups around 932 band (Ferreira et al., 2023). Here, sulfated polysaccharides (characterized by the S=O bond) could also be observed through several peaks in the 1258 to 933 cm^{-1} region, as previously found in the ethanolic extract of *G. birdiae* (Maciel et al., 2008). Other functional groups found in the extract were also reported in algae groups, such as in the brown alga *Sargassum wightii* (Rajeswari and Jeyaprakash, 2019), in red

macroalga *G. tenuistipitata* (Sobuj et al., 2021) and *Mastocarpus stellatus* (Yang et al., 2021). Among the applications of these detected biomolecules, free radical scavenging properties, antiviral, antibacterial, and antifungal activities can be mentioned (Yang et al., 2021). Thus, these properties demonstrate high value for compound production and enable their use as an antioxidant and agricultural products to reduce damage caused by oxidative stress and to combat phytopathogenic agents.

4.3. Nutritional characteristics of aqueous extract

Algae can have up to 43 % carbohydrates, 25.29 % proteins, and 1.8 % lipids relative to dry-weight tissues (Rohani-Ghadikolaei et al., 2012; Yang et al., 2021). It was expected that after the lyophilization process, these biomolecules could be resuspended at the desired concentration. Further, it was possible to identify via HPLC and GC-MS many organic acids, carbohydrates, and amino acids, among them butyric acid, propionic acid, and acetic acid, which phosphoric acid, lactic acid, and citric acid stand out (Tables 3 and 2). The use of some organic acids, such as citric acid and lactic acid, favors plant growth and an increase in chlorophyll content, in addition to increasing the activity of antioxidant enzymes to improve productivity in rice plants (Kundu et al., 2020).

Seaweeds from the Brazilian Northeast coast are known for the high predominance of macro and microminerals in their tissues (Silva et al., 2024), although these minerals are not described in aqueous extract. Here, the water-based extract of *G. birdiae* also presented high nutritional content, being rich in macronutrients (nitrogen, potassium, magnesium, calcium, and phosphorus), and micronutrients (zinc, iron, boron, manganese, and copper) that are fundamental in plant metabolism (Table 3). Which highlights its potential for use in agriculture as a supplementary source of nutrients. Additionally, potassium and sodium were quantified at higher concentrations in the extract. It can assist osmotic regulation, and ionic homeostasis, directly influencing the efficiency of water use. Furthermore, treatments with exogenous applications of signaling molecules, such as those containing sodium, potassium, and calcium can minimize the effects of oxidative stress in rice plants (Tahjib-Ui-Arif et al., 2019). This property hypothesizes the use of extract as a priming agent since spraying sodium on rice plants can minimize its deleterious effects caused by salinity (Subramanyam et al., 2019), as well as potassium nitrate priming increases rice grain yield (Das et al., 2022), and calcium chloride priming enhances stress tolerance in rice seedlings (Wang et al., 2022).

4.4. Algal aqueous extract has potential for use in rice plants

Natural products like plant biofertilizers and biostimulants have been utilized to boost agricultural productivity and economic growth (Fertahi et al., 2023). Among these, seaweed biomass stands out as an eco-friendly raw material, rich in compounds that enhance nutrient uptake and utilization while also improving plants' tolerance to stress (Chojnacka et al., 2021). Here, an experiment was conducted to try spraying algae extract on rice plants.

The benefits of seaweed extracts for foliar feeding are improved root system, chlorophyll content, antioxidant system, and senescence delay that increases vegetative growth (Vasantharaja et al., 2019). The presence of various polysaccharides and sugars, as confirmed in this study (Table 4), may play a role in plant growth by participating in sensing and signaling processes (Rolland et al., 2002). In this study, we observed a positive trend in variables directly linked to rice growth attributes (Fig. 4). The increase in the number of tillers and carboxylation efficiency, for instance, may directly impact the number of panicles and, therefore, the number of grains produced per plant (Huang et al., 2020). Besides, there was also a tendency to increase photosynthetic parameters (*A*, pigments, and total biomass), influencing the fixation of atmospheric carbon. The application of *G. edulis* seaweed 10 % sap at recommended NPK levels demonstrates the ecological alternative way to increase potato plant height, was due to the presence of indole acetic acid, kinetin, zeatin, and several mineral nutrients (Garai et al., 2021). Our extract was rich in phosphoric and citric acids, supporting its potential as a biofertilizer (Table 2). The foliar application of phosphoric and citric acids promotes growth, increases dry biomass, enhances photosynthetic pigments (chlorophyll and carotenoids), and improves yield in cotton plants (Ibrahim and El-Waraky, 2023).

Overall, there is an impact on the metabolism of rice plants sprayed with algal extract that resulted in two divergent rice metabolic profiles, and agronomic variables had a positive correlation (Fig. 4). The composition of the extract exhibited antioxidant components that reduced electrolyte leakage and cellular damage in treated plants. It contributed to the divergence of the groups, minimizing the daily environmental changes in which electrolyte leakage has a negative correlation with agronomic variables. It is corroborated by the *in vitro* antioxidant effect of *G. birdie* (Souza et al., 2011). Therefore, the development and application of seaweed extracts as bio-input with fertilizing and stimulating properties have been gaining prominence (van der Weide et al., 2022). On this hand, *G. birdiae* aqueous extracted presented interesting properties to be a candidate for commercial uses. One of its main characteristics is the increasing productivity under stress conditions caused by biotic and abiotic factors (Raja and Vidya, 2023). However, different percentages, periodicity, and ways to apply the extract remain to be tested.

5. Conclusions

The water-based *Gracilaria birdiae* extraction process was low-cost with minimal losses, producing a residue that can be further utilized. The different storage methods demonstrated high thermal stability, and the lyophilization process effectively preserved it. The composition of the extract contains organic molecules that can stimulate plant growth, enhance productivity, and potentially improve stress responses. Additionally, macro and micronutrients such as nitrogen, phosphorus, and potassium helped supplement nutrition. Rice plants submitted to foliar application tended to increase morphological and biochemical characteristics related to productivity. Future studies are needed to define ideal concentrations and periodicity for optimized application.

CRediT authorship contribution statement

Isabelle Mary Costa Pereira: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Pedro Higor Rocha Mariano:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Luan Victor Maia:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Stelamaris de Oliveira Paula-Marinho:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Rosilene Oliveira Mesquita:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Enéas Gomes Filho:** Writing – review & editing, Writing – original draft, Supervision, Resources. **Márjory Lima Holanda Araújo:** Writing – review & editing, Writing – original draft, Supervision, Data curation, Conceptualization. **Humberto Henrique de Carvalho:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2025.145770.

Data availability

No data was used for the research described in the article.

References

Aguiar, A.L.L. de, Araújo, M.L.H., Benevides, N.M.B., Mattos, A.L.A., Araújo, I.M. da S., Silva, E.M.C. da, 2023. Sequential extraction process and physicochemical characterization of R-phycoerythrin and agar from red macroalgae Gracilaria birdiae. Algal Res. 69. https://doi.org/10.1016/j.algal.2022.102920.

Al-Mamary, M.A., Moussa, Z., 2021. Antioxidant activity: the presence and impact of Hydroxyl groups in small molecules of natural and synthetic origin. In: Antioxidants - Benefits, Sources, Mechanisms of Action. https://doi.org/10.5772/intechopen.95616.

Albalasmeh, A.A., Berhe, A.A., Ghezzehei, T.A., 2013. A new method for rapid determination of carbohydrate and total carbon concentrations using UV spectrophotometry. Carbohydr. Polym. 97, 253–261. https://doi.org/10.1016/j.carbpol.2013.04.072.

Albuquerque, J.C.S., Araújo, M.L.H., Rocha, M.V.P., de Souza, B.W.S., de Castro, G.M.C., Cordeiro, E.M.S., Silva, J. de S., Benevides, N.M.B., 2021. Acid hydrolysis conditions for the production of fine chemicals from Gracilaria birdiae alga biomass. Algal Res. 53, 102139. https://doi.org/10.1016/j.algal.2020.102139.

Alvares, C.A., Stape, J.L., Sentelhas, P.C., Gonçalves, J.L. de M., Sparovek, G., 2014. Köppen's climate classification map for Brazil. Meteorol. Zeitschrift 22, 711–728. https://doi.org/10.1127/0941-2948/2013/0507.

Badmus, U., Hefft, D.I., Taggart, M., Boyd, K., 2024. Nutritional composition of selected seaweeds. In: Applications of Seaweeds in Food and Nutrition. Elsevier, pp. 101–113. https://doi.org/10.1016/B978-0-323-91803-9.00001-9.

Benítez-García, I., Dueñas-Ledezma, A.K., Martínez-Montaño, E., Salazar-Leyva, J.A., Carrera, E., Osuna Ruiz, I., 2020. Identification and quantification of plant growth regulators and antioxidant compounds in aqueous extracts of Padina durvillaei and ulva lactuca. Agronomy 10, 1–13.

Borburema, H.D.S., Almeida-Faria, G., Silveira, S.L.C., Gomes, V. de S., Nauer, F., Marinho-Soriano, E., 2025. Agarophyte seaweeds exhibit differential responses to marine heatwaves depending on the species and climatic season. J. Appl. Phycol. https://doi.org/10.1007/s10811-025-03460-w.

Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities od protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 246–254.

Bremner, J.M., 1996. Nitrogen-total. Methods of soil analysis: Part 3 Chemical methods,, 5, pp. 1085–1121. https://doi.org/10.2136/sssabookser5.3.c37.

Castro, T.A.T. de, Tavares, O.C.H., Torchia, D.F. de O., Pereira, E.G., Rodrigues, N.F., Santos, L.A., Vendramini, A.L. do A., da Mata, M.R., Viêgas, C.V., Berbara, R.L.L., García, A.C., 2024. Regulation of growth and stress metabolism in rice plants through foliar and root application of seaweed extract from Kappaphycus alvarezii (Rhodophyta). J. Appl. Phycol. https://doi.org/10.1007/s10811-024-03216-y.

Chan, P.T., Matanjun, P., Yasir, S.M., Tan, T.S., 2015. Antioxidant activities and polyphenolics of various solvent extracts of red seaweed, Gracilaria changii. J. Appl. Phycol. 27, 2377–2386. https://doi.org/10.1007/s10811-014-0493-1.

Chojnacka, K., Michalak, I., Dmytryk, A., Gramza, M., Słowiński, A., Górecki, H., 2021. Algal extracts as plant growth biostimulants. Proc. Karelian Res. Cent. Russ. Acad. Sci. 36. https://doi.org/10.17076/eb1383.

Das, D., Basar, N.U., Ullah, H., Attia, A., Salin, K.R., Datta, A., 2022. Growth, yield and water productivity of rice as influenced by seed priming under alternate wetting and drying irrigation. Arch. Agron Soil Sci. 68, 1515–1529. https://doi.org/10.1080/03650340.2021.1912320.

De Wrachien, D., Schultz, B., Goli, M.B., 2021. Impacts of population growth and climate change on food production and irrigation and drainage needs: a world-wide view. Irrig. Drain. 70, 981–995. https://doi.org/10.1002/ird.2597.

Dionisio-Sese, M.L., Tobita, S., 1998. Antioxidant responses of rice seedlings to salinity stress. Plant Sci. 135, 1–9. https://doi.org/10.1016/S0168-9452(98)00025-9.

DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356. https://doi.org/10.1021/ac60111a017.

Elouardi, M., Mabrouki, J., El Belghiti, M.A., 2023. Evaluation of algal absorbed CO2 sequestration techniques: a review. Euro-Mediterranean J. Environ. Integr. 8, 481–491. https://doi.org/10.1007/s41207-023-00379-x.

Estevam, A.C.T., De Almeida, M.C., De Oliveira, T.A., Florentino, E.R., Alonso Buriti, F. C., Porto, A.L.F., 2017. Comparison of dairy desserts produced with a potentially probiotic mixed culture and dispersions obtained from: gracilaria birdiae and Gracilaria domingensis seaweeds used as thickening agents. Food Funct. 8, 3075–3082. https://doi.org/10.1039/c7fo00776k.

FAO, 2022. The State of World Fisheries and Aquaculture 2022. FAO. https://doi.org/10.4060/cc0461en.

Fatimi, A., 2022. Seaweed-based biofertilizers: a patent analysis. Recent pat. Biotechnol. 16, 144–154. https://doi.org/10.2174/1872208316666220128105056.

Ferreira, M. de S., Ribeiro, F. de O.S., Dourado, F.F., Oliveira, A.C. de J., Araújo, T.D.S., Brito, L.M., Pessoa, C., de Lima, L.R.M., de Paula, R.C.M., Silva-Filho, E.C., da Silva, D.A., 2023. Production of galactan phthalates derivatives extracted from Gracilaria birdie: characterization, cytotoxic and antioxidant profile. Int. J. Biol. Macromol. 243, 125254. https://doi.org/10.1016/j.ijbiomac.2023.125254.

Fertahi, S., Elalami, D., Tayibi, S., Taarji, N., Lyamlouli, K., Bargaz, A., Oukarroum, A., Zeroual, Y., El Bouhssini, M., Barakat, A., 2023. The current status and challenges of biomass biorefineries in Africa: a critical review and future perspectives for bioeconomy development. Sci. Total Environ. 870, 162001. https://doi.org/10.1016/j.scitotenv.2023.162001.

Fleurence, J., 1999. Seaweed proteins: biochemical, nutritional aspects and potential uses. Trends Food Sci. Technol. 10, 25–28. https://doi.org/10.1016/B978-0-08-100722-8.00010-3.

Gadelha, C.G., Coutinho, Í.A.C., Pinheiro, S.K. de P., Miguel, E. de C., Carvalho, H.H. de, Lopes, L. de S., Gomes-Filho, E., 2021. Sodium uptake and transport regulation, and photosynthetic efficiency maintenance as the basis of differential salt tolerance in rice cultivars. Environ. Exp. Bot. 192, 104654. https://doi.org/10.1016/j.envexpbot.2021.104654.

Garai, S., Brahmachari, K., Sarkar, S., Mondal, M., Banerjee, H., Nanda, M.K., Chakravarty, K., 2021. Impact of seaweed sap foliar application on growth, yield, and tuber quality of potato (Solanum tuberosum L.). J. Appl. Phycol. 33, 1893–1904. https://doi.org/10.1007/s10811-021-02386-3.

Glauco, F., He, P., Chen, Z., 2024. Combine effects of multiple environmental factors on growth and nutrient uptake of euryhaline seaweed growth in integrated multitrophic aquaculture systems. Algal Res. 77, 103347. https://doi.org/10.1016/j.algal.2023.103347.

Guaratini, T., Lopes, N.P., Marinho-Soriano, E., Colepicolo, P., Pinto, E., 2012. Antioxidant activity and chemical composition of the non polar fraction of Gracilaria domingensis (Kützing) Sonder ex Dickie and Gracilaria birdiae (Plastino & Oliveira). Rev. Bras. Farmacogn. 22, 724–729. https://doi.org/10.1590/S0102-695X2012005000063.

Hoagland, D.R., Arnon, D.I., 1950. The water-culture method for growing plants without soil. Calif. Agric. Exp. Stn. 347, 1–22.

Holanda, T. de B.L., Bessa, É.F., Lima, F.E. de S., Lopes, F.L. de S., Martins, J.R.P., Benevides, N.M.B., Araújo, M.L.H., 2025. Growth and iota-carrageenan from the red seaweed Solieria filiformis (Rhodophyta, Solieriaceae) cultivated in the Ceara state coast, Brazil. Aquac. Int. 33. https://doi.org/10.1007/s10499-025-01864-6.

Huang, M., Shan, S., Cao, J., Fang, S., Tian, A., Liu, Y., Cao, F., Yin, X., Zou, Y., 2020. Primary-tiller panicle number is critical to achieving high grain yields in machine-transplanted hybrid rice. Sci. Rep. 10, 2811. https://doi.org/10.1038/s41598-020-59751-4.

Hummel, J., Selbig, J., Walther, D., Kopka, J., 2007. The golm metabolome database: a database for GC-MS based metabolite profiling. Current 75–95. https://doi.org/10.1007/4735_2007_0229.

Ibrahim, A., El-Waraky, E., 2023. Effect of spraying phosphoric acid and ascorbic acid on cotton productivity under delayed sowing date. Egypt. J. Agron. 45. https://doi.org/10.21608/agro.2023.182398.1352, 0–0.

Khan, N., Sudhakar, K., Mamat, R., 2022. Thermogravimetric analysis of marine macroalgae waste biomass as bio-renewable fuel. J. Chem. 1–9. https://doi.org/10.1155/2022/6417326, 2022.

Köppen, W., Geiger, R., 1928. Klimate der erde. Gotha: Verlag Justus Perthes, pp. 91–102. Wall-map 150cmx200cm.

Kundu, A., Raha, P., Dubey, A.N., Rani, M., Paul, A., Patel, R., 2020. Differential responses of rice (Oryza sativa L.) to foliar fertilization of organic potassium salts. J. Plant Nutr. 44, 1330–1348. https://doi.org/10.1080/01904167.2020.1862193.

Lisec, J., Schauer, N., Kopka, J., Willmitzer, L., Fernie, A.R., 2006. Gas chromatography mass spectrometry-based metabolite profiling in plants. Nat. Protoc. 1, 387–396. https://doi.org/10.1038/nprot.2006.59.

Lutz, A., 1985. Normas analíticas do Instituto Adolfo Lutz. Métodos Químicos E Físicos Para Analise De Alimentos, second ed. São Paulo.

Maciel, J.S., Chaves, L.S., Souza, B.W.S., Teixeira, D.I.A., Freitas, A.L.P., Feitosa, J.P.A., de Paula, R.C.M., 2008. Structural characterization of cold extracted fraction of soluble sulfated polysaccharide from red seaweed Gracilaria birdiae. Carbohydr. Polym. 71, 559–565. https://doi.org/10.1016/j.carbpol.2007.06.026.

Marinho-Soriano, E., 2017. Historical context of commercial exploitation of seaweeds in Brazil. J. Appl. Phycol. 29, 665–671. https://doi.org/10.1007/s10811-016-0866-8.

Mohammed, H.O., O'Grady, M.N., O'Sullivan, M.G., Hamill, R.M., Kilcawley, K.N., Kerry, J.P., 2021. An assessment of selected nutritional, bioactive, thermal and technological properties of brown and red Irish seaweed species. Foods 10, 2784. https://doi.org/10.3390/foods10112784.

Nabti, E., Jha, B., Hartmann, A., 2017. Impact of seaweeds on agricultural crop production as biofertilizer. Int. J. Environ. Sci. Technol. 14, 1119–1134. https://doi.org/10.1007/s13762-016-1202-1.

Nascimento, R.R. do, Edvan, R.L., Nascimento, K., dos, S., Alves Barros, D.M., Rocha, A. M., Dias e Silva, T.P., Santos, E.M., Miranda, R. de S., Biagiotti, D., Araújo, M.J. de, 2023. Fermentation profile, nutritional quality and microbial populations of melon plant biomass silage ensiled with corn bran. Agronomy 13, 1–13. https://doi.org/10.3390/agronomy13041049.

Peixoto, M.E.O., Chow, F., 2025. Biomass production of the red macroalga Gracilariopsis tenuifrons (Gracilariales) using a low-cost commercial fertilizer and its additional benefits for carbon sequestration. Rev. Bras. Bot. 48. https://doi.org/10.1007/s40415-025-01070-x.

Pessarrodona, A., Franco-Santos, R.M., Wright, L.S., Vanderklift, M.A., Howard, J., Pidgeon, E., Wernberg, T., Filbee-Dexter, K., 2023. Carbon sequestration and climate change mitigation using macroalgae: a state of knowledge review. Biol. Rev. (Camb.). https://doi.org/10.1111/brv.12990.

Pisano, R., Arsiccio, A., Capozzi, L.C., Trout, B.L., 2019. Achieving continuous manufacturing in lyophilization: technologies and approaches. Eur. J. Pharm. Biopharm. 142, 265–279. https://doi.org/10.1016/j.ejpb.2019.06.027.

Premarathna, A.D., Tuvikene, R., Fernando, P.H.P., Adhikari, R., Perera, M.C.N., Ranahewa, T.H., Howlader, M.M., Wangchuk, P., Jayasooriya, A.P., Rajapakse, R.P. V.J., 2022. Comparative analysis of proximate compositions, mineral and functional chemical groups of 15 different seaweed species. Sci. Rep. 12, 19610. https://doi.org/10.1038/s41598-022-23609-8.

Rai, S., Shukla, N., 2020. Biofertilizer: an alternative of synthetic fertilizers. Plant Arch 20, 1374–1379.

Raja, B., Vidya, R., 2023. Application of seaweed extracts to mitigate biotic and abiotic stresses in plants. Physiol. Mol. Biol. Plants 29, 641–661. https://doi.org/10.1007/s12298-023-01313-9.

Rajeswari, R., Jeyaprakash, K., 2019. Bioactive potential analysis of brown seaweed Sargassum wightii using UV-VIS and FT-IR. J. Drug deliv. Ther. 9, 150–153. https://doi.org/10.22270/jddt.v9i1.2199.

Rajkumari, N., Chowrasia, S., Nishad, J., Ganie, S.A., Mondal, T.K., 2023. Metabolomics-mediated elucidation of rice responses to salt stress. Planta. https://doi.org/10.1007/s00425-023-04258-1.

Ray, D.K., Sloat, L.L., Garcia, A.S., Davis, K.F., Ali, T., Xie, W., 2022. Crop harvests for direct food use insufficient to meet the UN's food security goal. Nat. Food 3, 367–374. https://doi.org/10.1038/s43016-022-00504-z.

Ribeiro, E.E.V., Nobre, I.G.M., Silva, D.R.O., da Silva, W.M.X., Sousa, S.K.O., Holanda, T. B.L., Lima, C.G., de Lima, A.C.A., Araújo, M.L.H., da Silva, F.L.F., Matos, W.O., 2024. Profile of inorganic elements of seaweed from the Brazilian Northeast coast. Mar. Pollut. Bull. 202. https://doi.org/10.1016/j.marpolbul.2024.116413.

Rohani-Ghadikolaei, K., Abdulalian, E., Ng, W.-K., 2012. Evaluation of the proximate, fatty acid and mineral composition of representative green, brown and red seaweeds from the Persian Gulf of Iran as potential food and feed resources. J. Food Sci. Technol. 49, 774–780. https://doi.org/10.1007/s13197-010-0220-0.

Rolland, F., Moore, B., Sheen, J., 2002. Sugar sensing and signaling in plants. Plant Cell 14, S185–S205. https://doi.org/10.1105/tpc.010455.

Rosemary, T., Arulkumar, A., Paramasivam, S., Mondragon-Portocarrero, A., Miranda, J., 2019. Biochemical, micronutrient and physicochemical properties of the dried red seaweeds Gracilaria edulis and Gracilaria corticata. Molecules 24, 2225. https://doi.org/10.3390/molecules24122225.

Roslee, A.N., Munajat, N.F., 2017. Comparative study on the pyrolysis behaviour and kinetics of two macroalgae biomass (Gracilaria changii and Gelidium pusillum) by thermogravimetric analysis. IOP Conf. Ser. Mater. Sci. Eng. 257, 012037. https://doi.org/10.1088/1757-899X/257/1/012037.

Silva, R.O., William, M.X., Araújo, M.L.H., Francisco, L.F., Matos, W.O., 2024. Profile of inorganic elements of seaweed from the Brazilian Northeast coast 202. https://doi.org/10.1016/j.marpolbul.2024.116413.

Sobuj, M.K.A., Islam, M.A., Islam, M.S., Islam, M.M., Mahmud, Y., Rafiquzzaman, S.M., 2021. Effect of solvents on bioactive compounds and antioxidant activity of Padina tetrastromatica and Gracilaria tenuistipitata seaweeds collected from Bangladesh. Sci. Rep. 11, 1–13. https://doi.org/10.1038/s41598-021-98461-3.

Souza, B.W.S., Cerqueira, M.A., Martins, J.T., Quintas, M.A.C., Ferreira, A.C.S., Teixeira, J.A., Vicente, A.A., 2011. Antioxidant potential of two red seaweeds from the Brazilian coasts. J. Agric. Food Chem. 59, 5589–5594. https://doi.org/10.1021/jf200999n.

Subramanyam, K., Laing, G. Du, Van Damme, E.J.M., 2019. Sodium selenate treatment using a combination of seed priming and foliar spray alleviates salinity stress in rice. Front. Plant Sci. 10, 1–17. https://doi.org/10.3389/fpls.2019.00116.

Sumayya, S.S., Lubaina, A.S., Murugan, K., 2020. Phytochemical, HPLC and FTIR analysis of methanolic extract from Gracilaria dura (C agardh) J agardh. J. Drug Deliv. Ther. 10, 114–118. https://doi.org/10.22270/jddt.v10i3.3996.

Tahjib-Ui-Arif, M., Sohag, A., Afrin, S., Bashar, K., Afrin, T., Mahamud, A.G.M., Polash, M., Hossain, M., Sohel, M., Brestic, M., Murata, Y., 2019. Differential response of sugar beet to long-term mild to severe salinity in a soil–pot culture. Agriculture 9, 223. https://doi.org/10.3390/agriculture9100223.

Taiz, L., Zeiger, E., 2013. Fisiologia Vegetal. Porto Alegre Artmed.

Torres, M.D., Flórez-Fernández, N., Domínguez, H., 2019. Integral utilization of red seaweed for bioactive production. Mar. Drugs 17, 314. https://doi.org/10.3390/md17060314.

van der Weide, R.Y., Elissen, H.J.H., Hol, S.J.E., Evenhuis, A., de Vos, R.C.H., van der Meer, I.M., van Geel, W.C.A., 2022. Agricultural applications of seaweed extracts : seaweeds for plant care: review and experiments in the Netherlands. https://doi.org/10.18174/629760.

Vasantharaja, R., Abraham, L.S., Inbakandan, D., Thirugnanasambandam, R., Senthilvelan, T., Jabeen, S.K.A., Prakash, P., 2019. Influence of seaweed extracts on growth, phytochemical contents and antioxidant capacity of cowpea (Vigna unguiculata L. Walp). Biocatal. Agric. Biotechnol. 17, 589–594. https://doi.org/10.1016/j.bcab.2019.01.021.

Wahlström, N., Harrysson, H., Undeland, I., Edlund, U., 2018. A strategy for the sequential recovery of biomacromolecules from red Macroalgae Porphyra umbilicalis kützing. Ind. Eng. Chem. Res. 57, 42–53. https://doi.org/10.1021/acs.iecr.7b03768.

Wang, Y., Shen, C., Jiang, Q., Wang, Z., Gao, C., Wang, W., 2022. Seed priming with calcium chloride enhances stress tolerance in rice seedlings. Plant Sci. 323, 111381. https://doi.org/10.1016/j.plantsci.2022.111381.

Wellburn, A.R., 1994. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J. Plant Physiol. 144, 307–313. https://doi.org/10.1016/S0176-1617(11)81192-2.

Yang, Y., Zhang, M., Alalawy, A.I., Almutairi, F.M., Al-Duais, M.A., Wang, J., Salama, E.-S., 2021. Identification and characterization of marine seaweeds for biocompounds production. Environ. Technol. Innov. 24, 101848. https://doi.org/10.1016/j.eti.2021.101848.

Zafar, A., Ali, I., Rahayu, F., 2022. Marine seaweeds (biofertilizer) significance in sustainable agricultural activities: a review. IOP Conf. Ser. Earth Environ. Sci. 974. https://doi.org/10.1088/1755-1315/974/1/012080.

Zekry, E., Nora, A., Youssef, H., Saber, H., Rushdi, M.I., Abdel, I.A.M., Darwish, A.G., Ramadan, U., 2022. Halimeda opuntia and Padina pavonica extracts improve growth and metabolic activities in maize under soil - saline conditions 3189–3203. https://doi.org/10.1007/s10811-022-02844-6.