

Comprehensive Analysis of *Chlorella vulgaris* and *Arthrospira platensis*: Algae for Food Well-Being and Sustainable Agriculture

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ABSTRACT: This study investigates the nutritional and bioactive properties of *Chlorella vulgaris* (chlorella) and *Arthrospira platensis* (spirulina) to explore their potential applications in food sector and sustainable agriculture. The study involved comprehensive metabolite profiling using nuclear magnetic resonance (NMR) spectroscopy, analysis of volatile organic compounds via gas chromatography (GC), and carotenoid quantification through high-performance liquid chromatography (HPLC). Risk elements and mineral content were determined using inductively coupled plasma (ICP) analysis, while the fatty acid profile was assessed by gas chromatography–mass spectrometry (GC–MS). Antioxidant activity was evaluated using DPPH and ABTS assays. The antimicrobial potential was assessed using disc diffusion and minimum inhibitory concentration (MIC) methods, along with antibiofilm activity tested against plant phytopathogens. Results showed that *C. vulgaris* contains high levels of sucrose (18.47 mg/g), while *A. platensis* has elevated glutamate (7.73 mg/g) and lactate (3.65 mg/g). The volatile compound analysis of *C. vulgaris* and *A. platensis* showed that both algae are predominantly composed of alcohols, *C. vulgaris* 63.56% and *A. platensis* 60.81%. *A. platensis* exhibited superior antioxidant efficacy compared to *C. vulgaris*. Both algae showed the largest zones of inhibition against *Xanthomonas arboricola* and the filamentous fungus *Monilia fructigena*. In addition, both species showed strong antibiofilm activity against *P. megaterium*. The results indicate that both algae are rich in valuable bioactive compounds with potential applications in sustainable agriculture and food production. Extracts from *C. vulgaris* and *A. platensis* demonstrated promising antimicrobial and antioxidant properties, highlighting their potential as natural biocontrol agents and alternatives to synthetic pesticides and additives. Their incorporation into agricultural systems could help reduce environmental impact and support the development of more sustainable food systems.

KEYWORDS: spirulina, chlorella, metabolite profiling, volatile compounds, antimicrobial activity, antioxidant activity

1. INTRODUCTION

Algae have long been a part of the human diet, valued for their potential as functional foods.¹ Recently, scientific interest in algae as nutritious and health-promoting sources has surged, particularly in *Chlorella vulgaris* (chlorella) and *Arthrospira platensis* (spirulina).¹ These nutrient-dense algae are recognized for their high protein content, up to 62% in *C. vulgaris* and 70% in *A. platensis*, and for providing all essential amino acids^{2,3} and also for containing all essential amino acids.⁴ They provide a variety of vitamins and minerals, iron, and calcium, along with unsaturated fatty acids and vitamins such as A, B₂, B₆, B₈, B₁₂, E, and K.⁵ *A. platensis* is a photosynthetic cyanobacterium characterized by phycocyanin as a major pigment, while *C. vulgaris* is a green microalga containing chlorophyll *a* and *b* as primary pigments, along with various carotenoids.⁴ Due to their rich nutrient profiles, algae are increasingly recognized not only as valuable dietary supplements but also as functional foods and nutraceuticals with potential health benefits.^{6,7}

Numerous studies have confirmed the efficacy of algae as functional foods, highlighting their substantial nutritional potential.^{5,8–10} However, their benefits extend beyond nutrition; these microorganisms are rich in bioactive

compounds with diverse applications, particularly in agriculture.¹¹ Recent advancements in biotechnology and environmental sciences have revealed innovative ways to utilize algae in addressing agricultural challenges.¹² Their biological activities can enhance soil health, promote plant growth, and offer natural alternatives for managing phytopathogenic microorganisms.¹³ This multifaceted potential positions algae as a vital resource that bridges nutrition and sustainable agricultural practices.¹⁴ Algae can synthesize a variety of biologically active compounds that exhibit antibacterial, antiviral, and antifungal properties.¹⁵ The secondary metabolites in *A. platensis* and *C. vulgaris* offer a range of biological activities that could benefit agricultural practices.¹⁶ Additionally, their antioxidant capacity helps mitigate oxidative stress in plants, promoting overall health and productivity.¹⁷ Moreover,

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volatile components extracted from these algae have demonstrated antimicrobial properties, providing a natural alternative for controlling pathogenic microorganisms affecting plant health.¹⁸

The main objectives of this study are to characterize the nutritional and bioactive compounds present in *Chlorella vulgaris* and *A. platensis*, including carotenoids and volatile organic compounds, and to evaluate their antioxidant, antimicrobial, and antibiofilm activities. Furthermore, this research aims to assess the potential applications of these microalgae extracts as natural biocontrol agents to promote sustainable agriculture.

2. MATERIAL AND METHODS

2.1. Characterization of Algae Samples. For this study, we selected powdered extracts of *Chlorella vulgaris* (chlorella) and *A. platensis*, (spirulina) both at a purity of 100%, sourced from certified organic suppliers. These extracts are labeled with the SI-EKO-002 certification, indicating that they were cultivated outside the European Union. The powders were imported from China and produced under strict quality assurance measures, using filtered natural water. They were obtained in 2022 from Futunatura, a Slovenian company located in Kranj, which adheres to the regulations outlined in the EC Organic Regulation. For optimal preservation, the powders were stored in airtight containers in a cool, dry, and dark environment, maintaining a temperature close to 20 °C.

2.2. Characterization of Algae Extracts Using NMR Spectroscopy. All reagents used were of analytical grade. Potassium dihydrogen phosphate (KH₂PO₄, 99%), deuterium oxide (D₂O, 99.9%), methanol-d₄ (MeOD, >99.8%), and methanol were obtained from VWR (Radnor, PA, USA). Sodium deuteroxide solution (NaOD, 40% w/v in D₂O, 99.5%) was purchased from Alfa Aesar (Kandel, Germany), and 3-(Trimethylsilyl) propionic-2,2,3,3-*d*₄ acid sodium salt (TMSP, 99%) from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water was produced by a Millipore Direct-Q 3 UV system (Millipore Corp., Bedford, MA, USA) or acquired from Merck KGaA (Darmstadt, Germany). Algae samples (50 mg each) were finely milled and extracted using a 1:1 v/v mixture of MeOD and D₂O, following the protocol described by Mascellani et al.¹⁹ NMR spectra were recorded on a Bruker Avance III spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) operating at 500.18 MHz proton frequency, equipped with a BBFO SmartProbe with *z*-axis gradients. Measurements were performed at 298 K using the “noesygppr1d” pulse sequence to suppress water signals. Acquisition parameters included a 4 s collection time, 64 K data points, 16 ppm spectral width, 1 s recycle delay, 0.1 s mixing time, and 128 scans. Automated tuning and matching maintained consistent receiver gain. Free induction decays were referenced to TMSP at 0.0 ppm. Data processing involved exponential apodization (0.3 Hz), zero filling, phase and baseline correction using Mnova software v.14.1.0 (Mestrelab Research, Santiago de Compostela, Spain). The processed data were analyzed using Chenomx NMR Suite v.9.02 (Chenomx, Edmonton, Canada) for quantification, employing the Chenomx library and customized signatures to suit the study requirements.¹⁹ NMR analyzes were performed as single measurements per sample due to the robustness and reproducibility of the method; therefore, technical replicates and calculation of standard deviations were not conducted.

2.3. Quantitative Elemental Profiling Using ICP-OES. A thorough analysis of various elements - specifically Ag, Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sr, and Zn - was performed on different microalgae samples using optical emission spectrometry with inductively coupled plasma (ICP-OES).²⁰ The preparation of these samples involved a mineralization process conducted via a microwave digestion system (Ethos UP, Milestone Srl, Sorisole, BG, Italy). This method included 0.5 g of powder algae and a solution of 5 mL of HNO₃ (≥69.0%, Trace SELECT, Honeywell Fluka, Morris Plains, USA), 1 mL of H₂O₂ (≥30%, Sigma-

Aldrich, Saint-Louis, Missouri, USA), and 2 mL of ultrapure water (18.2 MΩ cm; 25 °C, Synergy UV, Merck Millipore, France). The samples were subjected to a heating temperature of 200 °C for 15 min, followed by a gradual cooling to 50 °C over the next 15 min. After the mineralization step, filtration was performed using filter paper no. 390 (Munktell & Filtrak GmbH, Bärenstein, Germany), and the samples were diluted to a final volume of 50 mL with ultrapure water. Elemental concentrations were quantified with an ICP-OES system (700 Series, Agilent Technologies, USA), which utilized an axial argon plasma and included an automated sampler (SPS-3, Agilent Technologies, USA). Calibration was conducted with a multielement standard solution created from individual ICP element standards (Sigma-Aldrich Production GmbH, Switzerland). The established detection limits (in μg/kg) for each analyzed element were: Ag 0.3; Al 0.2; As 1.5; Ba 0.03; Ca 0.01; Cd 0.05; Co 0.2; Cr 0.15; Cu 0.3; Fe 0.1; K 0.3; Li 0.06; Mg 0.01; Mn 0.03; Mo 0.5; Na 0.15; Ni 0.3; Pb 0.8; Sb 2.0; Se 2.0; Sr 0.01; Zn 0.2. The method's precision and accuracy were validated using a certified reference material (CRM-ERM CE278 K, Sigma-Aldrich Production GmbH, Switzerland).

2.4. High-Performance Liquid Chromatography of Carotenoids. The goal of this investigation was to analyze the amounts of selected carotenoids and chlorophylls in algae extracts prepared with ethanol and hexane, utilizing high-performance liquid chromatography with photodiode array detection (HPLC-PDA). Pigments were identified by comparing their retention times and UV–vis absorption spectra to those of authenticated reference standards, following HPLC standard protocols. The analysis was conducted on a Shimadzu Prominence HPLC system, which was precisely calibrated to ensure optimal conditions. The mobile phase included three different components: (A) tetrahydrofuran, (B) a solution of water mixed with acetic acid at a 100:1 ratio, and (C) methanol. The system operated with a flow rate of 1 mL/min, a 20 μL injection volume, and maintained a column temperature of 35 °C. The elution process followed a linear gradient, initially reducing the proportion of component B from 10% to 8%, while simultaneously increasing component C from 87% to 89% over the first 6 min. Following that, the gradient was further modified over the next 9 min, decreasing component B to 0% and raising component C to 90%, finishing with a 5 min isocratic phase at 90% C. Presence or absence detection of fucoxanthin, violaxanthin, astaxanthin, lutein, zeaxanthin, lycopene, α -carotene, chlorophyll *b*, and chlorophyll *a* (Sigma-Aldrich, Steinheim, Germany) was carried out at a wavelength of 450 nm, with UV/vis spectral data gathered from 200 to 800 nm.²¹ Ethanol and hexane extracts were prepared for HPLC analysis. 50 g of powdered algae was added to 500 mL of ethanol or hexane and incubated for 24 h in the dark at 25 °C in a shaking incubator (GFL 3031, Burgwedel, Germany). After filtration through Whatman grade 2 filter paper, the biomass was again extracted with 500 mL of ethanol or hexane under the same conditions. The extraction cycle was repeated twice to ensure complete recovery of pigments. All extracts were combined and concentrated using a vacuum rotary evaporator (Witeg Labortechnik, Germany). Ten mg of the dehydrated algae extract was dissolved in 1 mL of methanol and filtered using a 0.45 μm filter (Millipore, Billerica, MA, USA). The chromatographic conditions were optimized with standard reference compounds dissolved in methanol at 1000 μg/mL. A stock solution was created for quantification, followed by dilution to prepare working solutions ranging from 100 μg/mL to 0.625 μg/mL, which were used for calibration curves. All standard solutions were kept at 4 °C. The concentrations of standard compounds in the algae extracts were calculated by analyzing peak areas and utilizing linear regression equations derived from the calibration curves. The analysis results (mg/g of extract) were presented as mean values of three independent experiments ± SD.

2.5. Analysis of Volatile Compounds in Algae Extracts. Volatile compounds were investigated following the procedures set forth by Issa-Issa et al.^{22,23} A Shimadzu GC2030 Gas Chromatograph, coupled with a TQ8040 NX triple quadrupole mass spectrometer and an AOC-6000Plus autosampler (Shimadzu Scientific Instruments,

Columbia, MD, USA), was utilized for the analyzes. For headspace analysis preparation, about 0.5 g of algae sample was combined with 5 mL of water and 0.5 g of sodium chloride, which facilitated the release of volatile compounds into the headspace. The headspace solid-phase microextraction (HS-SPME) was conducted with a DVB/CAR/PDMS fiber (Supelco, Bellefonte, PA, USA). Samples were incubated at 40 °C with agitation at 500 rpm for 20 min to simulate the effects of chewing. The gas chromatography process commenced at a temperature of 40 °C, maintained for 2 min, and subsequently increased by 3 °C per min until reaching 250 °C. Helium served as the carrier gas, set at a pressure of 50.4 kPa and a linear flow rate of 36.3 cm/s. Temperatures for the injection, ion source, and interface were fixed at 260 °C, 200 °C, and 250 °C, respectively, with an overall helium flow rate of 1.01 mL/min. The identification of volatile compounds employed a multifaceted approach, including: 1) calculating retention indices using a C₇–C₁₆ n-alkane standard mixture (Sigma-Aldrich, Steinheim, Germany), 2) comparing these indices with established standard values, and 3) correlating mass spectra with data from the NIST 14 and Wiley 229 libraries. All analyzes were conducted in triplicate, and the results were reported as the percentage of each compound's area relative to the total peak area with standard error (SE).

2.6. Analysis of Fatty Acids Profile. The powdered macroalgae *C. vulgaris* (150 mg) and *A. platensis* (195 mg), were extracted overnight with 6 mL of dichloromethane/methanol (2:1 v/v). The extracts were filtered and dried by vacuum evaporation, to obtain, respectively, 21.0 and 23.0 mg. Then, 1 mL of each extract was dried with nitrogen and in the presence of methanol transmethylated with BF₃. The extraction of the obtained fatty acid methyl esters (FAMES) after evaporation of methanol was carried out with n-hexane. The analyzes were performed using a gas chromatograph coupled to a Clarus 500 mass spectrometer model PerkinElmer (Waltham, MA, USA), equipped with a FID (flame ionization detector). A Varian Factor Four VF-5 capillary column was housed in the GC oven.²⁴ Two μ L of the extract was injected into the column in splitless mode. The gas chromatographic conditions were as follows: the injector was set at 280 °C and the oven temperature program started from 170 °C and increased up to 260 °C with a rate of 3 °C/min and held constant for 15 min. The identification of the volatile compounds was performed first through the comparison of the mass spectra with those present in the Wiley 2.2 and Nist 11 mass spectral library database and then through the calculation of the linear retention indices (LRI) thanks to a series of alkane standards (C₈–C₂₄). The calculated LRIs were then compared with those reported in the literature. The areas of individual peaks of the FID signal were used to calculate the relative concentrations of the components compared to the total area. The analyzes were performed in triplicate.

2.7. Biological Properties and Activities of Algae Extracts.

2.7.1. Algae Extract Preparation for Analysis of Biological Activity. To produce water extracts from powdered algae for subsequent analysis, the process began by adding 50 g of powdered algae to a 1 L glass container, followed by the introduction of 500 mL of distilled water. This mixture was incubated in a dark environment at a temperature of 25 °C for 24 h using a shaking incubator (GFL 3031, Burgwedel, Germany). After the incubation period, the solution was filtered using Whatman grade 2 filter paper (Germany). The remaining algae powder underwent a second extraction by soaking it in an additional 500 mL of distilled water, which was also incubated for 24 h. This extraction process was repeated twice more to guarantee complete extraction. For concentrating the extracts, a vacuum rotary evaporator (Witeg Labortechnik, Germany) was employed. The evaporation was conducted in a water bath maintained at 50 °C and at a pressure of 42 mbar, achieving a boiling point of 30 °C in the flask (KIMBLE, DWK Life Sciences, Rockwood, TN, USA). The concentrated extracts were then transferred into sealable glass containers with the aid of a metal spoon and stored in a dark environment at approximately 4 °C until further analysis. On the analysis day, a 500 mg/mL extract solution was prepared by diluting the concentrated extract with ultrapure water.²¹

2.7.2. Antioxidant Capacity. The antioxidant properties of algae water extracts were evaluated using radical scavenging assays, specifically targeting 2,2-diphenyl-1-picrylhydrazyl (DPPH[•]) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS^{•+}) radicals, sourced from Sigma-Aldrich, Taufkirchen, Germany.²⁵ The extracts were prepared at a concentration of 500 mg/mL in ultrapure water. For the DPPH[•] assay, a methanol solution of 0.025 g/L DPPH[•] was made and adjusted to an absorbance of 0.8 at 515 nm using a Glomax spectrophotometer (Promega Inc., Madison, WI, USA). The ABTS^{•+} radical cation was generated following established protocols, and the solution was diluted to achieve an absorbance of 0.7 at 744 nm. In the assays, 190 μ L of either the DPPH[•] or ABTS^{•+} solution was mixed with 10 μ L of the algae extract in a 96-well microtiter plate. The mixture was incubated in the dark at room temperature while shaking at 1000 rpm for 30 min. After incubation, absorbance was measured at 744 nm for ABTS^{•+} and 515 nm for DPPH[•]. The percentage of inhibition for both radicals was calculated using the formula $(A_0 - A_A)/A_0 \times 100$, where A_0 is the absorbance of the control in methanol and A_A is the absorbance of the sample. A Trolox equivalent antioxidant capacity (TEAC) calibration curve was created using Trolox dissolved in methanol (Uvasol for spectroscopy, Merck, Darmstadt, Germany) at concentrations from 0 to 100 μ g/mL. This approach offers a comprehensive analysis of the antioxidant activity present in the algae extracts, highlighting their potential health benefits.

2.8. Antimicrobial Activity.
2.8.1. Bacteria. The antimicrobial efficacy was assessed using the following bacterial strains: *Pseudomonas syringae* CCM 2868, *Agrobacterium radiobacter* CCM 2926, *Xanthomonas arboricola* CCM 1441, *Priestia (Bacillus) megaterium* CCM 2007, and *Pectobacterium carotovorum* CCM 1008. These strains were acquired from the Czech Collection of Microorganisms in Brno, Czech Republic, and were maintained in a lyophilized state at –18 °C. For the evaluation, the lyophilized strains were rehydrated and subsequently cultured in Mueller–Hinton Broth (MHB, Oxoid, Basingstoke, UK) at a temperature of 37 °C for 24 h, except for *P. syringae*, which was incubated at 30 °C. Following incubation, the cultures were calibrated to the 0.5 McFarland standard using a densitometer, which corresponds to approximately 1.5×10^8 colony-forming units (CFU) per milliliter. These standardized bacterial cultures were then prepared for the assessment of antimicrobial activity.²⁶

2.8.2. Microscopic Fungi. The investigation of antimicrobial activity focused on a variety of fungal strains, including *Trichoderma harzianum* CCM F-470, *Botrytis cinerea* F-314, *Fusarium solani* CCM 8014, and *Monilia fructigena* CCM F-300. These strains were sourced from the Czech Collection of Microorganisms. For the assays, the fungi were transferred onto Potato Dextrose Agar (PDA, Oxoid, Basingstoke, UK) with the aid of a sterile bacterial loop, ensuring each strain was inoculated in triplicate. The inoculated plates were incubated at a constant temperature of 21 °C for 5 days. Following this incubation, the fungal cultures were adjusted to a 0.5 McFarland standard using a densitometer, which corresponds to roughly 1.5×10^8 colony-forming units (CFU) per milliliter. These prepared cultures were subsequently utilized to evaluate their antimicrobial activity.

The selected microbial strains are relevant phytopathogens commonly found in agriculture. They were chosen for their economic importance, resistance potential, and frequent occurrence in plant diseases. This diverse panel allows for a comprehensive evaluation of the extracts' potential as natural biocontrol agents in sustainable crop protection.

2.8.3. Disk Diffusion Method. Microbial strains, including both bacterial and fungal species, were cultivated, and their concentrations were adjusted as specified in Section 2.8.1 and 2.8.2. For bacterial evaluations, 100 μ L of the suspension was inoculated onto Mueller–Hinton Agar (MHA, Oxoid, Basingstoke, UK). In contrast, fungal strains were evenly spread on PDA plates with a sterile cotton swab, creating three parallel streaks across the agar surface. Sterile 6 mm discs (Oxoid, Basingstoke, UK) were subsequently placed on the inoculated plates, each receiving 10 μ L of the algae extract prepared at

a concentration of 500 mg/mL. The agar plates underwent incubation for 24 h at 37 °C for bacterial strains (30 °C for *P. syringae*), while fungal strains were incubated at 21 °C for 5 days. Positive controls consisted of two antibiotics, cefoxitin and gentamicin (Oxoid, Basingstoke, UK), which are effective against both Gram-positive and Gram-negative bacteria, along with Itraconazole (BioMaxima, Lublin, Poland) to evaluate antifungal activity against filamentous fungi. Negative controls comprised discs treated with ultrapure water, which served as the solvent for the extracts, as well as blank discs with no substances applied. The diameters of the inhibition zones surrounding each disc were measured in triplicate, and the mean values with standard deviations were calculated in millimeters, ensuring that all measurements were performed in triplicate.²⁷

2.8.4. Minimum Inhibitory Concentration. To assess the minimum inhibitory concentration (MIC) of algae-derived water extracts against selected bacterial strains, MHB served as the culture medium. Each well in a 96-well microtiter plate was filled with 150 μ L of bacterial suspension. The initial wells received 150 μ L of algae extract at a concentration of 500 mg/mL, followed by serial dilutions to create concentrations ranging from 250 mg/mL down to 0.122 mg/mL. Control wells contained only MHB and those with MHB plus the solvent (ultrapure water) used for the extract. A growth control was established with bacterial cultures without algae extract. After a 24 h incubation period at 37 °C for all bacteria (except 30 °C for *P. syringae*), the MIC was measured using a Glomax plate spectrophotometer (Promega Inc., Madison, WI, USA) at 570 nm.²⁸ The MIC values indicating 50% growth inhibition (MIC₅₀) were calculated from dose–response curves generated from the inhibition data, analyzed with Microsoft Excel (version 2.73). The concentration–response relationship was represented graphically as scatter plots, with logarithmic trendlines for clarity. MIC₅₀ values were derived from these trendline equations, indicating the concentrations that resulted in 50% growth inhibition. Results were presented as mean \pm standard deviation based on triplicate experiments.

2.9. Antibiofilm Activity. To evaluate the antibiofilm activity (%), 96-well microtiter plates were incubated for 48 h at 37 °C (or 30 °C for *P. syringae*), contributing to a total incubation period of 72 h. Afterward, each plate underwent three wash cycles with 200 μ L of ultrapure water to eliminate any loosely attached bacterial cells. The plates were left to air-dry for 20 min, then treated with 200 μ L of a 0.01% crystal violet solution for 20 min. Excess dye was gently washed away with ultrapure water until the rinsewater appeared clear, and the plates were allowed to dry in the air for a minimum of 1 h. Following this, 200 μ L of 96% ethanol was added to each well, and the optical density (OD) of the stained biofilm was measured at 570 nm using a Glomax spectrophotometer. The OD readings provided a quantitative assessment of bacterial adhesion, which was instrumental in determining the antibiofilm effectiveness of the microalgae extract. The percentage of biofilm inhibition was calculated using the designated formula

$$\% \text{ OD} = \frac{(\text{OD}_{\text{control}} - \text{OD}_{\text{sample}})}{\text{AD}_{\text{control}}} \times 100$$

In this formula, % OD indicates the percentage of biofilm inhibition, OD_{control} represents the optical density of the biofilm in control wells (bacteria combined with extract solvent), and OD_{sample} denotes the optical density of the biofilm in the treated wells. Results are expressed as mean \pm standard deviation from triplicate experiments.

2.10. Statistical Analysis. Differences in values for different algae species were evaluated using one-way analysis of variance (ANOVA). Tukey's test was used to determine which specific values showed significant differences. A significance level of $p < 0.05$ was used. Statistical analyzes were performed using Astatsa software and findings were presented as mean \pm standard deviation (SD) for each microalgae species.

3. RESULTS

3.1. Metabolite Profiling in Microalgae by NMR Spectroscopy.

Table 1 presents the metabolite profile of *C.*

Table 1. Metabolite Profiling of Algae (mg/g)

metabolite/algae	<i>C. vulgaris</i>	<i>A. platensis</i>
acetate	1.02	4.13
alanine	2.92	0.68
formate	0.31	0.58
glutamate	4.51	7.73
glutamine	0.22	0.07
histidine	0.01	0.05
isoleucine	0.13	0.17
lactate	2.16	3.65
leucine	0.48	0.06
lysine	0.41	0.06
methionine	0.07	0.12
phenylalanine	0.13	0.10
proline	0.69	0.56
succinate	0.89	0.51
sucrose	18.47	0.06
threonine	0.33	0.10
tryptophan	0.01	0.02
valine	0.21	0.24

vulgaris and *A. platensis*. *C. vulgaris* showed significantly higher concentrations of acetate and lactate compared to *A. platensis*. Glutamate was present in lower concentrations in *C. vulgaris* than in *A. platensis*, yet it remains one of the dominant metabolites in both species. On the other hand, sucrose was found in much higher concentrations in *C. vulgaris* than in *A. platensis* (18.47 mg/g vs 0.06 mg/g), indicating differences in carbohydrate metabolism between the two species. Among amino acids, the most significant difference was observed in alanine and proline concentrations, with *C. vulgaris* exhibiting higher levels of both compared to *A. platensis*. Glutamine was present in small amounts in *C. vulgaris*, whereas *A. platensis* had an even lower concentration of this metabolite. Leucine and lysine were detected in both species but in different concentrations, with *C. vulgaris* showing the highest levels of these amino acids.

Algae are recognized as valuable sources of nutraceuticals and bioactive compounds with potential applications in agriculture and health.¹¹ This study compared the metabolite profiles of commercially available *C. vulgaris* and *A. platensis* using NMR spectroscopy, revealing key biochemical differences relevant to their bioactivity and agricultural potential. A prominent distinction was the significantly higher acetate concentration in *A. platensis* (4.13 mg/g) compared to *C. vulgaris* (1.02 mg/g). Additionally, *A. platensis* exhibited elevated glutamate levels (7.73 mg/g). Acetate is a central metabolic intermediate involved in carbohydrate and amino acid catabolism as well as fatty acid biosynthesis.²⁹ Its known antibacterial properties³⁰ suggest that the higher acetate content in *A. platensis* may enhance its antimicrobial efficacy, potentially supporting plant health by inhibiting phytopathogens. The observed glutamate levels in *A. platensis* are also notable, as glutamic acid has been associated with improved plant growth and stress resilience. For example, studies have shown that exogenous application of glutamate enhances onion yield and aids in garlic preservation.³¹ This suggests that *A. platensis* extracts might better promote plant vigor compared to

C. vulgaris. These findings indicate that *A. platensis* may offer superior benefits for promoting plant vigor compared to *C. vulgaris*. Moreover, the variability in metabolite concentrations between the two species is in line with previous reports emphasizing that algae biochemical profiles are strongly influenced by species type and extraction protocols. It is also important to consider that the levels of metabolites and potentially toxic compounds in microalgae can be influenced by various factors. These include species-specific genetic traits, growth conditions such as light intensity, temperature, nutrient availability, and cultivation methods.^{32,33}

These results highlight the significance of species-specific metabolite profiling in optimizing algae-based applications in agriculture. The higher levels of acetate and glutamate in *A. platensis* suggest a greater potential for use in plant biostimulants or protective formulations. As emphasized by Pantami et al.³⁴ precise extraction methods—such as those based on NMR spectroscopy—are essential for accurately capturing this metabolic diversity. Our study reinforces the need for detailed metabolomic assessments when selecting algae strains for specific agronomic purposes, contributing to the development of more sustainable and effective agricultural solutions.

3.2. Risk Elements and Mineral Content in *C. vulgaris* and *A. platensis*. The analysis of mineral, trace, and risk elements in *C. vulgaris* and *A. platensis*, summarized in Table 2,

Table 2. Mineral, Trace Element and Risk Elements Content (mg/kg)^a

element/algae	<i>C. vulgaris</i>	<i>A. platensis</i>
Co	0.59 ± 0.07 ^a	0.13 ± 0.05 ^b
Ca	1611 ± 160 ^a	2044 ± 127 ^a
Na	882.43 ± 15.08 ^a	9075 ± 561 ^b
K	4780 ± 114 ^a	10,431 ± 399 ^b
Mg	365.40 ± 3.64 ^a	302.49 ± 8.79 ^a
Al	88.93 ± 0.53 ^a	107.52 ± 18.60 ^a
Ag	ND	ND
Ba	15.07 ± 0.30 ^a	6.23 ± 1.40 ^b
Cd	ND	ND
Cr	0.65 ± 0.06 ^a	0.47 ± 0.09 ^a
Cu	3.43 ± 0.45 ^a	0.62 ± 0.20 ^b
Fe	827.95 ± 8.97 ^a	414.22 ± 26.64 ^b
Li	0.04 ± 0.00 ^a	0.81 ± 0.03 ^b
Mn	42.89 ± 0.52 ^a	16.75 ± 1.10 ^b
Ni	1.38 ± 0.28 ^a	1.91 ± 0.34 ^a
Pb	ND	ND
Sr	8.54 ± 0.70 ^a	90.09 ± 4.67 ^b
Zn	19.44 ± 0.48 ^a	5.83 ± 0.46 ^b
Se	1.10 ± 0.71 ^a	0.87 ± 0.23 ^a
As	ND	ND
Sb	0.84 ± 0.41 ^a	0.81 ± 0.33 ^a

^aND—not detected; values marked with the same uppercase letter in rows do not differ significantly $p > 0.05$.

highlights their nutritional profiles. Both algae were rich in essential minerals. *C. vulgaris* contained calcium, while *A. platensis* had an even higher concentration. *A. platensis* also significantly surpassed *C. vulgaris* in sodium and potassium levels. In terms of trace elements, *C. vulgaris* had a notably higher iron concentration than *A. platensis*, as well as more copper and manganese. Conversely, *A. platensis* contained more lithium compared to *C. vulgaris*.

Regarding safety, both algae tested negative for harmful elements like silver, cadmium, and arsenic. Their chromium and nickel levels were similar, but strontium levels differed significantly, with *A. platensis* containing much more. *C. vulgaris* had higher zinc content than *A. platensis*, while selenium and antimony levels were comparable in both.

The mineral and trace element profiles of *Chlorella vulgaris* and *A. platensis* offer valuable insights into their nutritional potential and possible contributions to health. *C. vulgaris* was found to be particularly rich in iron (827.95 mg/kg) and zinc (19.44 mg/kg), while *A. platensis* showed notably high concentrations of sodium (9075 mg/kg) and potassium (10,431 mg/kg). For example, calcium content in almonds, a well-known nut source, is approximately 269 mg/100 g, which is considerably higher than in dairy products like milk (120–130 mg/100 g).³⁵ Both species exhibited favorable safety profiles, with undetectable levels of risk elements such as cadmium, lead, silver, and arsenic. Additionally, both algae contained trace elements like manganese and selenium. Iron and zinc are essential micronutrients required for plant growth, photosynthesis, enzyme activation, and maintaining overall plant health.³⁶ The higher concentrations of sodium and potassium in *A. platensis* align with previous findings by Tibbetts et al.,³⁷ which also highlighted its potential as a mineral-rich supplement. Importantly, the absence of toxic metals reinforces their suitability for consumption. Algae are known to bioaccumulate heavy metals such as mercury, cadmium, lead, copper, and thallium from their environment,³⁸ making regular monitoring critical to ensure safety. The detection of manganese and selenium in both species further supports their functional value. While *C. vulgaris* may be particularly beneficial as a source of iron and zinc, *A. platensis* could serve as an effective source of sodium and potassium. Moreover, the presence of antioxidant-supporting elements such as manganese and selenium underscores the potential health-promoting properties of both strains.³⁹

3.3. Determination of Carotenoids and Chlorophylls in Ethanol and Hexane Extracts. The quantification of specific carotenoids and chlorophylls in ethanol and hexane extracts of *C. vulgaris* and *A. platensis* is summarized in Table 3, which details the concentrations measured in the extracts. In the hexane extract of *C. vulgaris*, lutein levels were detected at 11.19 mg/g, while chlorophyll *a* and α -carotene were present

Table 3. Concentrations of Specific Carotenoids and Chlorophylls in Ethanol and Hexane Extracts

sample extract	standard compound	R _t (min)	^c concentration (mg/g of extract)
<i>C. vulgaris</i> ^a H	lutein	12.645	11.19 ± 0.11 ^c
	chlorophyll <i>a</i>	22.601	0.81 ± 0.01
	α -carotene	25.988	1.02 ± 0.02
<i>C. vulgaris</i> ^b E	lutein	12.619	0.10 ± 0.01
	chlorophyll <i>b</i>	18.477	0.65 ± 0.01
	chlorophyll <i>a</i>	21.738	0.93 ± 0.02
<i>A. platensis</i> E	α -carotene	25.988	1.02 ± 0.01
	lutein	12.817	9.09 ± 0.10
	chlorophyll <i>b</i>	19.945	18.89 ± 0.12
<i>A. platensis</i> H	α -carotene	26.208	2.61 ± 0.02
	lutein	12.585	19.57 ± 0.11
	α -carotene	26.359	41.64 ± 0.32

^aH—hexane extract. ^bE—ethanol extract. ^cMeans values ± SD of three determinations.

Table 4. Volatile Compounds Content of the Extract with Water (% ± Standard Error, SE)^a

family	compound	retention time (min)	<i>C. vulgaris</i>	<i>A. platensis</i>
esters	methyl octanoate	8.164	0.32 ± 0.01 ^a	0.09 ± 0.01 ^b
	octyl acetate	8.914	1.81 ± 0.04 ^a	1.94 ± 0.04 ^a
	ethyl octanoate	10.610	0.16 ± 0.01 ^a	0.21 ± 0.01 ^a
	∑		2.29 ± 0.03^a	2.24 ± 0.04^a
aldehydes	hexanal	2.450	1.00 ± 0.02 ^b	3.13 ± 0.05 ^a
	<i>E</i> -2-heptenal	4.232	0.17 ± 0.01 ^a	0.10 ± 0.01 ^a
	benzaldehyde	4.400	2.86 ± 0.07 ^b	3.81 ± 0.10 ^a
	nonanal	7.602	1.90 ± 0.03 ^a	1.83 ± 0.03 ^a
	∑		5.93 ± 0.05^b	8.87 ± 0.07^a
alcohols	<i>Z</i> -2-pentanol	2.240	1.83 ± 0.04 ^a	1.21 ± 0.02 ^b
	2,3-butanediol (isomer 1)	2.294	5.36 ± 0.10 ^a	0.59 ± 0.01 ^b
	2,3-butanediol (isomer 2)	2.350	4.76 ± 0.08 ^a	0.11 ± 0.01 ^b
	hexanol	2.994	0.33 ± 0.01 ^b	1.27 ± 0.03 ^a
	2-ethyl-1-hexanol	5.593	11.16 ± 0.26 ^a	5.60 ± 0.13 ^b
	perillyl alcohol	5.776	7.13 ± 0.14 ^a	3.08 ± 0.06 ^b
	<i>E</i> -2-octenol	6.551	0.19 ± 0.01 ^a	0.61 ± 0.01 ^b
	1-octen-3ol	4.588	2.03 ± 0.03 ^b	4.97 ± 0.08 ^a
	3,4-dimethylcyclohexanol	7.897	14.31 ± 0.01 ^a	37.02 ± 0.01 ^a
	1-dodecanol	21.563	14.63 ± 0.24 ^a	5.14 ± 0.09 ^b
	∑		61.74 ± 0.15^a	59.60 ± 0.10^a
	acids	butanoic acid	2.190	2.41 ± 0.06 ^a
isovaleric acid		2.578	2.66 ± 0.04 ^a	0.07 ± 0.01 ^b
2-methylbutanoic acid		2.661	2.00 ± 0.03 ^a	0.84 ± 0.02 ^b
hexanoic acid		2.928	0.53 ± 0.02 ^a	0.05 ± 0.01 ^b
heptanoic acid		4.288	0.80 ± 0.04 ^a	0.24 ± 0.01 ^b
∑			8.40 ± 0.05^a	1.54 ± 0.02^b
ketones	butyrolactone	3.527	0.37 ± 0.02 ^a	0.22 ± 0.02 ^a
	6-methyl-5-hepten-2-one	4.681	0.68 ± 0.02 ^b	1.93 ± 0.04 ^a
	3,5-octadien-2-one	6.629	1.38 ± 0.03 ^a	0.45 ± 0.01 ^b
	3,5-octadien-2-one	7.307	1.10 ± 0.03 ^a	0.64 ± 0.02 ^b
	∑		3.53 ± 0.02^a	3.24 ± 0.02^b
terpenes	1-terpineol	7.740	0.65 ± 0.01 ^b	1.88 ± 0.03 ^a
	∑		0.65 ± 0.01^b	1.88 ± 0.03^a
alkanes	3,5,5-trimethyl-2-hexene	4.493	2.14 ± 0.04 ^a	1.32 ± 0.02 ^b
	∑		2.14 ± 0.02^a	1.32 ± 0.02^b
ionones	α -ionone	19.430	0.77 ± 0.01 ^a	0.21 ± 0.01 ^b
	β -ionone	21.668	2.21 ± 0.05 ^b	5.65 ± 0.13 ^a
	∑		2.98 ± 0.04^b	5.86 ± 0.08^a
pyrazines	2,5-dimethylpyrazine	3.574	9.08 ± 0.18 ^b	11.51 ± 0.23 ^a
	trimethylpyrazine	5.079	2.03 ± 0.04 ^a	2.22 ± 0.03 ^a
	2,5-dimethyl-3-ethylpyrazine	6.851	0.39 ± 0.02 ^b	1.00 ± 0.07 ^a
	∑		11.50 ± 0.08^a	14.73 ± 0.15^a
others	dimethyl sulfoxide	2.719	0.84 ± 0.02 ^a	0.70 ± 0.01 ^a
	∑		0.84 ± 0.02^a	0.70 ± 0.01^a

^aValues marked with the same uppercase letter in rows do not differ significantly $p > 0.05$.

in lower concentrations. The ethanol extract of *C. vulgaris* showed lower concentrations of lutein, chlorophyll *b*, and chlorophyll *a*, while the concentration of α -carotene remained unchanged. For *A. platensis*, the ethanol extract revealed a lutein concentration of **9.09 mg/g**, along with a significant concentration of chlorophyll *b* at **18.89 mg/g** and α -carotene at **2.61 mg/g**. Meanwhile, the hexane extract of *A. platensis* exhibited a higher lutein concentration of **19.57 mg/g**, while the α -carotene concentration reached **41.64 mg/g**.

Carotenoids are fat-soluble pigments produced by both *C. vulgaris* and *A. platensis*, with notable levels of lutein and α -carotene.⁴⁰ In our study, *A. platensis* exhibited higher concentrations of both compounds in ethanol and hexane extracts. In contrast, *C. vulgaris* showed comparatively lower

levels of these carotenoids. The higher carotenoid content in *A. platensis* is consistent with previous findings highlighting its rich pigment profile. Lutein and α -carotene are particularly valued for their roles in protecting cells from oxidative stress. These pigments, together with chlorophylls, contribute to the antioxidant potential of algae.⁴¹ The elevated levels of lutein and α -carotene in *A. platensis* suggest a greater potential for antioxidant-related applications, including supplements targeting oxidative stress. These findings reinforce the importance of solvent choice in pigment extraction and highlight *A. platensis* as a superior source of bioactive pigments compared to *C. vulgaris*.

3.4. Volatile Compounds. The analysis of volatile compounds in *C. vulgaris* and *A. platensis* reveals distinct

chemical profiles (Table 4). *C. vulgaris*, identified as an alcohol-rich type, contained a substantial 63.56% of volatile compounds, with significant contributions from 1-dodecanol (14.63%), 3,4-dimethylcyclohexanol (14.31%), and 2-ethyl-1-hexanol (11.16%). Following alcohols, 11.50% of the volatile profile consisted of pyrazines, making it the second most abundant group in *C. vulgaris*. *A. platensis* also exhibited a considerable alcohol content (60.81%) but displayed a more diverse profile, characterized by significant aldehydes (8.87%) and pyrazines (14.73%).

Volatile organic compounds (VOCs) in microalgae cultures are influenced by multiple abiotic and biotic factors, including temperature, light, nutrient levels, pH, salinity, and oxidative stress.⁴² In this study, *C. vulgaris* and *A. platensis* exhibited elevated concentrations of total alcohols, with 3,4-dimethylcyclohexanol being notably abundant among the volatile constituents. Overall, both *C. vulgaris* and *A. platensis* can be classified as alcohol-rich types. However, they exhibit a more complex composition with a notable presence of aldehydes and pyrazines. The presence of a wide array of volatile compounds in commercial *A. platensis* products, including several newly identified substances, aligns with previous reports emphasizing chemical diversity and the influence of cultivation and extraction methods.⁴³ These findings support the continued exploration of microalgae VOCs for functional food, pharmaceutical, and agricultural uses, highlighting the importance of production and extraction methods on the chemical composition and bioactivity of algae products.

3.5. Fatty Acids Content. GC/MS analyzes allowed the detection of eight fatty acids listed in Table 5. The ratio

Table 5. FAs Content (Percentage Mean Value \pm Standard Deviation) of the Transesterified Extract, as Determined by GC–MS

no	component ^a	LRI ^b	LRI ^c	(%) ^d	(%) ^e
1	myristic acid C14:0	1740	1748	0.5 \pm 0.02	0.4 \pm 0.02
2	pentadecanoic acid C15:0	1845	1844	0.2 \pm 0.02	
3	palmitoleic acid, C16:1n7	1926	1930	17.3 \pm 0.15	5.1 \pm 0.07
4	palmitic acid, C16:0	1971	1973	22.3 \pm 0.50	62.3 \pm 3.15
5	linoleic acid, C18:2n6	2111	2107	18.8 \pm 0.12	10.5 \pm 0.12
6	oleic acid, C18:1n9	2153	2152	35.7 \pm 1.05	14.1 \pm 0.15
7	elaidic acid C18:1n9	2170	2175		7.0 \pm 0.09
8	stearic acid, C18:0	2181	2188	5.2 \pm 0.06	0.6 \pm 0.03
	SUM			100.0	100.0
	saturated FAs			28.2	63.3
	unsaturated FAs			71.8	36.7

^aThe components are reported according to their elution order on apolar column (VF-5 ms). ^bLinear Retention Indices measured on apolar column. ^cLinear Retention indices from literature. ^dPercentage mean values of *C. vulgaris*. ^ePercentage mean values of *A. platensis*; not detected.

between the saturated and unsaturated fractions was inverted between the two algae samples. In fact, in *C. vulgaris* the unsaturated fraction prevailed (71.8%) over the saturated one (28.2%) while in *A. platensis* the saturated portion reached the highest average percentage value (63.3%). In *C. vulgaris* oleic acid was the major fatty acid (35.7%) followed by palmitic acid (22.3%) and palmitoleic acid (17.3%). On the other side, in *A.*

platensis, palmitic acid was the most abundant (62.3%) followed by oleic acid (14.1%) and linoleic acid (10.5%). Elaidic acid (7.0%) was detected only in *A. platensis*, while a small amount of pentadecanoic acid (0.2%) was found only in *C. vulgaris*.

GC–MS analysis revealed that *Chlorella vulgaris* contains a high proportion of unsaturated fatty acids (71.8%), mainly oleic acid (35.7%) and linoleic acid (18.8%), whereas *A. platensis* is dominated by saturated fatty acids (63.3%), primarily palmitic acid (62.3%). The fatty acid profile of *C. vulgaris* clearly shows a dominance of unsaturated fatty acids, which aligns with previous studies highlighting the nutraceutical value of *Chlorella* species due to their high unsaturated fatty acid content.⁴⁴ In contrast, *A. platensis* exhibits a lipid profile rich in saturated fatty acids, consistent with earlier findings emphasizing its high palmitic acid content.⁴⁵ Oleic acid, the main fatty acid in *C. vulgaris*, has been identified as the key compound responsible for antioxidative activity in *C. tenuifolia* seed oil, demonstrated in *Caenorhabditis elegans*.⁴⁶ Additionally, oleic acid is known to reduce the risk of cardiovascular diseases by alleviating oxidative stress and inflammation.⁴⁷ Conversely, palmitic acid, dominant in *A. platensis*, shows antitumor effects⁴⁸ and possesses significant antioxidant and antibacterial properties.⁴⁹

3.6. Evaluation of the Antioxidant Activity of Water Extracts of Algae.

The antioxidant potential of water extracts from *C. vulgaris* and *A. platensis* was assessed using the DPPH assay, which measures the ability to scavenge free radicals (Table 6). The findings are expressed as IC₅₀ values and their

Table 6. Antioxidant Activity of Water Extract by DPPH and ABTS Assay^a

algae	IC ₅₀ (mg/mL)	TEAC equivalent
	DPPH assay	
<i>C. vulgaris</i>	11.60 \pm 1.27 ^b	0.000256 ^b
<i>A. platensis</i>	0.38 \pm 0.02 ^a	0.007812 ^a
	ABTS assay	
<i>C. vulgaris</i>	1.29 \pm 0.30 ^b	0.001925 ^b
<i>A. platensis</i>	0.33 \pm 0.04 ^a	0.007563 ^a

^aValues marked with the same uppercase letter in columns do not differ significantly $p > 0.05$.

corresponding Trolox Equivalent Antioxidant Capacity (TEAC). *C. vulgaris* showed an IC₅₀ of 11.60 mg/mL and a TEAC equivalent of 0.000256, whereas *A. platensis* had a significantly lower IC₅₀ value of 0.38 mg/mL, equating to a TEAC of 0.007812. In comparison to Trolox, which has an IC₅₀ of 2.97 μ g/mL, both algae extracts demonstrated reduced antioxidant activity. Furthermore, the statistical evaluation revealed that values with the same uppercase letter in the respective columns indicate no significant differences ($p > 0.05$).

The antioxidant capabilities of the water extracts from *C. vulgaris* and *A. platensis* were evaluated using the ABTS assay, which quantifies the ability of extracts to neutralize free radicals (Table 6). The results are presented as IC₅₀ values along with their Trolox Equivalent Antioxidant Capacity (TEAC). *C. vulgaris* demonstrated an IC₅₀ of 1.29 mg/mL, corresponding to a TEAC value of 0.001925. *A. platensis*, on the other hand, exhibited a more favorable IC₅₀ of 0.33 mg/mL, translating to a TEAC of 0.007563. Both algae extracts showed lower antioxidant activity compared to Trolox, which has an IC₅₀ of

2.48 $\mu\text{g/mL}$. Additionally, statistical analysis indicated that values sharing the same uppercase letter in the columns are not significantly different ($p > 0.05$).

The antioxidant capacity measured by DPPH and ABTS assays revealed that *A. platensis* exhibited significantly higher antioxidant activity compared to *C. vulgaris*. These findings are consistent with previous reports showing strong antioxidant properties of *A. platensis*. Taghavi Takyar et al.⁵⁰ also observed that the antioxidant activity of *A. platensis* extracts increases with higher concentrations, indicating a dose-dependent effect. Similarly, El-Baky et al.⁵¹ found that microalgae extracts from *C. ellipsoidea* and *Spirulina maxima* reduced reactive oxygen species in wheat plants, supporting the role of these algae as effective natural antioxidants. Other studies similarly confirm the presence of antioxidant compounds in these species and their ability to mitigate oxidative stress. The presence of key antioxidant metabolites and trace elements further supports their use as multifunctional bioactive agents. These results highlight the potential of these microalgae extracts as sustainable, natural alternatives to synthetic antioxidants, contributing to improved health and agricultural productivity.

3.7. Evaluation of Antimicrobial Activity. The antimicrobial properties of water extracts from *C. vulgaris* and *A. platensis* were assessed using the disc diffusion method at a concentration of 500 mg/mL. The results, measured in millimeters (mm), are summarized below in Table 7. For

Table 7. Disc Diffusion Method for Water Extracts of Algae (500 mg/mL) in mm^a

microorganism	<i>C. vulgaris</i>	<i>A. platensis</i>
Bacteria		
<i>Priestia megaterium</i>	2.56 \pm 1.13 ^a	4.44 \pm 1.51 ^b
<i>Pseudomonas syringae</i>	7.22 \pm 0.44 ^a	1.89 \pm 0.93 ^b
<i>Xanthomonas arboricola</i>	2.33 \pm 0.50 ^b	6.33 \pm 0.71 ^a
<i>Agrobacterium radiobacter</i>	5.67 \pm 0.50 ^a	5.00 \pm 0.71 ^a
<i>Pectobacterium carotovorum</i>	ND	5.00 \pm 0.87 ^a
Fungi		
<i>Fusarium solani</i>	ND	ND
<i>Monilia fructigena</i>	5.13 \pm 0.83 ^b	3.00 \pm 0.50 ^a
<i>Trichoderma harzianum</i>	ND	ND
<i>Botrytis cinerea</i>	2.01 \pm 0.31 ^a	ND

^aND—not detected; Values marked with the same uppercase letter in rows do not differ significantly $p > 0.05$.

bacterial strains, *P. megaterium* displayed a zone of inhibition of 2.56 mm for *C. vulgaris*, while *A. platensis* showed a greater inhibitory effect with a measurement of 4.44 mm. In the case of *P. syringae*, both extracts exhibited inhibition, but *C. vulgaris* was stronger with 7.22 mm and *A. platensis* showed inhibition zone with 1.89 mm. Regarding *X. arboricola*, both *C. vulgaris* and *A. platensis* showed inhibition zones (2.33 mm, 6.33 mm). *A. radiobacter* showed similar inhibition with *C. vulgaris* at 5.67 mm and *A. platensis* at 5.00 mm. *P. carotovorum* was not detected (ND) for *C. vulgaris*, while *A. platensis* recorded an inhibition zone of 5.00 mm.

In fungal assessments, *M. fructigena* and *B. cinerea* showed a stronger response to *C. vulgaris* compared to *A. platensis*, while other fungi such as *F. solani*, and *T. harzianum* were not detected (ND) for both algae extracts.

The Table 8 presents the minimum inhibitory concentration (MIC₅₀) values for the water extracts of *A. platensis*, *C. vulgaris* and *A. platensis*, measured in mg/mL, against several

Table 8. Minimum Inhibitory Concentration (MIC₅₀) for Water Extracts of Algae in Mg/mL^a

microorganism	<i>C. vulgaris</i>	<i>A. platensis</i>
<i>Priestia megaterium</i>	42.83 \pm 10.36 ^b	3.19 \pm 0.08 ^a
<i>Pseudomonas syringae</i>	24.59 \pm 3.76 ^b	8.73 \pm 1.09 ^a
<i>Xanthomonas arboricola</i>	18.31 \pm 11.74 ^a	17.33 \pm 8.47 ^b
<i>Agrobacterium radiobacter</i>	20.26 \pm 0.17 ^b	1.67 \pm 0.05 ^a
<i>Pectobacterium carotovorum</i>	26.34 \pm 18.57 ^b	2.19 \pm 0.31 ^a

^aValues marked with the same uppercase letter in rows do not differ significantly $p > 0.05$.

microorganisms. For *P. megaterium*, *C. vulgaris* showed an MIC₅₀ of 42.83 mg/mL, whereas *A. platensis* had a significantly lower MIC₅₀ of 3.19 mg/mL. In the case of *P. syringae*, the MIC₅₀ for *C. vulgaris* was 24.59 mg/mL, compared to 8.73 mg/mL for *A. platensis*. Regarding *X. arboricola*, *C. vulgaris* recorded an MIC₅₀ of 18.31 mg/mL, while *A. platensis* displayed a slightly higher MIC₅₀ of 17.33 mg/mL. For *A. radiobacter*, the MIC₅₀ was 20.26 mg/mL for *C. vulgaris*, but just 1.67 mg/mL for *A. platensis*. Lastly, for *P. carotovorum*, *C. vulgaris* had an MIC₅₀ of 26.34 mg/mL, while *A. platensis* presented a notably lower value of 2.19 mg/mL.

Figure 1 demonstrates the antibiofilm activity of water extract *C. vulgaris*. *C. vulgaris* demonstrated significant antibiofilm activity against all tested bacteria. The strongest inhibition was observed for *P. megaterium* (96.2% at 250 mg/mL), with high efficacy maintained even at lower concentrations (>94%). For the other bacteria (*P. syringae*, *P. carotovorum*, *X. arboricola*, *A. radiobacter*), inhibition was concentration-dependent, with the highest activity at intermediate concentrations (125 mg/mL), showing inhibition rates between 65% and 76%. At the lowest concentration tested (31.25 mg/mL), antibiofilm activity decreased notably, especially for *P. syringae* (40.2%) and *A. radiobacter* (43.4%).

The water extract of *A. platensis* showed strong antibiofilm activity against *P. megaterium* (Figure 2), with inhibition rates of 96.0% and 95.3% at concentrations of 250 and 125 mg/mL, respectively. However, antibiofilm effects against *P. syringae*, *P. carotovorum*, *X. arboricola*, and *A. radiobacter* were considerably lower and concentration-dependent. Inhibition decreased sharply with lower extract concentrations, reaching near zero or even negative values at 62.5 and 31.25 mg/mL for most bacteria, indicating no antibiofilm activity or potential biofilm promotion at these low doses.

Sustainable agriculture currently relies heavily on intensive tillage, excessive fertilization, irrigation and chemical pesticides, leading to serious health and environmental problems. However, algae represent an important source of biomass and produce large quantities of bioactive compounds. These properties of algae could be used as biocontrol agents in the fight against pathogenic fungi that cause serious plant diseases.⁵² The antimicrobial activity of *C. vulgaris* and *A. platensis* against various human and fish pathogens has been well-documented, but their potential use in controlling plant diseases has received less attention. However, Viana et al.⁵³ tested the effects of *C. vulgaris* applied through irrigation as a biocontrol agent against *Fusarium oxysporum* in spinach. Their study demonstrated that incorporating *C. vulgaris* into irrigation solutions can effectively inhibit this pathogen, suggesting a promising approach for managing plant diseases. The application of *C. vulgaris* and *A. platensis* in managing plant diseases through antibiofilm activity presents a promising

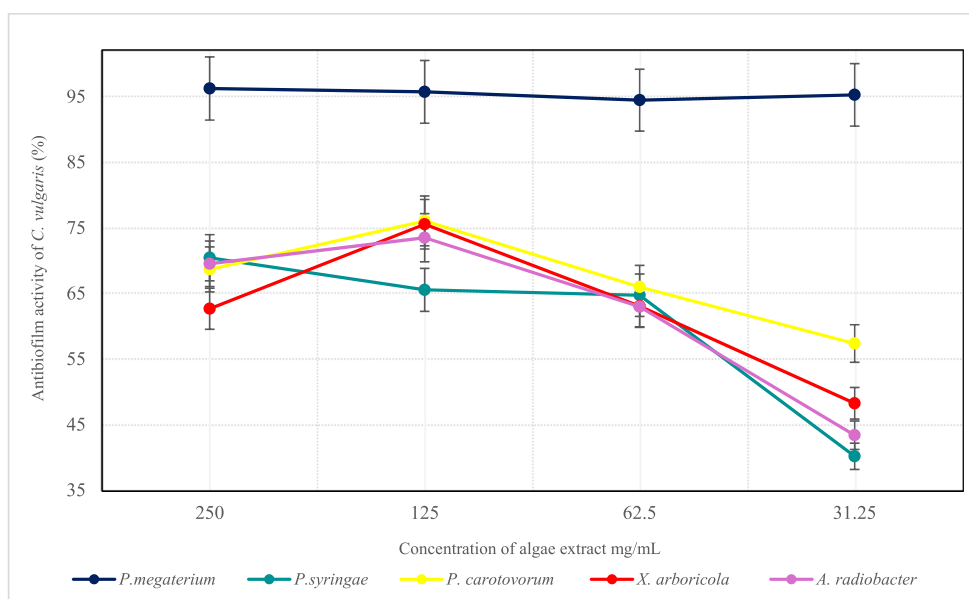


Figure 1. Antibiofilm activity of *C. vulgaris*.

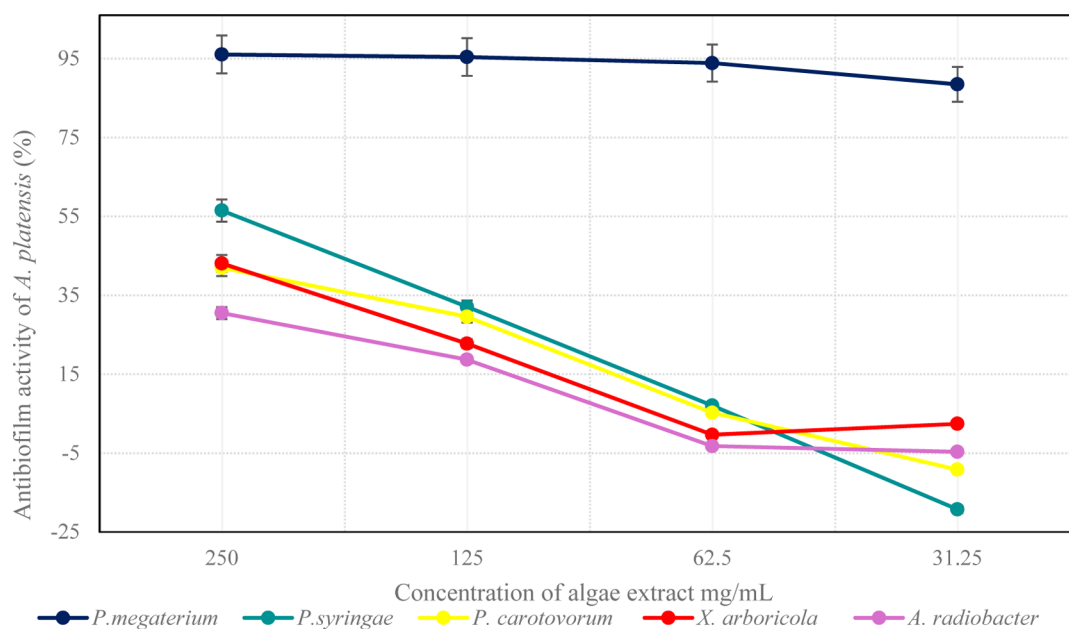


Figure 2. Antibiofilm activity of *A. platensis*.

area for further exploration.⁵⁴ Biofilms create a protective barrier for pathogens, increasing their resistance to treatments.⁵⁵ Supported by our findings, certain algae extracts have shown effective antibiofilm properties that could help in disrupting pathogen biofilms on plants.

In conclusion, the present study emphasizes the significant potential of *Chlorella vulgaris* (chlorella) and *A. platensis* (spirulina) in advancing sustainable agricultural practices. The comprehensive analysis of their nutritional and bioactive properties reveals that *C. vulgaris* is particularly rich in sucrose, which can serve as a valuable energy source for enhancing plant growth and development. In contrast, *A. platensis* is characterized by higher levels of glutamate, acetate and lactate, compounds that may contribute to improved nutrient assimilation and metabolic efficiency in plants. The volatile compound profiles indicate that both algae are primarily

composed of alcohols, suggesting that they possess diverse biochemical components that could positively influence plant health and resilience. A significant content of fatty acids suggests the high nutritional value of these algae. The superior antioxidant activity observed in *A. platensis* highlights its potential for protecting crops against oxidative stress, a common challenge in agricultural systems. The antimicrobial evaluation demonstrates that both algae exhibit strong inhibitory effects against major plant pathogens, including *X. arboricola* and the filamentous fungus *M. fructigena*. This suggests their utility in disease management, potentially reducing the reliance on synthetic pesticides and promoting healthier crop production. Furthermore, their remarkable antibiofilm activity could play a crucial role in mitigating biofilm-related challenges that compromise plant health and yield. Overall, this study positions *C. vulgaris* and *A. platensis* as

valuable bioresources for sustainable agriculture. Their rich nutritional profiles, antioxidant capabilities, and antimicrobial properties make them ideal candidates for developing eco-friendly agricultural inputs, such as natural fertilizers and biopesticides. Future research should focus on the practical applications of these algae in field trials and their integration into agricultural practices to enhance crop productivity, promote soil health, and contribute to environmentally sustainable farming systems. By leveraging the bioactive compounds found in these algae, agriculture can move toward more sustainable and resilient practices that benefit both crops and the environment.

■ ASSOCIATED CONTENT

Data Availability Statement

The data will be available upon request to the authors.

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsfoodscitech.5c00309>.

Representative chromatograms of ethanol and hexane extracts of *C. vulgaris* and *A. platensis*—Cv H-C. *vulgaris* hexane extract; Cv E-C. *vulgaris* ethanol extract; Ap E-A. *platensis* ethanol extract; Ap H-A. *platensis* hexane extract (Lut-lutein; Chl *a*-chlorophyll *a*; Chl *b*-chlorophyll *b*; α -Car- α -carotene) (PDF)

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