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Research Article

Phenolic Profile and Antioxidant Activity of Crude Extracts from Microalgae and Cyanobacteria Strains

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Aqueous and methanolic extracts of several microalgae (*Ankistrodesmus* sp., *Spirogyra* sp., *Euglena cantabrica*, and *Caespitella pascheri*) and cyanobacteria (*Nostoc* sp., *Nostoc commune*, *Nodularia spumigena*, *Leptolyngbya protospira*, *Phormidiochaete* sp., and *Arthrospira platensis*) were screened for their radical scavenging activity against the stable radical 1,1-diphenyl-2-picrylhydrazyl. Despite the fact that water was a more efficient solvent to extract greater amount of extractable substances, it seems that methanol was more efficient to extract a selected group of compounds with a higher antioxidant activity. In addition, the identification of 4 simple phenolics (gallic, syringic, protocatechuic, and chlorogenic acids) and the flavonoids (+) catechin and (−) epicatechin was carried out by using reverse phase high performance liquid chromatography. The strain *Euglena cantabrica* showed the highest concentration of phenolic compounds, particularly gallic and protocatechuic acids (5.87 and 2.97 mg per gram of dried biomass, resp.). Aqueous and methanolic extracts of microalgae *Euglena cantabrica* also exhibited the highest antioxidant activity, probably due to the presence of the high contents of phenolics.

1. Introduction

In living systems under stress conditions, the excessive generation of hydroxyl radical ($OH^{\bullet}$) and other highly reactive oxygen species (ROS) produces oxidative damage through the reaction of these species with many biomolecules including DNA [1]. Several studies on pharmacological research have evidenced that oxidative stress and increased amounts of free radicals are features of chronic diseases including cancer [2], aging, and neurodegenerative diseases such as Alzheimer's and Parkinson's [3, 4] and cardiovascular diseases such as atherosclerosis [5]. Phenolic compounds are secondary metabolites widely distributed in plants with well-known health benefits [6, 7]. These compounds are described as radical scavengers because they are donors of hydrogen atoms or electrons, producing stable radical intermediates. They can also inhibit iron-mediated oxyradical formation to prevent various processes of oxidative stress considering the origin of the above cited diseases [8]. Epidemiological studies have confirmed that consumption of diets rich in phenolic compounds may prevent the onset of many degenerative diseases [1, 9].

On the other hand, lipid oxidation is the greater cause of food quality deterioration [10]. Several reports have been focused on the enrichment of food products with seaweed extracts to evaluate their preservative properties and/or nutritional benefits [11, 12]. Extruded maize product enriched with red seaweed *Porphyra columbina* showed higher total phenolic content and antioxidant capacity than the extruded maize without seaweed [13]. Addition of edible seaweeds,

Sea Spaghetti *(Himanthalia elongata)*, Wakame *(Undaria pinnatifida)*, and Nori *(Porphyra umbilicalis)* to low-salt meat emulsion model systems, enriched the meat samples with soluble polyphenolic compounds thereby enhancing the antioxidant capacity of the systems [14]. Incorporation of four different seaweed extracts (cochayuyo, sea lettuce, ulte, and red luche) as part of the covering liquids of canned Atlantic salmon *(Salmo salar)* provided advantages in the preservation of the fish samples [15]. Rodríguez De Marco et al. [16] studied the effect of the incorporation of spirulina on nutritional quality of dried pasta concluding that these samples of pasta exhibited higher phenolic content and antioxidant capacity compared to control sample.

Despite the reported antioxidant properties of the phenolic compounds and the potential of microalgae and cyanobacteria as sources of these compounds [17], few studies have focused on their identification and quantification in microalgae [18–20] and on the role played by phenolics in microalgae defense mechanisms against high ROS levels [21–23]. Reports on the evaluation of antioxidant activity of microalgae and cyanobacteria extracts [24, 25] have also concluded that a high number of microalgal species produce a wide range of antioxidants, including carotenoids, polyunsaturated fatty acids, polysaccharides, or mycosporine-like amino acids (MAAs). These studies generally apply the Folin-Ciocalteu test to quantify the antioxidant capacity of samples from micro- or macroalgal cells without focusing on the identification of specific phenolic components, probably due to the assumed hypothesis that phenolic compounds are only terrestrial lignin-derivatives [21, 26, 27]. In fact, Waterman and Mole [28] defined polyphenolic secondary metabolites as compounds with a wide diversity of chemical structures, which are present in terrestrial plants and aquatic macrophytes (excluding microalgae).

However, pioneer papers show that microalgae and cyanobacteria also contain phenolic and cinnamic acid derivatives at μg levels on a dry weight basis [29, 30] and evidences for microalgae polyphenols synthesis are clear [21–23]. Therefore, it is necessary to improve accurate methodologies to detect and quantify phenolics in microalgae and cyanobacteria, which help to explain the role played by these compounds [22, 31]. The increased demand for healthy foods might then use a nontraditional alternative source of natural antioxidants and other ingredients, with potential benefits for consumers, based on microalgae and cyanobacteria [17, 32, 33].

The main objective of this work was to determine the antioxidant activity of extracts obtained from several microalgae and cyanobacteria strains with regard to their potential uses. Moreover, 6 phenolic compounds (gallic acid, (+) catechin, (−) epicatechin, syringic acid, protocatechuic acid, and chlorogenic acid) have been identified and quantified in the extracts by reverse phase high performance liquid chromatography (RP-HPLC). These compounds were selected because several reports have demonstrated that they are widely distributed in nature: algae [23, 34], common edible Mediterranean plants [35], and fruits and vegetables [36]. According to this, different strains of microalgae and cyanobacteria, most of them are bioprospected at the Canarian Archipelago and mainland Spain, identified, and deposited at the culture collection of the Spanish Bank of Algae (http://www.bea.marinebiotechnology.org), are being characterized to identify possible metabolites with interest for biotechnological applications.

2. Materials and Methods

2.1. Chemicals. Methanol (HPLC grade) and formic acid (analytical quality) were from Panreac (Barcelona, Spain). Water used through the entire study was purified on a Milli-Q system from Millipore (Bedford, MA, USA). The radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was from Sigma-Aldrich Chemie (Steinheim, Germany). Phenolic standards, gallic acid (GA), protocatechuic acid (PA), (−) epicatechin (E), chlorogenic acid (CA), syringic acid (SA), (+) catechin (C), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT), were purchased from Sigma-Aldrich Chemie (Steinheim).

The cartridges for solid phase extraction (SPE) were Chromabond Easy containing 500 mg of polar modified polystyrene divinylbenzene (particle size 93 μm) from Macherey-Nagel.

2.2. Algal Strains and Culture Conditions. Microalgae and cyanobacteria clonal strains were provided by the culture collection at the Spanish Bank of Algae (Table 1). Cultures were scaled up to 5-L flasks, under controlled conditions, at a light intensity of 100 μmol photons m^{-2} s^{-1} with a photoperiod of 16 : 8 (L : D), temperature at 23 ± 2°C, and continuous aeration supplied with CO_2 pulse addition at a rate of 1 min every hour. Biomass samples were harvested, concentrated, frozen at −80°C, and freeze-dried (6.5 L Labconco, USA) before extractions were carried out. For *Arthrospira platensis* (BEA 0016B) a second biomass sample was obtained, under outdoor conditions, in a modified culture medium enriched with urea (AA).

2.3. Preparation of Extracts for Antioxidant Activity Determination. Freeze-dried microalgae and cyanobacteria biomass (25 mg) were extracted with 2 mL of solvent (methanol and water were separately tested) for 40 min at room temperature by using a vortex stirrer (IKA/GENIUS 3). After centrifugation at 3000 rpm for 10 min (centrifuge HERAEUS Fresco 17), the supernatant was collected and evaporated to dryness in a rotary vacuum evaporator (Eppendorf, Concentrator Plus 5305). The amount of extractable substances was expressed as a percentage by weight of the freeze-dried biomass.

The extractions were performed in triplicate and concentrates were dissolved in methanol (10 mg ml^{-1}) to test the antioxidant capacity (in triplicate). Strains showing no antioxidant activity were also prepared at 40 mg mL^{-1} *(Nostoc commune*, *Arthrospira platensis*, and *Caespitella pascheri)*. In addition, *Euglena cantabrica* concentrate was also dissolved at 1 mg mL^{-1} and 5 mg mL^{-1}.

Methanol solutions of pure phenolic compounds were used as standards and prepared as follows: BHA and BHT at 1 mg mL^{-1} and 0.1 mg mL^{-1} and GA, C, E, SA, PA, and CA at 0.1 mg mL^{-1}.

Table 1: Microalgae and cyanobacteria strains assayed, indicating origin and culture medium for biomass production.

BEA code	Strain	Class	Culture medium	Geographical origin
BEA 0016B	*Arthrospira platensis*	Cyanophyceae	Zarrouk[a]	Chad, Lake Chad
BEA 0024B	*Nostoc commune*	Cyanophyceae	BG-11	Spain, Canary Islands, Gran Canaria. On a trunk of *Phoenix canariensis*
BEA 0661B	*Leptolyngbya protospira*	Cyanophyceae	BG-11	Spain, Canary Islands, Gran Canaria. Güigüí Ravine
BEA 0762B	*Phormidiochaete* sp.	Cyanophyceae	BG-11	Spain, Galicia, Ourense. Las Burgas
BEA 0854B	*Nodularia spumigena*	Cyanophyceae	BG-11	Spain, Vizcaya, Vitoria. Añana saltworks
BEA 1052B	*Nostoc* sp.	Cyanophyceae	BG-11	Spain, Canary Islands, Gran Canaria. Tirajana, Hondo ravine
BEA 0149B	*Caespitella pascheri*	Chlorophyceae	BBM[b]	Spain, Canary Islands, Gran Canaria. Guayadeque ravine
BEA 0536B	*Ankistrodesmus* sp.	Chlorophyceae	BG-11	Spain, Canary Islands, La Gomera. Las Rosas reservoir
BEA 0937B	*Euglena cantabrica*	Euglenophyceae	BBM[b]	Spain, Canary Islands, Gran Canaria. Charca de Maspalomas
BEA 0666B	*Spirogyra* sp.	Zygnematophyceae	BG-11	Spain, Canary Islands, Gran Canaria. Azuaje ravine.

[a]A second biomass sample of BEA 0016B (AA) *Arthrospira platensis* was obtained from a modified Zarrouk culture medium enriched with urea.
[b]BBM: Bold's basal medium including vitamins.

2.4. Free Radical Scavenging Activity on DPPH. The sample solution (20 μL of pure phenolic compound solutions or extracts prepared from algal samples) and 1 mL of DPPH (0.1 mM) were mixed. After 20 min incubation at 23°C in darkness, the inhibition of the DPPH was monitored against a methanol blank. The ability of the samples to scavenge DPPH radical was evaluated by measuring the decrease in absorbance (Abs) at 515 nm using a Shimadzu 1700 UV-Vis spectrophotometer. The decolorization percentage of DPPH was calculated by the following equation: RSA = 100 × [1 − (Abs of DPPH solution mixed with the sample)/(Abs of DPPH solution)] and half-life time ($t1/2$) was calculated as the time required for reducing initial concentration of DPPH by 50%. All the tests were performed in triplicate and the results were averaged.

2.5. Preparation of Extracts by Solid Phase Extraction (SPE) for the Phenolic Profile Analysis by RP-HPLC. Freeze-dried material (0.6 g) was mixed with Milli-Q water (60 mL) for 1 h at room temperature by using a multipoint magnetic stirrer (ANM-10006, Paris, France). The extract was filtered for removal of solid particles and after centrifugation at 3500 rpm for 10 min (ALC-4232), the supernatant was collected and hydrolysed at room temperature by addition of HCl (2 mol L^{-1}). After 60 min, hydrolysates were purified using solid phase extraction (SPE) according to a previously reported method with several modifications [22].

The SPE cartridge was equilibrated with 3 mL of methanol followed by 3 mL of Milli-Q water and samples were loaded into cartridges at a flow of 2.5 mL min^{-1}. The cartridge was rinsed with 2 mL of aqueous methanol solution (5%) and the analytes were recovered by washing the cartridge with 4 mL of methanol. After completion of eluent evaporation, methanol was added (300 μL) to the residue and the resulting solution was filtered using a 45 μm nylon syringe filter to be injected into the HPLC system. In these conditions, it was impossible to calculate concentrations for GA and PA that fall outside the range of the calibration curve in *Euglena cantabrica* extract. Therefore, 20 μL of the filtered solution obtained from this strain was also diluted with methanol making a total volume of 1 mL.

2.6. Determination of the Phenolic Profile by RP-HPLC. Chromatographic analysis was performed on a Liquid Chromatography Varian system, equipped with a ternary pump, an autosampler, and a diode array detector (DAD), connected to a computer installed Star software. The column (250 mm × 4.6 mm, 5 μm) and the guard column (10 mm × 4.6 mm, 5 μm) were reverse phase Pursuit XRs C18 (Varian, Barcelona). Two mobile phases were used: eluent A was water enriched with 0.1% formic acid and eluent B was methanol. The column operated at a flow rate of 1.0 mL min^{-1}, and 20 μL of each sample was separately injected. The applied elution conditions began with 15% B and increased up to 40% B in 13 min; it was then changed for 1 min to 40% B and a linear gradient from 40% to 30% B for 1 min. After that, it was returned to 40% B for 1 min and kept isocratic for 2 min. Finally, it was returned for 3 min to its initial condition in order to equilibrate pressures. Each standard phenolic compound was individually injected to check the retention times (RT). Monitoring was set at 270 nm (GA, PA, C, E, and SA) and 324 nm (CA) for quantification. Calibration curves were prepared in the range of concentrations between 1 and 100 μg mL^{-1} for determination of C, E, SA, and CA and between 1 and 800 μg mL^{-1} to quantify GA and PA. The linearity was estimated by linear regression analysis applying the least square method. Limits of detection (LOD) and limits of quantification (LOQ) were determined based on signal-to-noise ratio at 3 and 10, respectively.

Table 2: Extraction yields in methanol and water, expressed as a percentage by weight of the freeze-dried biomass.

	Methanol	Water
Cyanobacteria		
Nostoc commune	5.86	31.9
Nostoc sp.	7.03	34.0
Leptolyngbya protospira	8.30	26.4
Nodularia spumigena	20.3	63.2
Phormidiochaete sp.	6.43	14.4
Arthrospira platensis (AA)	21.8	57.2
Arthrospira platensis	28.1	54.6
Microalgae		
Euglena cantabrica	57.9	25.7
Caespitella pascheri	16.3	13.7
Spirogyra sp.	12.8	13.2
Ankistrodesmus sp.	18.3	30.2

3. Results and Discussion

3.1. Extraction Yields for Microalgae and Cyanobacteria. Yields for the assayed microalgae and cyanobacteria extractions were determined and presented in Table 2. The results obtained in the present study are consistent with previous reports, which have shown that the extraction yield is strongly dependent on the solvent polarity and extracts prepared with polar solvents gave the highest percentages of extractable substances [37, 38]. As can be observed in Table 2, water increased the efficiency of the extractions with two exceptions: extracts obtained from *Euglena cantabrica* and *Caespitella pascheri* showed the highest yield when methanol was used as solvent.

The highest yields were found in cyanobacteria strains *Nodularia spumigena* and *Arthrospira platensis* cultivated in the presence (AA) and absence of urea (63.2%, 57.2%, and 54.6%, resp.) and microalgae *Euglena cantabrica* (57.9%).

3.2. Free Radical Scavenging Activity on DPPH. From the results presented in Table 3, the cyanobacteria *Nostoc commune* and *Arthrospira platensis* did not inhibit DPPH radical, even at the highest tested concentration of 40 mg mL^{-1}, while eukaryotic *Caespitella pascheri* showed activity only when the extract was prepared at this higher concentration. The remaining cyanobacteria exhibited low values of capacity to scavenge free radical DPPH that ranged from 7.65% *(Leptolyngbya protospira)* to 27.89% (*Nostoc* sp.). Extracts from microalgae *Euglena cantabrica* displayed considerably stronger relative radical scavenging efficiencies than the other strains (100% inhibition when extracts were prepared with water and methanol at concentrations 10 mg mL^{-1} and 5 mg mL^{-1}) with a half-life ($t1/2$) lower than 2.1 s followed by methanolic extract of *Spirogyra* sp. (61.56%) with a $t1/2$ of 202 s.

Our results demonstrated that cyanobacteria strains (*Nostoc* sp., *Leptolyngbya protospira*, *Nodularia spumigena*, and *Phormidiochaete* sp.) and microalgae (*Euglena cantabrica*, *Caespitella pascheri*, *Spirogyra* sp., and *Ankistrodesmus* sp.) showed DPPH scavenging capacity when the samples were prepared at a higher concentration than the solutions of the standards BHA and BHT, the most widely used

Table 3: Relative radical scavenging activity (RSA) of samples expressed as % inhibition ± standard deviation of three measurements and half-life ($t1/2$) in seconds.

	Methanol RSA ($t1/2$)	Water RSA ($t1/2$)
Cyanobacteria		
Nostoc commune[a,b]	—	—
Nostoc sp.[b]	27.89 ± 0.01	—
Leptolyngbya protospira[b]	7.65 ± 0.01	—
Nodularia spumigena[b]	13.02 ± 0.02	—
Phormidiochaete sp.[b]	14.59 ± 0.01	9.14 ± 0.02
Arthrospira platensis (AA)[a,b]	—	—
Arthrospira platensis[a,b]	—	—
Microalgae		
Euglena cantabrica[b]	100 ± 0 (<2)	100 ± 0 (<2)
Euglena cantabrica[c]	100 ± 0 (2.1 ± 0)	100 ± 0 (2.1 ± 0)
Euglena cantabrica[d]	71 ± 0.4 (4 ± 0)	48.9 ± 0.2
Caespitella pascheri[a]	26.3 ± 0.2	—
Caespitella pascheri[b]	—	—
Spirogyra sp.[b]	61.56 ± 0.04 (202 ± 3)	43.37 ± 0.01
Ankistrodesmus sp.[b]	29.43 ± 0.00	8.3 ± 0.0
Synthetic preservatives		
BHA[d]	91 ± 2 (130 ± 9)	NE
BHT[d]	26 ± 2	NE

[a]Samples prepared at 40 mg mL^{-1}.
[b]Samples prepared at 10 mg mL^{-1}.
[c]Samples prepared at 5 mg mL^{-1}.
[d]Samples prepared at 1 mg mL^{-1}.
— means activity not detected.
NE: not evaluated.

Table 4: Methodological data for quantitative determination of phenolic standards analysed by RP-HPLC.

Phenolic compound	LOD[a] (μg mL^{-1})	LOQ[a] (μg mL^{-1})	Regression equation	Correlation coefficient (r)
GA	0.024261	0.080868	$y = 293189x - 420948$	0.9990
PA	0.014679	0.048930	$y = 234664x - 98186$	0.9995
C	0.113697	0.378993	$y = 49026x - 164952$	0.9997
CA	0.131857	0.439524	$y = 287357x - 26773$	0.9981
E	0.128127	0.427090	$y = 50853x - 377965$	0.9982
SA	0.027729	0.092429	$y = 288245x - 7411113$	0.9973

[a]Detection limits were calculated as signal-to-noise ratio of six determinations.

Table 5: Phenolic compounds detected in the samples in μg per gram of freeze-dried algal biomass.

	Phenolic compound					
	GA	PA	C	CA	E	SA
			Cyanobacteria			
Nostoc commune	71	—	—	2.16	—	—
			Microalgae			
Euglena cantabrica	5,872	2,970	71.4	78	7.1	—
Spirogyra sp.	91.4	—	—	—	—	—
Ankistrodesmus sp.	—	25.4	—	—	—	—

—: not detected.

food synthetic preservatives. At the same concentration of 1 mg mL^{-1}, *Euglena cantabrica* gave higher radical scavenging activity (71%) than BHT (26%) and lower activity and half-life (4 s) than BHA (91% with a half-life of 130 s). Our results align well with Rodríguez-García and Guil-Guerrero [33] who determined the antioxidant activity of the microalgal ethanolic extracts of *Porphyridium cruentum*, *Phaeodactylum tricornutum* and *Chlorella vulgaris* by means of the β-carotene-linoleate model system, where the activity of *C. vulgaris* extract was higher than those obtained for the other microalgal extracts tested and for the synthetics BHA and BHT.

The relative RSA of the pure standards (at concentration 0.1 mg mL^{-1}) was as follows: gallic acid: 94% ($t1/2$ 120 s); syringic acid: 38.1%; (+) catechin: 30.5%; (−) epicatechin: 31.8%; protocatechuic acid: 26.2%; chlorogenic acid: 26%; and BHA: 18.1% and BHT did not show activity (Figure 1).

Despite the fact that water was more efficient solvent to extract greater amount of extractable substances, it seems that methanol was more efficient to extract a selected group of compounds with a higher antioxidant activity (Table 3). These results corroborate well with earlier reports focused on the extraction of nine microalgae strains with different solvents (ethanol, aqueous ethanol, and water) to study the capacity of extracts to scavenge DPPH radical [39]. The authors concluded that the aqueous extract of all the tested strains exhibited lower scavenging activities than the alcoholic extracts. These same results were observed by Herrero et al. [19] optimizing the extraction of antioxidants from the chlorophyte *Dunaliella salina*. However, Rao et al. [25] reported that there was no major difference in the antioxidant activity of the green colonial microalga *Botryococcus braunii* extracted by different solvents (acetone, methanol, ethanol, chloroform/methanol (1 : 1 and 2 : 1, v/v), petroleum ether, hexane, and ethyl acetate).

3.3. Phenolic Profile by RP-HPLC. Phenolic compounds were identified by comparing retention times (RT) with those of standards and by extensive UV-Vis spectral analysis. An internal standard was used to avoid the matrix effect and to improve the precision of quantitative analysis. GA (RT: 6.15 min), PA (RT: 9.85 min), C (RT: 11.45 min), CA (RT: 12.97 min), E (RT: 14.97 min), and SA (RT: 15.75 min) were well resolved.

Limits of detection (between 0.01468 and 0.1319 μg mL^{-1}) and limits of quantification (between 0.04893 and 0.4395 μg mL^{-1}) were acceptable. All correlation coefficients for calibration curves gave values not less than 0.9973 (Table 4). Our methodology to determine the phenolic profile of microalgae and cyanobacteria offers adequate sensitivity.

Among cyanobacteria extracts, GA and CA were only identified in *Nostoc commune*. Phenolic constituents GA, C, E, SA, PA, and CA were not detected in *Nostoc* sp., *Leptolyngbya protospira, Nodularia spumigena, Phormidiochaete* sp., *Arthrospira platensis*, and the strain *Caespitella pascheri* (Table 5). Klejdus et al. [29] reported that phenolic compounds were more abundant in microalgae compared to cyanobacteria species. These authors related the lack of phenolic compounds in cyanobacteria with the different evolutionary states of microalgae and cyanobacteria, since microalgae are organisms more advanced with phenolic compounds involved in several stress adaptation mechanisms [22, 23, 29]. However, several reports have demonstrated that cyanobacteria produce a great variety of secondary bioactive metabolites [40, 41].

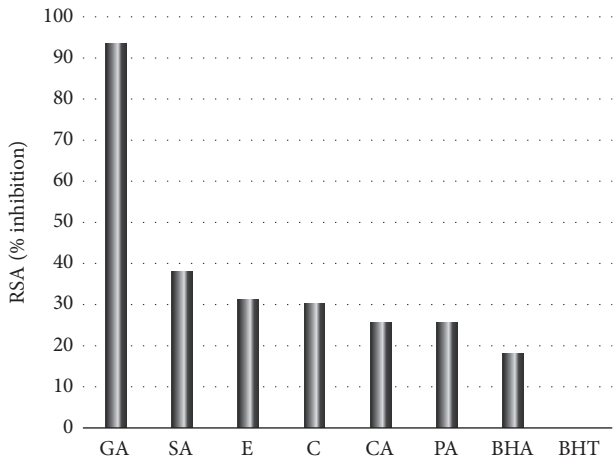

Figure 1: Relative radical scavenging activity (RSA) of pure phenolic compounds solutions at 0.1 mg mL^{-1}.

Among all tested microalgae, *Caespitella pascheri* did not show phenolic compounds. However, GA was identified in *Spirogyra* sp. and PA was detected in *Ankistrodesmus* sp. The extract derived from *Euglena cantabrica* exhibited the highest amount of GA and PA in comparison with the other strains in the present study (5,872 and 2,970 μg per gram of freeze-dried material, resp.) and also presented relevant quantities of compounds C, CA, and E (Table 5). The results here agree with recently published studies focused on the macroalgae *Himanthalia elongata* (Ochrophyta) which have shown relevant amounts of phenolic compounds increased to levels of mg per gram of freeze-dried algae [42], which seems to be common in many macroalgae species [43]. These contents of phenolic compounds are significantly higher compared to other previously published data [29, 30]. Onofrejová et al. [30] reported lower amounts of phenolic compounds extracted from in vitro culture of the microalgae *Spongiochloris spongiosa* (5.1 $\mu g\, g^{-1}$) and the cyanobacteria *Anabaena doliolum* (3.6 $\mu g\, g^{-1}$) and from food products including marine macroalgae *Undaria pinnatifida* (Wakame) and *Porphyra tenera* (Nori) (1.0 and 1.9 $\mu g\, g^{-1}$, resp.). Strains *Spirogyra* sp. and *Ankistrodesmus* sp. also showed higher contents of phenolic compounds (91.4 and 25.4 $\mu g\, g^{-1}$, resp.) compared to data for Nori and Wakame. Klejdus et al. [29] reported that *Spongiochloris spongiosa, Spirulina platensis*, *Anabaena doliolum*, *Nostoc* sp., and *Cylindrospermum* sp. contained phenolic compounds at μg levels per gram of biomass. These findings concord well with results in previous studies in our laboratory, where high contents of several phenolic compounds such as gentisic acid and (+) catechin were identified and quantified in the microalgae *Dunaliella tertiolecta* and *Phaeodactylum tricornutum* [22, 23].

As can be observed in Figure 1, GA is the most active compound in inhibiting DPPH radical, much more than the synthetic antioxidants BHA and BHT at the same concentration (0.1 mg mL^{-1}). Crude algae extracts consist of a wide variety of substances with active components at lower levels and with interfering constituents that decrease the antioxidant capacity [44, 45]. Therefore, antioxidant activities determined for some of the cyanobacteria strains and the eukaryotic *Caespitella pascheri* (Table 3) seem not to be related to their phenolic composition. However, the high content of GA and PA in *Euglena cantabrica* extracts may explain the radical scavenging activity observed when the extracts were prepared at the same concentration (1 mg mL^{-1}) as pure compounds BHA and BHT.

4. Conclusion

Results in this study confirmed that several cyanobacteria and microalgae were effective as scavengers of free radicals and this activity might be related to the phenolics compounds detected in some of the strains. Particularly, *Euglena cantabrica* displayed relevant quantities of phenolic compounds (gallic acid, protocatechuic acid, (+) catechin, chlorogenic acid, and (−) epicatechin) presenting a significantly high antioxidant capacity that could be considered interesting for different industrial applications. Further studies with a broader selection of microalgae and cyanobacteria and a variety of phenolic compounds are required to confirm new possibilities as those shown for the strains assayed here.

Additional Points

Practical Applications. Few investigations have focused on the quantification of phenolic compounds in microalgae and their potential application in food preservation to prolong the shelf-life by reducing oxidative deterioration and to improve the textural and sensory properties. On the other hand, diets rich in antioxidants have been long recommended to reduce the incidence of diseases caused by oxidative stress. The euglenoid *Euglena cantabrica* showed relevant amounts of gallic and protocatechuic acids at mg levels per g DW and a significant antioxidant activity probably due to the presence of the high contents of phenolics.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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