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Research Article

Oxidative Stress Index, Antioxidant Activity, and Phenolic Composition of Commercial *Spirulina* and *Undaria* sp. Extracts

Ticari Spirulina ve Undaria sp. Ekstraktlarının Oksidatif Stres İndeksi, Antioksidan Aktivitesi ve Fenolik Bileşimi

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Abstract

Aim: Microalgae (*Spirulina* sp.) and macroalgae (*Undaria* sp.) are widely marketed as functional foods due to their antioxidant potential. However, direct comparative data on their antioxidant capacity, phenolic composition, and oxidative stress indices remain limited. This study aimed to compare the antioxidant activities, total oxidant/antioxidant status, and phenolic profiles of commercial *Spirulina* and *Undaria* sp. extracts obtained with solvents of different polarity.

Material and Methods: Extracts were prepared using hexane, ethanol, and water. Antioxidant activities were assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), cupric reducing antioxidant capacity (CUPRAC), and metal chelation assays. Total phenolic (TPC), total flavonoid (TFC), and phenolic aldehyde contents were quantified spectrophotometrically. Phenolic identities and profiles were analyzed using LC–MS/MS in multiple reaction monitoring mode with authentic standards. Total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) were measured using commercial kits.

Results: *Spirulina* sp. ethanol and hexane extracts exhibited the strongest radical-scavenging and chelating activities, with lower IC_{50} values in DPPH and ABTS assays compared with *Undaria* sp. *Spirulina* sp. consistently showed higher TAS and lower OSI, indicating a more favorable oxidative balance. Phenolic profiling revealed vanillin, vanillic acid, and gentisic acid as dominant in *Spirulina* sp., whereas *Undaria* sp. was richer in high-molecular-weight phlorotannins but yielded lower TPC and TFC values. Solvent polarity strongly influenced outcomes: ethanol and hexane extracts provided higher phenolic content and stronger antioxidant activity than water.

Conclusion: *Spirulina* sp. extracts demonstrated superior antioxidant activity and phenolic diversity compared to *Undaria*, challenging the perception that brown algae always dominate in polyphenol content. By integrating classical assays with oxidative stress indices, this study highlights *Spirulina* sp.'s potential as a more effective source of functional antioxidants.

Keywords: *Spirulina*, *Undaria*, phenolic profile, antioxidant activity, oxidative stress index

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Öz

Amaç: Mikroalg (*Spirulina* sp.) ve makroalg (*Undaria* sp.), antioksidan potansiyelleri nedeniyle fonksiyonel gıda olarak yaygın şekilde pazarlanmaktadır. Ancak antioksidan kapasite, fenolik bileşim ve oksidatif stres indeksleri açısından doğrudan karşılaştırmalı veriler sınırlıdır. Bu çalışma, farklı polaritedeki çözücülerle elde edilen ticari *Spirulina* ve *Undaria* sp. ekstraktlarının antioksidan aktivitelerini, toplam oksidan/antioksidan durumlarını ve fenolik profillerini karşılaştırmayı amaçladı.

Gereç ve Yöntemler: Ekstraktlar hekzan, etanol ve su kullanılarak hazırlandı. Antioksidan aktiviteler 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), cupric reducing antioxidant capacity (CUPRAC) ve metal şelatlama testleriyle değerlendirildi. Toplam fenolik (TPC), toplam flavonoid (TFC) ve fenolik aldehit içerikleri spektrofotometrik olarak ölçüldü. Fenolik kimlikler ve profiller, gerçek standartlarla çoklu reaksiyon izleme modunda LC-MS/MS kullanılarak analiz edildi. Toplam antioksidan durum (TAS), toplam oksidan durum (TOS) ve oksidatif stres indeksi (OSI) ticari kitlerle belirlendi.

Bulgular: *Spirulina* sp.'nın etanol ve hekzan ekstraktları, *Undaria*'ya kıyasla daha güçlü radikal süpürücü ve şelatlama aktiviteleri gösterdi ve DPPH/ABTS testlerinde daha düşük IC_{50} değerlerine sahipti. *Spirulina* sp., tüm çözücü fraksiyonlarında daha yüksek TAS ve daha düşük OSI sergiledi. Fenolik profil analizleri *Spirulina* sp.'da vanilin, vanilik asit ve gentisik asit gibi küçük fenoliklerin baskın olduğunu gösterdi; *Undaria* sp. ise yüksek molekül ağırlıklı florotaninlere dayanıyordu. Çözücü polaritesi belirleyici oldu; etanol ve hekzan ekstraktları fenolik içerik ve antioksidan aktivite açısından suya üstünlük sağladı.

Sonuçlar: *Spirulina* sp. ekstraktları, fenolik çeşitliliği ve antioksidan kapasitesiyle *Undaria*'dan üstün bulunmuş, kahverengi alglerin her zaman polifenolce daha zengin olduğu yönündeki algıya meydan okumuştur. Klasik testlerin OSI ile birlikte kullanılması, *Spirulina* sp.'nın fonksiyonel gıda ve nutrasötik potansiyelini daha güçlü şekilde ortaya koymaktadır.

Anahtar Kelimeler: *Spirulina*, *Undaria*, fenolik profil, antioksidan aktivite, oksidatif stres indeksi

Introduction

Oxidative stress – an imbalance between reactive oxygen species (ROS) production and antioxidant defenses – is a crucial factor in aging and the pathogenesis of many chronic diseases (1). Excess ROS can oxidatively damage lipids, proteins, and DNA, triggering cellular dysfunction and contributing to conditions such as cancer, cardiovascular disease, neurodegenerative disorders, and diabetes (2). The human body's endogenous antioxidants often cannot fully neutralize ROS under pathological or environmental stress, and thus dietary antioxidants are thought to play an important preventive role (3). Regular consumption of antioxidant-rich foods (including algae) has been linked to reduced oxidative damage and better health outcomes, spurring interest in algae-derived supplements as strategies to mitigate oxidative stress-related diseases (4, 5).

Spirulina (Arthrospira spp.) and *Undaria* pinnatifida (commonly known as wakame) are widely recognized as nutrient-dense algae with significant applications in functional foods and dietary supplements due to their rich composition of proteins, vitamins, minerals, and diverse bioactive compounds (6-9). *Spirulina* sp. is notably high in protein (up to 70% dry weight) and contains essential micronutrients alongside antioxidant compounds such as phycocyanin, carotenoids, and enzymatic antioxidants (e.g., superoxide dismutase, catalase) (6, 7). Similarly, *Undaria* pinnatifida, a brown macroalga traditionally consumed in East Asia, is rich in fucoidan, fucoxanthin, and polyphenols, which have demonstrated antioxidant, anti-inflammatory, anticancer, and antidiabetic effects (8). These attributes support the growing scientific and commercial interest in both algae as potent natural ingredients for managing oxidative stress and promoting metabolic health. Despite the well-documented antioxidant properties of *Spirulina* sp. and the established bioactive potential of *Undaria* pinnatifida, there is a notable lack of comparative studies examining these microalgal and macroalgal products side by side using unified analytical frameworks. Most prior research has focused on either *Spirulina* sp. or macroalgae individually, often emphasizing specific bioactivities or single-method antioxidant evaluations (10).

To the best of our knowledge, no study so far has directly compared a commercial *Spirulina* sp. product with a commercial

Undaria sp. product in terms of both classical antioxidant assay outcomes and integrated oxidative stress index (OSI) – defined as the ratio of total oxidant status (TOS) to total antioxidant status (TAS) – based oxidative stress indices. This represents a critical knowledge gap, given the increasing consumer availability of algae-based health products and the need to validate their antioxidant efficacy on a common scale. The present study aims to perform a comprehensive comparative evaluation of the antioxidant capacity and phytochemical composition of commercial *Spirulina* sp. and *Undaria* sp. products.

Material and Methods

Sample preparation and extraction

The soxhlet extraction was carried out to obtain the extracts and both microalgae samples were extracted with different solvents according to their increasing polarity: hexane, ethanol and distile water for 6 h. A vacuum by an evaporator was used to get the hexane and ethanol extracts and a freeze-drier for the water extracts. All extracts were stored at +4 °C until analysis.

Phenolic compounds

The phenolic compounds in the extracts of micro and macroalgae were identified using a Shimadzu LC-20AD HPLC system (Kyoto, Japan). Separation of phenolic compounds was performed on an INERTSIL ODS-3V C18 column (5 µm, 4.6 × 250 mm i.d.) maintained at 30 °C. The mobile phase consisted of water with 0.05% glacial acetic acid (Phase A) and acetonitrile (Phase B). The gradient program was as follows: 8% B for 0–0.10 min; 8% to 10% B from 0.10 to 2 min; 10% to 30% B from 2 to 27 min; 30% to 56% B from 27 to 37 min; and 56% to 8% B from 37 to 45 min. The analysis was carried out at a flow rate of 1.0 mL/min, with an injection volume of 20 µL, and detection was conducted using a 20A UV-Vis detector at 280 nm (11). Each experiment was repeated three times. Calibration curves were generated by injecting known concentrations of standard compounds (catechin, caffeic acid, rutin, trans-p-coumaric acid, and trans-resveratrol) for the quantitative determination of phenolic compounds.

Total phenolic (TPC) and total flavonoid (TFC) contents

The Folin Ciocalteu method was applied for TPC of the *Spirulina* sp. and *Undaria* sp. extracts and the aluminum nitrate method was for TFC of the *Spirulina* sp. and *Undaria* sp. extracts (12, 13). Solutions containing 1 mg of sample were completed to 4.6 mL with ultrapure water and 100 µL FCR and 3 min were added to this mixture. Then 300 µL of 2% Na2CO3 solution was added. After the mixture was shaken for 2 hours at room temperature, the absorbance of the samples was read at 760 nm. The total phenolic amounts of the extract were determined with the equation obtained from the standard gallic acid chart: Absorbance=0.0123 [gallic acid (µg)] - 0.0155, (r2, 0.9931).

Solutions containing 1 mg of sample were made up to 4.8 mL with ethanol, and 100 µL of 1 M potassium acetate and 100 µL of 10% aluminum nitrate solution were added to this mixture. Examples 40 min. After waiting at room temperature, the absorbance was read at 415 nm. The total flavonoid amounts of the extracts were determined with the equation obtained from the standard quercetin graph: Absorbans=0.0156 [quercetin (µg)] - 0.0112 (r2, 0.9985).

Targeted LC–MS/MS phenolic profiling

Extracts were analyzed by LC–MS/MS in multiple reaction monitoring (MRM) mode to confirm phenolic identities and to screen a broader targeted panel. An authenticated phenolic standards mixture was injected to establish retention times and transitions, and extract identities were confirmed by co-matching retention times and transition ratios.

Sample preparation: 50 mg of each extract was dissolved in 1.0 mL acetonitrile–methanol–water (1:1:1, v/v/v) with vortexing, with insoluble residues dispersed in an ultrasonic bath if present. After adding 0.8 mL hexane for phase separation and centrifugation (7000 rpm, 5 min), the polar subphase was collected, diluted 1:4 with the initial mobile phase, and filtered through a 0.22–0.25 µm membrane prior to injection.

LC–MS/MS conditions: Analyses were performed on an Agilent 6460 Triple Quadrupole LC/MS system (ESI) using a Poroshell 120 EC-C18 column (50 × 4.6 mm, 2.7 µm) at 30 °C. Injection volume was 4.0 µL, flow rate 0.400 mL/min, and total method time 40 min. Mobile phases were A: water with 0.1% formic acid and 5 mM ammonium formate, and B: acetonitrile with 0.1% formic acid. The gradient was programmed as follows: 85%A/15%B (0.0 min) → 75/25 (5.0 min) → 25/75 (15.0 min) → 0/100 (16.0–20.0 min) → re-equilibration to 85/15 (22.0–40.0 min). Data were acquired in MRM (positive and negative ion modes), targeting a panel of phenolic acids (e.g., gallic, caffeic, p-coumaric, ferulic, vanillic, syringic, chlorogenic), flavonoids (e.g., catechin, quercetin, kaempferol, rutin, naringenin, luteolin), and stilbenes (resveratrol, polydatin).

TAS measurement

The total antioxidant status measurements of *Spirulina* sp. and *Undaria* sp. ethanolic/methanolic/hexan extracts were performed using the Total Antioxidant Status kit (Rel Assay

Diagnostics, Turkey) with a detailed technical method developed by Erel (14). In this method, the antioxidant activity of the samples was evaluated against the rapid free radical reactions initiated by hydroxyl radicals. The TAS value was calculated as mmol Trolox equivalent/L, with trolox used as teh calibrator.

TOS measurement

The total oxidant status measurements of *Spirulina* sp. and *Undaria* sp. ethanolic/methanolic/hexan extracts were determined using the Total Oxidant Status kit (Rel Assay Diagnostics, Turkey) according to the method developed by Erel (15). The spectrophotometrically measurable color intensity is related to the total oxidant molecule content in the sample and is directly proportional. The TOS value was calculated as μmol H2O2 equivalent/L, with hydrogen peroxide used as the calibrator.

Measurement of OSI

The oxidative stress index (OSI), expressed as the percentage ratio of TOS levels to TAS levels, was calculated to determine the oxidative balance status of the extracts. The mmol value of TAS levels was converted to μmol, as in the TOS test. OSI (random unit: AU) was calculated and expressed as a percentage using the following formula after determining the TOS and TAS results: OSI = (TOS / TAS) × 100.

Antioxidant activity

Antioxidant activities of the ethanolic/methanolic/hexan extracts of *Spirulina* sp. and *Undaria* sp. were tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) cation radical scavenging, cupric reducing antioxidant capacity (CUPRAC) activity and metal chelating activity assays (16). Ascorbic acid, BHT, BHA and EDTA were used as standards. The IC50 value (50% inhibition activity) was calculated using the graph plotted between the percentage of antioxidant activity (inhibition%) and the concentration (μg/mL) of the extracts. The A0.50 value (concentration having 0.50 absorbance) was calculated using the graph plotted between the absorbance and the concentration (μg/mL) of the extracts. Results were given as IC50 values and inhibition percentage (%) at 400 μg/mL concentration for radical scavenging assays; A0.50 values and absorbance at 400 μg/mL concentration CUPRAC assay.

Statistical analysis

The data analysis was conducted using SPSS 27.0 for Windows (Statistical Package for Social Sciences). Descriptive statistics were applied to continuous variables, with the mean ± standard deviation (SD) values reported. A significance threshold of 0.05 was used for interpreting the analysis results, and the findings were presented with a 95% confidence interval.

Results

In vitro antioxidant activities

Across extracts, free-radical scavenging (DPPH, ABTS), reducing power (CUPRAC), and metal-chelating activities varied by both species and solvent. At 400 μg/mL, DPPH inhibition was high in ethanol extracts of both algae (Spirulina sp.–SE: 77.16% with IC_{50} = 49.25 μg/mL; *Undaria*–UE: 81.14% with IC_{50} = 56.61 μg/mL), while DPPH activity was not observed for hexane and water extracts within the tested range (>400 μg/mL for IC_{50}). As expected, reference antioxidants (BHT/BHA) showed stronger DPPH performance (IC_{50} ≈ 23–24 μg/mL). ABTS scavenging was robust in nearly all extracts and exceeded 100% for some polar/less-polar matrices (e.g., *Spirulina* sp.-SW 109.45% and *Undaria*-UH 107.41% at 400 μg/mL), with IC_{50} values spanning ~25–44 μg/mL (lowest—thus strongest—generally in less-polar extracts; e.g., *Spirulina* sp.-SH 25.34 μg/mL). Standards BHT/BHA again outperformed extracts (IC_{50} ≈ 12.9–13.1 μg/mL). For CUPRAC (reducing capacity), *Undaria* sp. extracts exhibited higher absorbance than *Spirulina* sp. at the same concentration (e.g., UE 6.70; UH 7.08; UW 5.08 vs. SE 1.24; SH 2.46; SW 4.49 at 400 μg/mL). A_0.50 values for CUPRAC were lower (better) in *Spirulina* sp. ethanol (156.19 μg/mL) but generally higher (weaker) for the other *Spirulina* sp. solvents than for *Undaria*, reflecting matrix- and solvent-dependent differences in reducing constituents. Positive controls (BHT/BHA) yielded A_0.50 ≈ 27 μg/mL, as expected for strong reductants.

Metal chelation was moderate-to-high across extracts, peaking in *Spirulina* sp.-SW (67.96%), *Undaria*-UW (65.99%), and *Undaria*-UH (63.88%) at 400 μg/mL; corresponding IC_{50} values clustered around ~30–37 μg/mL. EDTA, as the chelation standard, showed the highest activity (90.21%; IC_{50} = 4.79 μg/mL). Ethanol extracts of both algae performed strongly in DPPH, whereas ABTS activity was broadly strong across solvents and could surpass 100% at the fixed test concentration, likely reflecting assay chemistry and the presence of both hydrophilic and lipophilic antioxidants. *Undaria* sp. tended to show higher CUPRAC absorbance (greater reducing power) than *Spirulina* sp. at 400 μg/mL, while metal chelation was most pronounced in water and hexane extracts, consistent with contributions from non-phenolic ligands (Table 1).

Table 1. In-vitro antioxidant activities of *Spirulina* sp. and *Undaria* sp. extracts determined by DPPH• and ABTS•$^+$ radical-scavenging, CUPRAC reducing power, and metal-chelation assays at 400 µg/mL, with IC_{50} (or A_0.50 for CUPRAC) values summarized.

Extracts	DPPH• test		ABTS•+ test		CUPRAC test		Metal chelation test	
	Inhibition (%)^a	IC_{50} (µg/mL)^b	Inhibition (%)^a	IC_{50} (µg/mL)^b	Absorbance^a	A_0.50 (µg/mL)^b	Inhibition (%)^a	IC_{50} (µg/mL)^b
Spirulina sp.								
Ethanol	77.16 ± 0.28	49.25 ± 0.54	85.61 ± 0.72	31.83 ± 0.57	1.24 ± 0.07	156.19 ± 0.25	57.78 ± 0.40	29.06 ± 0.33
Hexane	—	> 400	78.54 ± 0.23	25.34 ± 0.46	2.46 ± 0.18	160.24 ± 0.71	62.55 ± 0.92	33.77 ± 0.22
Water	—	> 400	109.45 ± 0.44	41.55 ± 0.62	4.49 ± 0.56	169.87 ± 0.14	67.96 ± 0.73	37.44 ± 0.85
Undaria sp.								
Ethanol	81.14 ± 0.63	56.61 ± 0.39	91.34 ± 0.97	37.51 ± 0.11	6.70 ± 0.17	173.06 ± 0.28	59.87 ± 0.67	30.66 ± 0.12
Hexane	—	> 400	107.41 ± 0.33	40.92 ± 0.77	7.08 ± 0.53	167.90 ± 0.71	63.88 ± 0.59	33.03 ± 0.54
Water	—	> 400	112.85 ± 0.71	44.27 ± 0.87	5.08 ± 0.16	172.66 ± 0.81	65.99 ± 0.84	35.01 ± 0.77
Standards								
BHT	86.23 ± 0.21	23.17 ± 0.28	85.46 ± 0.38	13.07 ± 0.49	2.61 ± 0.28	27.64 ± 0.06	—	—
BHA	87.37 ± 0.31	24.12 ± 0.42	88.05 ± 0.68	12.88 ± 0.31	2.96 ± 0.09	26.95 ± 0.02	—	—
EDTA	—	—	—	—	—	—	90.21 ± 0.28	4.79 ± 0.12

a: Inhibition percentage of the extract at a concentration of 400 µg/mL. b: IC50 values are given as the mean ± SD of three parallel measurements. c: Absorbance of the extract at a concentration of 400 µg/mL. d: A0.50 values are given as the mean ± SD of three parallel measurements.

Oxidative status

Spirulina sp. extracts exhibited higher TAS and lower OSI than *Undaria*, indicating a more favorable net antioxidant balance in *Spirulina* sp. under the tested conditions. Specifically, *Spirulina* sp.-SE showed TAS = 8.741 mmol/L and TOS = 7.188 µmol/L, yielding OSI = 0.082, while *Spirulina* sp.-SH and *Spirulina* sp.-SW displayed similarly low OSI values (0.067 and 0.086, respectively). In contrast, *Undaria* sp. extracts presented lower TAS (UE 5.610; UH 5.443; UW 6.377 mmol/L) and higher OSI (UE 0.120; UH 0.128; UW 0.115), driven by relatively higher TOS (≈ 6.77–7.34 µmol/L) and lower TAS compared with *Spirulina* sp.. Overall, the lowest OSI values were observed in *Spirulina* sp. hexane/ethanol/water (≈ 0.067–0.086), whereas *Undaria* sp. showed consistently higher OSI (≈ 0.115–0.128) (Table 2).

LC–MS/MS phenolic profiling

LC–MS/MS analysis in MRM mode identified multiple phenolic acids (vanillic, gentisic, syringic, salicylic, and p-coumaric acids; plus the phenylpropanoid acid trans-cinnamic acid), a flavonoid (naringenin), and phenolic aldehydes (vanillin and hydroxybenzaldehyde) across the extracts (Table 3).

The encoded heatmap (Figure 1A) highlights the consistent abundance of vanillin, vanillic acid, and gentisic acid across all samples. These three compounds were the most dominant phenolics detected, with vanillin showing the highest encoded abundance in every extract. Naringenin, representing the flavonoid class, was consistently detected but only at low abundance, indicating limited flavonoid content across all samples. Figure 1B provides a count of detected phenolics per extract, offering a broader view of phenolic diversity. The ethanol and hexane extracts of *Spirulina* sp. and the hexane and water extracts of *Undaria* sp. yielded the highest number of detected compounds. In contrast, the water extract of *Spirulina* sp. exhibited the lowest diversity, with only six compounds identified. Figures 1A and 1B emphasize the influence of solvent polarity on phenolic profiling. While certain key phenolics were consistently present, overall diversity and detectability were markedly higher in organic solvent-based extractions (ethanol and hexane), underscoring their superior efficiency compared with water.

The TPC, TFC, and phenolic aldehyde contents of *Spirulina* sp. and *Undaria* sp. extracts were also quantified (Figure 2). Among all samples, the hexane extract of *Spirulina* sp. exhibited the highest TPC (11.6 µg/mg extract), followed by the ethanol extract of *Spirulina* sp. and the water extract of *Undaria* sp. In contrast, the water extract of *Spirulina* sp. showed the lowest TPC (~6.3 µg/mg), suggesting lower extraction efficiency of polar solvents for phenolics. TFC values were generally low, with the ethanol and hexane extracts of Spirulina sp. displaying the highest flavonoid content (~2.4–2.5 µg/mg). Notably, the ethanol extract of *Undaria* sp. did not yield detectable levels of flavonoids. Phenolic aldehydes were most abundant in the hexane extract of *Spirulina* sp. (~111 µg/mg), followed by ethanol extracts of both species. The *Spirulina* water extract again showed the lowest aldehyde levels (~27 µg/mg), further indicating limited efficiency in extracting bioactive phenolics. These findings are visually summarized in the heatmap (Figure 2, bottom right), clearly distinguishing extract groups based on their phenolic composition. Overall, *Spirulina* sp., particularly its ethanol and hexane extracts, showed a richer phenolic profile than *Undaria*. Solvent polarity thus played a critical role in determining both the quantity and diversity of

Table 2. TAS, TOS, and OSI values of *Spirulina* sp. and *Undaria* sp. by ethanol, hexane, and water extracts.

Extract	TAS (mmol/L)	TOS (µmol/L)	OSI
Spirulina sp.			
Ethanol	8.741 ± 0.287	7.188 ± 0.325	0.082 ± 0.003
Hexane	7.903 ± 0.322	5.334 ± 0.547	0.067 ± 0.007
Water	7.996 ± 0.194	6.885 ± 0.436	0.086 ± 0.003
Undaria sp.			
Ethanol	5.610 ± 0.285	6.774 ± 0.711	0.120 ± 0.015
Hexane	5.443 ± 0.339	6.983 ± 0.158	0.128 ± 0.003
Water	6.377 ± 0.408	7.341 ± 0.284	0.115 ± 0.014

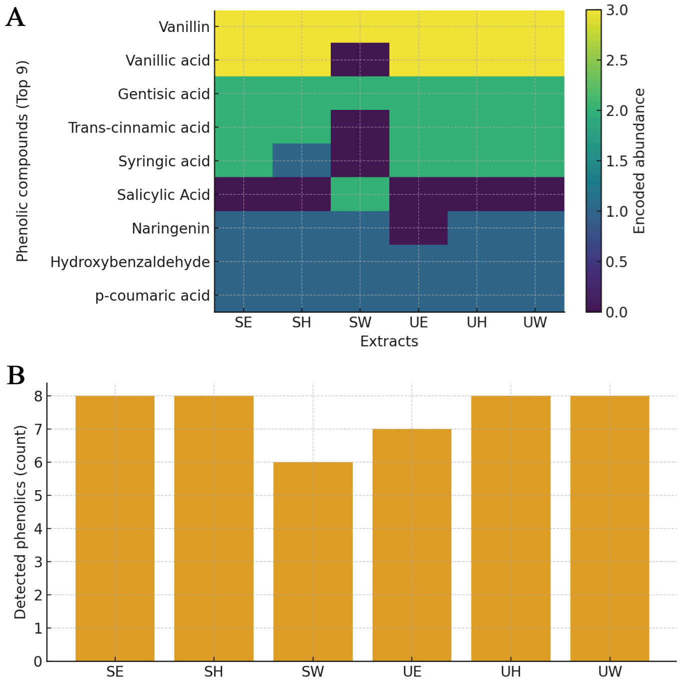
Values are mean ± SD; n = 6; technical replicates = 3. Abbreviations: TAS, total antioxidant status; TOS, total oxidant status; OSI, oxidative stress index.

Table 3. Phenolic profiling of commercial *Spirulina* sp. and *Undaria* sp. extracts.

Phenolic profiling	RT (min)	UE	SE	UH	SH	SW	UW
Gallic acid	2.32	0	0	0	0	0	0
Chlorogenic acid	2.50	0	0	0	0	0	0
Catechin	2.77	0	0	0	0	0	0
Epigallocatechin	3.03	0	0	0	0	0	0
Gentisic acid	2.87	18.2	17.4	18.2	18.6	20.7	19.3
Caffeic acid	3.26	0	0	0	0	0	0
Syringic acid	3.43	3.3	3.6	3.5	3.1	0	3.6
Vanillic acid	3.47	26.8	31.9	28.7	34.3	0	28.8
Rutin	3.88	0	0	0	0	0	0
Isoquercitrin	4.43	0	0	0	0	0	0
Polydatin	4.42	0	0	0	0	0	0
Hydroxybenzaldehyde	4.49	1.4	1.6	1.3	1.7	0.6	1.3
P-coumaric acid	4.25	0.7	1.5	1.2	1.8	1.2	1.1
Sinapic acid	5.26	0	0	0	0	0	0
Vanillin	4.81	189.6	211.6	171.7	221.0	52.2	169.5
Trans-ferulic acid	5.76	0	0	0	0	0	0
Taxifolin	5.87	0	0	0	0	0	0
Salicylic acid	7.62	0	0	0	0	9.8	0
O-coumaric acid	7.82	0	0	0	0	0	0
Baicalin	7.97	0	0	0	0	0	0
Protocatehuic ethyl ester	8.46	0	0	0	0	0	0
Protocatechuic acid	8.42	0	0	0	0	0	0
Kaempferol	10.13	0	0	0	0	0	0
Trans-cinnamic acid	10.55	16.1	18.8	15.3	23.8	0	15.7
Naringenin	11.25	0	2.4	1.2	2.4	0.8	1.3
Morin	12.03	0	0	0	0	0	0
Quercetin	11.97	0	0	0	0	0	0
7-hydroxyflavone	11.97	0	0	0	0	0	0
Chrysin	13.59	0	0	0	0	0	0
Luteolin	14.10	0	0	0	0	0	0
Biochanin a	13.79	0	0	0	0	0	0
5-hydroxyflavone	15.72	0	0	0	0	0	0
Diosgenin	20.99	0	0	0	0	0	0

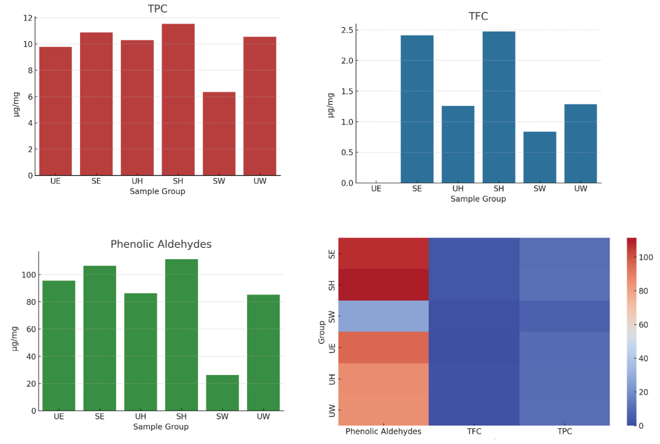
Spirulina sp. Ethanol (SE), hexane (SH), and water (SW) extracts. *Undaria* sp. ethanol (UE), hexane (UH), and water (UW) extracts.

extracted phenolic compounds.



SE, *Spirulina sp.* ethanol extract; SH, hexane extract; SW, water extract.
UE, *Undaria sp.* ethanol extract; UH, hexane extract; UW, water extract.

Figure 1. Phenolic profiling of commercial *Spirulina* sp. and *Undaria* sp. extracts. A) Encoded abundance heatmap for the Top-9 phenolics across ethanol, hexane, and water extracts (0 = not detected, 1 = +, 2 = ++, 3 = +++). B) Number of detected phenolics per extract out of the 33-compound targeted panel.



SE, *Spirulina sp.* ethanol extract; SH, hexane extract; SW, water extract.
UE, *Undaria sp.* ethanol extract; UH, hexane extract; UW, water extract.

Figure 2. Quantitative analysis of total phenolic, flavonoid, and phenolic aldehyde contents in *Spirulina* sp. and *Undaria* sp. extracts, with heatmap clustering revealing solvent-dependent variation.

Discussion

The present study compared the antioxidant capacity, phenolic composition, and oxidative stress indices of *Spirulina* sp. and *Undaria* sp. extracts, revealing several noteworthy patterns. First, *Spirulina* sp. extracts – particularly those obtained with ethanol and hexane – demonstrated strong in vitro antioxidant activity, with lower IC_{50} values in DPPH and ABTS assays compared with *Undaria*. *Undaria* sp. extracts were also active, and while *Spirulina* sp. ethanol extract performed better in CUPRAC based on $A_0.50$ values, *Undaria* sp. exhibited higher absorbance at the fixed concentration (400 µg/mL), indicating stronger reducing power under those conditions. For metal chelation, both algae showed moderate-to-high activity with similar IC_{50} values, suggesting broadly comparable chelating capacity. Second, *Spirulina* sp. consistently exhibited a higher TAS and correspondingly lower OSI than *Undaria* sp. across all solvent fractions, indicating a more favorable antioxidant/oxidant balance. Third, phenolic profiling showed that *Spirulina* sp. extracts (especially ethanol and hexane) contained a greater abundance and diversity of phenolic compounds relative to Undaria. Notably, vanillin, vanillic acid, and gentisic acid were identified as dominant phenolics in *Spirulina* sp. extracts, whereas *Undaria*'s profile was less diverse. In line with this, TPC and TFC were highest in *Spirulina*'s ethanol and hexane extracts, alongside elevated levels of phenolic aldehydes (e.g., vanillin). Finally, the results underscore that solvent polarity significantly influenced extract performance: ethanol and hexane extracts outperformed aqueous extracts in both antioxidant assays and phenolic yields for both algae.

In vitro assays confirmed that *Spirulina* sp. exhibits consistently stronger antioxidant activity than Undaria, particularly in radical-scavenging and metal-chelation assays. Its ethanol extract showed lower IC_{50} values in DPPH and ABTS tests (IC_{50} ≈ 0.78 mg/mL), consistent with earlier studies on *Spirulina* platensis, while Undaria pinnatifida showed weaker activity (IC_{50} ≈ 1.51 mg/mL for a subcritical water extract) (17-22). This difference is attributed to *Spirulina*'s rich composition of pigments and diverse phenolics, while *Undaria*'s effects rely more on sulfated polysaccharides and phlorotannins (23, 24). Interestingly, *Undaria* sp. showed higher CUPRAC absorbance at fixed concentrations, likely due to phlorotannins' strong reducing capacity, as noted in metabolomics studies of brown algae (25). Both algae chelated metals, with Spirulina sp. slightly stronger, likely due to phenolics and proteins forming stable complexes (26). TAS, TOS, and OSI values further confirmed this pattern: *Spirulina* sp. consistently showed higher TAS and lower OSI across solvents, indicating a more favorable antioxidant/oxidant balance (27). In vivo, *Spirulina* sp. extracts rich in vanillic acid and pyrogallol reduced MDA and increased antioxidant enzymes in diabetic rats (28). While *Undaria* sp. offers health benefits, its higher OSI may stem from lower vitamin/pigment levels or a greater burden of oxidizable components (29-31). Overall, *Spirulina* sp. not only excels in scavenging and chelation but also produces extracts inherently skewed toward antioxidative dominance, whereas *Undaria*'s antioxidant activity is offset by a relatively higher oxidant load.

LC–MS/MS profiling showed that *Spirulina*'s ethanol and hexane extracts contained a diverse range of phenolic compounds—particularly vanillin, vanillic acid, and gentisic acid—in higher abundance than those found in *Undaria*. Despite the general perception that brown algae are richer in polyphenols, *Spirulina* sp. stands out for its variety of low-molecular-weight phenolics. Previous studies have identified vanillin, benzoic acid, gallic acid, catechin, quercetin, and even curcumin-like compounds in *Spirulina* sp. (28, 32). Its high TPC, especially in non-polar fractions (26), confirms the presence of classical phenolic antioxidants, with some compounds possibly derived from the shikimate pathway or microbial associations. In contrast, Undaria's phenolic profile is dominated by high-molecular-weight phlorotannins, which are often difficult to quantify using standard HPLC methods. Shen et al. identified 12 polyphenolic compounds in several brown algae, including *Undaria*, such as vanillic, gallic, ferulic acids, epicatechin, and multiple phlorotannins (25). Our results also confirmed the presence of 4-hydroxybenzoic acid in aqueous extracts and flavonoids like naringenin in ethanolic extracts (33). However, due to the limited reactivity of phlorotannins in Folin-based assays, *Undaria*'s phenolic content may appear lower than it truly is (22). Overall, while *Spirulina* sp. contains a wider array of identifiable small phenolics, *Undaria*'s antioxidant potential largely resides in its abundant phlorotannins.

The extraction solvent strongly influenced recovery of phenolics and antioxidants in both species. Ethanol extracts of *Spirulina* sp. and *Undaria* sp. consistently showed the highest TPC and antioxidant activity, owing to ethanol's ability to dissolve a broad range of phenolics, flavonoids, and pigments (33, 34). In *Spirulina*, ethanol extracted both hydrophilic and moderately lipophilic antioxidants, while hexane preferentially recovered carotenoids and other non-polar compounds that still contributed to notable radical-scavenging activity. Water extracts were least effective, consistent with reports showing alcoholic extracts reaching ~50% DPPH inhibition compared with ~11% for hexane and ~34% for water (34). *Undaria* sp. followed a similar trend: ethanol and hexane extracts yielded the highest polyphenol and flavonoid levels (e.g. naringenin, phlorotannins), while aqueous extracts contained primarily polar phenolic acids like 4-hydroxybenzoic acid but lacked lipophilic or polymeric antioxidants (33). Interestingly, *Spirulina*'s hexane extract was substantially more active than *Undaria*'s, likely reflecting its richer pigment and lipophilic antioxidant content. These patterns emphasize that the phenolic and antioxidant profiles observed are not only species-dependent, but also highly solvent-dependent—supporting the use of mixed-polarity or sequential extraction strategies to maximize bioactive recovery.

This study has several limitations that should be acknowledged. First, only two commercially available algae products were analyzed, and the results may not represent all *Spirulina* or *Undaria* sources, which can vary by cultivation, processing, and geographic origin. Second, LC–MS/MS was used to identify a targeted set of phenolic compounds; however, the analysis did not cover all possible metabolites, and a broader untargeted metabolomic approach could provide additional insights. Third, the antioxidant assays used were in vitro chemical tests; while informative, they do not fully replicate biological conditions. Fourth, in vivo validation was beyond the scope of this study, and the physiological relevance of the observed differences in OSI, TPC, and phenolic diversity requires further confirmation in animal or clinical models. Finally, solvent choice strongly influenced extract outcomes, and the limited number of solvents tested may not capture the full spectrum of bioactive recovery achievable with more advanced extraction strategies.

In conclusion, this study showed that *Spirulina* sp. extracts, especially ethanol and hexane fractions, had stronger antioxidant activity and lower OSI than *Undaria. Spirulina* sp. was richer in low-molecular-weight phenolics such as vanillin and vanillic acid, whereas Undaria's activity was mainly linked to phlorotannins. Solvent polarity strongly influenced outcomes, with ethanol and hexane outperforming water. Overall, these findings indicate that *Spirulina* sp. can rival, and in some aspects surpass, brown algae in antioxidant potential, supporting its value as a functional food and nutraceutical source.

Ethical Approval

None.

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Conflicts of Interest

Authors declare that they have no conflicts of interest.

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