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Marine Macroalgae Extracts: Assessment of their Potential Application in Health and Wellness †

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Abstract: Algae are sustainable sources of bioactive compounds widely used in health and wellness applications, though supporting evidence is limited. This study characterized and compared aqueous extracts and purified fractions from *Fucus vesiculosus*, *Gracilaria sp.*, and *Ulva sp*. Total Phenolic Content (TPC) by *Folin-Ciocalteu* method, antioxidant activity by DPPH method, and the HPLC-DAD chromatographic profiles of extracts and fractions were compared. *Fucus vesiculosus* and *Gracilaria sp.* exhibited the highest TPC and antioxidant activity. Fractions without mucilage showed an enrichment in TPC and chromatographic profiles, particularly the polysaccharide-free extract of *Gracilaria sp.*, highlighting its promising applications and the need for future studies.

Keywords: Marine algae; *Fucus vesiculosus; Gracilaria sp.; Ulva sp.*; Bioactive compounds; Health and well-being

1. Introduction

The oceans, the largest reserves of biodiversity host marine algae, photosynthetic eukaryotes that play key roles in aquatic ecosystems, producing oxygen and supplying essential nutrients to coastal areas [1,2,3]. In addition, marine algae represent a sustainable, diverse, and still largely unexplored source of bioactive compounds, such as proteins and peptides, polysaccharides, lipids, vitamins, pigments, phenolic compounds [1,4,5]. These metabolites have been associated with a range of beneficial biological properties. However, despite increasing interest, scientific evidence about many claimed properties and their bioactives remains scarce. Macroalgae are taxonomically divided in three main groups: brown (*Phaeophyceae*), red (*Rhodophyta*), and green (*Chlorophyta*). Their chemical composition and biological activity are influenced by both species and environmental/geographical factors [5].

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Among the diversity of macroalgae, species of biotechnological interest stand out, such as *Fucus vesiculosus* (brown), *Ulva sp.* (green), and *Gracilaria sp.* (red). *Fucus vesiculosus* (bladderwrack) is rich in fucoidans, phlorotannins, and fucoxanthin, compounds with antioxidant, anti-inflammatory, anti-obesity, anti-aging, antimicrobial, anticancer, anticoagulant, and antidiabetic activities. Due to these properties, it has been extensively studied for applications in food products, biofertilizers, drugs, and cosmetics [5,6]. *Gracilaria sp.* (old woman's hair) is widely valued for the production of agar, a polysaccharide used in the food, cosmetic, and pharmaceutical industries. *Gracilaria sp.* also contains compounds with high added value, such as proteins, lipids, phenolic compounds, and phycobiliproteins, displaying antioxidant, anti-inflammatory, anticancer, and antimicrobial activities, among others [7]. *Ulva sp.* (sea lettuce) contains as main bioactive component ulvan, a polysaccharide, associated with a wide variety of biological activities, including anti-inflammatory, anticoagulant, anticancer, antioxidant, and antihyperlipidemic properties, demonstrating high potential for applications in medicine, cosmetics, functional foods, and other industries [8].

Despite their potential as sources of health-promoting compounds, comparative studies across algal groups and evaluations of purification strategies remain scarce, limiting the validation of their potential applications. In this context, the present study aimed to characterize and compare aqueous extracts and purified fractions from representative brown (*Fucus vesiculosus*), red (*Gracilaria sp.*), and green (*Ulva sp.*) algae, with emphasis on total phenolic content (TPC), antioxidant activity, and chromatographic profiles of compounds, to provide insights into their potential for health and wellness applications.

2. Material and Methods

2.1. Chemical

Acetonitrile (≥ 99.9%) and methanol (≥ 99.9%) from Honeywell (Charlotte, USA). Ultrapure water from Merck KGaA (Darmstadt, Germany). Trifluoroacetic acid (TFA) from PanReac (Barcelona, Spain). Phloroglucinol, *Folin-Ciocalteu* reagent, and sodium carbonate from Sigma-Aldrich, Merck KGaA (Darmstadt, Germany). 2,2-diphenyl-1-picrylhydrazyl (DPPH) and absolute ethanol (>99.8%) from Thermo Fisher Scientific (Waltham, USA) and distilled water. All other chemicals and reagents were of analytical grade.

2.2. Preparation of Marine Algae Extracts

Three species of macroalgae were used: *Fucus vesiculosus* (Batch F1MKG1.5 - 8/F1.0181024, Expiration Date 09/30/2027), *Ulva sp.* (Batch U1MKG1.5 - 8/G1.0181024, Expiration Date 06/30/2026) and *Gracilaria sp.* (Batch G1MKG1.5 - 8/G1.0181024, Expiration Date 10/31/2027). The biomass was supplied in flakes by the company ALGAplus® (Ílhavo, Portugal; https://www.algaplus.pt/), which is certified for sustainable and organic macroalgae cultivation.

Aqueous extracts were prepared from the macroalgal biomass in distilled water at concentrations of 100 g/L for *Fucus vesiculosus* and 100 g/1.5L for *Gracilaria sp*. and *Ulva sp.* The suspensions were autoclaved at 121 °C for 15 minutes, cooled and filtered to obtain the total extracts. An aliquot of each extract was frozen at -20 ºC for subsequent purification. The remaining extracts were frozen at -20 °C and subsequently lyophilized. The dried extracts were stored at -20 ºC until further analysis.

2.3. Purification of Extracts by Ethanol Precipitation

The aqueous extracts were purified to remove mucilages by ethanol precipitation. Absolute ethanol (99.8%) was added to the reserved aliquot of the aqueous extracts at a 4:1 ratio, and the mixtures was kept on ice for 2 minutes. The solutions were centrifuged at 22,553× *g* for 15 minutes yielding two fractions: a polysaccharide-free extract and a polysaccharide fraction. The polysaccharide-free extracts were dried by evaporation (40 °C, pressure of 175 mbar, and variable rotation) and subsequently stored at -20 °C until further use. The polysaccharide fractions were dried by lyophilization and also stored at -20 °C until further use.

2.4. Characterization of Marine Algae Extracts

For all analyses, stock solutions of the total aqueous extracts and purified fractions were prepared by dissolving 10 mg of extract in 1 mL of a solvent mixture of acetonitrile (ACN) and 0.05% trifluoroacetic acid (TFA) in water (50:50, v/v), obtaining a final concentration of 10 mg/mL.

2.4.1. Quantification of the Total Phenolic Content

The total phenolic content (TPC) was determined by the *Folin-Ciocalteu* colorimetric method, following an adapted protocol from Coelho *et al* [5].

Results were expressed in phloroglucinol equivalents (PGE) per g of dry extract (mg PGE/g of dry extract). For analysis, the prepared solutions were incubated under orbital shaking for 1 hour at 4°C, and the absorbance was measured at 760 nm in a UV-VIS spectrophotometer (JASCO Inc., Easton, Maryland, EUA). Quantification was performed against a calibration curve ($Abs_{760\ nm}$ = 7.758 PGE + 0.006, R^2 = 0.9918) using phloroglucinol as standards (0–2000 μg/mL). All assays were performed in duplicate.

2.4.2. Determination of Antioxidant Activity

The antioxidant activity (AA) was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) redial scavenging method, following an adapted protocol from Coelho *et al* [5].

The solutions to be analysed (10 mg/mL) were mixed with a 0.002% (w/v) DPPH solution in methanol and incubated for 30 min at room temperature in the dark. The decrease in absorbance was measured at 517 nm using a UV-VIS spectrophotometer. Antioxidant activity was expressed as the percentage of DPPH radical inhibition, calculated from the absorbance of the samples relative to a methanol control. All assays were performed in duplicate.

2.4.3. Analysis of the Chromatographic Profile of Extracts by High-Performance Liquid Chromatography

The chromatographic profile of compounds in the extracts and purified fractions was analyzed by High-Performance Liquid Chromatography (HPLC). The analysis was performed on an HPLC-DAD Elite LaChrom® system (HITACHI, VWR, Tokyo, Japan), equipped with a reverse phase column, ACE 3 C18 (150x4.6mm (ACE-111-1546) from ACE), an L-2200 Autosampler (injector), an L-2300 Column Oven, and an L2455 Diode Detector (VWR, Radnor, PA, USA).

The analysis was performed by injecting 25 μL of the sample, and the separation was achieved using a gradient composed of ACN (solution A) and 0.05% TFA in water (solution B), as described: 0 min (100% B), 30 min (30% A, 70% B), 40 min (80% A, 20% B), 45 min (80% A, 20% B), 50 min (30% A, 70% B), 52 min (0% A, 100% B), 55 min (0% A, 100% B). The flow rate was set at 0.8 mL/min, and chromatograms were acquired between 200

and 600 nm using a diode array detector (DAD). The chromatographic profiles of the extracts and the purified fractions were represented as the maximum absorbance values at the scanned wavelengths.

3. Results

3.1. Yields of Total Extracts and Purified Fractions

Total extracts were obtained from the biomass of *Fucus vesiculosus*, *Gracilaria sp.* and *Ulva sp.*, according to the procedure described in Section 2.2. After drying, the yields of the extracts were calculated and are summarized in Table 1. Among the studied species, *Gracilaria sp.* exhibited the highest yield of total extract (28.2%), followed by *Ulva sp.* (21.6%) and *Fucus vesiculosus* (18.7%).

Table 1. Yields of dry total extracts obtained from algae biomass.

Total Extract	Dry Extract (mg)	Biomass (mg)	Yield (% m/m)
Fucus vesiculosus	34.1	182	18.7
Gracilaria sp.	62.7	222	28.2
Ulva sp.	28.7	133	21.6

The total extracts were subsequently purified using ethanol precipitation to remove mucilage (Section 2.3), yielding two fractions: a polysaccharide-free extract and a polysaccharide fraction. The yields of these fractions are presented in Tables 2 and 3.

Table 2. Yields of dry polysaccharide-free extracts obtained from purification.

Polysaccharide-free Extract	Dry Extract (mg)	Biomass (mg)	Yield (% m/m)
Fucus vesiculosus	22	182	12.4
Gracilaria sp.	35.2	222	15.9
Ulva sp.	11.6	133	8.72

Table 3. Yields of dry polysaccharide fractions obtained from purification.

Polysaccharide Fraction	Dry Extract (mg)	Biomass (mg)	Yield (% m/m)
Fucus vesiculosus	14.6	182	8.0
Gracilaria sp.	13.7	222	13.7
Ulva sp.	18.3	133	13.8

Overall, *Gracilaria sp.* yielded the highest amounts in both the aqueous extract (Table 1) and the extract without polysaccharides (Table 2), whereas *Ulva sp.* and *Fucus vesiculosus* showed lower yields The yield of the aqueous extract was generally higher than those of the purified fractions.

3.2. Quantification of the Total Phenolic Content

The total phenolic content was determined using the *Folin–Ciocalteu* method as describer in Section 2.4.1, and the results are expressed as mg of phloroglucinol equivalents

(PGE) per g of dry extract (mg PGE/g of dry extract). TPC was seen to varied significantly between species and different types of extracts, with the results shown in Figure 1.

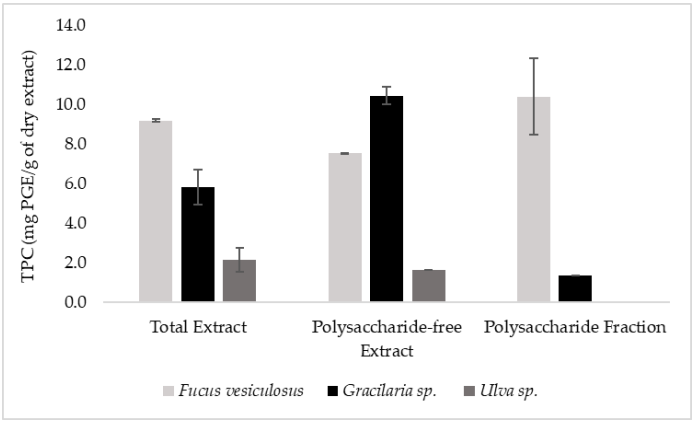

Figure 1. Total phenolic content in mg PGE/g dry extract of total extracts polysaccharide-free extracts, and polysaccharide fractions from *Fucus vesiculosus*, *Gracilaria sp.*, and *Ulva sp.*

The polysaccharide-free extract of *Gracilaria sp.* exhibited the highest TPC (10.4± 0.4 mg PGE/g of dry extract). In *Fucus vesiculosus*, the highest TPC value was obtained in the polysaccharide fraction (10± 2mg PGE/g of dry extract), while in *Ulva sp.*, the TPC was very low or below the detection limit in all extract and fractions analyzed.

3.3. Determination of Antioxidant Activity

The antioxidant activity (AA) of the extracts and purified fractions was measured using the DPPH radical scavenging assay, as described in Section 2.4.2. AA varied significantly between species and different types of extract (Figure 2).

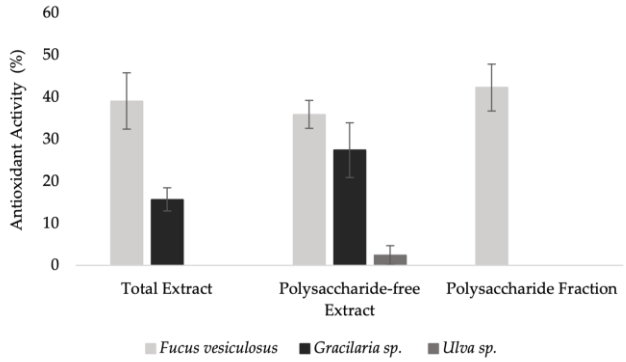

Figure 2. Antioxidant Activity of total extracts, polysaccharide-free extracts, and polysaccharide fractions from *Fucus vesiculosus*, *Gracilaria sp.*, and *Ulva sp.*

The polysaccharide fraction of *Fucus vesiculosus* showed the highest antioxidant activity (42.25 ± 5.59%). For *Gracilaria sp.*, the polysaccharide-free extract showed the highest AA (27.4 ± 6.51%). The extract and purified fractions from *Ulva sp.* displayed the lowest or undetectable AA.

These results are consistent with the TPC findings (Figure 1), since the extracts with the highest phenolic content in each species also demonstrated the greatest AA.

3.4. Analysis of the Chromatographic Profile of Extracts by HPLC-DAD

The chromatographic profiles of the extracts and purified fractions from *Fucus vesiculosus* (Figure 3), *Gracilaria sp.* (Figure 4), and *Ulva sp.* (Figure 5), were obtained by HPLC-DAD. The profiles revealed distinct peaks corresponding to compounds with variations

in peak number and intensity among the different types of extracts and macroalgae species

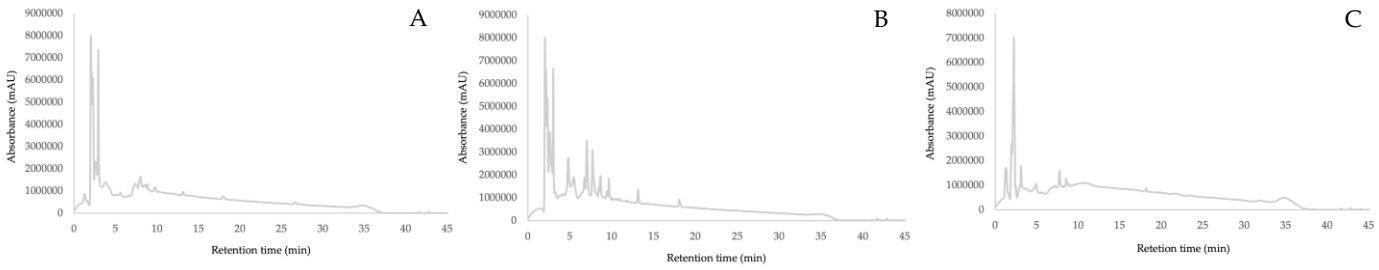

Figure 3. Chromatographic profile of (A) total Extract, (B) polysaccharide-free extract, and (C) polysaccharide fraction of *Fucus vesiculosus*.

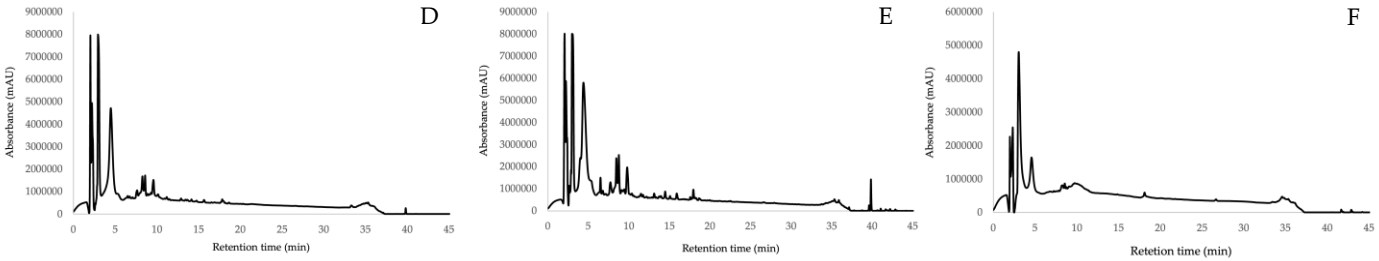

Figure 4. Chromatographic profile of (D) total extract, (E) polysaccharide-free extract, and (F) polysaccharide fraction of *Gracilaria sp*.

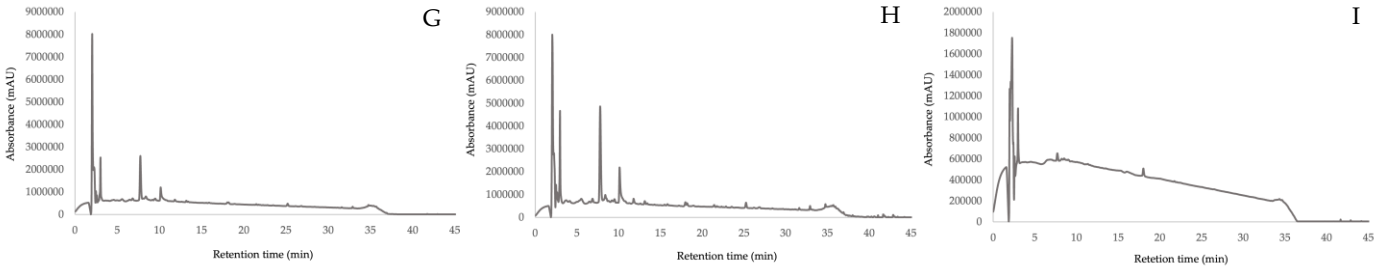

Figure 5. Chromatographic profile of (G) total Extract, (H) polysaccharide-free extract, and (I) polysaccharide fraction of *Ulva sp*.

In general, the chromatographic profiles supported the TPC results obtained. For example, for *Gracilaria sp*. the polysaccharide-free extract (E), which had the highest TPC, displayed more intense peaks than the total extract (D) and the polysaccharide fraction (F). *Ulva sp*. extract (G) and purified fractions (H and I), with very low TPC, showed fewer or smaller peaks in the chromatographic profile. In *Fucus vesiculosus* both the extract (A) and the purification fractions, polysaccharide free extract (B) and polysaccharide fraction (C), which exhibited high TPC and antioxidant activity, showed intense peaks. However, each displayed a distinct chromatographic pattern, reflecting the separation of different compound during purification process.

4. Discussion

This study investigated the extraction total phenolic composition and antioxidant potential of three macroalgae species, *Fucus vesiculosus*, *Gracilaria sp.*, and *Ulva sp.* Extraction yields differed notably among species, with *Gracilaria sp.* providing the highest yield

in both the total extract and polysaccharide-free extract, while *Fucus vesiculosus* and *Ulva sp.* yielded lower amounts. These variations likely reflect differences in the overall composition of compounds present in the macroalgal biomass. Parameters such as TPC, AA, and the chromatographic profile of total extracts and purified fractions were determined, allowing to understand both variability in metabolite composition among species and the impact of different extraction and purification methods on their potential functionality. These findings demonstrate that both species and extraction/purification methods strongly influence the phenolic profile, with important implications for the bioactive potential of the extracts. The results for TPC are consistent with previous studies, which report higher TPC in red and brown algae extracts (*Gracilaria sp.* and *Fucus vesiculosus*, respectively) compared to green algae (*Ulva sp.*) [9]. Herein, the polysaccharide-free extract of *Gracilaria sp.* showed higher TPC than previously reported [9], indicating that the extraction and mucilage removal process used in this study was particularly effective in enriching phenolic compounds for this extract purified fraction. This enrichment likely contributed to the enhanced AA observed in the polysaccharide-free fraction from *Gracilaria sp*, highlighting the role of both phenolic content and composition in determining the bioactive potential of macroalgal extracts, as also reported in other studies [9]. In contrast, green algae showed insignificant or undetectable AA, in agreement with its lower TPC. The results obtained for the AA, *Fucus vesiculosus* showed the highest value across all extracts, followed by *Gracilaria sp.*, especially in the extract without polysaccharides. These observations reinforce the evidence of a positive correlation between AA and TPC, since extracts with higher TPC generally exhibited stronger AA [1].

The chromatographic profiles obtained are consistent with the TPC values. Notably, the polysaccharide-free extract of *Gracilaria sp.* showed both the highest TPC (10.4 mg PGE/g of dry extract) and an enrichment of compounds in the chromatogram compared to the total extract. These results highlight the importance of optimized and selective extraction processes capable of removing polysaccharides and concentrating bioactive metabolites.

Nevertheless, the data indicate that the relationship between TPC and AA in not strictly linear. For example, *Gracilaria sp.* exhibited higher TPC than *Fucus vesiculosus* but lower AA, suggesting that AA depends not only on the total amount of phenolic compounds, but also on their qualitative composition and structural diversity. Different classes of phenolic compounds display different reducing capacities and radical-scavenging efficiencies, which could explain the observed differences. These findings underscore the need for future studies to characterize the specific phenolic profiles and interactions that influence bioactivity in macroalgal extracts and purified fractions, in order to comprehensively evaluate their potential for applications in functional foods, nutraceuticals, or other bioactive formulations.

5. Conclusions

The brown alga *Fucus vesiculosus*, red alga *Gracilaria sp.*, and green alga *Ulva sp.* revealed distinct profiles of TPC and AA, with *Fucus vesiculosus* and *Gracilaria sp.* exhibiting the highest TPC. Aqueous extraction followed by mucilage removal through ethanol precipitation effectively increased both TPC and AA, due to the enrichment of bioactive compounds in the polysaccharides free extract, particularly in the red algae *Gracilaria sp.* This purified fraction represents a promising source of bioactive and antioxidant compounds to be further explored and evaluated for incorporation into dermatological formulations, food supplements, and other innovative health-related applications, as its high phenolic

content and antioxidant activity may provide protective and preventive benefits for human health.

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