




## Article

# Antioxidant and Anti-Inflammatory Potential of Seaweed Extracts as Functional Ingredients

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**Abstract:** The use of seaweed as a source of bioactive extracts has received increased attention from the market in recent years—particularly for nutraceutical applications. In this context, this study evaluated the nutraceutical application of seaweed biomass and extracts from three seaweeds from Portugal: *Ulva* sp., *Laminaria ochroleuca*, and *Chondrus crispus*. For each of the said seaweeds, four different extracts were obtained using GRAS solvents—acetone (A), ethanol (E), ethanol–water (1:1) (EW), and one polysaccharide-rich extract (P) using water and further precipitation with ethanol. The bioactive potential of the extracts was assessed in terms of antioxidant capacity (ABTS<sup>•+</sup>, DPPH<sup>•</sup>, •NO, O<sub>2</sub><sup>•−</sup> scavenging, and ORAC-FL assay) and anti-inflammatory capacity (COX inhibition and human red blood cell membrane stabilisation). Furthermore, the biochemical profile was determined for the raw biomass and extracts to better comprehend their possible applications as nutraceuticals. The results show that all extracts have antioxidant potential. Five extracts (*L. ochroleuca* E, EW, and P and *Ulva* sp. E and P) showed anti-inflammatory capacity. Overall, *L. ochroleuca* EW extract exhibited the most promising potential as both an antioxidant and anti-inflammatory and is an interesting candidate nutraceutical ingredient.

**Keywords:** GRAS solvents; polysaccharides; antioxidant capacity; anti-inflammatory; radical scavenging



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## 1. Introduction

In 2019, the seaweed biomass market was estimated at USD 5.9 billion, with the human consumption application segment accounting for about 80% [1]. In terms of consumption, seaweeds have been a part of the Asian diet for a long time, but in Western countries, they are still limited and mainly linked to sushi, other Asian cuisines, and functional foods [1].

The concept of “functional foods” was coined in response to a widespread interest in certain foods that may benefit one’s health. Food and nutrition research has progressed beyond detecting and fixing nutritional deficiencies to developing foods that promote optimal health and lower illness risk [1]. Seaweeds are known for their high contents of polysaccharides, minerals, and vitamins. However, more recently, bioactive compounds, such as polyphenols, peptides, lipids, and pigments, have drawn attention due to several bioactivities, such as antioxidant, anti-inflammatory, anti-tumour, anti-obesity and anti-hypertensive effects [1,2]. Therefore, seaweeds now appear to be a great potential source of bioactive compounds as ingredients in functional foods [2].

Antioxidant compounds are gaining attention due to their potential to prevent several oxidative-stress-associated diseases, such as inflammation—which occurs when the balance between antioxidants and reactive oxygen species (ROS) is disrupted because of either the depletion of antioxidants or the accumulation of ROS [3].

Furthermore, there is a growing interest in natural antioxidants as replacements for synthetic ones due to increased safety concerns [4]. Many synthetic antioxidants (e.g., butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT)) used as food preservatives are considered to have carcinogenic and/or toxic effects on animal models [4]. Thus, algal antioxidant compounds have a great potential for improving the oxidative stability of food products [5].

In fact, crude seaweed extracts (acetic, aqueous, methanolic, and ethanolic) have already been shown to have interesting antioxidant properties [6,7], and the presence of phenolic compounds, pigments, polysaccharides, fatty acids, and peptides in seaweed extracts is linked to these activities [8]. For the extraction of bioactive compounds to be used in the food industry, food GRAS (Generally Recognised as Safe) solvents with lower environmental impact and toxicity (such as water, ethanol, and acetone) are required.

Seaweeds are very diverse organisms and are commonly organised into three groups according to their pigments: green, red, and brown, which have potentially different biochemical characteristics. Seaweed communities on the Portuguese coast are also remarkably diverse in terms of species, since this region is a biogeographical transition zone (between the Mediterranean and the Atlantic regions) due to the presence of a latitudinal temperature gradient, with overlapping cold- and warm-water seaweed species [9]. Since at least the 14<sup>th</sup> century, seaweeds have been harvested for use as fertilizers [10], although they are a natural and significant resource that is yet underexplored and investigated, particularly in terms of their economic potential. Within the three groups of green, red, and brown seaweeds, *Ulva* sp., *Chondrus crispus* and *Laminaria ochroleuca*, respectively, represent the most frequent species (>25% coverage) on the rocky shores of Northern Portugal [10].

Therefore, due to the worldwide trend towards the use of natural compounds in the food/nutraceutical market [1], this work aimed to preliminarily assess the potential of the use of seaweeds from the Northern Portugal coast in the food market as either (i) a nutrient-rich ingredient, (ii) a source of nutraceutical ingredients, namely, those with antioxidant and anti-inflammatory potential, and (iii) a source of antioxidant compounds to be used as food preservatives.

## 2. Materials and Methods

### 2.1. Seaweed Biomass Source

Biomass from three seaweed species, *Ulva* sp. (Chlorophyta), *Chondrus crispus* (Rhodophyta), and *Laminaria ochroleuca* (Phaeophyceae), were produced in enclosed systems for this study. *Ulva* sp. and *C. crispus* were produced in a pilot land-based system at CIIMAR (Portugal) in 400 L tanks receiving water from fish tanks to increase the nutrient concentration and yield. *L. ochroleuca* was produced in a pilot open-water installation and settled off Sagres (Portugal) in an oyster production farm. Seaweed biomass was dried at 60 °C for 48 h and stored in dry conditions.

### 2.2. Nutritional Value of Seaweed Biomass

Protein, fat, ash, moisture, and carbohydrate contents were evaluated using AOAC-based methods: the Kjeldahl assay based on 990.03 AOAC for proteins, the Soxhlet extraction method based on 945.16 AOAC for fat, and the 942.05 AOAC method for ash, and for carbohydrates, the content was estimated by subtracting the other components (Carbohydrates = 100% – Proteins % – Fat % – Water % – Ash %); finally, for moisture content, the 2000 AOAC method was used.

### 2.3. Bioactive Compound Extraction

In order to obtain extracts rich in different groups of potential bioactive compounds, for each seaweed, four different extracts were obtained with organic GRAS solvents—acetone (A), ethanol (E), a mixture of ethanol: water (1:1, *v/v*) (EW), and a fourth aqueous extract that was produced to obtain a polysaccharide-rich extract (P).

The extractions were performed in triplicate using 250 mg of dry biomass with a final solvent volume of 15 mL. In order to improve the extraction rate, cells were crushed using a bead beater (Precellys Homogenizer, Bertin, France) using 3 cycles with 5 mL of solvent each (6 series of 8000 rpm for 30 s with a 45 s pause) and 670 mg of ceramic beads to maximise cell disruption. Extracts were centrifuged at  $2000\times g$  for 10 min, and the supernatant was dried in a rotavapor system. EW extract was further freeze-dried to remove the water.

Polysaccharides were precipitated using water extract and cold ethanol ( $-20\text{ }^{\circ}\text{C}$ ) in a 1:3 ratio [11]. The solution was centrifuged at  $2000\times g$  for 10 min, and the polysaccharide phase was dried in a dry oven ( $60\text{ }^{\circ}\text{C}$ ). All extracts were stored in low humidity (desiccator) in the dark until further analyses.

### 2.4. Antioxidant Capacity Assessment

The antioxidant capacity of each extract was assessed by five different assays. Four scavenging assays were performed: two that measure total activity against synthetic radicals (ABTS $\bullet^{+}$  and DPPH $\bullet$ ) and another two that measure activity against biological reactive species ( $\text{O}_2^{\bullet-}$ , and  $\bullet\text{NO}$ ). Additionally, the oxygen radical absorbance capacity (ORAC-FL) assay was also determined. All extracts were resuspended in 20% DMSO at a concentration of  $4\text{ mg mL}^{-1}$  and further diluted for each assay. The antioxidant capacity was calculated from Trolox equivalent curves and expressed as  $\text{mg}_{\text{TE}}\text{ g}^{-1}$ . The assays were performed in triplicate.

#### 2.4.1. ABTS $\bullet^{+}$ Radical Scavenging

The ABTS $\bullet^{+}$  scavenging assay was performed as described by Granados-Guzman et al. [12] for all of the extracts at a final concentration of  $500\text{ }\mu\text{g mL}^{-1}$ . In summary,  $63\text{ }\mu\text{L}$  of each sample was transferred to a 96-well plate and mixed with  $180\text{ }\mu\text{L}$  of ABTS $\bullet^{+}$ . The reaction was incubated for 6 min in darkness, and the absorbance was read at 734 nm. The extract absorbance interference was reduced by measuring a blank using distilled water instead of ABTS $\bullet^{+}$ .

#### 2.4.2. DPPH $\bullet$ Radical Scavenging

The DPPH $\bullet$  scavenging assay was performed as described by Bobo-García et al. [13] for all of the extracts at a final concentration of  $500\text{ }\mu\text{g mL}^{-1}$ . Briefly,  $63\text{ }\mu\text{L}$  of each sample was transferred to a 96-well plate and mixed with  $180\text{ }\mu\text{L}$  of DPPH $\bullet$ . Samples were incubated for 30 min in darkness, and the absorbance was read at 515 nm. The extract absorbance interference was reduced by measuring a blank using methanol instead of DPPH $\bullet$ .

#### 2.4.3. $\text{O}_2^{\bullet-}$ Radical Scavenging

The  $\text{O}_2^{\bullet-}$  scavenging assay was performed as described by Pinho et al. [14] for all of the extracts at a final concentration of  $500\text{ }\mu\text{g mL}^{-1}$ . The reaction was performed using  $50\text{ }\mu\text{L}$  of each sample transferred to a 96-well plate together with  $50\text{ }\mu\text{L}$  of NADH ( $166\text{ }\mu\text{M}$ ),  $150\text{ }\mu\text{L}$  of NBT ( $43\text{ }\mu\text{M}$ ), and  $50\text{ }\mu\text{L}$  of PMS ( $2.7\text{ }\mu\text{M}$ ). Samples were incubated for 5 min in darkness, and the absorbance was read at 560 nm. The extract interference was reduced by measuring a blank using phosphate buffer ( $19\text{ }\mu\text{M}$ , pH 7.4) instead of PMS.

#### 2.4.4. NO Radical Scavenging

The •NO scavenging assay was performed as described by Pinho et al. [14] for all of the extracts at a final concentration of 500 µg mL<sup>-1</sup>. Briefly, 100 µL of each dilution was transferred to a 96-well plate together with 100 µL of SNP (20 mM) and incubated for 60 min with 20 µmol<sub>photons</sub> m<sup>-2</sup> s<sup>-1</sup> of fluorescent light. Then, 100 µL of Griess reagent (1.0% sulphanilamide and 0.1% N-(1)-naphthylethylenediamine in 2% phosphoric acid) was added to the solution and incubated for 10 min in darkness, and the absorbance was read at 562 nm. The extract interference was reduced by measuring a blank using 2% phosphoric acid instead of the Griess reagent.

#### 2.4.5. Oxygen Radical Absorbance Capacity (ORAC-FL) Assay

The ORAC-FL assay was performed as described by Davalos et al. [15] for all of the extracts in a range of concentrations from 1.5 to 80 µg mL<sup>-1</sup>. First, 20 µL of each dilution was transferred to a 96-well plate together with 120 µL of fluorescein (70 nM) and incubated for 15 min at 37 °C. After, 60 µL of AAPH solution (12 mM) was added. The fluorescence was then recorded every minute for 80 min. A blank was prepared using phosphate buffer instead of the extract solution, and eight calibration solutions using Trolox (1–8 µM) were used for the standard curve. ORAC-FL values were obtained by regression equations between the net area under the fluorescence decay curve (AUC) and antioxidant concentration. ORAC-FL values were expressed as Trolox equivalents using the standard curve calculated for each assay.

### 2.5. Anti-Inflammatory Capacity Potential Assessment

#### 2.5.1. Human Red Blood Cell (HRBC) Membrane Stabilisation by Heat Induction

The HRBC assay was performed according to Moualek et al. [16]. Fresh blood was collected intravenously from a healthy human volunteer into heparinised tubes to prevent coagulation. Blood was centrifuged at 1250× g for 5 min and washed three times with an equal volume of sodium phosphate buffer (PBS, 10 mM, pH 7.4). HRBC was reconstituted in a 40% (v/v) suspension with isotonic PBS. Salicylic acid (3.6 mM) was used as a positive control, and 20% DMSO (in PBS) was used as a negative control. The assay solution consisted of 2% HRBC mixed with the same volume of extract diluted in 20% DMSO at a final concentration of 500 µg mL<sup>-1</sup> and incubated for 20 min at 54 °C. Then, the solution was cooled in a tap water bath and centrifuged at 1250× g for 5 min. The absorbance of the supernatant was measured spectrophotometrically at 540 nm using a microplate reader (Thermofisher GO, Waltham, MA, USA). The inhibition of HRBC degradation was calculated for each extract and expressed as a percentage of inhibition (%).

#### 2.5.2. Cyclooxygenase (COX-2) Enzymatic Activity

The inhibition of COX-2 enzymatic activity was tested in triplicate using the COX inhibitor screening assay kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions. Dried extracts were resuspended in DMSO and tested at a concentration of 500 µg mL<sup>-1</sup>.

### 2.6. Biochemical Characterisation of Extracts

#### 2.6.1. Soluble Proteins

The soluble protein content was determined by the Bradford method [17] following the manufacturer's protocol (Biorad, Hercules, CA, USA). The quantification was performed using 10 mg of extract solubilised in 1 mL of phosphate buffer (50 mM, pH 7), and bovine serum albumin (BSA) (Alfa Aesar, Ward Hill, MA, USA) was used to obtain a standard curve. The protein content was expressed as a percentage of extract weight (%). The assay was performed in triplicate.

### 2.6.2. Lipids

The content of lipids was quantified gravimetrically using a chloroform/methanol (2:1, *v/v*) extraction [18] using 100 mg of extract in 5 mL of solvent. Afterward, the non-polar fraction was collected, dried, and weighed to determine the lipid content of the extract. The content of lipids was expressed as a percentage of extract weight (%). The assay was performed in triplicate.

### 2.6.3. Carbohydrates

The carbohydrate content was quantified spectrophotometrically through the phenol-sulphuric acid method [19]. The quantification was performed using 10 mg of extract resuspended in sulphuric acid (1 M). Glucose (Alfa Aesar, Ward Hill, MA, USA) was used to obtain a standard curve. The carbohydrate content was expressed as a percentage of extract weight (%). The assay was performed in triplicate.

### 2.6.4. Phenolic Compounds

The total phenolic compound content was determined by the Folin-Ciocalteu method [20]. The quantification was performed using 10 mg of extract resuspended in methanol-water (4:1, *v/v*). Gallic Acid (Sigma-Aldrich, St. Louis, MO, USA) was used to obtain a standard curve, and thus, the phenolic compound content was expressed as a percentage of extract weight (%). The assay was performed in triplicate.

## 2.7. Statistical Analysis

Experimental data were analysed using GraphPad Prism V. 7.0. A Shapiro-Wilk test of normality was performed before the analysis of variance (ANOVA) to confirm the normal distribution of the residuals. A two-way ANOVA with Tukey's multi-comparison test was used to find differences between the antioxidant capacity, anti-inflammatory capacity, and biochemical composition between extracts. A principal component analysis was performed using GraphPad Prism V. 9.0 using the data from bioactive screening and biochemical profiles from all evaluated extracts.

## 3. Results

### 3.1. Nutritional Characterisation of Seaweed Biomass

The dried biomass of the three studied seaweeds was evaluated in terms of nutritional value (Table 1). In terms of moisture, *Ulva* sp. held the highest content ( $11.7 \pm 0.5\%_{DW}$ ), while *L. ochroleuca* presented the lowest value, which was 2-fold lower than *Ulva* sp. In addition, *L. ochroleuca* biomass contained a low protein content ( $8.9 \pm 0.2\%_{DW}$ ) compared to the other two seaweeds with ca.  $25\%_{DW}$  (2.7-fold higher).

**Table 1.** Nutritional characterisation of dry seaweed biomass (average  $\pm$  standard deviation,  $n = 3$ ). Lowercase letters indicate statistical differences between species for the same parameter ( $p < 0.05$ ).

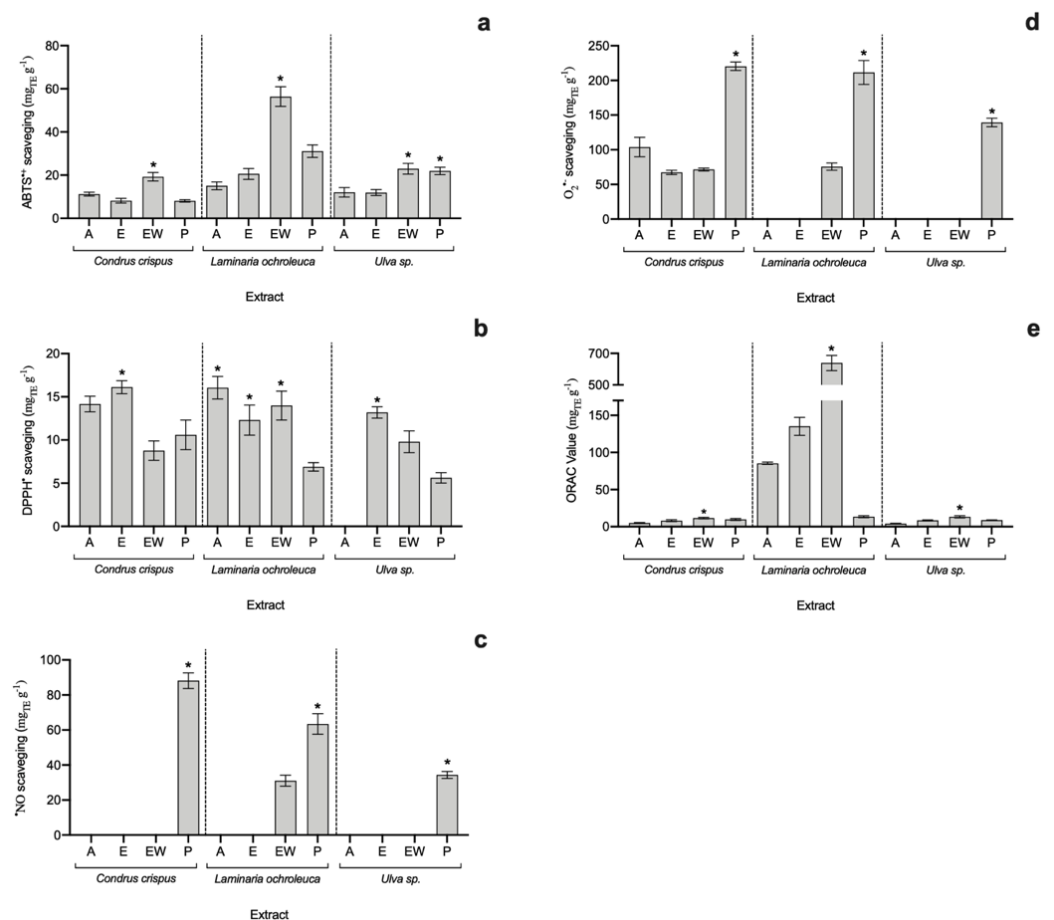
Algae	Content (%)				
	Moisture	Protein	Fat	Ash	Carbohydrates
<i>Chondrus crispus</i>	$8.1 \pm 0.1^a$	$24.4 \pm 0.5^a$	$0.4 \pm 0.1^a$	$19.4 \pm 0.1^a$	$47.7 \pm 0.6^a$
<i>Laminaria ochroleuca</i>	$5.6 \pm 0.1^b$	$8.9 \pm 0.2^b$	$0.5 \pm 0.1^a$	$38.2 \pm 0.4^b$	$46.7 \pm 0.6^a$
<i>Ulva</i> sp.	$11.7 \pm 0.5^c$	$25.0 \pm 0.1^a$	$0.3 \pm 0.1^a$	$27.2 \pm 0.2^c$	$35.8 \pm 0.2^b$

In terms of fat, all three seaweeds had a low content, no more than 0.6%, as usually found in these organisms [21]. As expected, the main component of the biomass from all three species was carbohydrates, with *C. crispus* and *L. ochroleuca* being the ones with the highest amount (ca. 47%). *L. ochroleuca* showed a high ash content ( $38.2 \pm 0.4$ ), which was two times higher than *C. crispus*.



### 3.2. Antioxidant Capacity of Seaweed Extracts

It is known that some radicals, such as  $\bullet\text{NO}$  and  $\text{O}_2^{\bullet-}$ , are responsible for triggering non-desirable metabolic pathways, some of them resulting in inflammation and, in the worst scenario, tumourigenesis [22]. In addition, it was found that synthetic antioxidants used in the food industry to act as food preservatives may cause tumourigenesis [23]. Thus, it is crucial to find antioxidant compounds from natural sources to avoid the use of synthetic ones that are also able to block the action of said  $\bullet\text{NO}$  and  $\text{O}_2^{\bullet-}$  radicals. For that reason, the antioxidant capacity of seaweed extracts was measured using five methods, allowing a comprehensive evaluation of the bioactive potential of these organisms (Figure 1). While total radical scavenging capacity evaluated by  $\text{ABTS}^{\bullet+}$  and  $\text{DPPH}^{\bullet}$  assays may indicate the potential of extracts to be used in food conservation, the specific antioxidant assays ( $\bullet\text{NO}$  and  $\text{O}_2^{\bullet-}$ ) reveal their nutraceutical potential. Moreover, the ORAC-FL assay has been recommended for the total antioxidant evaluation of foods and beverages [24].



**Figure 1.** Antioxidant capacity of seaweed extracts in terms of (a)  $\text{ABTS}^{\bullet+}$ , (b)  $\text{DPPH}^{\bullet}$ , (c)  $\bullet\text{NO}$ , and (d)  $\text{O}_2^{\bullet-}$  scavenging assays and (e) ORAC-FL assay (average  $\pm$  standard deviation,  $n = 3$ ). Extracts: acetone (A), ethanol (E), ethanol–water (EW) and polysaccharides (P). The asterisk symbol (\*) indicates the highest value per species ( $p < 0.05$ ).

When it comes to total radical scavenging capacity, the EW and E extracts of all analysed algae exhibited the highest potential, particularly the EW of *L. ochroleuca* ( $56.41 \pm 4.56 \text{ mg}_{\text{TE}} \text{g}^{-1}$  for  $\text{ABTS}^{\bullet+}$  and  $13.99 \pm 1.68 \text{ mg}_{\text{TE}} \text{g}^{-1}$  for  $\text{DPPH}^{\bullet}$ ), which was 1.8-fold higher than the second-best extract, *L. ochroleuca* P (in ABTS assay), but not statistically different ( $p > 0.05$ ) from *L. ochroleuca* A in the  $\text{DPPH}^{\bullet}$  assay.

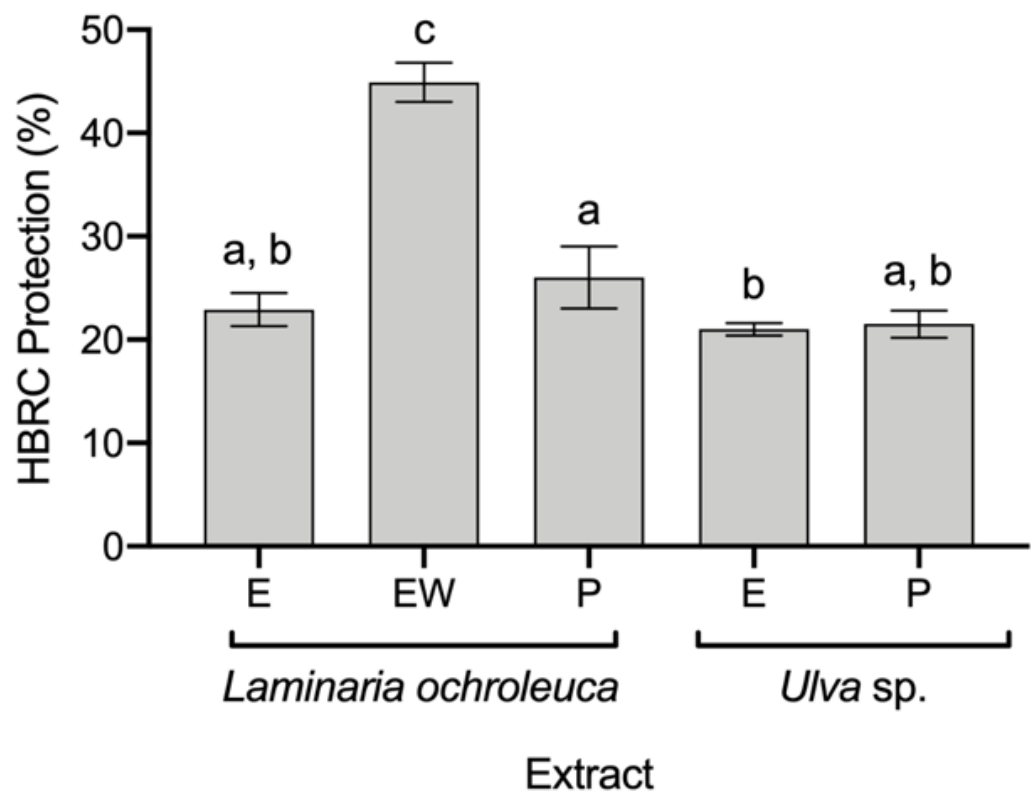
Polysaccharide-rich extracts (P) from all three species attained the highest antioxidant values against  $\bullet\text{NO}$  and  $\text{O}_2^{\bullet-}$  radicals, revealing their nutraceutical potential. Additionally, looking at specific activity against  $\bullet\text{NO}$ , apart from P extracts, only *L. ochroleuca* EW showed

scavenging capacity ( $31.06 \pm 3.16 \text{ mg}_{\text{TE}} \text{ g}^{-1}$ ), while the highest value was achieved by *C. crispus* P extract ( $88.21 \pm 4.46 \text{ mg}_{\text{TE}} \text{ g}^{-1}$ ). Regarding the  $\text{O}_2^{\bullet-}$  scavenging assay, the highest value was found in *C. crispus* P ( $220.38 \pm 6.04 \text{ mg}_{\text{TE}} \text{ g}^{-1}$ ) and *L. ochroleuca* P ( $211.44 \pm 17.20 \text{ mg}_{\text{TE}} \text{ g}^{-1}$ ) extracts, with no statistical differences ( $p > 0.05$ ).

Finally, concerning the oxygen radical absorbance capacity (ORAC-FL), although all extracts seem to have an antioxidant effect, *L. ochroleuca* showed much higher potential than the other species, with a special highlight for *L. ochroleuca* EW extract ( $638.90 \pm 48.30 \text{ mg}_{\text{TE}} \text{ g}^{-1}$ ), which was 4.7-fold higher than the second-best extract (*L. ochroleuca* E).

### 3.3. Anti-Inflammatory Potential of Seaweed Extracts

Although all extracts were evaluated for HRBC protection against thermal degradation, only five extracts had protective effects (Figure 2): *L. ochroleuca* E, EW, and P extracts and *Ulva* sp. E and P extracts. The highest protective effect was found in *L. ochroleuca* EW extract, with  $44.9 \pm 1.6\%$  of protection, almost twice the protection afforded by the other extracts, with ca. of 23% of protection. Furthermore, for the COX-2 inhibition assay, only *L. ochroleuca* EW extract was evaluated, resulting in an inhibition of  $32.8 \pm 2.42\%$  of the enzymatic activity at a concentration of  $500 \mu\text{g mL}^{-1}$ .



**Figure 2.** Anti-inflammatory capacity of seaweed extracts in terms of their protection of human red blood cells (HRBCs) from thermal degradation (average  $\pm$  standard deviation,  $n = 3$ ). Extracts: ethanol (E), ethanol: water (EW), and polysaccharides (P). <sup>a–c</sup> Different letters above bars mean statistical differences ( $p < 0.05$ ).

### 3.4. Biochemical Composition of Seaweed Extracts

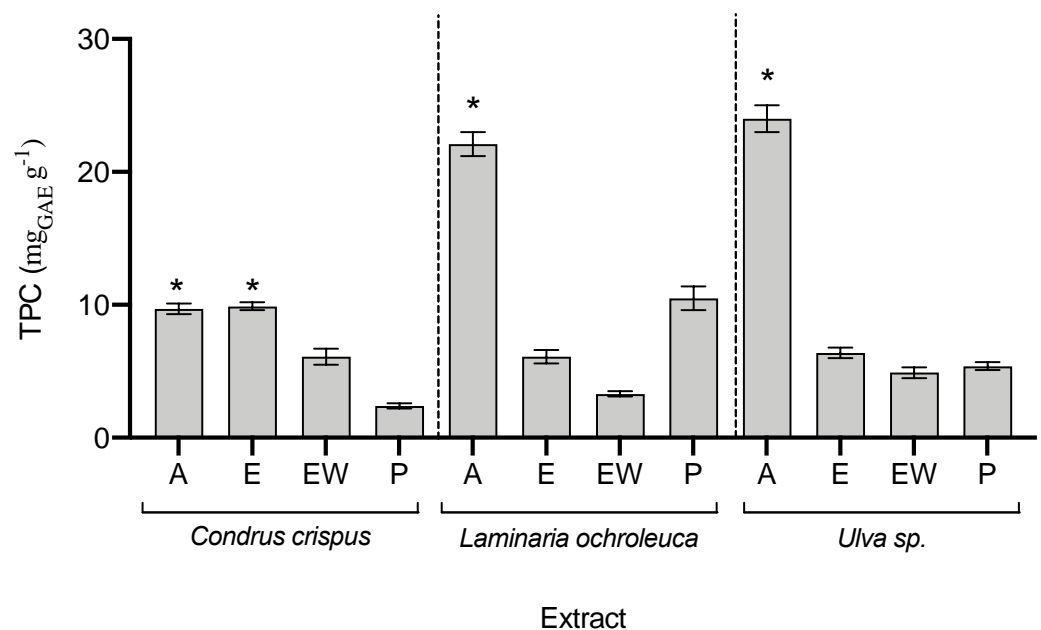
To better understand the potential of the seaweed extracts, their biochemical composition was determined (Table 2). For all of the different algal species, the extracts' biochemical compositions followed a clear trend according to the solvent used in the extraction and its polarity: i.e., proteins and carbohydrates were better extracted with polar solvents, particularly with an aqueous counterpart, and lipids were better extracted with non-polar solvents, such as acetone and ethanol.

In terms of proteins, the EW system allowed the extraction of the highest content in all three species (ca. 13%<sub>DryExtract</sub>), while the carbohydrate content was higher in the polysaccharide-rich extract (P) at ca. 70%<sub>DryExtract</sub>. Although the total amount of lipids in the raw biomass was extremely low, the highest value was found in acetonic extracts.

Regarding the phenolic compound content (Figure 3), acetonic extracts had the highest phenolic values in the three species, being two-fold higher than the content found in the other extracts. As phenolic compounds are usually associated with a high antioxidant capacity, a correlation was attempted with the five antioxidant assays; however, no correlation was found ( $p > 0.05$ ).

**Table 2.** Biochemical composition of seaweed extracts (average  $\pm$  standard deviation,  $n = 3$ ). Extracts: acetone (A), ethanol (E), ethanol–water (EW), and polysaccharides (P). Bold letters indicate the highest value in each biochemical group in each species. <sup>a–i</sup> Lowercase letters indicate statistical differences between species for the same parameter ( $p < 0.05$ ).

Algae	Extract	Content (% <sub>Dry Extract</sub> )		
		Proteins	Carbohydrates	Lipids
<i>Chondrus crispus</i>	A	8.9 $\pm$ 0.6 <sup>a</sup>	10.2 $\pm$ 1.0 <sup>a</sup>	<b>30.0 <math>\pm</math> 0.8 <sup>a</sup></b>
	E	10.4 $\pm$ 0.3 <sup>b</sup>	11.3 $\pm$ 1.0 <sup>a</sup>	19.0 $\pm$ 0.2 <sup>b</sup>
	EW	<b>13.2 <math>\pm</math> 0.7 <sup>c</sup></b>	40.2 $\pm$ 1.5 <sup>b</sup>	24.8 $\pm$ 2.5 <sup>c</sup>
	P	8.2 $\pm$ 0.8 <sup>a</sup>	<b>73.3 <math>\pm</math> 6.0 <sup>c</sup></b>	1.0 $\pm$ 0.1 <sup>d</sup>
<i>Laminaria ochroleuca</i>	A	11.3 $\pm$ 0.9 <sup>b</sup>	10.7 $\pm$ 0.8 <sup>a</sup>	<b>52.4 <math>\pm</math> 5.1 <sup>e</sup></b>
	E	8.9 $\pm$ 0.5 <sup>a</sup>	5.7 $\pm$ 0.5 <sup>d</sup>	14.2 $\pm$ 1.0 <sup>f</sup>
	EW	<b>13.1 <math>\pm</math> 0.5 <sup>c</sup></b>	27.3 $\pm$ 0.6 <sup>e</sup>	1.2 $\pm$ 0.1 <sup>d</sup>
	P	6.4 $\pm$ 0.3 <sup>d</sup>	<b>68.4 <math>\pm</math> 3.9 <sup>c</sup></b>	0.1 $\pm$ 0.1 <sup>g</sup>
<i>Ulva sp.</i>	A	9.2 $\pm$ 0.6 <sup>a</sup>	10.7 $\pm$ 1.0 <sup>a</sup>	<b>61.0 <math>\pm</math> 0.1 <sup>h</sup></b>
	E	9.8 $\pm$ 0.5 <sup>a, b</sup>	12.8 $\pm$ 1.2 <sup>a</sup>	24.5 $\pm$ 0.3 <sup>c</sup>
	EW	<b>13.5 <math>\pm</math> 0.9 <sup>c</sup></b>	23.8 $\pm$ 2.0 <sup>f</sup>	3.2 $\pm$ 0.1 <sup>i</sup>
	P	5.7 $\pm$ 0.3 <sup>e</sup>	<b>67.8 <math>\pm</math> 3.1 <sup>c</sup></b>	0.1 $\pm$ 0.1 <sup>g</sup>

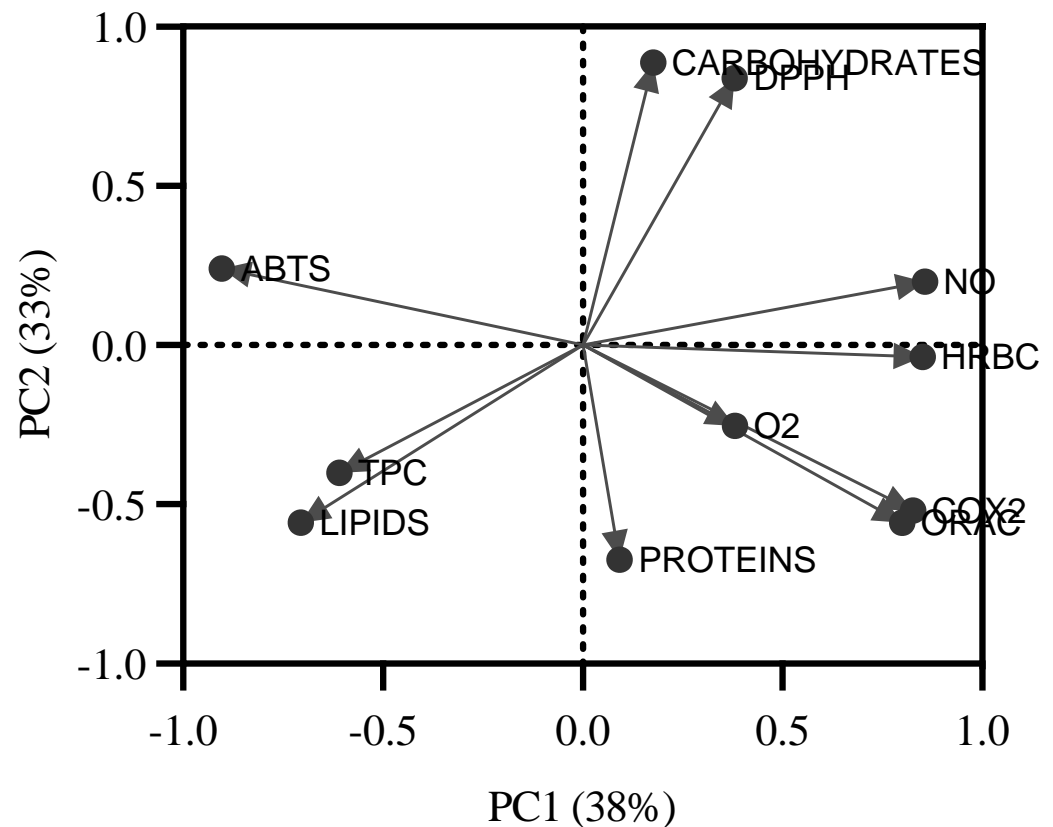


**Figure 3.** Total phenolic compounds (TPC) in seaweed extracts (average  $\pm$  standard deviation,  $n = 3$ ). Extracts: acetone (A), ethanol (E), ethanol–water (EW), and polysaccharides (P). The asterisk symbol (\*) indicates the highest value per species ( $p < 0.05$ ).



### 3.5. Principal Component Analysis

As no direct correlation was found between the biochemical profile and the bioactive potential, a principal component analysis was performed to gain a better understanding of the influence of biochemical groups on the bioactive capacity of the extracts (Figure 4). Two principal components explain 71% of the variation in the variables. The first principal component (PC1) explains 38% of the total variation, while the second (PC2) explains 33%. Based on the polarity of compounds, PC1 splits the data into lipophilic and hydrophilic compounds. Notably, most of the bioactive potential is related to hydrophilic groups, while ABTS<sup>•+</sup> antioxidant potential would be more related to lipophilic compounds.



**Figure 4.** Results of the principal component analysis applied to all values of antioxidant capacity of seaweed extracts (ABTS<sup>•+</sup>, DPPH<sup>•</sup>, •NO, O<sub>2</sub><sup>•−</sup>, and ORAC-FL), anti-inflammatory capacity (COX-2 and HRBC), and the biochemical profile (proteins, carbohydrates, lipids, and total phenolic compounds (TPC)).

### 4. Discussion

As functional ingredients, extracts can be used in two different ways: as a source of a specific bioactive capacity or as a source of a specific compound (or a group of compounds). Here, both evaluations were considered: first, the antioxidant and anti-inflammatory capacity of the seaweed extracts, and second, the biochemical composition of the extracts.

The antioxidant potential of seaweeds has been proposed for a long time; for example, Yan et al. [25] screened the potential of 27 seaweeds from China in terms of DPPH<sup>•</sup> scavenging potential. The authors used successive solvent extraction (chloroform > ethyl acetate > acetone > methanol > water) to use the whole biomass. Similar to the present study, the potential of different extracts depended not only on the species but also on the solvent choice. *Desmarestia viridis* (Ochrophyta) was highlighted as holding a high antioxidant potential in methanol, acetone, and ethyl acetate extracts, although it showed almost no activity in the chloroform fraction.

As mentioned before, the evaluation of antioxidant capacity should consider different mechanisms of action and groups of compounds. As extracts are complex matrices, the synergetic effect of molecules also dramatically impacts the final bioactive capacity. While ABTS<sup>•+</sup>, DPPH<sup>•</sup>, O<sub>2</sub><sup>•−</sup>, and •NO assays are based on scavenging capacity, ORAC-FL is based on the inhibition of peroxy-radical-induced oxidation and the protection of the degraded compound.

Special attention must be given to *L. ochroleuca* EW extract. Its great potential was revealed in total antioxidant assays, namely, the ABTS<sup>•+</sup>, DPPH<sup>•</sup>, and ORAC-FL assays, which revealed an antioxidant capacity of up to 5-fold higher than the second-best extract. This may indicate its great potential to be used to replace synthetic food preservatives, particularly in the beverage segment [24]. In addition, the interesting results of *L. ochroleuca* EW extract in O<sub>2</sub><sup>•−</sup> and •NO radical tests indicate that its application as a component in food or beverage compositions could be polyvalent.

The bioactive potential of *L. ochroleuca* has been exploited before, as it contains a diverse composition of phenolic compounds, amino acids, pigments, and polysaccharides in a special fucoidan extract [26]. *L. ochroleuca* extracts have already been associated with depressant and analgesic effects on the central nervous system [27], hypoglycaemic capacity [28], and anti-inflammatory [29], antioxidant, and anti-tumour properties [26].

The EW extract may have an extra advantage in extracting both hydrophilic and lipophilic compounds. In comparison to other extracts of *Laminaria* species, the results of antioxidant capacity obtained in this study with EW extracts of *L. ochroleuca* seem to be higher than (for ABTS<sup>•+</sup> and ORAC assays) or comparable to (DPPH<sup>•</sup> assay) others reported before, as observed in Table 3. It also must be noted that the anti-inflammatory values obtained for both assays are not very different from other specific extracts (Table 3). Hence, as in this study, a potential synergetic effect of these compounds may have contributed to the increased antioxidant potential of EW extract.

**Table 3.** Comparison of the obtained antioxidant (mg<sub>TE</sub> g<sup>−1</sup>) and anti-inflammatory (% of inhibition) results of EW extracts of *L. ochroleuca*, marked in shade, with other reported values of *Laminaria* species or brown algae.

Algae	Concentration (mg <sub>TE</sub> g <sup>−1</sup> )			HRBC (%)	COX (%)
	ABTS <sup>•+</sup>	DPPH <sup>•</sup>	ORAC		
<i>Laminaria ochroleuca</i>	EW: <b>56.41 ± 4.56</b>	EW: 13.99 ± 1.68	EW: <b>638.90 ± 48.30</b>	EW: 44.9 ± 1.6	EW: 32.8 ± 2.42
<i>Laminaria</i> sp.	W: 32 <sup>1</sup>	EW: 5–20 <sup>2</sup>	P: 312.12 <sup>3</sup>	FE: 52–88 <sup>4</sup>	SE: 47 <sup>5</sup>

EW—ethanol-water; P—polysaccharides; W—water; FE—fucoidan extract from brown algae; SE—sterol extracts.  
<sup>1</sup> [24]; <sup>2</sup> [30]; <sup>3</sup> [31]; <sup>4</sup> [32]; <sup>5</sup> [33].

On the other hand, when it comes to specific radicals, such as O<sub>2</sub><sup>•−</sup> and •NO, polysaccharide extracts showed the highest potential, regardless of the species. In particular, *C. crispus* P revealed its high potential; it was the best in scavenging •NO and, together with *L. ochroleuca* P, the best in scavenging O<sub>2</sub><sup>•−</sup>. In addition, polysaccharides from seaweeds have been associated with anticoagulant, antithrombotic capacity, antimicrobial, anti-inflammatory, and antioxidant potential [26].

Regarding the anti-inflammatory capacity of extracts, only a few extracts had a protective effect in the HRBC assay. *L. ochroleuca* EW was shown to be the best extract, and its potential was evaluated for a more specific mechanism of action: the inhibition of the COX-2 enzyme. COX enzymes are involved in the production of prostaglandins and the induction of inflammatory reactions, and inhibiting these enzymes can alleviate symptoms such as pain and fever [34]. *L. ochroleuca* EW inhibited the enzyme's activity by 30%, which indicates a potential that should be explored in more detail.

Inflammation is a protective reaction to an injury caused by physical trauma, toxic substances, or pathogens. Anti-inflammatory mechanisms comprise enzymes and signalling transcription factors. COX-1 and COX-2 enzymes have been extensively studied in inflammatory disorders and have been associated with illness incidence [35]. Moreover, lysosomal enzymes are released during the inflammatory process, causing tissue damage. These enzymes' extracellular activity is thought to be linked to acute or chronic inflammation. Lysosomal membrane stabilisation is vital in regulating the inflammatory response by restricting the release of lysosomal components. The HRBC membrane is comparable to the lysosomal membrane, and the extract's stability implies that lysosomal membranes will be stabilised [36].

The anti-inflammatory effect of a *Laminaria* species was described by Lee et al. [37] in a cell line study. The authors used *Laminaria japonica* aqueous extract to inhibit UVB-induced inflammation in the human keratinocyte (HaCat) cell line. The extract acted by maintaining the levels of nc 886 gene expression, subsequently inhibiting the production of the inflammatory response.

Regarding the nutritional composition of raw biomass, the species *Ulva* sp. and *C. crispus* were shown to be great sources of protein (25%), while *L. ochroleuca* could be used as a source of carbohydrates (47%) and minerals due to its ash content (38%), mainly containing twice the value reported before for this species [38]. However, the high ash content in *Laminaria* species has been reported before [39], where *Laminaria digitata* also showed 38%, suggesting the use of this seaweed as a food supplement of some mineral trace elements.

The biochemical profile of the extracts was aimed not only at providing insight into their nutritional values but also at correlating some groups of compounds and their bioactive potential, as many studies have reported a positive correlation between antioxidant capacity and total phenolic compounds. However, in this study, the extracts with higher phenolic contents (A extracts) did not show great potential, except for *L. ochroleuca* A in the DPPH• assay. This poor correlation was also found by Heo et al. [40], who evaluated different enzymatic extracts from seven brown seaweeds from Korea. The authors found that extracts from the same species with similar contents of phenolic compounds had completely different antioxidant capacities, and they proposed that other compounds, such as low-molecular-weight polysaccharides, proteins, or peptides, may influence the antioxidant potential, which can be associated with the results observed in the principal component analysis, which related hydrophilic compounds to most of the bioactive assays. Barbosa et al. [21] evaluated the fatty acid profile of *L. ochroleuca* from Northern Portugal, indicating a possible similarity to the one used in the present study. Although present at a low concentration, this seaweed was shown to have polyunsaturated fatty acids (PUFAs) with known antioxidant capacity, such as linoleic acid,  $\gamma$ -linolenic acid, and EPA (eicosapentaenoic acid), ranging from 70 to 160 mg kg<sub>DW</sub><sup>-1</sup>.

Furthermore, in terms of application, seaweed extracts have been attracting the attention of the natural cosmetic industry, and many claims are already associated with extracts and raw biomass used in cosmetic formulations, including antioxidant, anti-ageing, photoprotective, and anti-inflammatory effects, among others [23]. Thus, in addition to nutraceutical applications, the extract evaluated here can also be used in other industries, such as cosmeceutical (cosmetic with nutraceutical potential).

## 5. Conclusions

*Chondrus crispus*, *Laminaria ochroleuca*, and *Ulva* sp. showed the potential to be exploited in the food and food supplement markets. While *Ulva* sp. and *C. crispus* showed great potential to be used as a source of protein, *L. ochroleuca* could be used as a source of carbohydrates and minerals.

In terms of extracts as functional ingredients, five extracts (*L. ochroleuca* E, EW, and P and *Ulva* sp. E and P) showed anti-inflammatory potential, thus revealing their potential for nutraceutical applications. *L. ochroleuca* EW extract is highlighted due to its great polyvalent potential. In addition to its great total antioxidant capacity, indicative of its high potential to replace synthetic food preservatives, particularly in the food and beverage segment, it was also revealed to have antioxidant potential against  $O_2^{\bullet-}$  and  $\bullet NO$  radicals and anti-inflammatory potential in the inhibition of COX-2. Hence, *L. ochroleuca* seems to be a promising seaweed to be exploited due to its polyvalent potential for application as (i) a nutrient-rich ingredient, particularly in minerals, (ii) a source of nutraceutical ingredients due to its antioxidant and anti-inflammatory potential, and (iii) a source of antioxidant compounds to be used as food preservatives.

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