Abstract: *Sargassum muticum* was processed by hydrothermal extraction under previously optimized non-isothermal conditions (up to 187 °C). The alginate free crude hydrolysate was further concentrated by ultrafiltration, operating in diafiltration mode to produce an extract (SmE) enriched in the fucoidan and the phlorotannin fractions and with low mineral content and antioxidant capacity equivalent to that of Trolox. In order to explore the potential of this concentrated product for food or feed additive, the in vivo antioxidant potential was assessed. Male Sprague–Dawley rats were fed SmE dissolved in distilled water at doses of 0.5, 1.0 or 2.0 g kg\(^{-1}\), administered via an intragastric tube daily for three weeks. The weight and organ gain was not significantly affected in the different groups in relation to the control group fed a standard diet. Serum glucose was significantly lowered in the groups receiving the higher SmE doses, liver GPx levels were reduced and liver TBARS levels decreased in rats administered the extract, but no effect on SOD activity in either liver or erythrocytes was observed.

Keywords: *Sargassum muticum*; autohydrolysis; ultrafiltration; diafiltration; serum parameters antioxidant enzymes; lipid peroxidation

1. Introduction

Marine algae, containing bioactive compounds not present in terrestrial plants and beneficial to human health, are a source of phytochemical active compounds (such as polysaccharides, sterols, carotenoids and tocopherols) with antiviral, antimicrobial, antifungal, anticoagulant and antioxidant properties. Sulphated polysaccharides are widespread in marine algae, especially in brown seaweeds. The biological activities of sulphated polysaccharides include anticoagulant, antioxidant, antiviral, anticancer and immunomodulating activities [1–4]. Algicin acid has been reported to be capable of decreasing cholesterol levels, exerting an antihypertensive effect, preventing absorption of toxic products and protecting the surface membranes of the stomach and intestine [5]. Phlorotannins, polymers of phloroglucinol, which are present in brown algae, are valuable antioxidant compounds [6].

Algae are exposed to light and high concentrations of oxygen, leading them to produce oxidative agents, but algae also generate compounds that protect against oxidation [7]. Among the compounds present in algae, antioxidants have probably attracted the most interest. Brown algae possess potential antioxidant activity, and different in vitro studies have demonstrated that *Sargassum* spp. has compounds conferring it potent antioxidant properties [8–11]. Although scarce, the in vivo antioxidant activity of *Sargassum* spp. extracts have been explored. The consumption of *Sargassum polycystum* extracts lowers the risk of hyperglycaemia, dyslipidaemia and atherogenesis in mammalian models of type 2 diabetes [12,13].
*Sargassum muticum*, an invasive brown algae on the Atlantic coasts, represents an important environmental problem and can also affect recreational activities in coastal areas. Eradication trials were unsuccessful, and periodic removal of specimens has proven effective in preventing further spread. Therefore, valorization of the removed biomass has been proposed. Hydrothermal processing offered higher biomass solubilization yields than other intensification methods [14] and conforms a suitable strategy for the extraction and depolymerization of the fucoidan fraction [15,16]. Further processing with ultrafiltration membranes allowed the concentration in bioactives and the elimination of minerals in the concentrated product [17]. Some fractions could be especially enriched in bioactives [18].

Reactive oxygen species (ROS) cause damage to biomolecules and participate in the development of many disorders and diseases. ROS may be highly damaging, as they can attack biological macromolecules (namely lipids, proteins and DNA), induce oxidation and cause membrane damage, enzyme inactivation and DNA damage [19]. Oxidative stress is considered to play a pivotal role in the pathogenesis of aging and several degenerative diseases such as atherosclerosis, cardiovascular disease, type 2 diabetes and cancer [20]. ROS scavenging or detoxifying mechanisms involve enzymatic and non-enzymatic antioxidants, either those produced in the body (i.e., endogenous antioxidants) [21] or those supplied in the diet [22]. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) act by catalyzing the decomposition of oxidants and free radicals and exerting protective effects against cellular damage [23]. Methods of increasing antioxidant capacity have been suggested as being potentially useful for preventing or delaying the adverse actions of ROS.

In this study, the in vivo antioxidant properties of *Sargassum muticum* hydrothermal extracts were evaluated by examining the effects on the antioxidant enzyme activity in growing male Sprague–Dawley rats fed with a diet supplemented with these extracts. The activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in liver and in red blood cells were determined and the total amounts of lipid peroxidation products in liver were assayed. The effects of the algal extract on serum parameters and body weight of rats was also determined.

2. Results and Discussion

2.1. Extract Composition and Characteristics

The concentrated *Sargassum muticum* extract evaluated in this study was produced according to the scheme shown in Figure 1. The hydrothermal extract produced by hydrothermal processing in hot pressurized water media was further concentrated in membranes during successive washing cycles to selectively remove low molecular weight components. The salt content in the concentrated product (SmE) obtained after the ultrafiltration stage operating in the diafiltration mode was reduced by almost six times, to less than 2% of the dry weight. The arsenic content in the product was lowered to a safe-to-use concentration (0.4 µg g⁻¹) from the original value of the autohydrolysis crude extract, which was 29.4 µg g⁻¹. The fucoidan content accounted for more than 50% of the product dry weight, being fucose the most abundant constituent, followed by galactose, glucose, xylose and mannose; with an average sulfation of 7%. Along the diafiltration process the phenolic content of the concentrated product could be increased up to 10–15%. The SmE product showed antiradical properties equivalent to 20% of those presented by Trolox [17]. The fractions with molecular weight in the range of 50–100 kDa and that with more than 100 kDa contained higher phenolic and sulfate content. Both could be responsible for the biological properties, in particular for cytotoxicity against tumoral cells and antioxidant capacity. However, those fractions under 5 kDa contribute in lower extent to these properties.
Figure 1. Flowchart of the extraction and concentration of the *Sargassum muticum* concentrated extract (SmE).

The Fourier transform infrared spectroscopy (FT-IR) spectra of the concentrated product (Figure 2a) shows characteristic absorption bands in the following regions: 2923 cm\(^{-1}\), corresponding to C–H stretching; at 1629 cm\(^{-1}\), corresponding to carbohydrate O–C–O asymmetric stretching vibrations; 1421 cm\(^{-1}\), corresponding to C–OH deformation vibration; and 1384 cm\(^{-1}\), corresponding to C–C–H and O–C–H deformation. Shoulders at 1260 cm\(^{-1}\) correspond to S=O stretching vibration of the sulfate. The bands in the 1064 cm\(^{-1}\) region correspond to C–O and C–C stretching vibrations, and those in the 820 cm\(^{-1}\) region correspond to the C–O–S vibration of sulfate groups.

The GPC chromatogram (Figure 2b) shows that the concentrated product is distributed in fractions higher than 50 kDa and a smaller fraction between 5 and 12 kDa, whereas the original hydrolysate included a wider distribution of different molecular weight fractions, also corresponding to low polymerization degrees [16].

![Figure 1](image1.png)

**Figure 1.** Flowchart of the extraction and concentration of the *Sargassum muticum* concentrated extract (SmE).

![Figure 2](image2.png)

**Figure 2.** (a) The Fourier transform infrared spectroscopy (FT-IR) spectra of the concentrated product and (b) gel permeation chromatography (GPC) chromatogram of hydrolysate after autohydrolysis (blue line) and concentrated product (green line).
2.2. Effect of Administration

2.2.1. Influence on Weight

Administration of *S. muticum* extracts did not affect the rate of increase in body weight of rats during the growth period (Table 1) and there were very few modifications considering the individual relative weight of organs in response to the administration of all three doses. However, in a similar study, Zaragozá et al. [24] observed an increase in the relative weight of the kidneys of rats administered in a dose of 750 mg kg\(^{-1}\) of *Fucus vesiculosus* extracts, whereas administration of 200 mg kg\(^{-1}\) did not modify this parameter. In another study, the administration of an *Ulva rigida* extract to Wistar rats during a period of five weeks did not alter the body weight nor the triglycerides when compared to the control group, although the total cholesterol levels were lower [25]. In the present study, no significant differences in either the total cholesterol levels or in the different fractions between the control and treated groups were observed. However, although both the level of glycaemia and triglycerides in plasma decreased in the rats fed with the *Sargassum* extract, the reduction was not significant. Tas et al. [25] suggested that flavonoids and other phytochemicals may lower plasma levels of glucose as the serum concentration of insulin increases. Daech et al. [26] reported that the ability of polysaccharides to reduce glycaemia levels may be due to modulation of the immune system and because they can improve the production of short-chain fatty acids by the intestinal microbiota thereby inhibiting the absorption of intestinal glucose.

Table 1. Trophic balance in rats control and *Sargassum muticum* extract treated groups. Values are mean ± SEM. Number of animals per group = 6.

<table>
<thead>
<tr>
<th>Trophic Balance</th>
<th>Control</th>
<th>0.5 g kg(^{-1})</th>
<th>1.0 g kg(^{-1})</th>
<th>2.0 g kg(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain (g day(^{-1}))</td>
<td>6.79 ± 0.15</td>
<td>7.02 ± 0.11</td>
<td>6.93 ± 0.11</td>
<td>6.79 ± 0.22</td>
</tr>
<tr>
<td>Liver/body weight (%)</td>
<td>3.30 ± 0.04</td>
<td>3.43 ± 0.07</td>
<td>3.35 ± 0.08</td>
<td>3.42 ± 0.04</td>
</tr>
<tr>
<td>Kidney/body weight (%)</td>
<td>0.86 ± 0.00</td>
<td>0.79 ± 0.02 *</td>
<td>0.79 ± 0.02 *</td>
<td>0.81 ± 0.01 *</td>
</tr>
<tr>
<td>Intestinal mucosa/body weight (%)</td>
<td>0.85 ± 0.01</td>
<td>0.87 ± 0.03</td>
<td>0.74 ± 0.02</td>
<td>0.80 ± 0.05</td>
</tr>
<tr>
<td>Heart/body weight (%)</td>
<td>0.41 ± 0.00</td>
<td>0.41 ± 0.00</td>
<td>0.42 ± 0.01</td>
<td>0.39 ± 0.01</td>
</tr>
<tr>
<td>Lung/body weight (%)</td>
<td>0.55 ± 0.01</td>
<td>0.48 ± 0.03</td>
<td>0.59 ± 0.02 *</td>
<td>0.53 ± 0.07</td>
</tr>
<tr>
<td>Stomach/body weight (%)</td>
<td>0.62 ± 0.03</td>
<td>0.59 ± 0.02</td>
<td>0.57 ± 0.02</td>
<td>0.64 ± 0.02</td>
</tr>
</tbody>
</table>

* Significant difference from the control group at \(p < 0.05\).

2.2.2. Influence on Serum Parameters

Seaweed supplementation can lower blood levels of triglycerides in diabetes patients [27]. This effect may occur via the inhibition of fatty acid synthesis by fermentation products or by changes in intestinal motility [28]. Increased levels of high-density lipoprotein and decreased levels of triglycerides are positive effects that may reduce the incidence of cardiovascular diseases.

In this respect, the reduction in lipid peroxidation induced by the ingestion of the *Sargassum* extract may reduce the deleterious effects due to free radicals. No significant differences in the levels of the other serum parameters analyzed were observed in relation to administration of *S. muticum* extract (Table 2).
The total amount of lipid peroxidation products was assayed by quantifying thiobarbituric acid reactive substances. Variations in catalase activity were not observed in either liver or erythrocytes of rats administered the Sargassum extract under conditions of stress, but possibly do not have any effect under conditions of no stress.

In a similar study, erythrocytic TBARS levels of diabetic patients treated with seaweed extracts were lower than in controls; catalase and GSH-Px activities were higher in the treated group than in controls, but SOD was not affected by the seaweed supplement [27].

### Table 2. Serum parameters in control rats and Sargassum muticum treated groups. Values are mean ± SEM. Number of animals per group = 6.

<table>
<thead>
<tr>
<th>Serum Parameters</th>
<th>Control</th>
<th>0.5 g kg⁻¹</th>
<th>1 g kg⁻¹</th>
<th>2 g kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO total (mg dL⁻¹)</td>
<td>121.50 ± 3.96</td>
<td>118.17 ± 3.24</td>
<td>118.50 ± 5.36</td>
<td>114.83 ± 2.96</td>
</tr>
<tr>
<td>HDL (mg dL⁻¹)</td>
<td>49.50 ± 1.30</td>
<td>48.80 ± 1.53</td>
<td>48.10 ± 2.09</td>
<td>45.33 ± 1.15</td>
</tr>
<tr>
<td>LDL (mg dL⁻¹)</td>
<td>51.70 ± 2.94</td>
<td>50.70 ± 1.71</td>
<td>45.08 ± 2.27</td>
<td>46.43 ± 2.14</td>
</tr>
<tr>
<td>Triglycerides (mg dL⁻¹)</td>
<td>105.75 ± 6.21</td>
<td>98.23 ± 6.23</td>
<td>86.50 ± 5.70</td>
<td>97.17 ± 8.19</td>
</tr>
<tr>
<td>BUN (mg dL⁻¹)</td>
<td>11.25 ± 0.34</td>
<td>11.33 ± 0.76</td>
<td>13.00 ± 0.71</td>
<td>12.00 ± 0.95</td>
</tr>
<tr>
<td>Total protein (g dL⁻¹)</td>
<td>5.40 ± 0.05</td>
<td>5.57 ± 0.08</td>
<td>5.50 ± 0.11</td>
<td>5.37 ± 0.09</td>
</tr>
<tr>
<td>Albumin (g dL⁻¹)</td>
<td>4.15 ± 0.058</td>
<td>4.30 ± 0.05</td>
<td>4.13 ± 0.12</td>
<td>4.13 ± 0.03</td>
</tr>
<tr>
<td>GOT (UI L⁻¹)</td>
<td>127.50 ± 8.14</td>
<td>157.83 ± 11.46</td>
<td>167.50 ± 24.46</td>
<td>164.67 ± 19.81</td>
</tr>
<tr>
<td>GPT (UI L⁻¹)</td>
<td>23.25 ± 2.28</td>
<td>21.83 ± 4.00</td>
<td>26.00 ± 1.71</td>
<td>29.00 ± 3.97</td>
</tr>
<tr>
<td>Glucose (mg dL⁻¹)</td>
<td>100.80 ± 3.28</td>
<td>95.83 ± 3.06</td>
<td>90.78 ± 2.76</td>
<td>82.40 ± 2.55</td>
</tr>
</tbody>
</table>

Serum parameter abbreviations: CHO, total cholesterol; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; BUN, blood urea nitrogen; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase.

### 2.2.3. Influence on Antioxidant Biomarkers

**Enzymes**

Antioxidants can act by ROS scavenging or inhibiting lipid peroxidation. Endogenous antioxidant enzymes such as SOD, GPx and CAT provide protection against ROS. Among marine algae, the Sargassaceae family has been found to exhibit the highest radical scavenging activity [29].

In the present study, we determined the activities of the antioxidant enzymes SOD, GPx and CAT in liver and red blood cells of the rats in relation to administration of the S. muticum extract. The total amount of lipid peroxidation products was assayed by quantifying thiobarbituric acid reactive substances (TBARS), which are often used as oxidation markers.

Data in Table 3 summarize the influence of the extract on the lipid peroxidation and antioxidant enzymes. Results indicate a significant reduction of 62.68% in liver GPx (U·mg⁻¹ prot.) and of 45.27% in TBARS levels for the SmE fed rats at 0.5 and 1 g Kg⁻¹, respectively, but no effect was shown on SOD activity in either liver or erythrocytes. In a similar study, erythrocytic TBARS levels of diabetic patients supplemented with seaweeds were lower than in controls; catalalase and GSH-Px activities were higher in the treated group than in controls, but SOD was not affected by the seaweed supplement [27].

### Table 3. Activity of lipid peroxidation and antioxidants enzymes in control rats and Sargassum muticum extract-treated groups. Values are mean ± SEM. Number of animals per group = 6.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Control</th>
<th>0.5 g kg⁻¹</th>
<th>1.0 g kg⁻¹</th>
<th>2.0 g kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD hemolized (U·mg⁻¹ Hb)</td>
<td>11.33 ± 2.02</td>
<td>9.00 ± 1.81</td>
<td>14.46 ± 2.78</td>
<td>13.27 ± 3.57</td>
</tr>
<tr>
<td>SOD liver (U·mg⁻¹ prot.)</td>
<td>35.09 ± 1.80</td>
<td>34.55 ± 1.24</td>
<td>32.71 ± 1.18</td>
<td>35.51 ± 0.96</td>
</tr>
<tr>
<td>GPx hemolized (U·mg⁻¹ Hb)</td>
<td>1422.02 ± 162.87</td>
<td>1036.83 ± 183.74</td>
<td>1568.59 ± 212.84</td>
<td>1368.04 ± 283.80</td>
</tr>
<tr>
<td>GPx liver (U·mg⁻¹ prot.)</td>
<td>1306.18 ± 39.35</td>
<td>536.75 ± 69.13 *</td>
<td>467.49 ± 35.90 *</td>
<td>528.55 ± 44.6 *</td>
</tr>
<tr>
<td>CAT hemolized (μmol H₂O₂·mg⁻¹ Hb)</td>
<td>752.60 ± 95.72</td>
<td>630.82 ± 143.89</td>
<td>894.28 ± 167.33</td>
<td>726.53 ± 182.14</td>
</tr>
<tr>
<td>CAT liver (μmol H₂O₂·mg⁻¹ Hb)</td>
<td>1551.92 ± 38.42</td>
<td>1450.28 ± 37.02</td>
<td>1460.20 ± 61.52</td>
<td>1458.94 ± 35.48</td>
</tr>
<tr>
<td>TBARS liver (nmol MDA·mg⁻¹ prot)</td>
<td>2.43 ± 0.21</td>
<td>1.33 ± 0.09 *</td>
<td>1.53 ± 0.08 *</td>
<td>1.82 ± 0.12</td>
</tr>
</tbody>
</table>

* Significant difference from the control group at p < 0.05.

Catalase affects cellular resistance to toxicity induced by hydrogen peroxide and it is responsible for eliminating H₂O₂. The ROS scavenging activity of SOD is therefore consistent with the action of CAT because SOD generates H₂O₂, which must be scavenged by CAT [24,30]. However, significant variations in catalase activity were not observed in either liver or erythrocytes of rats administered the Sargassum extracts. The antioxidant effects of diverse substances may be particularly low under conditions of stress, but possibly do not have any effect under conditions of no stress.
Other Antioxidant Compounds

Potential antioxidant compounds are widely distributed in seaweeds and act by scavenging ROS and inhibiting peroxidation.

Guerra Dore et al. [31] tested various doses of isolated fucan from Sargassum vulgare and observed anticoagulant, antithrombotic and anti-inflammatory activity, but low antioxidant activity. In a similar way, Kellogt and Lila [32] tested the ability of fractionated extracts of marine algae to scavenge the DPPH radical. The red alga Pyropia fallax and the green alga Ulva lactuca did not show any detectable antioxidant activity. However, some species of brown algae have been found to display a high capacity to scavenge ROS and lower NO production. Marudhupandi et al. [33] found that the antioxidant activity of fucoidan depended on the sulphate content of the extract. There appears to be some variability in the antioxidant capacity of algal extracts and their sulphate content [34].

Some products with low phenolic content have shown antioxidative effects. It is therefore possible that other compounds may also contribute to the antioxidant properties of seaweeds [35]. Muroney et al. [36] did not observe any differences in the levels of oxidation of extrahepatic tissue in pigs fed diets supplemented with extracts of Laminaria spp. This led these authors to consider that three weeks of dietary supplementation was not sufficient to induce significant changes. The findings of a recent study by Zhao et al. [6] indicated that phlorotannin extracted from Sargassum hemiphyllum exerts antioxidant activity against induced oxidative damage in the liver, kidney and brain.

In the present study, the fact that the administration of the Sargassum extract significantly decreased lipid peroxidation may have led to a parallel reduction in the mechanisms of defense against oxidation. Further studies are required in order to determine if polysaccharides and/or phlorotannins from algal extracts display antioxidant activity, how the action is exerted and whether the effect is only manifested under conditions of induced stress.

3. Materials and Methods

3.1. Materials

Specimens of Sargassum muticum were collected by hand from Mourisca beach (Alcabre, North-West of Spain) in June 2012 and stored at −18 °C until use. Sampling was carried out in June because the concentrations of phenolics and fucoidan in this species are maximal in Summer [11,16,37]. Before use, the algal material was defrosted, cleaned, rinsed with tap water, and minced.

3.2. Extraction and Concentration

Briefly, the autohydrolysis process was carried out as follows: Four hundred g of dried equivalent algal material was mixed with water, at a liquid: Solid ratio of 30:1 (w/w, dry basis), and was heated in a 12 L stainless steel reactor PARR 4843 (Parr Instr. Co., Moline, IL, USA) until reaching a temperature of 187 °C (Simpson severity factor of autohydrolysis process = 3.46). Once the target temperature was reached, the reactor was immediately cooled, and the liquid and solid phases were separated by vacuum filtration with a Büchner funnel. The process was repeated until 50 L of liquid extract was obtained.

Consecutively, the liquid extract obtained in the autohydrolysis process was subjected to membrane concentration. Firstly, it was passed through a spiral polymeric microfiltration membrane (diameter 60.7 mm, length 101.6 cm; Iberlact, Spain) to remove macroparticles. The remaining alginate of the permeate was precipitated by adding 1% of dry equivalent CaCl₂ dihydrate (99%, ACS reagent, Acros Organics™, Belgium) and stirring overnight. Then, the supernatant (4 L) was concentrated to half volume by passing it through a 1KDa vertical spiral regenerated cellulose membrane (diameter 56 mm, length 39.9 cm, Prep/Scale TFF 6 Cartridge; EMD Millipore, Burlington, MA, USA). The permeate was stored and the process was repeated in order to concentrate all the liquid extract obtained by autohydrolysis. Permeates from repeated batches were pooled, as were the concentrates. Finally, the pooled concentrate was mixed with water (1:1) and further subjected to repeated diafiltration.
through the same (cleaned) 1 KDa membrane to remove salts. Diafiltration was monitored by
determining the salt content of the retentate, monitored by comparing the pH of the liquid extract
against a pH–CaCl₂ concentration standard curve. The process was stopped after seven diafiltration
cycles, when the concentration of the retentate reached values lower than 2 g CaCl₂ equivalent L⁻¹.

The final retentate (R7) was freeze-dried and stored until analysis. A flow chart of the process is shown
in Figure 1.

3.3. Experimental Animals and Diets

The study followed the general guidelines for the care and use of laboratory animals,
as recommended by the Council of European Communities (Council of European Communities,
1986). All experiments were approved by the Ethics Committee of the University of Santiago de
Compostela (15007AE/09/FUN 01/FIS [02]). Male Sprague-Dawley rats of initial body weight 90–100 g,
obtained from the Animal Service Centre of the University of Santiago de Compostela, were housed in
an air-conditioned room, which was maintained at a temperature of approximately 22 °C, with a 12 h
light/12 h dark cycle and free access to food and water. The rats were fed a standard powdered A04
diet (Scientific Animal Food & Engineering, Augy, France) and were divided into four groups. After an
acclimation period of five days before the start of the experiment, the control group was administered
distilled water via an intragastric tube. The other three groups were administered the
S. muticum extract, dissolved in distilled water to produce doses of 0.5, 1.0 or 2.0 g kg⁻¹, also via an intragastric
tube. The extracts (or distilled water) were administered daily for three weeks.

At the end of the experimental period, the rats were fasted overnight and killed by decapitation
with a guillotine. The liver, kidney, stomach, lungs and heart were removed immediately, rinsed with
cold 0.9% (w/v) NaCl buffer, dried and weighed. The intestines were also extracted and scraped to
obtain the intestinal mucosa.

3.4. Serum Biochemical Parameters

At the end of the experimental period, a sample of blood was obtained from each rat and
centrifuged at 1300 g for 10 min. The serum obtained was used for biochemical determinations with a
Spotchem SP-4410 autoanalyser.

3.5. Preparation of Tissues for Measurement of Antioxidant Levels

3.5.1. Preparation of Liver Samples

Samples of liver were homogenized (1:4 w/v) in ice-cold 0.25 M sucrose solution, in an Ultraturrax
T-25 homogenizer (Ika, Germany). The homogenate was centrifuged at 100,000× g for 60 min at 4 °C
in a Beckman ultracentrifuge. The supernatant fraction was decanted and used to determine SOD
and GPx activities. For the catalase assay, the homogenate containing 1 mL of 0.9% (w/v) NaCl was
centrifuged at 1000× g for 10 min at 4 °C. Protein concentrations in the supernatants were measured
using bovine serum albumin as the reference standard [38].

3.5.2. Blood Sample Preparation

Immediately after the rats were decapitated, blood samples were collected in heparinized tubes.
The heparinized blood was centrifuged for 10 min at 3000× g and 4 °C to obtain erythrocytes. The buffy
coat on the erythrocyte sediment was separated carefully after the plasma was removed. The erythrocyte
sediment was washed three times with two volumes of a cooled isotonic NaCl solution to remove the
plasma remnant. After each procedure, the erythrocyte-saline mixture was centrifuged at 3000× g for
10 min at 4 °C. The erythrocytes were then lysed with cold distilled water (1:1), vortexed for 1 min, and
stored in a refrigerator at 4 °C for 15 min. The cell debris was removed by centrifugation at 4500× g for
15 min. The haemolysate was stored at −80 °C until being assayed. The haemoglobin concentration in
the haemolysate was determined by the cyanmethemoglobin method [39].
3.6. Measurement of Lipid Peroxidation Levels

Lipid peroxidation was assayed by the thiobarbituric acid reactive substances (TBARS) procedure [40]. Samples (0.5 mL) previously treated with 25 µL of butylated hydroxytoluene (1% w/v in glacial acetic acid) were mixed with 0.2 mL of sodium laurylsulphate (8% w/v), 1 mL of acetic acid (20% w/v) and 1 mL of 0.8% (w/v) 2-thiobarbituric acid, and the mixture was heated at 95 °C for 30 min. The resulting chromogen was extracted with 3 mL of n-butyl alcohol and centrifuged at 1000×g for 10 min. The absorbance of the organic phase was measured at 532 nm in a spectrophotometer, with 1, 1, 3, 3-tetraethoxy propane as the reference standard.

3.7. SOD Measurement

SOD (EC 1.15.1.1) activity was determined by a modified version of the method described by Beauchamp and Fridovich [41]. The activity was measured with the RANSOD test kit (Randox Laboratories Ltd., Crumlin, UK), which is based on the ability of the test substance to prevent formation of formazan from 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) by superoxide radicals generated by xanthine oxidase/xanthine. Formazan formation was recorded spectrophotometrically at 505 nm. Inhibition of 50% of the INT oxidation after reduction of the sample was defined as one unit of SOD activity.

3.8. Measurement of GPx Activity

GPx (EC 1.11.1.9) activity was assayed with Ransel test kits (Randox Laboratories Ltd., UK) [42]. Conversion of NADPH to NADP⁺ was monitored continuously in a spectrophotometer at 340 nm for 3 min. GPx activity was expressed as moles of NADPH oxidized to NADP⁺ per minute per milligram of protein of the sample.

3.9. Measurement of Catalase Activity

Catalase (EC 1.11.1.6) activity was determined spectrophotometrically by a modified version of the method described by Aebi [43], based on the measurement of the hydrogen peroxide substrate remaining after the catalase reaction. Catalase first converts hydrogen peroxide to water and oxygen, and the enzymatic reaction is stopped with sodium azide. An aliquot of the reaction mixture is then assayed for the remaining amount of hydrogen peroxide, by a colorimetric method that uses a substituted phenol (3, 5-dichloro-2-hydroxybenzenesulfonic acid), which oxidatively couples to 4-aminantipyrine in the presence of hydrogen peroxide and horseradish peroxidase (HRP). The amount of red quinoneimine dye (N-(4-antipyryl)-3-chloro-5-sulfonate-p-benzoquinone-monoimine) produced was quantified at 520 nm. The concentration of hydrogen peroxide used in this assay (50 mm) provides a measurable signal, but does not cause inactivation of the enzyme. The catalase activity was expressed as micromoles of H₂O₂ decomposed per minute per milligram of protein.

4. Conclusions

*Sargassum muticum* algal material is a potential feedstock that can be processed by autohydrolysis and ultrafiltration with membranes to obtain fucoidan and phlorotannin-rich extracts, with low mineral content, antiradical capacity equivalent to that of Trolox, and in vivo antioxidant potential in animal studies (decreased serum glucose, reduced liver GPx and TBARS levels with no body and organ weight gain).

5. Statistical Analysis

Data were expressed as mean values and the corresponding standard errors (SEM). Statistical analysis was carried out with SPSS, version 17.0, for Windows (Chicago, IL, USA). The results were analyzed by one-way analysis of variance (ANOVA) followed by a post hoc Tukey’s test (when significant differences were detected). Differences were considered significant at *p* < 0.05.

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