Food Research International 191 (2024) 114641

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Exploring the fate of phlorotannins from *Laminaria digitata* across the gastrointestinal tract: Insights into susceptibility and bioactivity prior and post gastrointestinal digestion

Marcelo D. Catarino [a], Carlos J. García [b], Rocío García-Villalba [b], Artur M.S. Silva [a], Débora A. Campos [c], Maria Manuela Pintado [c], Bruno Neves [d], Susana M. Cardoso [a,*], Francisco A. Tomás-Barberán [b]

[a] *LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal*

[b] *Research Group on Quality, Safety and Bioactivity of Plant Foods. Department of Food Science and Technology, CEBAS-CSIC, PO Box 164, Espinardo, Murcia 30100, Spain*

[c] *CBQF-Centro de Biotecnologia e Química Fina–Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal*

[d] *Department of Medical Sciences and Institute of Biomedicine—iBiMED, University of Aveiro, 3810-193 Aveiro, Portugal*

ARTICLE INFO

Keywords:
Laminaria digitata
Phlorotannins
Brown macroalgae
Gastrointestinal tract
Marine bioactives
Antioxidant
Anti-inflammatory

ABSTRACT

Phlorotannins are phenolic compounds exclusive from brown macroalgae endowed with promising bioactive properties. However, considering that diet is their main route of entrance to our system, gastrointestinal digestion might affect such bioactive properties. Here, phlorotannin extracts obtained from *Laminaria digitata* were submitted to simulated gastrointestinal digestion to evaluate its impact on their antioxidant and anti-inflammatory properties. Overall, a reduction of the total phlorotannin content along the gastrointestinal tract was noticed, although the antioxidant activity measured *in vitro* via NO$^{\bullet}$ and O$_2^{\bullet-}$ scavenging assays, maintained almost the same. The crude extract (70 % v/v acetone) exhibited superior inhibition of NO$^{\bullet}$ release on lipopolysaccharide-stimulated cells after digestion. In contrast, the opposite occurred to the phlorotannin-purified extract, indicating that the digestive process favors the anti-inflammatory properties of the former but not the latter. Data collected from UHPLC-MS analysis revealed that the fuhalol and carmalol-type compounds were completely absent from the digested phlorotannin-purified extract, which could partly explain its lower anti-inflammatory activity compared with its non-digested counterpart. Overall, this study contributes to a better understanding of the impact of gastrointestinal digestion on the bioactivity profile of *L. digitata* phlorotannins, demonstrating that fuhalols and carmalols are particularly susceptible to the digestive process.

1. Introduction

Laminaria digitata, commonly known as oarweed, is a species of brown seaweeds widely distributed along the cold North Atlantic and Arctic oceans (Baweja, Kumar, Sahoo, & Levine, 2016), being one of the most economically important species produced/harvested in Europe and pointed out as one of the key seaweeds to be grown in 2030 (Vincent, Stanley, & Ring, 2020). This species has primarily been used as a source of alginate. However, it can also be used as a food source of great nutritional value, containing multiple potentially beneficial bioactive compounds (Purcell-Meyerink, Packer, Wheeler, & Hayes, 2021). Indeed, *L. digitata* stands out as a prolific source of phlorotannins, offering a rich reservoir of these bioactive compounds.

Phlorotannins constitute a class of structurally unique phenolic compounds that can only be found in brown seaweeds. Their biogenesis is attributed to the Golgi apparatus and the endoplasmic reticulum, which has been seen to produce small vesicles called physodes, known to be the reservoir of soluble phlorotannins (Erpel, Mateos, Pérez-Jiménez, & Pérez-Correa, 2020). The phloroglucinol monomeric unit is synthesized via the acetate-malonate pathway, and its condensation gives rise to chains and net-like structures with diverse molecular weights that can range from 126 Da to 650 kDa. Depending on the linkages established

---

* Corresponding author.
E-mail address: susanacardoso@ua.pt (S.M. Cardoso).

https://doi.org/10.1016/j.foodres.2024.114641
Received 22 February 2024; Received in revised form 11 June 2024; Accepted 11 June 2024

between units (phenyl, ether, or dibenzodioxin) and the presence of additional OH groups they can be divided into fucols, phlorethols, fucophlorethols, eckols, fuhalols and carmalols as exemplified in Fig. 1 (Shrestha, Zhang, & Smid, 2021).

Phlorotannins serve essential physiological roles in algae as integral components of the cell wall and protective agents against several biotic and abiotic stresses (e.g., grazing, bacteria, UV radiation, oxidative stress, or heavy metals) (Li, Fu, Duan, Liu, Xu, & Gao, 2017). Moreover, these compounds have attracted considerable research interest lately, and they hold a broad range of promising health benefits with great potential for application in food, pharma, cosmetics, and other industries. In fact, over the last years, much research has been done addressing the capacity of phlorotannins to regulate relevant physiological processes that affect body functions, metabolism, inflammation, and cell proliferation. Antioxidant, anti-inflammatory, antidiabetic, anticancer, antibacterial, and anti-aging are some potential health benefits identified (Meng, Mu, Sun, & Garcia-Vaquero, 2021).

However, their ability to act as effective bioactive agents *in vivo* must consider the extent of biotransformation they may suffer during their passage through the gastrointestinal tract, especially considering that, in general, the main point of entrance of polyphenols into our organism is through diet. While the bioavailability of polyphenols from terrestrial plants and the mechanisms underlying their beneficial effects *in vivo* are quite well understood, there is still very limited knowledge in this field of phlorotannins. Some studies have reported a progressive decrease in the phlorotannin concentrations along the *in vitro* gastrointestinal digestion (GID), paralleled by the loss of antioxidant activity (Catarino; et al., 2022; Catarino; et al., 2021; Vazquez-Rodriguez et al., 2021). Contrastingly, a previous study from our group demonstrated that after submitting a phlorotannin extract from *Himanthalia elongata* to an *in vitro* GID model, it was able to inhibit the $NO^{\bullet}$ release on Raw 264.7 cells stimulated with lipopolysaccharide (LPS) more effectively than its equivalent non-digested sample. We hypothesized that the digestive process might induce structural changes in the compounds that favor their anti-inflammatory properties.

In this context, this work aims to study how the GID can influence the bioactive properties (antioxidant and anti-inflammatory) and phlorotannin profile of a phlorotannin extract from *L. digitata*, and how the differences in terms of phlorotannin composition between digested and non-digested extracts can explain the different bioactive properties.

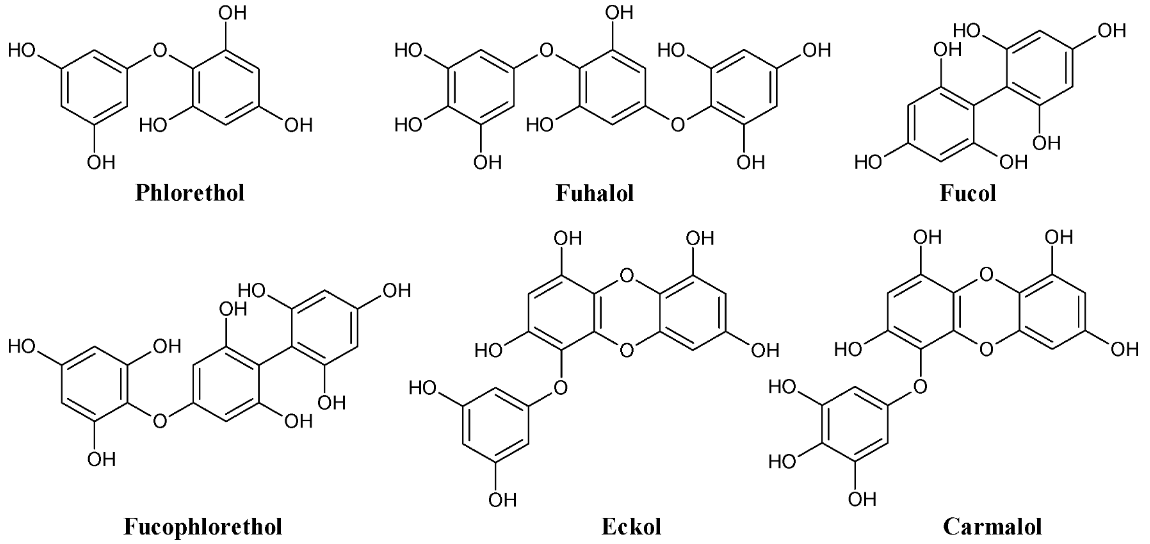
2. Material and methods

2.1. Extraction procedure

The extraction was performed as previously described (Chouh, Nouadri, Catarino, Silva, & Cardoso, 2022). Briefly, 30 g of dried *L. digitata* powder (Algamar, Pontevedra, Spain) was dispersed in 2.1 L of 70 % acetone (Fisher, Pittsburgh, PA, USA), containing 1 % glacial acetic acid (Fisher, Pittsburgh, PA, USA), under constant agitation and at room temperature for 3 h. The mixture was subsequently filtered through cotton and then through a G4 glass filter, generating the crude extract. This extract was concentrated in a rotary evaporator to approximately 250 mL and defatted, using *n*-hexane (1:1, v/v; Fisher, Pittsburgh, PA, USA) several times—until a colorless non-polar fraction was obtained—and the aqueous phase was subjected to liquid–liquid extraction with ethyl acetate (1:1, v/v; Fisher, Pittsburgh, PA, USA) three times, to obtain a phlorotannin-purified fraction. The solvent was finally removed by rotary evaporation. Both samples were freeze-dried and stored at − 20 °C until further use.

2.2. Simulation of the gastrointestinal digestion

The simulation of the GID of the *L. digitata* samples was performed following the method described elsewhere (Bonifacio-Lopes et al., 2022). To simulate the oral digestion, 1 g of dried sample (crude extract or ethyl acetate fraction) was suspended in 20 mL of distilled water, followed by the adjustment of the pH to between 5.6 and 6.9, with 1 M $NaHCO_3$ (Sigma-Aldrich, St. Louis, MO, USA), before the addition of 0.6 mL min^{-1} of α-amylase (Sigma-Aldrich, St. Louis, MO, USA) at 100 U/mL. Enzymatic digestion was carried out during 2 min of mastication, at 37 °C and 200 rpm. Before moving to the next compartment, the pH of the mouth digest was adjusted to 2.0, using 1 M HCl (Fisher, Pittsburgh, PA, USA), and then mixed with a simulated gastric juice consisting of 25 mg mL^{-1} of pepsin (Sigma-Aldrich, St. Louis, MO, USA) added at a ratio of 0.05 mL mL^{-1} of mouth digest. Incubation was carried out over 60 min at 37 °C and 130 rpm. Finally, for intestinal digestion, the pH of the gastric digest was adjusted to 6.0, using 1 M $NaHCO_3$, prior to the addition of a simulated intestinal juice consisting of 2 g/L of pancreatin (Sigma-Aldrich, St. Louis, MO, USA) and 12 g/L bile salts (Sigma-Aldrich, St. Louis, MO, USA) at a ratio of 0.25 mL mL^{-1} of the gastric digest. The samples were then incubated for 120 min, at 37 °C and 45 rpm, to mimic a long intestinal-digestion process. Before and after each step of the digestion, *i.e.*, oral, gastric and intestinal steps, aliquots of 1



Phlorethol

Fuhalol

Fucol

Fucophlorethol

Eckol

Carmalol

Fig. 1. Representation of the different classes of phlorotannins.

mL were taken, freeze-dried and stored until further use.

2.3. Quantification of total phlorotannins

The estimation of the total phlorotannin content in the samples was performed according to the 2,4-dimethoxybenzaldehyde (DMBA) colorimetric method, as described before (Amarante, Catarino, Marcal, Silva, Ferreira, & Cardoso, 2020). For this method, DMBA (Sigma-Aldrich, St. Louis, MO, USA) at 2 % (w/v) was mixed with HCl (Fisher, Pittsburgh, PA, USA) 6 % (v/v), both prepared in glacial acetic acid (Fisher, Pittsburgh, PA, USA), in a proportion of 1:1. Next, 250 μL of this solution was mixed with 50 μL of each sample (250 μg mL^{-1} solubilized in DMSO) in a 96-well plate which was incubated in the dark and at room temperature for 60 min. Finally, the absorbance was read at 515 nm and the phlorotannin content was determined using a regression equation of the phloroglucinol (Sigma-Aldrich, St. Louis, MO, USA) linear calibration curve. The results were expressed as g phloroglucinol equivalents/100 g extract.

2.4. Radical scavenging experiments

The radical scavenging activity of the samples was analyzed via nitric oxide radical and superoxide radical ($NO^{\bullet}$ and $O_2^{\bullet-}$, respectively) scavenging methods, following the previously described procedures (Catarino; et al., 2022). For $NO^{\bullet}$, 100 μL of six different sample concentrations were mixed with 100 μL of sodium nitroprusside (3.33 mM in 100 mM sodium phosphate buffer pH 7.4; Acros Organics, Hampton, NH, USA) and incubated for 15 min under a fluorescent lamp (Tryun 26 W). Next, 100 μL of Griess reagent (0.5 % sulfanilamide from Acros Organics, Hampton, NH, USA, and 0.05 % *N*-(1-naphthyl)ethylenediamine dihydrochloride from VWR, Radnor, PA, USA, in 2.5 % H_3PO_4 from Fisher, Pittsburgh, PA, USA) were added to the mixture, which was incubated for another 10 min at room temperature (RT) in the dark. The absorbance was then measured at 562 nm, and the $NO^{\bullet}$ scavenging capacity was calculated as the sample concentration capable of scavenging 50 % of the radical (IC_{50}).

For the $O_2^{\bullet-}$, 75 μL of six different sample concentrations were mixed with 100 μL of 300 μM β-NADH (Sigma-Aldrich, St. Louis, MO, USA); 75 μL of 200 μM nitroblue tetrazolium (NBT; Sigma-Aldrich, St. Louis, MO, USA); and 50 μL of 15 μM phenazine methosulfate (PMS; Sigma-Aldrich, St. Louis, MO, USA), in a 96-well plate. After 5 min, the absorbances at 560 nm were recorded, and the IC_{50} was calculated. Ascorbic acid and gallic acid (Sigma-Aldrich, St. Louis, MO, USA) were the reference compounds for $NO^{\bullet}$ and $O_2^{\bullet-}$, respectively.

2.5. Cellular experiments

2.5.1. Cell culture

Raw 264.7 cells, a mouse leukemic monocyte macrophage cell line (ATCC TIB-71), were cultured according to the conditions previously described (Catarino, Silva, Cruz, Mateus, Silva, & Cardoso, 2020), *i.e.*, in Dulbecco's modified eagle (DMEM; Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10 % fetal bovine serum (Gibco, Paisley, UK), 100 U/mL penicillin and 100 μg/mL streptomycin (Sigma-Aldrich, St. Louis, MO, USA), at 37 °C in a humidified atmosphere of 95 % air and 5 % CO_2. Along with the experiments, the medium was replaced every two days, and cells were monitored by microscopy to detect any morphological change.

2.5.2. Cell viability

The effect of each sample on cell viability was evaluated according to the AlamarBlue assay as commonly performed in our lab (Amarante et al., 2020). Briefly, cells (6×10^4 cells/well) were plated in 96-well plates and allowed to adapt overnight, after which serial dilutions of each sample reconstituted in DMEM with 0.5 % dimethyl sulfoxide (DMSO; Fisher, Pittsburgh, PA, USA) were added. The concentration of DMSO used was shown to have minimal impact on Raw 264.7 viability (Jamalzadeh et al., 2016). After 24 h incubation, 50 μM resazurin (Sigma-Aldrich, St. Louis, MO, USA) was added to the cells, 3 h before recording the absorbance at 570 nm, with a reference wavelength of 620 nm, using a standard spectrophotometer. The results were expressed relative to untreated cell viability.

2.5.3. Inhibition of lipopolysaccharide-stimulated NO• production

The effects of the extracts on the lipopolysaccharide (from Escherichia coli—serotype 026:B6; Sigma-Aldrich, St. Louis, MO, USA) −triggered nitrite production in Raw 264.7 cells was measured using a colorimetric reaction, with Griess reagent, as described elsewhere (Catarino et al., 2020). For this, cells were plated and treated with the *L. digitata* samples, as described above in Section 2.5.2. After 1 h of sample exposure, 50 ng/mL of lipopolysaccharide were added to each well (except for the negative control) and incubated for 24 h. The supernatants were then collected and mixed with equal volumes of Griess reagent. After 30 min, in the dark, the absorbance was recorded at 550 nm, and the percentage of nitrite production was calculated based on the LPS-treated cells (positive control).

2.6. HPLC-DAD-ESI-MS^n analysis

Analyses of the ethyl acetate fraction before and after gastrointestinal digestion (intestinal phase) were carried out on an Agilent 1100 HPLC system equipped with vacuum degasser (G1322), autosampler (G1313A), binary pump (G1322A) and ultraviolet–visible diode array detector (UV–Vis DAD) (Agilent Technologies, Aldbronn, Germany). The HPLC system was coupled in series to the ion trap (IT) mass spectrometer, model Esquire 1100, equipped with an electrospray interface (ESI) (Agilent Technologies, Aldbronn, Germany). Phlorotannins were separated on a reverse phase Poroshell 120 EC- C18 column (100 × 3 mm, 2.7 μm particle size), operating at room temperature and a flow rate of 0.3 mL min^{-1}. A volume of 15 μL of sample was injected. The mobile phases used were water with formic acid (1 %) (phase A) and acetonitrile (phase B) and the solvent gradient changed according to the following conditions: 0 min, 1 % B, 0 to 12 min, 1–10 % B; 12 to 27 min, 10–50 % B; 27–28 min, 50 % B; 28 to 29 min, 50 to 1 % B and maintained at 1 % till 33 min. The UV–Vis spectra were acquired in the range of 200 to 600 nm.

The mass spectrometer used nitrogen as a drying and nebulizing gas with the following parameters: nebulizer pressure was set at 65 psi, dry gas flow 11 L/min, and dry gas temperature 350 °C. Mass spectrometry data was acquired in the negative ionization mode. The capillary voltage was set at 4 kV. Mass scan (MS) and MS/MS spectra were measured in the *m/z* 100–1200 range at a scan speed of 13000 *m/z*/s, and the target mass was 745. The maximum accumulation time of the ion trap and the number of MS repetitions to obtain the MS average spectra were set at 50 and 3 ms, respectively. Compound stability was set at 15 %. Conditions for automatic MS/MS were: width of the isolation, 4.0; fragmentation amplitude, 1.00 V; and number of parents, 3. The identification of the compounds was carried out using their elution order in the HPLC chromatograms, molecular weight, and their MS/MS fragments.

3. Results

3.1. Total phlorotannin content of the L. digitata extract and the purified fraction

The extract obtained from *L. digitata* with 70 % acetone accounted for approximately 26 % of the dry algal material, comprising a total phlorotannin content of 1.86 g phloroglucinol equivalents/100 g (Table 1). Interestingly, the total phlorotannin content herein estimated was substantially higher compared to what has been reported previously, in which the total phlorotannin content ranged between 0.1 to 0.5 g phloroglucinol equivalents/100 g (Heffernan, Smyth, Soler-Villa,

Table 1
Extraction yield (as % w/w of algal powder for crude extract and % w/w of crude extract for the fractions) and total phlorotannin content of *L. digitata* crude extract and further fractions.

Sample	Yield (%)	TPhC (g PGE/100 g)
CRD	26.10 ± 0.90	1.86 ± 0.14
HEX	10.10 ± 1.43	0.33 ± 0.17
EtOAc	18.29 ± 2.02	6.20 ± 0.55
AQ	67.99 ± 8.24	0.21 ± 0.01

CRD: Crude extract; HEX: Hexane fraction; EtOAc: Ethyl acetate fraction; AQ: Aqueous residue; TPhC: Total phlorotannin content; PGE: phloroglucinol equivalents.

Fitzgerald, & Brunton, 2015). This means that the total phlorotannin content values herein obtained are 4–6 times higher than those previously reported for this species. However, it should be noted that, in such studies, the extractions were conducted under different conditions. None of them included 70 % acetone, which has been repeatedly shown to be the best solvent for phlorotannins extraction (Koivikko, Loponen, Honkanen, & Jormalainen, 2005; Wang, Jonsdottir, & Olafsdottir, 2009).

After the liquid–liquid partitioning step, an ethyl acetate fraction with approximately three times more total phlorotannin content (6.20 g phloroglucinol equivalents/100 g sample) was obtained, which was an expected result since ethyl acetate has been commonly described as the solvent that retains the highest concentrations of phlorotannins in this kind of procedures (Liu & Gu, 2012; Wang et al., 2012).

3.2. *Phlorotannin stability and antioxidant activity throughout the gastrointestinal tract*

The effects of the gastrointestinal digestion in crude extract and ethyl acetate fraction in terms of total phlorotannin content and antioxidant activity were evaluated after each compartment of the gastrointestinal tract, *i.e.*, after oral, gastric and intestinal digestions. As depicted in Fig. 2A, both samples displayed a progressive decrease in the total phlorotannin content, representing a loss of approximately 40 % and 30 % for crude extract and ethyl acetate fraction, respectively, between the non-digested and digested samples at the intestinal phase. The transformation and metabolization of polyphenols along the gastrointestinal tract are a well-documented occurrence and have been demonstrated several times in various land plant phenolics (Quan et al., 2018; Sanchez-Velazquez, Mulero, Cuevas-Rodriguez, Mondor, Arcand, & Hernandez-Alvarez, 2021), and more recently in a few studies involving phlorotannins (Catarino; et al., 2022; Catarino; et al., 2021; Corona et al., 2016).

As expected, the antioxidant activity of the ethyl acetate fraction was more than twice as high as that of the crude extract at all times, which is coherent with total phlorotannin content differences observed between the two samples. However, it was interesting to note that the loss of phlorotannins along the gastrointestinal tract was accompanied by a loss of antioxidant activity in both $NO^{\bullet}$ (Fig. 2B) and $O_2^{\bullet-}$ (Fig. 2C) experiments at the intestinal phase only for the crude extract. In turn, although there is a slight decreasing trend in the antioxidant activity of the ethyl acetate fraction, this maintained a relatively constant scavenging effect throughout the different gut compartments, contrasting with previous observations from other species (Catarino; et al., 2022; Catarino; et al., 2021).

It is also interesting to note that when comparing with previous works, the ethyl acetate fraction from *L. digitata* herein studied was the least affected by the gastrointestinal digestion in terms of total phlorotannin content, *i.e.*, while previous works reported losses of approximately 50 % in *H. elongata* (Catarino; et al., 2022) and 75 % in *F. vesiculosus* (Catarino; et al., 2021), here the loss only accounted for 30 %. The differences in terms of phlorotannin profiles between each species could explain this. For example, the majority of the compounds detected on *F. vesiculosus* had polymerization degrees ranging between 4 and 8 phloroglucinol units, with compounds belonging mainly to the group of fucol, phlorethols or fucophlorethols (Catarino et al., 2019), while those found in *H. elongata* were predominantly oligomers with 7 to 12 phoroglucinol units with a significant presence of carmalols (Catarino; et al., 2022).

The compounds found in the *L. digitata* sample herein studied ranged between 3 and 9 phloroglucinol units with a significant presence of fuhalols as discussed below. It is also important to note that the distinction between fucols, phlorethols, and fucophlorethols via MS is difficult as these only differ on the type of linkages established between phloroglucinol units. Therefore, it is possible that these three species also have different compositions in these three groups. Naturally, all these differences in terms of phlorotannin composition are likely to contribute to the different responses to the gastrointestinal digestion and to the different observations in terms of antioxidant properties.

3.3. *Toxicity and anti-inflammatory properties along the gastrointestinal tract*

Inflammation is a common event occurring in virtually all intestinal diseases, including inflammatory bowel disease (Chron's disease, ulcerative colitis, microscopic colitis), irritable bowel disease, celiac disease, cancer (e.g., gastric, colon, rectal), and several others. Since sound $NO^{\bullet}$ scavenging effects were found in the experiments *in chemico*, the anti-inflammatory activity of both crude extract and ethyl acetate fraction were tested in a biological system of inflammation, *i.e.*, in Raw 264.7 macrophages stimulated with the Toll-like receptor 4 (TLR-4) agonist, lipopolysaccharide, to understand how non-digested and digested samples (at the intestinal phase) can display differences in terms of anti-inflammatory activity.

As depicted in Fig. 3, under normal conditions and the absence of lipopolysaccharide (negative control cells), the levels of $NO^{\bullet}$ were almost neglectable. In contrast, high levels of this radical were denoted in the LPS-treated cells, indicating the activation of macrophages. Upon

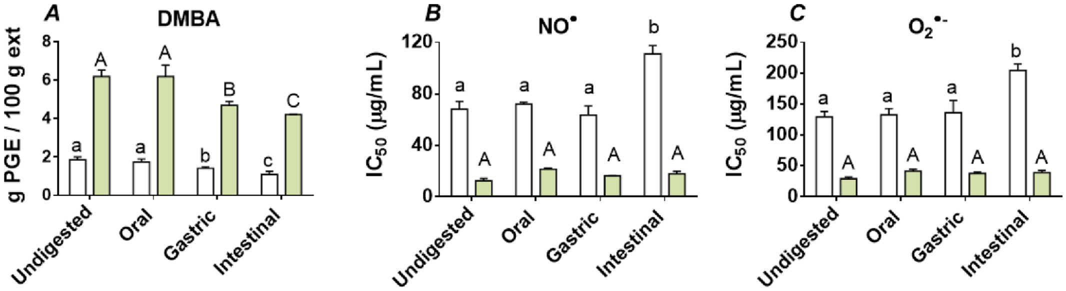


Fig. 2. Total phlorotannin content (graph A) and antioxidant activity (graphs B and C) of *L. digitata* CRD (white bars) and EtOAc fraction (green bars) through the different stages of GID. Different letters (a–c) indicate significant differences between the means of the white bars ($p < 0.05$). Different capital letters (A–C) indicate significant differeces between the means of the green bars ($p < 0.05$). PGE – phloroglucinol equivalents; ext – extract.

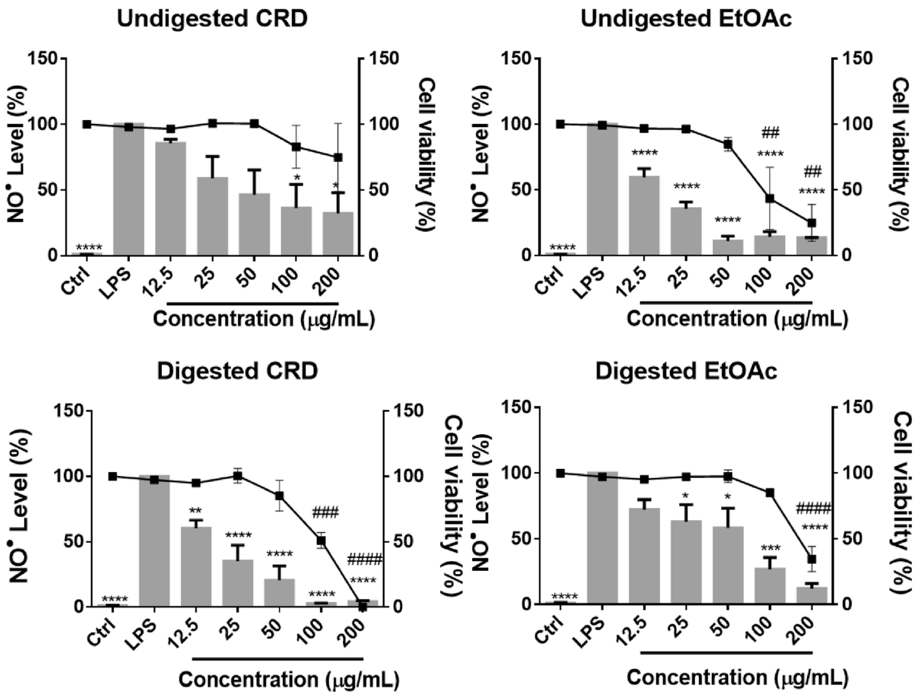


Fig. 3. Effects of *L. digitata* crude extract (CRD) and the respective purified ethyl acetate fraction (EtOAc) on the NO$^{\bullet}$ production (grey bars) and viability (■) of LPS-stimulated Raw 264.7 cells. Data represent the mean ± SEM from at least three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p < 0.0001$, indicate that NO$^{\bullet}$ production is significantly different from the positive control (with LPS), and $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ and $^{\#\#\#\#}p < 0.0001$ indicate that cell viability is statistically different from the negative control (CTRL, without LPS), as determined by one-way ANOVA, followed by Dunnet's post-hoc test.

treatment with the different *L. digitata* samples, each caused an apparent dose-dependent inhibitory effect of the LPS-induced NO$^{\bullet}$ release.

When looking at the non-digested samples, the inhibitory effect was much more pronounced in the ethyl acetate fraction than in the crude extract, as the former reduced the NO$^{\bullet}$ release by almost 90 % at 50 μg mL^{-1}. The latter reached approximately 50 % inhibition for the same concentration. For higher concentrations, both non-digested crude extract and ethyl acetate fraction revealed a reduction in cell viability of more than 20 %, which suggests that, for concentrations above 50 μg mL^{-1}, these samples might exert cytotoxic properties and consequently compromise the readings for the NO$^{\bullet}$ release. Interestingly, after the gastrointestinal digestion, contrasting results were observed, with the crude extract demonstrating higher inhibitory effects than the ethyl acetate fraction. In this case, for a concentration of 50 μg mL^{-1}, the crude extract was able to inhibit approximately 80 % of the LPS-induced NO$^{\bullet}$ while for the ethyl acetate fraction to achieve identical results, it requires double the concentration, even though the total phlorotannin content of the latter is four times higher. These results demonstrate that the correlation between phlorotannin concentration and inhibition of NO$^{\bullet}$ release does not apply in this case. That crude extract might contain other compounds that, upon gastrointestinal digestion, can exert more potent anti-inflammatory activity than phlorotannins. Interestingly, these results also contrast with those observed in the NO$^{\bullet}$ scavenging experiment, *i.e.*, after digestion, the scavenging properties of the ethyl acetate fraction remained constant, while those of the crude extract were considerably lower. Nevertheless, it is important to consider that several radical scavenging-independent mechanisms in cells, such as transcriptional activity, gene and/or protein expressions, enzyme activity, and several others can affect the release of NO$^{\bullet}$ (Lawrence, 2009). Upon GID, the compounds existing in both crude extract and ethyl acetate fraction may suffer modifications that can either enhance or diminish their ability to interfere with these mechanisms, thus explaining the contrasting observations.

Previous studies conducted with *H. elongata* reported that the extracts submitted to the gastrointestinal digestion were more effective at inhibiting the LPS-stimulated NO$^{\bullet}$ release than their non-digested counterparts (Catarino; et al., 2022), which is, in fact, consistent with the results here observed for the crude extract. The hypothesis was that the gastrointestinal digestion contributed to the breakdown of complex phlorotannins' structures and/or the formation of specific metabolites that might exert more potent inhibitory effects on the NO$^{\bullet}$ production/release. However, the results herein obtained show that ethyl acetate fraction from *L. digitata* does not follow the same behavior and, in this case, the gastrointestinal digestion did not favor the anti-inflammatory properties of this sample. As mentioned before, the phlorotannin profile observed for *H. elongata* has several differences in terms of total phlorotannin content and composition compared with the one herein observed for *L. digitata*, which could explain different sensitivities to gastrointestinal digestion and translate into different bioactive effects.

3.4. Characterization of the non-digested and digested phlorotannin-rich fraction

To further elucidate the impact of the gastrointestinal digestion on the phlorotannin profile of *L. digitata* and how these changes may influence the anti-inflammatory activity observed, an HPLC-ESI-MS^{n} analysis was conducted on the non-digested and digested ethyl acetate fraction (at the intestinal phase), as this was the sample with the higher phlorotannin content.

One of the most evident changes observed was the significant reduction of the prominent peak agglomerate that typically elutes at the end of the chromatogram (Figure S1) and is commonly associated with polymeric phlorotannins eluting all together. This suggests that gastrointestinal digestion may have played a role in the breakdown of these compounds or the formation of complexes between phlorotannins and other components of the gastrointestinal juices.

Overall, a total of 30 phlorotannins with polymerization degrees ranging from 3 to 9 phloroglucinol units were tentatively identified in the non-digested ethyl acetate fraction of *L. digitata*. In turn, 25 compounds with the same polymerization degree range were found in the digested ethyl acetate fraction. All of the compounds tentatively identified presented deprotonated molecular ions well documented in the literature as well as MS/MS fragmentation patterns consistent with previous works, showing typical losses of −18, − 44, −126 and −142 Da (corresponding to water, ethanal, phloroglucinol and *hydroxy*-phloroglucinol units, respectively) and combinations of these moieties (Catarino et al., 2022).

The majority of the compounds identified have been previously described in *L. digitata* or species belonging to the same genus (Vissers, Caligiani, Sforza, Vincken, & Gruppen, 2017; Wekre, Holmelid, Underhaug, Pedersen, Kopplin, & Jordheim, 2023). However, a few compounds have been newly detected in the studied algal sample. This was the case of dioxinodehydroeckol ([M−H]$^-$ at *m/z* 369), which, although frequently described in Laminariales, it usually is associated with species from the genus *Ecklonia* and *Eisenia*. Nevertheless, other species outside of this order, including species from *Fucus* (Catarino et al., 2021; Lopes et al., 2018)*, Silvetia* (Vazquez-Rodriguez et al., 2021), *Sargassum* (Li et al., 2017) and *Ascophyllum* (Agregan, Munekata, Franco, Dominguez, Carballo, & Lorenzo, 2017) have already been found to be sources of this compound as well.

The fuhalol-type phlorotannins herein detected, *i.e.*, hydroxytrifuhalol and hydroxytetrafuhalol ([M−H]$^-$ at *m/z* 405 and 529, respectively), are another example of compounds that have not been described in *Laminaria* seaweeds before. However, the presence of other fuhalols in *L. digitata* has been recently reported (Vissers et al., 2017).

Two carmalol-type phlorotannins, namely diphlorethohydroxycarmalol and tetraphlorethohydroxycarmalol ([M−H]$^-$ at *m/z* 511 and 759, repectively), have also been newly identified in the non-digested sample. Interestingly, carmalols are usually characteristic of the genus *Ishige* (Aln, Yoon, Kim, Kim, Shin, & Kim, 2006; Fernando, Kim, Sanjeewa, Oh, Jeon, & Lee, 2017; Heo & Jeon, 2009; Heo et al., 2010) although they have been already described in three other distinct species including *H. elongata* (Catarino; et al., 2022), *S. fusiforme* (Li et al., 2017) and *Carpophyllum maschalocarpum* (Li & Glombitza, 1991).

An additional phlorotannin derivative ([M−H]$^-$ at *m/z* 447) has been identified based on its MS/MS fragmentation pattern, which revealed the appearance of product ions and neutral losses typical of phlorotannins. Indeed, the MS^2 of this compound yielded the product ions at *m/z* 247, 373, and 321 (Table 2), indicating a phloroglucinol dimer, a phloroglucinol trimer, and a loss of a phloroglucinol unit, respectively. When performing MS^3 of the significant product ion, *i.e.*, the [M−H]$^-$ at *m/z* 373, it was possible to find the appearance of the fragment ions at *m/z* 247, 229, and 125, corresponding to a phloroglucinol dimer, a fucol unit, and a phloroglucinol unit, respectively.

The comparison of non-digested and digested ethyl acetate fractions (Figure S2 to S5) allowed us to conclude that the fuhalol and carmalol-type compounds (peaks 5, 12, 13, 19, 23, and 25) that were present in the non-digested sample were almost entirely absent in the digested sample (Table 2). The only exception was hydroxytrifuhalol ([M−H]$^-$ at *m/z* 405), which was present in both samples. However, the isomer detected in the digested ethyl acetate fraction (peak 1) did not match that of the non-digested ethyl acetate fraction (peak 13), as perceived by their distinct elution times (3.4 min and 13.8 min, respectively). This suggests that fuhalols and carmalols, *i.e.*, phlorotannins containing extra OH groups (a pyrogallol group), are particularly susceptible to oxidative degradation under the conditions faced during the gastrointestinal digestion, causing them to degrade, transform or even precipitate as they cross the upper gastrointestinal digestion.

Interesting changes were observed for other compounds as well. This was the case of the trimer with [M−H]$^-$ at *m/z* 373, with two isomers detected in the non-digested ethyl acetate fraction (eluting at 6.5 and 12.0 min) and an additional isomer, eluting at 4.1 min (Table 2), was found in the digested sample. An identical pattern was observed for the pentamer with [M−H]$^-$ at *m/z* 621, which exhibited two isomers in the non-digested sample but three isomers upon gastrointestinal digestion.

These observations suggest that the conditions faced along the gastrointestinal tract tend to favor the occurrence of certain isomeric forms of phlorotannins to the detriment of others, possibly resulting from the modifications on the fuhalols and carmalols, which would explain their absence in the digested ethyl acetate fraction, and/or breakdown of higher molecular weight compounds present in the extract that were not detected in this HPLC-MS analysis.

Naturally, the changes observed in the phlorotannin profile of the non-digested and digested ethyl acetate fraction, as well as in other co-extracted compounds that were not considered, are very likely to be responsible for the different anti-inflammatory responses experienced between cells treated with non-digested versus digested ethyl acetate fraction. As mentioned above, it was expected that gastrointestinal digestion would contribute to the breakdown of higher molecular compounds into lower molecular phlorotannins that, in turn, would exert a more potent anti-inflammatory effect on the LPS-stimulated cells. However, even though the gastrointestinal digestion contributed to the formation of specific lower molecular weight isomers that were not present in the non-digested sample, this did not translate into an increase in the anti-inflammatory effects.

Notably, the disappearance of the fuhalols and carmalols from the digested ethyl acetate fraction might have a critical impact on the results seen in the cellular experiments. Indeed, diphlorethohydroxycarmalol was described as the main active phlorotannin from *I. okamurae*, responsible for its prominent radical scavenging activity and protective effects on H_2O_2-stressed cells (Heo et al., 2009), as well as a very effective anti-inflammatory agent capable of suppressing a broad range of inflammatory responses (Fernando et al., 2017). Likewise, three fuhalols isolated from *S. carpophyllum* were found to be effective inhibitors of total IκB degradation in immunoglobulin E-sensitized rat basophilic leukemia cells, strongly reducing the allergic response (Matsui, Ito, Itoigawa, & Shibata, 2022). Therefore, the absence of the fuhalol and carmalol-type compounds in the digested ethyl acetate fraction might partly explain the loss of anti-inflammatory activity observed when comparing the digested and non-digested samples.

4. Conclusions

Overall, this work contributes to disclosing new insights about the impact of the gastrointestinal digestion in the bioactive properties of phlorotannins. As expected, phlorotannins can be deemed sensitive to the conditions faced through the gastrointestinal tract as reflected by the significant loss of total phlorotannin content in the digested extracts. However, compared to other species, *L. digitata* phlorotannins showed higher resistance. This study also made clear that the group of carmalols and fuhalols are susceptibel to upper gastrointestinal digestion as evidenced by the UHPLC-MS data. Considering that these groups of compounds have been previously demonstrated to be critical players in the anti-inflammatory properties of other seaweeds, their disappearance after digestion could partly justify the loss of the anti-inflammatory effect observed in the cellular experiments on the purified extract. Nevertheless, this activity loss was not always observed and was not proportional to the extent of the total phlorotannin loss, indicating that these are not the only compounds contributing to the activities observed and that metabolites from the digestion might also have an important role.

Further studies about the formation of these metabolites should be considered especially targeting the ones resulting from the degradation of fuhalols and carmalols. It would also be essential to elucidate which portion of these compounds is capable of crossing the gut barrier and which portion would proceed to the large intestine, as they may also interfere with the gut microbiota activity and be transformed into new bioactive metabolites.

CRediT authorship contribution statement

Marcelo D. Catarino: Writing – original draft, Validation,

Table 2
Assignment of the compounds present in the *L. digitata* non-digested and digested EtOAc samples.

Peak	RT (min)	[M−H]⁻ *m/z*	MS/MS fragments[a]	Tentative assignment	Non-digested	Digested
3	5.2	369	MS^2 [369]: **281**, 129, 325, 309, 173, 353 MS^3 [281]: **129,** 263	Dioxinodehydroeckol	✓	x
3	5.2	447	MS^2 [447]: **373**, 429, 289, 247, 321 MS^3 [373]: **247,** 229, 125, 189	Phlorotannin derivative	✓	x
1	3.4	405	MS^2 [405]: **191**, 387, 173, 213 MS^3 [191]: **111**, 173, 155	Hydroxytrifuhalol	x	✓
13	13.8	405	MS^2 [405]: **373**, 345, 191 MS^3 [373]: **345**, 289, 139	Hydroxytrifuhalol	✓	x
5	6.7	529	MS^2 [529]: **485**, 467, 359, 327 MS^3 [485]: **215,** 229, 309, 259, 139	Hydroxytetrafuhalol	✓	x
19	18.4	529	MS^2 [529]: **497**, 403, 387, 469, 357, 263, 205 MS^3 [497]: **469**, 343, 371, 329, 425, 247, 231, 203	Hydroxytetrafuhalol	✓	x
12	13.0	653	MS^2 [653]: **609**, 591, 465 MS^3 [609]: **483**, 591, 573, 467, 449, 565	Hydroxypentafuhalol	✓	x
23	21.1	511	MS^2 [511]: **387**, 351, 245, 261, 201, 369, 325 MS^3 [387]: **245**, 261, 369, 313, 217, 231, 341, 139	Diphlorethohydroxycarmalol	✓	x
25	23.1	759	MS^2 [759]: **617**, 245, 351, 495, 741, 369, 385, 229, 635, 511, 475 MS^3 [617]: **369**, 477, 245, 383, 423	Tetraphlorethohydroxycarmalol	✓	x
2	4.1	373	MS^2 [373]: **355**, 247, 311, 201, 289, 329, 141 MS^3 [355]: **245**, 245, 229, 215, 311, 269, 283, 121	Phloroglucinol Trimer	x	✓
4	6.5	373	MS^2 [373]: **355**, 311, 231, 329, 293, 141, 215, 161 MS^3 [355]: **309**, 225, 139, 243, 195, 287, 119, 161	Phloroglucinol Trimer	✓	✓
10	12.0	373	No MS2 signal	Phloroglucinol Trimer	✓	✓
2	4.1	497	MS^2 [497]: **479**, 331, 435, 461, 353, 383, 371 MS^3 [479]: **461,** 435, 353, 391, 417, 309, 339, 325, 231	Phloroglucinol Tetramer	x	✓
5	6.7	497	MS^2 [497]: **249**, 479, 339, 231, 205, 189, 161 MS^3 [249]: **205,** 229, 163, 219, 189	Phloroglucinol Tetramer	✓	x
7	8.6	497	MS^2 [497]: **479**, 353, 371, 339, 335, 435, 309, 229 MS^3 [479]: **339**, 353, 229, 461	Phloroglucinol Tetramer	x	✓
9	10.3	497	MS^2 [497]: **353**, 479, 335, 371, 309, 435, 229 MS^3 [205]: **205,** 229, 163, 219, 189	Phloroglucinol Tetramer	✓	✓
18	17.8	497	MS^2 [497]: **265**, 373, 487, 353, 455, 309, 139 MS^3 [265]: **139**, 245, 123, 177	Phloroglucinol Tetramer	✓	✓
6	7.4	621	MS^2 [621]: **603**, 585, 455, 559, 331, 477, 373, 289, 515 MS^3 [603]: **585,** 559, 477, 313, 353, 519	Phloroglucinol Pentamer	x	✓
13	13.8	621	MS^2 [621]: **373**, 603, 495, 461, 325 MS^3 [373]: **355**, 329, 311, 231, 139, 287, 245	Phloroglucinol Pentamer	✓	✓
14	14.6	621	MS^2 [621]: **603**, 461, 335, 479, 353, 373, 229 MS^3 [603]: **461**, 353, 479, 355, 585, 229, 307, 559, 249, 283, 433	Phloroglucinol Pentamer	✓	✓
8	8.9	745	MS^2 [745]: **709**, 727, 583, 579, 331, 247, 683, 619, 413, 455, 539 MS^3 [709]: **583,** 443, 461, 567, 665	Phloroglucinol Hexamer	✓	✓
13	13.0	745	MS^2 [745]: **727**, 709, 455, 579, 289, 353, 497, 353, 433, 547 MS^3 [727]: **709**, 437, 585, 683, 603, 519, 501	Phloroglucinol Hexamer	✓	✓
17	17.0	745	MS^2[745]:**497**, 353, 479, 335, 371, 513, 601, 727, 309, 457, 247 MS^3[497] **353**, 335, 479, 371, 309, 229, 435, 247	Phloroglucinol Hexamer	✓	✓
19	18.4	745	MS^2 [745]: **727**, 603, 339, 229, 479, 355, 371, 461, 497 MS^3 [727]: **603**, 339, 353, 585, 229, 461, 477, 373	Phloroglucinol Hexamer	✓	✓
20	19.2	745	MS^2 [745]: **371**, 727, 603, 477, 355, 619, 583, 511, 449, 411, 229 MS^3 [371]: **343**, 245, 323, 353, 231, 163, 139	Phloroglucinol Hexamer	✓	✓
11	12.6	869	MS^2 [869]: **851**, 833, 585, 603, 477, 725 MS^3 [851]: **833**, 585, 707, 353, 601, 441, 725, 619, 335, 461, 369, 307, 477	Phloroglucinol Heptamer	✓	✓
15	15.2	869	MS^2 [869]: **851**, 833, 579, 455, 413, 707, 269 MS^3 [851]: **833**, 707, 725, 417, 583, 435, 567, 643	Phloroglucinol Heptamer Heptamer	✓	✓
16	16.2	869	MS^2 [869]: **833**, 851, 703 MS^3 [833]: **707**, 583, 567, 815, 691, 461	Phloroglucinol Heptamer	✓	✓
22	20.5	869	MS^2 [869]: **851**, 725, 601, 707, 461, 353, 495 MS^3 [851]: **727**, 603, 833, 707, 461, 335,	Phloroglucinol Heptamer	✓	✓
23	21.1	869	MS^2 [869]: **725**, 851, 353, 477, 635, 601, 371, 311, 415, 323, 581, 495 MS^3 [725]: **707**, 581, 351, 443, 601, 475,	Phloroglucinol Heptamer	✓	✓
19	18.4	993	MS^2 [993]: **975**, 957, 621 MS^3 [975]: **957**, 443, 583, 725, 709, 745, 831, 461, 549, 369, 353, 309	Phloroglucinol Octamer	✓	✓
21	20.3	993	MS^2 [993]: **957**, 975, 621, 373, 603 MS^3 [957]: **583**, 831, 707, 693, 815, 555, 443	Phloroglucinol Octamer	✓	✓
23	21.1	1117	MS^2 [1117]: **1099**, 1081, 851, 973, 745, 707, 601, 477, 353 MS^3 [1099]: **1081**, 833, 955, 353, 973, 477, 583, 691, 725, 707, 493, 849, 601, 937, 549, 459, 621, 371	Phloroglucinol Nonamer	✓	✓
24	21.6	1117	MS^2 [1117]: **1099**, 745, 1081, 603, 497, 479, 621, 355, 727 MS^3 [1099]: **1081**, 975, 585, 537, 707, 831, 955, 601, 351, 495	Phloroglucinol Nonamer	✓	✓

[a] Product ions are organized according to their relative abundance with the most abundant ion highlighted in bold; RT – retention time.

Investigation, Formal analysis, Data curation, Conceptualization. **Carlos J. García:** Writing – review & editing, Investigation, Formal analysis. **Rocío García-Villalba:** Writing – review & editing, Validation. **Artur M.S. Silva:** Writing – review & editing, Resources. **Débora A. Campos:** Writing – review & editing, Validation. **Maria Manuela Pintado:** Writing – review & editing, Validation, Supervision, Resources. **Bruno Neves:** Writing – review & editing, Validation, Investigation, Formal analysis. **Susana M. Cardoso:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Data curation, Conceptualization. **Francisco A. Tomás-Barberán:** Writing – review & editing, Validation, Supervision, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by FCT/MCTES (LA/P/0008/2020 DOI 10.54499/LA/P/0008/2020, UIDP/50006/2020 DOI 10.54499/UIDP/50006/2020 and UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020) through national funds, by the European Union (FEDER funds through COMPETE POCI-01-0145-FEDER-031015) and national funds (FCT, Fundação para a Ciência e Tecnologia) through project PTDC/BAA-AGR/31015/2017, “Algaphlor—brown algae phlorotannins: from bioavailability to the development of new functional foods”. Marcelo D. Catarino would like to acknowledge the research contract funded by FCT/MCTES (LA/P/0008/2020 DOI 10.54499/LA/P/0008/2020, UIDP/50006/2020 DOI 10.54499/UIDP/50006/2020 and UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020) through national funds, and Susana M. Cardoso thanks FCT/MCTES for the funding provided through the Institutional Call to Scientific Employment Stimulus.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2024.114641.

References

Agregan, R., Munekata, P. E. S., Franco, D., Dominguez, R., Carballo, J., & Lorenzo, J. M. (2017). Phenolic compounds from three brown seaweed species using LC-DAD-ESI-MS/MS. *Food Research International, 99*, 979–985. https://doi.org/10.1016/j.foodres.2017.03.043

Aln, M. J., Yoon, K. D., Kim, C. Y., Kim, J. H., Shin, C. G., & Kim, J. (2006). Inhibitory activity on HIV-1 reverse transcriptase and integrase of a carmalol derivative from a brown alga, Ishige okamurae. *Phytotherapy Research, 20*(8), 711–713. https://doi.org/10.1002/ptr.1939

Amarante, S. J., Catarino, M. D., Marcal, C., Silva, A. M. S., Ferreira, R., & Cardoso, S. M. (2020). Microwave-assisted extraction of phlorotannins from *Fucus vesiculosus*. *Marine Drugs, 18*(11). https://doi.org/10.3390/md18110559

Baweja, P., Kumar, S., Sahoo, D., & Levine, I. (2016). Chapter 3 - biology of seaweeds. In J. Fleurence, & I. Levine (Eds.), *Seaweed in health and disease prevention* (pp. 41–106). San Diego, CA, USA: Academic Press.

Bonifacio-Lopes, T., Catarino, M. D., Vilas-Boas, A. A., Ribeiro, T. B., Campos, D. A., Teixeira, J. A., & Pintado, M. (2022). Impact of circular brewer’s spent grain flour after in vitro gastrointestinal digestion on human gut microbiota. *Foods, 11*(15). https://doi.org/10.3390/foods11152279

Catarino, M. D., Fernandes, I., Oliveira, H., Carrascal, M., Ferreira, R., Silva, A. M. S., … Cardoso, S. M. (2021). Antitumor activity of *Fucus vesiculosus*-derived phlorotannins through activation of apoptotic signals in gastric and colorectal tumor cell lines. *International Journal of Molecular Sciences, 22*(14), 7604. https://doi.org/10.3390/ijms22147604

Catarino, M. D., Pires, S. M. G., Silva, S., Costa, F., Braga, S. S., Pinto, D. C. G. A., … Cardoso, S. M. (2022). Overview of phlorotannins’ constituents in fucales. *Marine Drugs, 20*(12), 754. https://doi.org/10.3390/md20120754

Catarino, M. D., Silva, A., Cruz, M. T., Mateus, N., Silva, A. M. S., & Cardoso, S. M. (2020). Phlorotannins from *Fucus vesiculosus*: modulation of inflammatory response by blocking NF-κB signaling pathway. *International Journal of Molecular Sciences, 21*(18), 6897. https://doi.org/10.3390/ijms21186897

Catarino, M. D., Silva, A. M. S., Mateus, N., & Cardoso, S. M. (2019). Optimization of phlorotannins extraction from *Fucus vesiculosus* and evaluation of their potential to prevent metabolic disorders. *Marine Drugs, 17*(3), 162. https://doi.org/10.3390/md17030162

Catarino, M. D., Circuncisao, A. R., Neves, B., Marcal, C., Silva, A. M. S., Cruz, M. T., & Cardoso, S. M. (2022). Impact of gastrointestinal digestion on the anti-inflammatory properties of phlorotannins from *Himanthalia elongata*. *Antioxidants, 11*(8), 1518. 10.3390/antiox11081518.

Catarino;, M. D., Marcal, C., Bonifacio-Lopes, T., Campos, D., Mateus, N., Silva, A. M. S., . . . Cardoso, S. M. (2021). Impact of phlorotannin extracts from F*ucus vesiculosus* on human gut microbiota. *Marine Drugs, 19*(7), 375. 10.3390/md19070375.

Chouh, A., Nouadri, T., Catarino, M. D., Silva, A. M. S., & Cardoso, S. M. (2022). Phlorotannins of the brown algae *Sargassum vulgare* from the mediterranean sea coast. *Antioxidants, 11*(6), 1055. https://doi.org/10.3390/antiox11061055

Corona, G., Ji, Y., Anegboonlap, P., Hotchkiss, S., Gill, C., Yaqoob, P., … Rowland, I. (2016). Gastrointestinal modifications and bioavailability of brown seaweed phlorotannins and effects on inflammatory markers. *British Journal of Nutrition, 115*(7), 1240–1253. https://doi.org/10.1017/S0007114516000210

Erpel, F., Mateos, R., Pérez-Jiménez, J., & Pérez-Correa, J. R. (2020). Phlorotannins: From isolation and structural characterization, to the evaluation of their antidiabetic and anticancer potential. *Food Research International, 137*. https://doi.org/10.1016/j.foodres.2020.109589

Fernando, I. P. S., Kim, H. S., Sanjeewa, K. K. A., Oh, J. Y., Jeon, Y. J., & Lee, W. W. (2017). Inhibition of inflammatory responses elicited by urban fine dust particles in keratinocytes and macrophages by diphlorethohydroxy-carmalol isolated from a brown alga Ishige okamurae. *Algae, 32*(3), 261–273. https://doi.org/10.4490/algae.2017.32.8.14

Heffernan, N., Smyth, T. J., Soler-Villa, A., Fitzgerald, R. J., & Brunton, N. P. (2015). Phenolic content and antioxidant activity of fractions obtained from selected Irish macroalgae species (Laminaria digitata, Fucus serratus, Gracilaria gracilis and Codium fragile). *Journal of Applied Phycology, 27*(1), 519–530. https://doi.org/10.1007/s10811-014-0291-9

Heo, S. J., & Jeon, Y. J. (2009). Evaluation of diphlorethohydroxycarmalol isolated from Ishige okamurae for radical scavenging activity and its protective effect against H2O2-induced cell damage. *Process Biochemistry, 44*(4), 412–418. https://doi.org/10.1016/j.procbio.2008.12.005

Heo, S. J., Ko, S. C., Kang, S. M., Cha, S. H., Lee, S. H., Kang, D. H., … Jeon, Y. J. (2010). Inhibitory effect of diphlorethohydroxycarmalol on melanogenesis and its protective effect against UV-B radiation-induced cell damage. *Food and Chemical Toxicology, 48*(5), 1355–1361. https://doi.org/10.1016/j.fct.2010.03.001

Jamalzadeh, L., Ghafoori, H., Sariri, R., Rabuti, H., Nasirzade, J., Hasani, H., & Aghamaali, M. R. (2016). Cytotoxic effects of some common organic solvents on MCF-7, RAW-264.7 and human umbilical vein endothelial cells. *Avicenna Journal of Medical Biochemistry, In press*(In press). 10.17795/ajmb-33453.

Koivikko, R., Loponen, J., Honkanen, T., & Jormalainen, V. (2005). Contents of soluble, cell-wall-bound and exuded phlorotannins in the brown alga Fucus vesiculosus, with implications on their ecological functions. *Journal of Chemical Ecology, 31*(1), 195–212. https://doi.org/10.1007/s10886-005-0984-2

Lawrence, T. (2009). The nuclear factor NF-κB pathway in inflammation. *Cold Spring Harbor Perspectives in Biology, 1*(6). https://doi.org/10.1101/cshperspect.a001651

Li, Fu, X., Duan, D., Liu, X., Xu, J., & Gao, X. (2017). Extraction and identification of phlorotannins from the brown alga, Sargassum fusiforme (harvey) setchell. *Marine Drugs, 15*(2). 10.3390/md15020049.

Li, & Glombitza, K.-W. (1991). Carmalols and phlorethofuhalols from the brown alga carpophyllum-maschalocarpum. *Phytochemistry, 30*(10), 3417-3421. Doi 10.1016/0031-9422(91)83220-F.

Liu, H., & Gu, L. (2012). Phlorotannins from brown algae (Fucus vesiculosus) inhibited the formation of advanced glycation endproducts by scavenging reactive carbonyls. *Journal of Agricultural and Food Chemistry, 60*(5), 1326–1334. https://doi.org/10.1021/jf204112f

Lopes, G., Barbosa, M., Vallejo, F., Gil-Izquierdo, A., Andrade, P. B., Valentao, P., … Ferreres, F. (2018). Profiling phlorotannins from Fucus spp. of the Northern Portuguese coastline: Chemical approach by HPLC-DAD-ESI/MSn and UPLC-ESI-QTOF/MS. *Algal Research-Biomass Biofuels and Bioproducts, 29*, 113–120. https://doi.org/10.1016/j.algal.2017.11.025

Matsui, T., Ito, C., Itoigawa, M., & Shibata, T. (2022). Three phlorotannins from Sargassum carpophyllum are effective against the secretion of allergic mediators from antigen-stimulated rat basophilic leukemia cells. *Food Chemistry, 377*. https://doi.org/10.1016/j.foodchem.2021.131992

Meng, W. H., Mu, T. H., Sun, H. N., & Garcia-Vaquero, M. (2021). Phlorotannins: A review of extraction methods, structural characteristics, bioactivities, bioavailability, and future trends. *Algal Research-Biomass Biofuels and Bioproducts, 60*. https://doi.org/10.1016/j.algal.2021.102484

Purcell-Meyerink, D., Packer, M. A., Wheeler, T. T., & Hayes, M. (2021). Aquaculture production of the brown seaweeds Laminaria digitata and Macrocystis pyrifera: applications in food and pharmaceuticals. *Molecules, 26*(5). https://doi.org/10.3390/molecules26051306

Quan, W., Tao, Y. D., Lu, M., Yuan, B., Chen, J., Zeng, M. M., … He, Z. Y. (2018). Stability of the phenolic compounds and antioxidant capacity of five fruit (apple, orange, grape, pomelo and kiwi) juices during in vitro-simulated gastrointestinal digestion. *International Journal of Food Science and Technology, 53*(5), 1131–1139. https://doi.org/10.1111/ijfs.13682

Sanchez-Velazquez, O. A., Mulero, M., Cuevas-Rodriguez, E. O., Mondor, M., Arcand, Y., & Hernandez-Alvarez, A. J. (2021). In vitro gastrointestinal digestion impact on stability, bioaccessibility and antioxidant activity of polyphenols from wild and commercial blackberries (Rubus spp.). *Food & Function, 12*(16), 7358–7378. https://doi.org/10.1039/d1fo00986a

Shrestha, S., Zhang, W., & Smid, S. D. (2021). Phlorotannins: A review on biosynthesis, chemistry and bioactivity. *Food Bioscience, 39*. https://doi.org/10.1016/j.fbio.2020.100832

Vazquez-Rodriguez, B., Santos-Zea, L., Heredia-Olea, E., Acevedo-Pacheco, L., Santacruz, A., Gutierrez-Uribe, J. A., & Cruz-Suarez, L. E. (2021). Effects of phlorotannin and polysaccharide fractions of brown seaweed Silvetia compressa on human gut microbiota composition using an in vitro colonic model. *Journal of Functional Foods, 84*. https://doi.org/10.1016/j.jff.2021.104596

Vincent, A., Stanley, A., & Ring, J. (2020). Hidden champion of the ocean - Seaweed as a growth engine for a sustainable European future.

Vissers, A. M., Caligiani, A., Sforza, S., Vincken, J. P., & Gruppen, H. (2017). Phlorotannin composition of *Laminaria digitata*. *Phytochemical Analysis, 28*(6), 487–495. https://doi.org/10.1002/pca.2697

Wang, T., Jonsdottir, R., Liu, H. Y., Gu, L. W., Kristinsson, H. G., Raghavan, S., & Olafsdottir, G. (2012). Antioxidant capacities of phlorotannins extracted from the brown algae Fucus vesiculosus. *Journal of Agricultural and Food Chemistry, 60*(23), 5874–5883. https://doi.org/10.1021/jf3003653

Wang, T., Jonsdottir, R., & Olafsdottir, G. (2009). Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. *Food Chemistry, 116*(1), 240–248. https://doi.org/10.1016/j.foodchem.2009.02.041

Wekre, M. E., Holmelid, B., Underhaug, J., Pedersen, B., Kopplin, G., & Jordheim, M. (2023). Characterization of high value products in the side-stream of Laminaria hyperborea alginate production-Targeting the phenolic content. *Algal Research-Biomass Biofuels and Bioproducts, 72*. https://doi.org/10.1016/j.algal.2023.103109