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In Vitro Potential of Antioxidant Extracts from *Gracilaria gracilis* Cultivated in Integrated Multi-Trophic Aquaculture (IMTA) for Marine Biobased Sector

Simona Manuguerra [1,†], Rosaria Arena [1], Eleonora Curcuraci [1,†], Giuseppe Renda [1,2], Maxime Rannou [1,3], Claire Hellio [3], Concetta Maria Messina [1,*] and Andrea Santulli [1]

[1] Laboratory of Marine Biochemistry and Ecotoxicology, Department of Earth and Marine Sciences DiSTeM, University of Palermo, Via Barlotta 4, 91100 Trapani, Italy; simona.manuguerra@unipa.it (S.M.); rosaria.arena@unipa.it (R.A.); eleonora.curcuraci@unipa.it (E.C.); pepperenda76@gmail.com (G.R.); maxime.rannou@unipa.it (M.R.); andrea.santulli@unipa.it (A.S.)

[2] Istituto di Biologia Marina, Consorzio Universitario della Provincia di Trapani, Via G. Barlotta 4, 91100 Trapani, Italy

[3] CNRS, IRD, Ifremer, LEMAR, Univ Brest, IUEM, F-29280 Plouzane, France; claire.hellio@univ-brest.fr

* Correspondence: concetta.messina@unipa.it

† These authors contributed equally to the manuscript.

Abstract: This study aimed to evaluate the antioxidant activity of bioactive compounds extracted from *Gracilaria gracilis* cultivated in an integrated multi-trophic aquaculture (IMTA) system by different extraction solvents and to investigate the potential capacity of the extracts in cellular systems against environmental pollutants. The global yields, total polyphenol contents, and antioxidant activity were assessed on *G. gracilis* by DPPH radical scavenging activity, comparing the antioxidant extraction efficiency of the different solvents (ethanol 80%, acetone 70%, N-hexane, and water). Ethanol extract, granted by the highest extractive yield and antioxidant capacity, was tested in vitro in the *Sparus aurata* fibroblast (SAF-1) cell line to evaluate its protective role against oxidative stress induced by the chemical flame retardant 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47). The results demonstrate that the cells pretreated with *G. gracilis* extract were protected against oxidative stress and had improved cell viability, cellular antioxidant defense system, and cell cycle control, as demonstrated by the gene expression on some biomarkers related to the cell cycle (*p53*) and oxidative stress (*nrf2*, *sod*, and *cat*). These results confirm that bioactive compounds obtained from seaweeds cultivated in IMTAs could contribute to producing high-value ingredients that are able to counteract environmental stressors, for the growth of the marine biobased industrial sector and the expansion of new value chains.

Keywords: *Gracilaria gracilis*; bioactive compounds; antioxidant defense system; integrated multi-trophic aquaculture (IMTA); environmental stressors

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1. Introduction

Capture fisheries and aquaculture production are important sources of food and income worldwide for hundreds of millions of people [1,2]. While the growth curve of fishery catches has flattened out over the past two decades, the aquaculture sector has rapidly grown, reaching around 9 million tons (excluding algae) in 2021, matching the fishing sector production [1].

Although the aquaculture sector contributes significantly to the production of animal proteins and to improving access to food sources, reducing food insecurity for the world's growing population, aquaculture is also an industrial productive process that faces numerous challenges, including the production of huge amounts of waste rich in nutrients, but whose utilization and possible applications are limited by the high salt content [3–5]. To reduce the amount of waste nutrients produced by aquaculture plants, a wide number of systems have been tested, including integrated multi-trophic aquaculture (IMTA) [5,6].

IMTA is based on the selection of organisms from different trophic levels living in similar environmental conditions providing by-products, including waste, of one aquatic species as an input (fertilizer, food) to another [2,3,7,8]. By reducing nutrient loading and increasing biomass production, IMTA guarantees an effective and economical use of resources, decreasing the negative effects of aquaculture on the marine environment, lowering production costs, and achieving better economic viability [5,9].

Wastes produced in IMTA provide a valuable source for the biomass growth of marine organisms rich in bioactive compounds, such as antioxidant compounds, which fuel the potential of transforming marine waste and by-products into resources [10,11].

It is known that plants and algae are rich in secondary metabolites with bioactive properties, such as polysaccharides, polyunsaturated fatty acids, polyphenolic compounds, antioxidants, peptides, essential vitamins and minerals, which enable them to adapt to different environmental conditions [12–15]. The extraction of these marine natural bioactive compounds with different techniques represents a constantly evolving and expanding industry sector to reduce the use of non-natural substances in the cosmeceutical, pharmaceutical, and agro-food sectors [10,16–19].

Macroalgae belonging to the genus *Gracilaria*, considered an important source of lipids, proteins, carbohydrates, phycobiliproteins, phenols, and phytochemicals, are widely cultivated in IMTA systems. Several studies have highlighted the antimicrobial, antiparasitic, antihypertensive, antioxidant, and anti-inflammatory properties of *Gracilaria* spp. bioactive compounds with potential applications in the pharmaceutical and biotechnological fields [20–24].

Natural antioxidant produced by marine algae possess scavenger properties against reactive oxygen species (ROS), the overproduction of which can lead to oxidative stress, responsible for DNA, protein, and lipid damage [12], and the modulation of cellular pathways related to cell cycle, cell metabolism, and energy balance [25–28].

Cells contain a complex endogenous enzymatic antioxidant defense system, such as catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH), aimed to protect cells and tissues from oxidative damages and counteract the toxicity of ROS [29]. Although natural antioxidants extracted from macroalgae, such as polyphenols, are able to prevent oxidative stress [27,30,31], few studies have reported the potential effects of *Gracilaria* spp. against ROS [32].

Recent evidence from animal and cell line models suggests that oxidative stress can be also induced by chemical environmental contaminants [26,28,33,34]. The intensification of human activities and the increasing release of pollutants, even at low concentrations (ng/g–µg/g) into marine ecosystems, have affected the quality and well-being of wildlife and humans by the transfer of pollutants through the food web [35–41]. Among the emerging contaminants, 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47), a bioaccumulative flame retardant, highly persistent and carcinogenic, is widely distributed in the marine environment [26–28,42,43].

Previous in vitro studies have demonstrated the protective effect of certain synthetic antioxidants against BDE-47-induced oxidative stress [26,27,44]. Given this, our study was designed to evaluate the protective effect of natural extracts obtained from *G. gracilis* cultivated in IMTA on the *Sparus aurata* fibroblast (SAF-1) cell line exposed to different concentrations of BDE-47. The global yields, total polyphenol contents, and antioxidant activity assessed by DPPH radical scavenging activity were analyzed, comparing the antioxidant extraction efficiency of solvents with different polarities.

In addition, the antioxidant activity of G. *gracilis* extract against BDE-47 in the SAF-1 cell line was evaluated at the molecular level by the gene expression related to the cell cycle (*p53*), oxidative stress, and antioxidant defense system (*nrf2*, *sod*, and *cat*) to detect molecular pathway alterations and early exposure biosensors, which is useful for environmental biomonitoring.

2. Materials and Methods

2.1. Sample Collection and Processing

Gracilaria gracilis samples were grown in the hatchery pond of a Recirculating Aquaculture System facility of sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) located in Sicily (Italy). *G. gracilis*, grown along a long line fixed approximately 20 cm below the water surface of the hatchery pond, was collected in triplicate during the summer season. After removing the epiphytes, the samples were dried for 48 h at 40 °C and homogenized. A dried powder of *G. gracilis* with a fraction between 250 and 500 µm was extracted by different solvents (N-hexane, water, acetone 70%, and ethanol 80%) to assess the yields in the total polyphenols.

2.1.1. Extraction by Solvents

G. gracilis phenolic compounds were extracted using solvents with different polarities, such as ethanol 80%, acetone 70%, N-hexane, and water, according to Messina et al. [45]. The extraction yield (*w*/*w*) was calculated by evaporating one aliquot of each extract (N-hexane, ethanol 80%, acetone 70%, and water) in a rotary vacuum evaporator and weighing it, as described by Messina et al. [45]. The following equation was used (1):

$$\text{Yield of extract (\%)} = \text{weight of extract/weight of sample} \times 100 \quad (1)$$

2.1.2. Total Polyphenol Contents

The polyphenol contents of the extracts were determined spectrophotometrically according to the method of Folin and Ciocalteu [46]. Analysis was performed, according to the method of Dhouibi et al. [47], on a 96-well microplate. All samples were analyzed in triplicate. The absorbance was measured at 725 nm, using a spectrophotometer (Multiskan, Thermo-Fisher Scientific, Waltham, MA, USA). The total phenolic contents were expressed as mg of gallic acid/mg of sample weight.

2.1.3. DPPH Radical Scavenging Activity

The antioxidant activity was evaluated by the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, using the method by Bernatoniene et al. [48], with some modifications. Synthetic antioxidant gallic acid (GAE, Merck KGaA, Darmstadt, Germany) was used as a reference compound, and standard curves were prepared at different concentrations (0.05–1 mg/mL). Each determination was carried out in three replicates. The absorbance was read at 517 nm after 30 min (Multiskan, Thermo-Fisher Scientific), and the antioxidant activity was calculated according to Formula (2):

$$\text{Scavenging effect (\%)} = [1 - (\text{absorbance of sample-absorbance of blank})/\text{absorbance of control}] \times 100 \quad (2)$$

The antioxidant activity is expressed as the amount of antioxidant required to achieve 50% inhibition of the radical activity (IC50) [47].

2.2. Protective Effect of G. gracilis Extract against the Pro-Oxidant BDE-47 in the SAF-1 Cell Line

SAF-1 Cell Culture and Treatment

The *Sparus aurata* fibroblast (SAF-1) cell line (ECACC No. 00122301) was cultured at 25 °C in Leibovitz L-15 medium supplemented with 2% L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin, and 15% fetal bovine serum (all reagents from Merck KGaA, Darmstadt, Germany). The cells were seeded in 96-well plates (Nunc, Darmstadt, Germany) (10,000 cells/well) and incubated for 24 h.

The protective effect of the ethanol extract of *G. gracilis* against oxidative stress induced by BDE-47 was assessed on SAF-1 cells. Untreated cells were cultivated in a specific culture medium as a control. One group of SAF-1 cells was exposed for 24 h to an ethanol extract of *G. gracilis* at concentrations of 0.005–0.01–0.02 µg/mL (*G. gracilis*). Another group of untreated SAF-1 cells was exposed for 48 h to BDE-47 (50–100 µM) (BDE-47). The last group of SAF-1 cells was pretreated for 24 h with ethanol extract of *G. gracilis*

(0.005–0.01–0.02 µg/mL), and then exposed for 48 h to BDE-47 (50 and 100 µM) (*G. gracilis* + BDE-47), according to a standardized protocol [27,28,44].

The BDE-47 standard was provided by SPECTRA (Rome, Italy), and the stock solution at a concentration of 25 mM was prepared by dissolving the powder compound in dimethylsulfoxide DMSO (Merck KGaA, Darmstadt, Germany).

The extract (dissolved in ethanol) was diluted in a culture medium at concentrations of 0.005–0.01–0.02 µg/mL and at a final solvent concentration not exceeding 0.1% (*v*/*v*).

The viability of the cells was measured as described by Espinosa et al. [27], using the MTT assay according to Mosmann [49]. The optical densities (ODs) at 570 nm with background subtraction at 690 were determined in a microplate reader (Multiscan-Sky Microplate Reader, Thermo-Scientific^{TM}, Waltham, MA, USA). The percentage of viability was determined according to Formula (3):

$$\text{Viability \%} = (\text{OD of the test sample}/\text{OD of the control sample}) \times 100 \quad (3)$$

OD measurements were performed in six replicates.

2.3. Gene Expression Assay

SAF-1 cells (500,000 cells/well) were incubated in triplicate (Nunc, Germany). After 24 h, the cells were pretreated with ethanol extract of *G. gracilis* (0.02 µg/mL) and then exposed to BDE-47 (100 µM) for 48 h. The medium was removed, the cells were washed using PBS, and 1 mL of PUREzol (Bio-Rad, Hercules, CA, USA) was added. The PUREzol containing the RNA from cells was obtained and stored at −80 °C prior to analyses.

Quantitative Reverse Transcription PCR

The total RNA was isolated from SAF-1 cells using an RNA extraction kit (Aurum Total RNA Fatty and Fibrous Tissue Kit (Bio-Rad, Hercules, CA, USA)). Then, 1 µg of the total RNA was converted into cDNA using the 5x iScript Reaction Mix Kit (Bio-Rad, Hercules, CA, USA). RT-qPCR was performed in a 20 µL reaction system using the 1x IQ SYBR Green Supermix (Bio-Rad, Hercules, CA, USA). The relative mRNA level of a target gene was quantified using the comparative cycle threshold ($2^{-\Delta\Delta CT}$) method [50]. The β-actin and 18s of the endogenous reference and the relative quantification of (*p53*, *nrf2*, *sod* and *cat*) gene expression was evaluated after normalization with the reference genes and compared to the control group. Data processing and statistical analyses were performed using CFX Manager 3.1 Software (Bio-Rad, Hercules, CA, USA). The primers used are shown in Table 1.

Table 1. Gilthead seabream primer sequences used for real-time PCR.

Genes	F/R Primer Sequence (5′–3′)
p53	CCTCATCCTCATCATCGCCT
	AGCTCGTTGAATTTGCAGGG
nrf2	GTTCAGTCGGTGCTTTGACA
	CTCTGATGTGCGTCTCTCCA
sod	CCATGGTAAGAATCATGGCGG
	CGTGGATCACCATGGTTCTG
cat	TTCCCGTCCTTCATTCACTC
	CTCCAGAAGTCCCACACCAT
β-actin	GTCATGGATTCCGGTGATGG
	TGGTGAAGGAGTAGCCACGC
18s	TGTGCCGCTAGAGGTGAAATT
	GCAAATGCTTTCGCTTTCG

2.4. Statistical Analysis

Statistical differences among the groups were assessed by one-way ANOVA, followed by the SNK or Games–Howell test, depending on the homogeneity of the variables. The normality of the variables was confirmed using the Shapiro–Wilk test, and the homogeneity of variance, by the Levene test. The significance level was 95% in all cases ($p < 0.05$). All the data were analyzed by the computer application SPSS for Windows® (version 20.0, SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Characterization of the Antioxidant Power

In light of the growing interest in seaweeds as a source of natural antioxidants, this study aimed to assess the global yield, the total polyphenol content, and the antioxidant activity of the marine red macroalga *G. gracilis* cultivated in an IMTA system, by comparing different extraction methods.

Since it is known that the extraction yield, polyphenol content, and antioxidant activity of extracts are influenced both by the matrices analyzed and the polarity of the used solvent, it is important to define which solvent is the best for the extraction of antioxidant compounds [51,52]. In fact, our results show that the highest percentage yields were obtained by the water (7.40 ± 0.23%) and ethanol 80% extractions (6.70 ± 0.47%), followed by acetone 70% (5.80 ± 0.70%) and N-hexane (0.31 ± 0.00%) extractions (Figure 1), highlighting that the *G. gracilis* global yield was affected by the extraction method. The yield of extracts increased with increasing solvent polarity, in agreement with Chan et al. [51], whose ethanol and water extractions led to similar extraction yields in *G. changii* wild-collected from the mangrove area of Santubong [51].

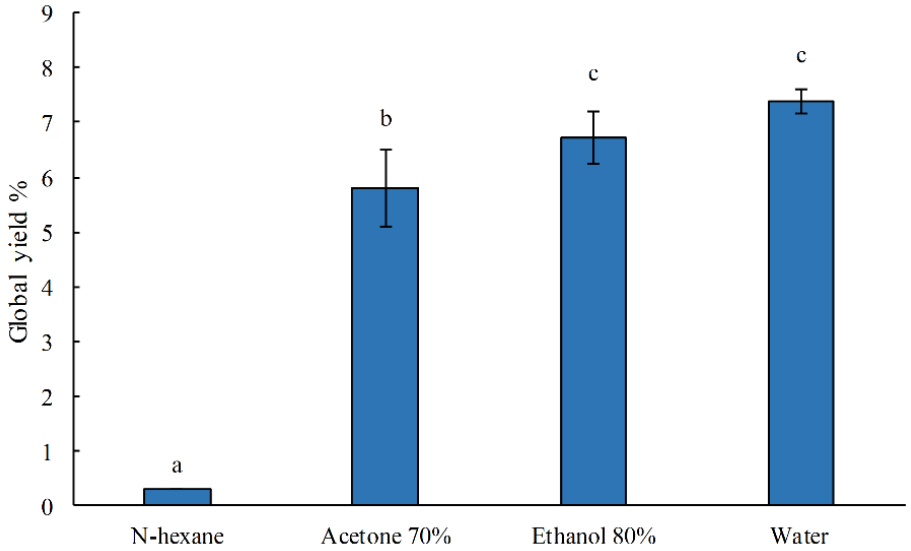


Figure 1. Extract yields (%) obtained with different solvents (N-hexane, acetone 70%, ethanol 80%, and water) from dried *G. gracilis*. Bars represent the mean ± SEM ($n = 3$). Lowercase letters indicate significant differences among different solvents ($p < 0.05$).

Polyphenols are products of secondary metabolism, with interesting biological activities having a key role in the defense against environmental stress [53], countering the oxidative stress that leads to ROS generation [53,54]. The obtained results show that the total polyphenol content was significantly higher in the ethanol 80% (0.16 ± 0.02 mg GAE/g DW) and water extracts (0.11 ± 0.01 mg GAE/g DW), followed by the acetone 70% (0.03 ± 0.003 mg GAE g/DW) and N-hexane (0.004 ± 0.001 mg GAE/g DW) extracts (Figure 2). This suggests that ethanol is an efficient solvent for polyphenolic acid [45,47,52,55,56]. Water and ethanol are the most commonly used solvents for the

extraction of antioxidant compounds due to their low toxicity and potential use in the nutraceutical and cosmeceutical sectors [16].

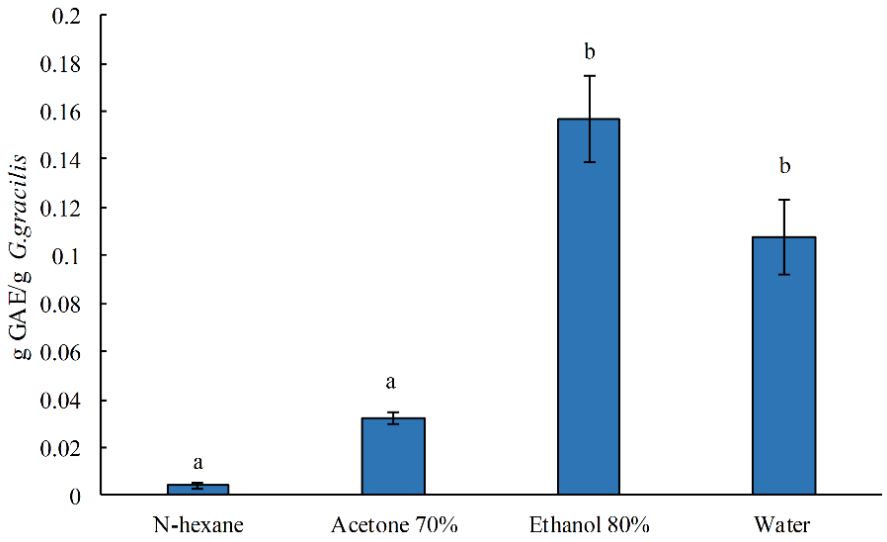


Figure 2. Total polyphenol content (mg GAE/g DW) in *G. gracilis* extracted by N-hexane, acetone 70%, ethanol 80%, and water. Bars represent the mean ± SEM (n = 3). Lowercase letters indicate significant differences among the different solvents ($p < 0.05$).

The DPPH assay measures the ability of an antioxidant to inhibit the DPPH free radicals.

The *G. gracilis* extracts obtained by N-hexane and ethanol 80% showed higher antioxidant activity (DPPH IC50) (8.30 ± 2.80 mg DW/mL and 10.46 ± 3.17 mg DW/mL, respectively) ($p < 0.05$) than those obtained by water (28.25 ± 5.80 mg DW/mL) and acetone 70% (37.7 ± 9.34 mg DW/mL) (Figure 3).

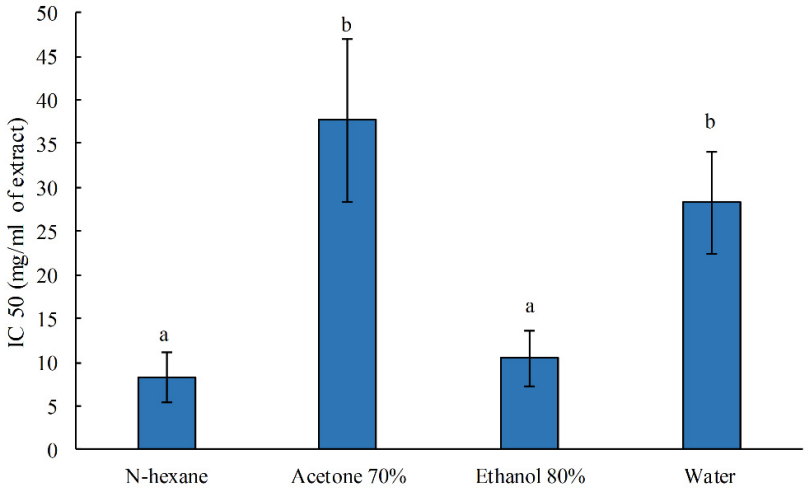


Figure 3. DPPH radical scavenging activity (IC 50, mg DW/mL) in *G. gracilis* by N-hexane, acetone 70%, ethanol 80%, and water. Bars represent the mean ± SEM (n = 3). Lowercase letters indicate significant differences among the different solvents ($p < 0.05$).

Our results are in agreement with those of Chan et al. [51], who showed that the radical scavenging activity of wild-collected *G. changii* extracts decreased as the solvent polarity increased. The water and acetone extracts showed the lowest radical scavenging activity compared to ethanol and ethyl acetate extracts [51]. Vega et al. demonstrated that hydroethanolic extracts from marine red macroalgae cultivated in IMTA showed the lowest scavenger activity compared to wild-collected species [16].

Polyphenols produced by *G. gracilis* showed antibacterial and antioxidant activities, as reported by Afonso et al. [57], highlighting their possible use as an additive for functional fish feeds. This was confirmed by Passos et al. [24], whose study showed that supplementing compounds extracted from *G. gracilis* in the diet of a farmed fish species enhanced fish health and growth. These results suggest that red seaweeds cultivated in an IMTA system are able to adapt and to grow under different integrated culture conditions, as reported by Ashkenazi et al. [58], and to produce bioactive compounds that might have a significant antioxidant capacity and potential use in different sectors [16,23,58].

3.2. Cytoprotective Potential of Ethanol Extract of *G. gracilis* in SAF-1 Cells Exposed to BDE-47

The *G. gracilis* ethanol extract was selected to evaluate its protective role against oxidative stress induced by BDE-47 in vitro in the *S. aurata* fibroblast (SAF-1) cell line.

The ethanol extracts of *G. gracilis* at concentrations of 0.005–0.01–0.02 µg/mL tested in the SAF-1 cell line did not significantly affect the cell viability compared to the untreated cells (Figure 4). The SAF-1 cells untreated and exposed to BDE-47 (50 µM) did not affect the cell viability after 48 h of incubation, whereas the SAF-1 cells not pretreated and exposed to the highest BDE-47 concentration (100 µM) resulted in a significant decrease in cell viability (up to 30%) ($p < 0.05$) after 48h, compared to the control (Figure 4).

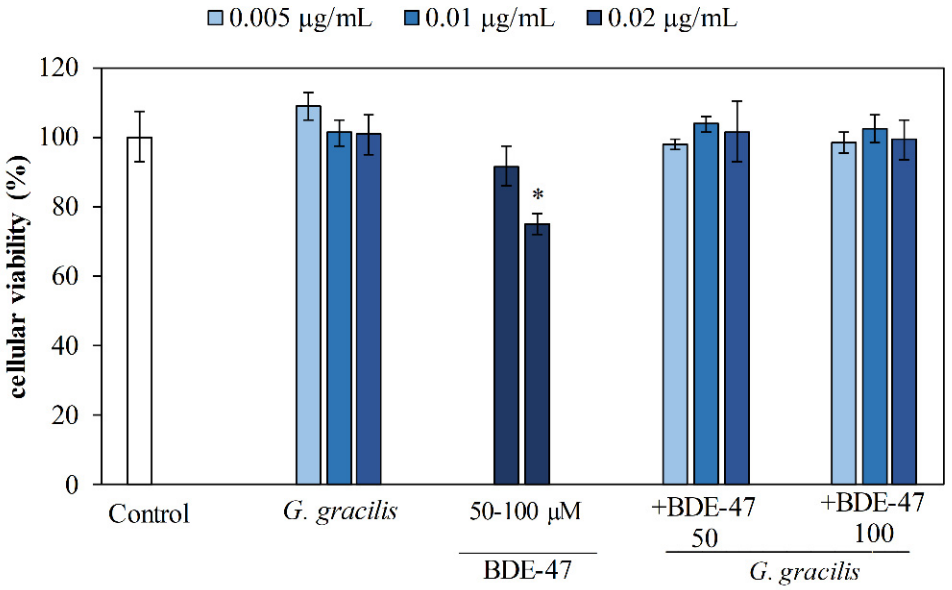


Figure 4. Cellular viability (%) of SAF-1 cells measured, comparing the control cells and cells exposed to different concentrations of BDE-47, with the cells pretreated for 24 h with different concentrations of *G. gracilis* extracts (0.005–0.01–0.02 µg/mL) and then exposed to different concentrations of BDE-47 (50–100 µM) for 48 h. The bars represent the mean ± SEM ($n = 6$). Statistical differences ($p < 0.05$) among the groups are indicated by "*".

The SAF-1 cells pretreated with the ethanol extracts of *G. gracilis* (0.005–0.01–0.02 µg/mL) and then exposed to 50–100 µM of BDE-47 (G. *gracilis* + BDE-47) showed a protective effect against BDE-47 oxidative stress induction compared to the cells exposed to 100 µM of BDE-47 without any pretreatment (BDE-47) (Figure 4), suggesting that *G. gracilis* extract could prevent and counter cellular oxidative stress. The cytoprotective effect of *G. gracilis* extract against BDE-47 oxidative stress induction further confirms its antioxidant power. The *G. gracilis* extracts showed the same ability as synthetic antioxidants (β-carotene and gallic acid) to counteract emerging contaminant inducing oxidative stress in seabream fibroblast cell lines [26], highlighting that bioactive compounds extracted by macroalgae represent a bioavailable source of antioxidants [27,28,43,44,59,60].

Evaluation of Molecular Biomarkers Related to Cell Cycle and Oxidative Stress by Gene Expression Analysis

One of the main effects induced by exposure to chemical contaminants is the overproduction of ROS in cells, causing oxidative stress [26,61]. This stress condition influences cellular homeostasis and the antioxidant defense system, modifying some biochemical pathways that might induce cellular transformations [26,28,44]. Indeed, previous studies have suggested that BDE-47-induced oxidative stress led to cytotoxicity and apoptosis [26,28,44]. Since the molecular mechanisms by which BDE-47 affects the cell cycle, the cellular response to stress, and the antioxidant defense system remain unclear, the modulation of molecular markers related to the cell cycle (*p53*) and oxidative stress (*nrf*2, *sod*, and *cat*) was analyzed by gene expression analysis in SAF-1 cells (Figure 5).

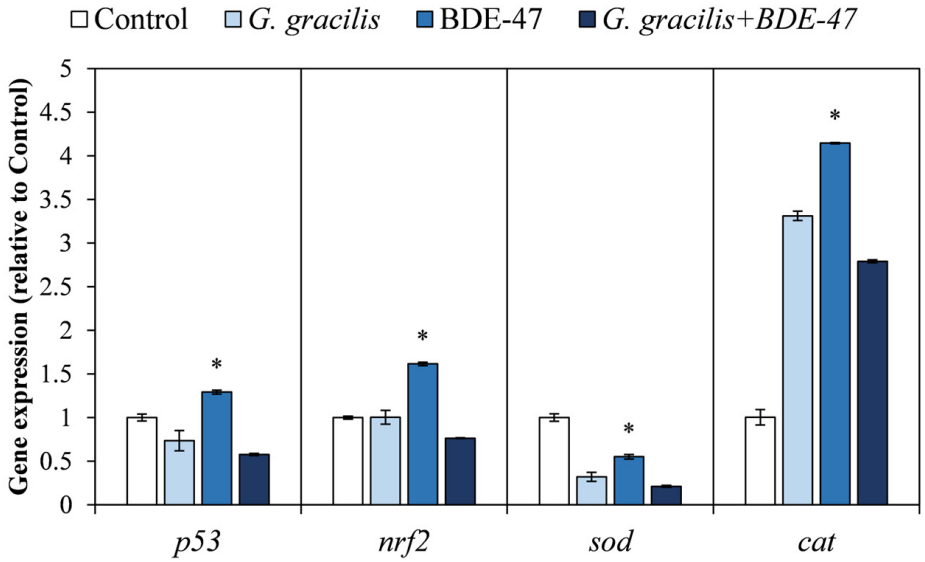


Figure 5. Relative gene expressions of genes related to *p53*, *nrf2*, *sod*, and *cat* in *S. aurata* SAF-1 cells not treated (Control), pretreated with *G. gracilis* (*G. gracilis*), and pretreated with *G. gracilis* extracts (0.02 µg/mL), and then exposed to BDE-47 (100 µM) (*G. gracilis* + BDE-47). The bars represent the mean ± SEM ($n = 6$). Statistical differences ($p < 0.05$) among the groups compared to the control are indicated by "*".

Tumor suppressor protein p53 plays a critical role in cell-cycle control, apoptosis, and in response to DNA damage [62]. After its activation by several stimuli, p53 regulates the expression of many target genes related to cell-cycle arrest and apoptosis induction, inhibiting cell proliferation [63,64]. In our study, the *p53* mRNA levels were significantly up-regulated ($p < 0.05$) in cells exposed to BDE-47 (BDE-47) compared to the untreated cells (control) (Figure 5), suggesting that oxidative stress induced by BDE-47 caused DNA damage in SAF-1 cells [26,27]. The obtained results show that pre-incubation with *G. gracilis* extract before exposure to BDE-47 (*G. gracilis* + BDE-47) significantly decreased the *p53* mRNA levels compared to the cells exposed to BDE-47 without any pretreatment (BDE-47) ($p < 0.05$) (Figure 5), highlighting that *G. gracilis* extract exhibited a strong cytoprotective activity against BDE-47-induced oxidative injury.

Nuclear factor erythroid 2-related factor 2 (Nrf2), a redox sensitive transcription factor, is activated in response to a wide range of oxidative stimuli [26,44,61]. After its activation, it translocates into the nucleus and activates the antioxidant responsive elements (AREs) of cytoprotective genes coding for antioxidant enzymes, stress protein, and phase II detoxification enzymes [61].

In this study, the up-regulation of the *nrf*2 gene observed in cells exposed to BDE-47 (BDE-47) (Figure 5) suggests a rapid antioxidant response in decreasing oxidative toxicity induced by environmental pollutants [26]. Persistent nuclear accumulation of Nrf2 may

have dangerous effects, such as free radical damage, apoptosis, and tumorigenesis [65]. In the SAF-1 cells pretreated with *G. gracilis* extract and then exposed to BDE-47 (*G. gracilis* + BDE-47), the mRNA expression levels of *nrf2* significantly decreased ($p < 0.05$) (Figure 5), showing a high protective capacity of the macroalga extract against BDE-47 oxidative stress induction. Furthermore, the decrease in *nrf2* expression could be related to its signaling deactivation and to the restoration of cellular redox homeostasis [61].

Chen et al. [66], in accordance with our results, reported a decrease in nrf2 expression when Neuro-2a cells were pretreated with N-acetyl-l-cysteine (NAC) and then exposed to BDE-47, highlighting the ability of NAC to alleviate the oxidative stress and block apoptosis.

In addition, Nrf2 countered oxidative stress induced by environmental pollutants, through the transcription of cytoprotective genes or antioxidant proteins [61]. SOD and CAT are the major antioxidant enzymes to protect cells against oxidative stress. SOD converts superoxide anion (O_2^-) produced by mitochondria to the less reactive hydrogen peroxide (H_2O_2) [67], while CAT detoxifies H_2O_2 to H_2O and O_2 [68]. High levels of SOD lead to an increase in intracellular H_2O_2, inducing DNA damage and tumor promotion [69]. A significant decrease in *sod* and an increase in *cat* mRNA levels ($p < 0.05$) were observed in cells exposed to BDE-47 (BDE-47) (Figure 5), suggesting that the overproduction of free radicals induced by BDE-47 affected the antioxidant system and led to oxidative cellular stress [27,61]. The pre-incubation of SAF-1 cells with *G. gracilis* extract and then exposed to BDE-47 (*G. gracilis* + BDE-47) significantly decreased the *sod* and *cat* mRNA levels ($p < 0.05$) compared to the cells exposed to BDE-47 without any pretreatment (BDE-47) (Figure 5). Furthermore, the cells pre-incubated with the ethanol extract and not exposed to BDE-47 further decreased the *sod* mRNA levels, suggesting that the ethanol extract of *G. gracilis*, could exert a preventive effect on BDE-47-induced oxidative stress, reducing the superoxide anion production and improving the antioxidant defense system [70]. The obtained results confirm that the pretreatment with *G. gracilis* extracts, before exposure to BDE-47, protected the SAF-1 cells against oxidative stress, improving the cellular antioxidant defense system and cell cycle control.

4. Conclusions

This study highlights that *G. gracilis* cultivated in IMTA could be a good source of antioxidants, as demonstrated by its antioxidant activity and determined by its polyphenol content. Hydroalcoholic solvent extraction (ethanol 80%) showed a higher extractive yield and polyphenol rate. Polyphenol compounds obtained from ethanol extract of *G. gracilis* showed protective capacity against oxidative stress induced by the pro-oxidant BDE-47 in the SAF-1 cell line. These results are supported by gene expression analysis, highlighting the antioxidant capacity of *G. gracilis* extracts against chemical contaminant inductors, which modulate molecular markers related to the cell cycle and oxidative stress. Furthermore, the biomarkers used as an early warning in this study to predict the effects of pro-oxidant contaminants at lower levels of biological organization could be adopted for environmental biomonitoring.

Given the high potential of bioactive compounds in counteracting the oxidative stress responsible for cell damage in all living organisms, including fish, induced by different environmental conditions, the extraction and isolation of bioactive compounds from marine matrices could be promoted for their potential applications in nutraceutical and aquaculture fields.

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