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# Pharmacological and Cosmeceutical Potential of Seaweed Beach-Casts of Macaronesia

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**Abstract:** Seaweed beach-casts are a seasonal phenomenon that regularly deposits tons of algae biomass on beaches, which are usually disposed of in landfills. The present work aimed to contribute to the valorization of this biomass by studying bioactivities that reveal its potential in the pharmaceutical and/or cosmeceutical industries. Methanol and ethanol extracts and fractions from 15 beach-casts biomass were tested for a range of bioactivities. Eight of the most active samples exhibit $IC_{50}$ values between 11.38 µg/mL and 19.28 µg/mL and selectivity indexes higher than 3.8, against NCI-H1299 (lung cancer) and T47D (breast cancer). Concerning antiaging potential, all the extracts tested presented UV protection, with SPF values above 30, like the standard sunscreen. Regarding aging-related enzymes, 24 samples were tyrosinase inhibitors exhibiting $IC_{50}$ values from 9.01 µg/mL to 200.09 µg/mL. Eight extracts inhibited collagenase ($IC_{50}$ < 248.76 µg/mL), two of them more actively than positive control EDTA ($IC_{50}$ = 59.26 µg/mL). In addition to UV protection, the cumulative effect of antityrosinase and anticollagenase activities shown by the samples suggest that they may play a significant role in preventing skin photoaging. The results obtained demonstrate the high potential of beach-cast seaweed biomass as sources of cosmetic bioproducts with antiaging effects, and of selective cytotoxic metabolites.

**Keywords:** beach-cast seaweeds; biomass valorization; anticancer; antiaging; antityrosinase; UV protection

## 1. Introduction

Beach-cast seaweeds, consisting in the accumulation of large amounts of algae detached from the bottom of the sea or coast platforms, are documented on beaches of many coasts around the world [1,2]. This is a natural and seasonal phenomenon which is mainly influenced by climate, wind conditions, eutrophication, and more importantly, the occurrence of considerable wave episodes; due to climate change, the number of beach-casts has been increasing dramatically over recent years [3,4].

The biomass deposited during beach-cast phenomena is unpleasant for beach users and affects the tourism industry [5,6], mainly because tourists often interpret stranded natural litter as lowering beach quality, especially if the material starts to decompose [7]. The beach-cast phenomenon has a huge impact in the Canary Islands (Spain), particularly on the island of Gran Canaria, which receives many swimmers, visitors and holidaymakers all year round, where rotting seaweed masses can become a nuisance affecting local businesses and the tourism industry. To understand the magnitude of this phenomenon, the amount of seaweed beach-casts varies from 400 to 1200 tons per year in Las Canteras beach in Grand Canaria, occurring with more incidence from November to March [8]. These beach casts are always variable mixtures of different species of seagrass and seaweed, and in the case of Gran Canaria island, the main species were reported to be *Cymodocea nodosa* (Angiosperm), *Cymopolia barbata* (Chlorophyta), *Halopithys incurva* (Rodophyta), *Halopteris scoparia* (formerly *Stypocaulon scoparium*), *Lobophora variegata, Treptacantha abies-marina* (formerly *Cystoseira abies-marina*), *Dictyota* sp. (Ochrophyta, Phaeophyceae), with *L. variegata* being the most frequent and abundant seaweed [8].

The management of all the biomass from a single beach-cast is difficult and costly, and, most of the time, the solution is to collect it from the beach and to dispose of it in landfills without further studies or valorization. However, seaweeds are gaining increasing attention in many industries like fuel, plastics, cosmetics, pharmaceutical, and food, essentially because they produce a wide variety of structurally and functionally diverse metabolites [9,10]. These compounds display attractive biological activities (i.e., antioxidant, antimicrobial, anticancer, etc.) with various applications, many of which are already commercially exploited [10–12]. Due to all potential applications of seaweeds and their metabolites, it is illogical to waste this valuable biomass; in fact, the biomass obtained from beach-cast holds a large interest, and some works have proved that it can be valorized as a source of biochemicals, feed, food, fertilizer and fuel [13,14].

In view of the above-mentioned, the present work evaluated samples from different beach-casts with distinct seaweed composition, assaying several biological activities of crude and fractionated extracts, aiming to establish new potential applications to valorize seaweed beach-cast biomass converting it into new products.

## 2. Materials and Methods

### 2.1. Harvesting, Treatment and Processing of Seaweed Beach-Casts

Different beach-casts arrived in the shore of Playa de Las Canteras beach (Las Palmas de Gran Canaria, Spain) were collected from 2016 to 2018 and treated as described below. The seaweed identity of each beach-cast was studied, showing different compositions and proportions of seaweeds, e.g., *Lobophora variegata*, *Dictyota* sp. and *Asparagopsis taxiformis* (Rhodophyta), among others, with some of the beach-casts being composed almost exclusively by *L. variegata*. The species composition of the beach-casts analyzed is presented in Table S1 (Supplementary Material).

A method was developed specifically for the treatment and processing of beach-casts removed from Las Canteras beach [15]. These were harvested with a machine, together with important amounts of sand, small stones (<8 mm), other impurities and materials. The washing process, necessary to clean the seaweed biomass, was carried out only with seawater, in order to avoid as much as possible, the loss of desirable and sought bioactive compounds, and to avert hindering any other possible applications of this biomass. After washing, each beach-cast batch, around 150–200 kg of fresh weight, was subjected to drying until a moisture content of less than 15% was obtained. The amount of biomass per surface required for drying was 6 kg fresh weight per square meter to accelerate dehydration in less than 24 h, and to lessen possible decomposition processes. The outdoor dryer modules were of 16 $m^2$ area, placed under the shade using a shading mesh. Drying was also achieved by the action of continuous natural wind. After 24 h, the dried samples were subsequently crushed and ground to a fine powder, stored at room temperature in the dark.

### 2.2. Algal Biomass Extraction with Organic Solvents

Seaweed beach-cast dried samples were used to obtain extracts for bioactivities determinations. Two solvents were individually used, 96% food grade analytical pure ethanol, and methanol (Honeywell, Riedel-de Haen, Charlotte, NC, USA). The extraction method was adapted from Nunes et al. [16] with some modifications. One hundred grams of seaweed beach-cast material, ground to about 200 mesh particle size was mixed with 1 L of the corresponding solvent and this suspension was placed in sonication, during 20 min, using a sonic bath (Fisherbrand, FB11209, Houston, TX, USA). The extraction continued further by placing this mixture in an automated extractor (Tecnolab, Timatic, Spello, Italy) programmed to run six extraction cycles at 8.5 bar pressure, during 1 h.

After filtration, each liquid extract was transferred to a rotary evaporator (Heidolph, Hei-Vap, Schwabach, Germany) to concentrate the extract. Finally, this concentrate was dried in the oven, at 40 °C and the solid extract was weighted and stored at −20 °C. The extraction yields are presented in Table S1 (Supplementary Material). Seaweed extracts were further evaluated for their biological activities.

Some of the most active extracts were submitted to fractionation, which was conducted according to the following methodology. One gram of extract (methanol or ethanol) was initially mixed with 50 mL of hexane. Suspension was vortexed and sonicated in the ultrasound bath for 10 min. Afterwards, samples were centrifuged at 7200× *g*, at 20 °C for 10 min. The supernatant was transferred and concentrated on a rotary evaporator at 40 °C, being completely dried in an oven at 40 °C and then stored at −20 °C. The remaining residue from centrifugation was dried in an oven at 40 °C and then mixed with dichloromethane (50 mL), following the same procedure described above. Two other solvents were sequentially used, following the same procedure, namely chloroform and methanol: water (1:1, v/v). Fractionated seaweed extracts were further analyzed for their bioactivities. The fractions yields are presented in Table S2 (Supplementary Material).

### 2.3. Preparation of Stock Solutions of Extracted Samples for Conducting Bioassays

Dried extracts and fractions were aseptically dissolved in sterile dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Madrid, Spain) at a final concentration of 40 (antimicrobial and anticancer assays) or 50 (remaining assays) mg/mL. In some cases, when samples did not dissolve properly, the concentration was lowered to 20 mg/mL and occasionally a mixture of sterile DMSO-water (1:1) was used. These were properly mixed using a vortex at maximum speed, and when needed also applying a sonication bath for 3–4 min. Stock solutions were stored at −20 °C until use.

### 2.4. Antibacterial Activity

The antibiotic activity of all extracts and fractions was individually determined employing four bacterial species, two Gram-positive i.e., *Bacillus subtilis* and *Staphylococcus aureus*, and two Gram-negative i.e., *Escherichia coli* and *Salmonella enterica*.

The different bacterial strains were individually grown in 125 mL Erlenmeyer flasks containing 10 mL of sterile liquid nutrient medium TSB (Tryptic Soy Broth) (Scharlab, Barcelona, Spain), to which an inoculum of 5 µL from a previous fresh overnight culture was added. Finally, the bacteria were incubated at 37 °C with orbital shaking at 100 rpm in the dark for 24 h.

For conducting the bioassays and to get a range of 1–5 × $10^5$ CFU/mL two single dilutions of $10^{-4}$ and $10^{-5}$ of the overnight cultures were freshly prepared and individually used.

All samples were tested at two final concentrations of 50 µg/mL and 100 µg/mL, freshly prepared in sterile TSB medium under aseptic conditions from sample stocks stored at −20 °C.

Bioassays following the microdilution method similar to published reports (e.g., Balouiri et al. [17] were performed in standard 96-well flat bottom plates (VWR, Barcelona, Spain). Each well was loaded individually with 100 µL of the prepared bacterial culture diluted to obtain 1–5 $10^5$ CFU/mL. To this, a further 100 µL of the freshly prepared doubled concentrated samples in TSB was added.

Three replicate wells for each sample and concentration were seeded, and the microplates were incubated for 24 h at 37 °C.

A vehicle containing the same amount of DMSO used to dissolve the extracts, a positive control containing 50–100 units of the antibiotic mixture streptomycin/tetracycline (Sigma-Aldrich, Madrid, Spain), and a blank were also set up in triplicates, and incubated under the same conditions.

Finally, the absorbance of each well was measured at 600 nm using a MultiSkan Go spectrophotometer (Thermo Fisher Scientific, MA, US).

The blank reading value was subtracted from all mean values to obtain the normalized values. Cell survival rate of the bacterial population following treatment was calculated using the normalized values and the following formula:

$$\text{Survival (\%)} = [(\text{sample absorbance value} \times 100)/(\text{normalized value of positive control})] \quad (1)$$

### 2.5. Antifungal Activity

Antifungal bioassays were conducted employing two yeast species, *Candida albicans* and *Saccharomyces cerevisiae*, evaluating cell viability following a microdilution method similar to the one described in Section 2.4 above, with small variations.

The yeast strains were grown in 10 mL of liquid YPD medium (Yeast extract Peptone Dextrose)(Sigma-Aldrich, Madrid, Spain) following inoculation of 5 µL from a fresh culture as described above. These were grown under the same conditions described above but during 48 h. Similarly, culture dilutions were aseptically prepared but employing fresh yeast culture dilutions of $10^{-2}$ and $10^{-3}$ of each individual species.

Likewise, the corresponding blanks, vehicle, and positive control (100–200 mM $CuSO_4$ solution), were prepared. The absorbance data were obtained and processed, calculating the percentage of cell viability as described for the antibacterial bioassay.

### 2.6. Anticancer Activity

Antitumor activity of samples was evaluated employing four human tumor cell lines: T47D (breast cancer), DU145 (prostate cancer), NCI-H1299 (lung carcinoma) and HT29 (colorectal cancer). A nontumor Vero cell line from kidney epithelial cells of the African green monkey was also used.

Each algal extract and fraction was initially tested at two sample concentrations to allow the detection of a high to moderately-high anticancer activity [18], at final concentrations of 20 µg/mL and 50 µg/mL, freshly prepared by diluting from sample stocks kept at −20 °C.

The determination of the cytotoxic activity of the samples was performed following the MTT (tetrazolium salts) method [19]. The selected tumor cell lines were grown in 75 $cm^2$ Nunc$^{TM}$ culture flasks (Thermo Fisher Scientific, Roskilde, Denmark), with the corresponding nutrient medium i.e., T47D and NCI-H1299 with RPMI medium; DU145 with DMEM medium, and HT29 with McCoy's medium (Biowest, Miami, FL, US). To perform the assay, 100 µL of cells with the appropriate nutrient medium were seeded in a 96-well plate (5000 cell/well for DU145 and 10,000 cells/well for the other three cell lines), similar to the method described by Tolosa et al. [20]. To determine the concentration of viable cells for the bioassays, an ADAM cell counter (NanoEnTek Inc., Waltham, MA, USA) was used following the manufacturer's instructions. Plates were incubated at 37 °C, 95% humidity and 5% $CO_2$, for 24–72 h depending on the time division of the cell line.

Assays were performed in triplicate for both concentrations and controls. A 5.43 µg/mL (10 µM) solution of the commercial anticancer drug adriamycin was used as positive control, together with vehicle and negative controls.

After 48 h incubation, 10 µL of freshly prepared MTT solution tempered in the incubator was added to each well (final concentration: 0.3 mg/mL) and allowed to react for 1.5–2 h, followed by solubilization and homogenization of formazan. Finally, absorbance was recorded at 595 nm using the above-mentioned MultiSKan Go spectrophotometer.

Analysis of the collected results was conducted by calculating the mean value and standard deviation of the three replicates. The data blank was subtracted to the absorbance values of treated cells, positive and negative controls. The percentage of cell survival was calculated as:

$$\text{Survival (\%)} = [(\text{Abs untreated cells} - \text{Blank}) \times 100]/\text{mean (Abs untreated cells} - \text{Blank})] \quad (2)$$

Samples displaying the lowest cell survival rates were reassayed in the same manner as above but in a range of concentrations up to 80 µg/mL, obtained by serial dilution, in order to allow the determination of the $IC_{50}$ values, i.e., the concentration that causes a 50% growth inhibition of the cell population.

### *2.7. Anticholinesterasic Activity*

The assay for measuring acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activity was modified from the assay described by Ellman et al. [21] and Arruda et al. [22]. Briefly, 3 mM 5,5′-dithiobis [2-nitrobenzoic acid](DTNB, 5 µL), 75 mM acetylthiocholine iodide (ATCI, 5 µL) or butyrylthiocholine iodide (BuTCI, 5 µL), sodium phosphate buffer 100 mM (pH 8.0, 110 µL), and sample or standard (donepezil) dissolved in buffer containing no more than 2.5% DMSO were added to the wells, and serial dilutions were carried out to obtain concentrations ranging between 0.293 µg/mL and 150 µg/mL (0.010–5.0 µg/mL for donepezil (0.026–13 µM), followed by 0.25 U/mL AChE or BuChE (10 µL). The absorbance was then read at 415 nm every 2.5 min for 7.5 min in a Bio Rad Model 680 Microplate Reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA). For each concentration, enzyme activity was calculated as a percentage of the velocities compared to that of the assay using buffer without any inhibitor. Every experiment was done in triplicate.

### *2.8. Antiaging Activity*

#### 2.8.1. DPPH Radical Scavenging Activity

Antioxidant activity was assayed by the DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging assay [23]. Serial dilutions of studied extracts, fractions or reference compounds (Trolox 100 µg/mL (400 µM)) were carried out in 96-well microplates, at different concentrations, ranging between 0.244 µg/mL and 250 µg/mL in methanol. DPPH dissolved in methanol was added to the microwells, yielding a final concentration of 45 µg/mL, and the absorbance at 515 nm was measured with a BioRad Microplate Reader Model 680 (Bio-Rad Laboratories, Inc., Hercules, CA, USA), after 30 min in the dark. In each assay, a control was prepared, in which the sample or standard was substituted by the same amount of solvent. Percentage of antioxidant activity (% AA) was calculated as:

$$\%\ \text{AA} = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})]/(\text{Abs}_{\text{control}})] \times 100 \quad (3)$$

where, $\text{Abs}_{\text{control}}$ is the absorbance of the control and $\text{Abs}_{\text{sample}}$ is the absorbance of the alga extract or standard. All assays were carried out in triplicate and results expressed as $IC_{50}$, i.e., as the concentration yielding 50% scavenging of DPPH, calculated by interpolation from the % AA vs. concentration curve.

#### 2.8.2. ABTS Radical Scavenging Assay

The method of Re et al. [24] was adopted to perform ABTS radical scavenging assay. The stock solutions included a 7 mM ABTS solution (2,2′ Azinobis-(3 ethylbenzothiazoline 6 sulfonic acid)) and a 2.4 mM potassium persulfate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12–16 h at room temperature in the dark. The solution was then diluted by mixing 1 mL ABTS solution with the amount of methanol necessary to obtain an absorbance of 0.7 at 734 nm. Serial dilutions of studied extracts, fractions or reference compounds (trolox, 100 µg/mL (400 µM)) were carried out in 96-well microplates, at different concentrations, ranging between 0.244 µg/mL and 250 µg/mL in methanol. ABTS solution was then

added to the microwells, and after 8 min of incubation the absorbance was recorded at 405 nm with a BioRad Microplate Reader Model 680 (Bio-Rad Laboratories, Inc., Hercules, CA, USA). In each assay, a negative control was prepared, in which the sample or fraction was substituted by the same amount of solvent. Percentage of antioxidant activity (% AA) was calculated as:

$$\text{Chelating effect (\%)} = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})]/(\text{Abs}_{\text{control}})] \times 100 \quad (4)$$

where $\text{Abs}_{\text{control}}$ is the absorbance of ABTS radical + methanol; $\text{Abs}_{\text{sample}}$ is the absorbance of ABTS radical + sample/standard.

All assays were carried out in triplicate and results expressed as $IC_{50}$, i.e., as the concentration yielding 50% scavenging of ABTS, calculated by interpolation from the % AA vs. concentration curve.

#### 2.8.3. Ferric Chelating Activity Assay

The $Fe^{2+}$ chelating ability of the extracts was measured by the ferrous iron-ferrozine complex method [25]. Briefly, the reaction mixture containing 2 mM $FeCl_2$ (10 µL), 5 mM ferrozine (10 µL) and 100 µL of serial concentrations of alga extracts or fractions (ranging between 0.244 µg/mL and 250 µg/mL in methanol) were mixed in a 96-well plate and incubated for 10 min at 27 °C. The absorbance was recorded employing a BioRad Microplate Reader Model 680 (Bio-Rad Laboratories, Inc., Hercules, CA, USA) at 550 nm. The absorbance of the control was determined by replacing the extract with methanol. EDTA, Ethylenediamine tetraacetic acid (0.195–100 µg/mL (0.290–297 µM)) was used as a positive control. The ability of the sample to chelate ferrous ion was calculated:

$$\text{Chelating effect (\%)} = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})]/(\text{Abs}_{\text{control}})] \times 100 \quad (5)$$

where $\text{Abs}_{\text{control}}$ is the absorbance of the ferrozine-ferrous iron complex + methanol; $\text{Abs}_{\text{sample}}$ is the absorbance ferrozine-ferrous iron complex + sample/standard.

All assays were carried out in triplicate and results expressed as $IC_{50}$, i.e., value which was the concentration of the sample that chelated 50% of the ferrous iron, calculated by interpolation from the % chelating effect vs. concentration curve.

#### 2.8.4. Inhibition of β-Carotene-Bleaching Assay

The antioxidant activity of each extract or fraction was evaluated by the β-carotene linoleate model system, adapted from the methods described by Barreira et al. [26] and Lu et al. [27]. Briefly, a solution of β-carotene was prepared by dissolving 2 mg of β-carotene in 10 mL of chloroform. A volume of 2 mL of this solution was pipetted into a 100 mL round-bottom flask. After the chloroform was removed at 40 °C under vacuum, 40 mg of linoleic acid, 400 mg of Tween 80 emulsifier, and 100 mL of distilled water were added to the flask with vigorous shaking. Aliquots (2.4 mL) of this emulsion were transferred into different test tubes containing 0.1 mL of different concentrations (0.244–250 µg/mL in methanol) of the extracts or the reference compound gallic acid (1.5 mM). The tubes were shaken and incubated at 50 °C in a water bath. As soon as the emulsion was added to each tube, the absorbance was measured at 470 nm using a spectrophotometer.

Absorbance readings were then recorded after 120 min. A blank, devoid of β-carotene, was prepared for background subtraction. Lipid peroxidation (LPO) inhibition was calculated using the following equations:

$$\text{Degradation rate (DR)} = \ln a/b \times 1/t, \quad (6)$$

where "$a$" is the initial absorbance, "$b$" is the absorbance at 120 min and "$t$" is the total test time (120 min). The antioxidant activity was presented as percent of inhibition using the following formula:

$$\text{\% AA} = (\text{DR}_{\text{control}} - \text{DR}_{\text{sample}})\ \text{DR}_{\text{control}} \times 100 \quad (7)$$

The extract concentration providing 50% antioxidant activity ($IC_{50}$) was calculated from the graph of antioxidant activity percentage vs. concentration.

#### 2.8.5. Determination of Sun Protection Factor (SPF)

The sun protection factor (SPF) of the extracts or fraction was determined by in vitro method using UV-Visible spectrophotometer [28,29]. Standard sunscreen lotion (Darphin Soleil SPF 30), or the extracts were taken at the concentration of 250 µg/mL in ethanol 50% (v/v). Thereafter, absorbance values of each sample were spectrophotometrically determined from 290–320 nm, in 5 nm intervals, and taking 50% ethanol as blank. Each measurement was done in triplicate. SPF was calculated by using the equation derived by Mansur et al. [28] and Santos et al. [29]:

$$SPF_{sample} = CF_{\cdot 290}\Sigma^{320}EE(\lambda)xI(\lambda)xA(\lambda) \quad (8)$$

where correction factor CF = 10, EE ($\lambda$) = erythemogenic effect of radiation of wavelength $\lambda$, I($\lambda$) = intensity of solar light of wavelength $\lambda$, A($\lambda$) = spectrophotometric absorbance values at wavelength λ. EE($\lambda$) × I($\lambda$) values determined by Sayre et al. [30] are as follows (Table 1):

**Table 1.** EE($\lambda$) × I($\lambda$) value of each of the wavelengths used to determine the sun protection factor (SPF).

| Wavelength ($\lambda$) | EE($\lambda$) × I($\lambda$) Value |
|---|---|
| 290 | 0.0150 |
| 295 | 0.0817 |
| 300 | 0.2874 |
| 305 | 0.3278 |
| 310 | 0.1864 |
| 315 | 0.0837 |
| 320 | 0.0180 |

#### 2.8.6. Inhibition of Extracellular Matrix ECM and Skin-Degrading Enzymes

##### Hyaluronidase Inhibition Assay

The methods described by Ndlovu et al. [31] and Zhou et al. [32] were applied for the hyaluronidase inhibition assay. The following was added into 2 mL test tubes: 50 µL of calcium chloride (12.5 mM), 50 µL of test samples or sodium aurothiomalate diluted in 100 mM acetate buffer; pH 3.5 (with concentrations ranging between 15.6–250 µg/mL), and 25 µL hyaluronidase (0.5 mg/mL). The tubes were incubated in a water bath (37 °C; 20 min) after which 50 µL of the substrate hyaluronic acid (0.25 mg/mL in 100 mM acetate buffer; pH 3.5) was added and the tubes incubated for further 40 min. A volume of 25 µL of $KBO_2$ $4H_2O$ (800 mM) was added to all tubes which were placed in a water bath (100 °C) for 3 min, left to cool to room temperature and 800 µL of DMAB (4-dimethylaminobenzaldehyde) (4 g DMAB in 40 mL acetic acid and 5 mL 10 N HCl) was added. The tubes were then incubated for 20 min and the contents transferred to respective wells in a 96-well plate. Absorbance was detected at 585 nm Bio Rad Model 680 Microplate Reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Percentage of hyaluronidase inhibition was calculated as:

$$\%\ \text{hyaluronidase inhibition} = [(Abs_{control} - Abs_{sample})]/(Abs_{control})] \times 100 \quad (9)$$

where $Abs_{control}$ is the absorbance of buffer + hyaluronidase; $Abs_{sample}$ is the absorbance of buffer + hyaluronidase + sample/standard.

All assays were carried out in triplicate and results expressed as $IC_{50}$, i.e., as the concentration yielding 50% of hyaluronidase inhibition, calculated by interpolation from the % hyaluronidase inhibition vs. concentration curve.

### Tyrosinase Inhibition Assay

The extracts were assayed by an adaptation of the tyrosinase inhibition method described by Shimizu et al. [33] and modified by Manosroi et al. [34]. Briefly, 25 µL of tyrosinase enzyme solution (135 U/mL), 25 µL of ten serial concentrations of the extracts (0.488 µg/mL to 250 µg/mL dissolved in 100 mM phosphate buffer, pH 6.8 containing no more than 2.5% DMSO) and 100 µL phosphate buffer were mixed in a 96-well plate, and incubated at 37 °C for 20 min. Then, 50 µL of 1.66 mM of tyrosine solution in 100 mM phosphate buffer (pH 6.8) were added. The enzyme activity was measured at 490 nm every 10 min for 30 min in a Bio Rad Model 680 Microplate Reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Kojic acid at 0.293–150 µg/mL (1.03 µM–1.06 mM) was used as positive control. The experiments were done in triplicate. For each concentration, enzyme activity was calculated as a percentage of the velocities compared to that of the assay using buffer without any inhibitor. The $IC_{50}$ value, which was the concentration of the sample that inhibited 50% of the enzyme activity, was determined by interpolation from the % tyrosinase inhibition vs. concentration curve.

### Elastase Inhibition Assay

The extracts were assayed by the method described by Ndlovu et al. [31] with some modifications. Briefly, 25 µL of elastase enzyme solution (0.3 U/mL), 50 µL of ten serial concentrations of the extracts or fractions (0.488 µg/mL to 250 µg/mL dissolved in 100 mM HEPES buffer, pH 7.5 containing no more than 2.5% DMSO) and 125 µL HEPES buffer were mixed in a 96-well plate and incubated at room temperature for 20 min. Then, 50 µL of N-Methoxysuccinyl-Ala-Ala-Pro-Val-p-nitroanilide (1 mM) were added. The enzyme activity was measured at 405 nm in the moment of substrate addition and after 40 min of incubation at 25 °C in a Bio Rad Model 680 Microplate Reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA). N-Methoxysuccinyl-Ala-Ala-Pro-chloromethyl ketone at 0.019–20 µg/mL (0.039–40 µM)) was used as positive control. The experiments were done in triplicate. For each concentration, enzyme activity was calculated as a percentage of the velocities compared to that of the assay, using buffer without any inhibitor. The $IC_{50}$ value, which was the concentration of the sample that inhibited 50% of the enzyme activity, was determined interpolation from the % elastase inhibition vs. concentration curve.

### Collagenase Inhibition Assay

An adaptation of the method of Moore and Stein [35] with modifications by Mandl et al. [36] was used to determine anticollagenase activity. The following was added to 2 mL test tubes: 25 µL of collagenase solution (0.8 U/mL), 25 µL TES buffer (50 mM) with 0.36 mM calcium chloride (pH 7.4) and 50 µL of test sample or the reference compound EDTA (with concentrations ranging between 15.6–250 µg/mL). The tubes were incubated in a water bath at 37 °C for 20 min. Thereafter, 50 µL of a 1 mM N-[3-(2-Furyl)acryloyl]-Leu-Gly-Pro-Ala (FALGPA) solution was added to the tubes and incubated further for 60 min at 37 °C. To all tubes, 200 µL of a solution containing equal volumes of a 1.6 mg/mL Tin chloride (II) solution in 200 mM citrate buffer (pH 5), and 50 mg/mL ninhydrin solution in DMSO was added. All tubes were placed in a water bath (100 °C) for 5 min and left to cool to room temperature before adding 200 µL of 50% isopropanol to each tube. Contents in the tubes were then transferred to respective wells in 96-well plates. Absorbance was detected at 550 nm Bio Rad Model 680 Microplate Reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Percentage of collagenase inhibition was calculated as:

$$\% \text{ collagenase inhibition} = [(Abs_{control} - Abs_{sample})]/(Abs_{control})] \times 100 \quad (10)$$

where $Abs_{control}$ is the absorbance of buffer + collagenase; $Abs_{sample}$ is the absorbance of buffer + collagenase + sample/standard.

All assays were carried out in triplicate and results expressed as $IC_{50}$, i.e., as the concentration yielding 50% of collagenase inhibition, calculated by interpolation from the % collagenase inhibition vs. concentration curve.

## 3. Results and Discussion

The 50 samples. i.e., 30 extracts obtained from 15 seaweed beach-cast (Table S1 Supplementary Material) and 20 fractions (Table S2 Supplementary Material) thereof obtained by solubility solvent fractionation (Table S2 Supplementary Material), were tested for a wide range of biological activities in order to assess the potential of the beach-cast biomass to be converted into added value products. The results obtained are presented and discussed below.

### 3.1. Antibacterial and Antifungal Activities

Antibiotic resistance, increasingly recognized as a growing global health issue, hinders the treatment of certain types of infections and can be a cause of death. Antibiotic drugs are losing effectiveness at an alarming rate, mainly due to their misuse in humans and animals [37]. In order to slow this trend, antibiotics should be used only when needed, and the discovery of new antimicrobial drugs is urgently demanded to solve such resistance [38]. Furthermore, it is known that Gram-positive bacteria are more sensitive to antibiotic compounds because these lack the extra outer layer of lipopolysaccharides and proteins in their cell membrane complex structure. Contrarily, this structure is present in Gram-negative bacteria, which often prevents transport and inclusion of antibiotic drugs, providing higher resistance and protection of Gram-negative bacteria against antibiotic molecules [39].

This study attempted to add a contribution to the quest of new antibiotic products by evaluating the seaweed beach-casts extracts and fractions following a microdilution assay for determining cell survival, conducted employing four species of bacteria (*B. subtilis*, *E. coli*, *S. aureus*, *S. enterica*). However, no activity was recorded for any of the beach-cast extracts against the four bacterium strains assayed at tested concentration of 100 μg/mL.

Antifungal activity was also evaluated following a variation of the microdilution cell inhibition method determining cell survival and/or growth inhibition of two yeasts, i.e., *S. cerevisiae* and *C. albicans*, but no antifungal activity was recorded for any of the beach-cast samples at the tested concentration of 100 μg/mL.

Finally, all samples were also evaluated against a battery of eight major phytopathogenic fungi (Aspergillus fumigatus, Botrytis cinerea, Fusarium solani, Geotrichium candidum, Screrotinia sclerotiorum, Rhizoctonia solani, Trichoderma viride, Verticillium dahliae) performing a growth inhibition assay on solid agar growth medium aseptically loaded with 100 μg/mL of each individual alga extracts contained in small Petri dishes; however, no inhibition results were obtained.

### 3.2. Anticancer Activity

All beach-cast seaweeds extracts and fractions were evaluated for their cytotoxic activity against four cell lines representing the most frequent cancer types in our population, i.e., lung (NCI-H1299), prostate (DU145), breast (T47D), and colon (HT29) [40].

The samples were assayed against the NCI-H1299 cell line, a non-small-cell lung cancer, one of the most aggressive forms of cancers with few effective therapeutic drugs available. Twenty-one samples (fourteen extracts and seven fractions) showed interesting results (Figure 1), namely some samples which displayed cell survival values of 27–31%.

For the 16 samples displaying the lowest cell survival (Figure 1), the $IC_{50}$ values (concentration that causes a 50% growth inhibition of the cell population) were calculated (Table 2) to learn more about their cytotoxic potential. The results showed very promising samples, since six of the $IC_{50}$ values are near or below 20 μg/mL, an established threshold to consider an extract active, used in this study as a selective value to discard samples with low or no activity [18], four of them exhibiting $IC_{50}$ values between 11.38 μg/mL to 13.13 μg/mL, which means high cytotoxic activity. A report with

*Pylaiella littoralis* (Ochrophyta, Phaeophyceae) seaweed extracts showed anticancer activity against various cancer cell lines, including NCI-H1299, reporting large cell survival values of ca. 80% for this cell line, but higher antiproliferative activity (ca. 20% cell survival) for the HT-29 colon cancer cell line. However, unlike the present study, this investigation assayed high extract concentrations of 100 µg/mL [41]. Phycocyanin, a pigment extracted from *Arthrospira platensis* (formerly *Spirulina platensis*) (Cyanobacteria) but which also exists in red macroalgae, was reported to inhibit NCI-H1299 cell growth by inducing cell morphological changes, apoptosis, inhibition on cell migration and cell cycle arrest [42].

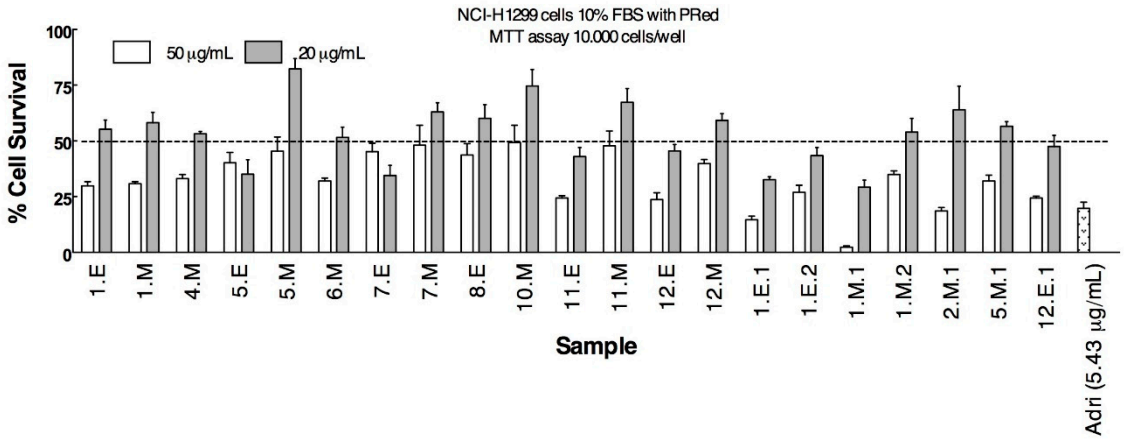


**Figure 1.** Cell survival against NCI-H1299 lung cancer cell line of samples (beach-cast extracts and fractions) tested at two concentrations (20 µg/mL and 50 µg/mL). Adriamycin at 5.43 µg/mL was used as reference.

**Table 2.** $IC_{50}$ values of samples showing anticancer activity against different cancer cell lines studied.

| | $IC_{50}$ (µg/mL) | | | | |
|---|---|---|---|---|---|
| **Sample** | **Lung NCIH1299** | **Prostate DU145** | **Breast T47D** | **Colon HT29** | **Vero** |
| 1.E | 33.77 | 36.80 | 19.28 | | 74.29 |
| 1.E.1 | 17.27 | 23.60 | 17.04 | 46.20 | 26.50 |
| 1.E.2 | 12.28 | - | 14.74 | 32.14 | 63.20 |
| 1.M | 24.60 | 28.50 | 16.04 | - | 80.00 |
| 1.M.1 | 12.50 | 25.40 | 21.90 | 24.40 | 59.30 |
| 1.M.2 | 27.60 | - | 33.60 | | >80.00 |
| 2.E | - | - | 22.85 | - | >80.00 |
| 2.M.1 | 23.90 | - | 39.60 | | >80.00 |
| 4.M | 26.43 | - | - | - | >80.00 |
| 5.E | 25.45 | - | - | - | >80.00 |
| 5.M.1 | 33.30 | - | 23.11 | | >80.00 |
| 6.M | 28.13 | - | - | - | >80.00 |
| 7.E | 31.51 | - | - | - | >80.00 |
| 10.E | 30.92 | - | 80.30 | - | >80.00 |
| 10.M | - | - | 77.25 | | >80.00 |
| 11.E | 13.13 | - | 38.60 | - | >80.00 |
| 12.E | 11.38 | - | 33.87 | - | >80.00 |
| 12.E.1 | 20.77 | 39.30 | 20.04 | 47.90 | 53.10 |

Most cancer drugs display low selectivity for the target cancerous cells generating cytotoxicity to healthy ones, with many unwanted and often severe side effects to patients [43]. Thus, finding compounds or samples with attractive selectivity indexes (SI) would assist in developing more selective cancer drugs.

Selectivity index was calculated for the most active samples to determine the prevalence of the samples to inhibit more specifically cancer cells instead of normal cells. The SI values were calculated

applying the formula SI = $IC_{50}$ Vero cell line/$IC_{50}$ cancer cell line for all strongest cancer inhibiting samples of this study. An SI value lower than 2 is considered to induce general toxicity to normal cells, while those with larger SI values present selectivity for the indicated cancer cells [44].

As shown in Table 3, 13 samples showed SI larger than 2, with some of them as high as 7. The most interesting were the four samples 1.M.1, 1.E.2, 11.E and 12.E, which exhibit the lowest $IC_{50}$ values (11.38 μg/mL to 13.13 μg/mL) and the highest SI values (from 4.7 to higher than 7.0), indicating a very promising cytotoxic action allied with an attractive selectivity of these samples to attack and inhibit growth of lung cancer cells compared with control Vero cells.

**Table 3.** Selectivity index (SI) values of samples showing anticancer activity against the cancer cell line studied.

| Sample | Lung NCIH1299 | Prostate DU145 | Breast T47D | Colon HT29 |
|---|---|---|---|---|
| 1.E | 2.20 | 2.02 | 3.85 | |
| 1.E.1 | 1.53 | 1.12 | 1.55 | 0.57 |
| 1.E.2 | 5.14 | - | 4.28 | 1.97 |
| 1.M | 3.25 | 2.80 | 4.99 | - |
| 1.M.1 | 4.74 | 2.33 | 2.71 | 2.43 |
| 1.M.2 | 2.89 | - | 2.38 | |
| 2.E | - | - | 3.50 | - |
| 2.M.1 | 3.34 | - | 2.02 | |
| 4.M | 3.02 | - | - | - |
| 5.E | 3.14 | - | - | - |
| 5.M.1 | 2.40 | - | 3.46 | |
| 6.M | 2.84 | - | - | - |
| 7.E | 2.53 | - | - | - |
| 10.E | 2.58 | - | 0.99 | - |
| 10.M | - | - | 1.03 | |
| 11.E | 6.09 | - | 2.07 | - |
| 12.E | 7.03 | - | 2.36 | - |
| 12.E.1 | 2.55 | 1.35 | 2.65 | 1.11 |

Concerning the DU145 prostate cell line, five samples (1.E; 1.M; 1.E.1; 1.M.1 and 12.E.1) (Figure 2) displayed around 50% or lower cell survival; particularly for the highest tested concentration (50 μg/mL), although these results were not as effective as those obtained with the anticancer drug adriamycin used as positive control (38% cell survival at 5.43 μg/mL) (Figure 2).

Taskin et al. [45] report inhibition of DU-145 cell growth by 90% by algae extracts, but those authors employed extremely high extract concentrations (100–200 μg/mL) therefore this activity can only be considered as moderate or weak, i.e., much less interesting than the one found in the present study

The six samples that exhibited the highest values of cell growth inhibition were chosen to determine their $IC_{50}$ values (Table 2). The recorded $IC_{50}$ values were higher than 20 μg/mL, which means that this cell line is less sensitive to the samples than the NCI-H1299 cell line. The best results obtained were for two samples with $IC_{50}$ around 23–25 μg/mL, indicating a moderate but close to high anticancer activity. Cell inhibition of the DU145 prostate cell line has also been reported when evaluating purified carotenoids (fucoxanthin) obtained from alga samples, achieving cell viability of ca. 5% when evaluated at a concentration of 20 μM [46].

Regarding selectivity index (Table 3), only two samples (1.M; 1.M.1) exhibited SI values highest than 2 (2.80 and 2.33), a result less impactful than those obtained against the NCIH1299 cell line but still positive.

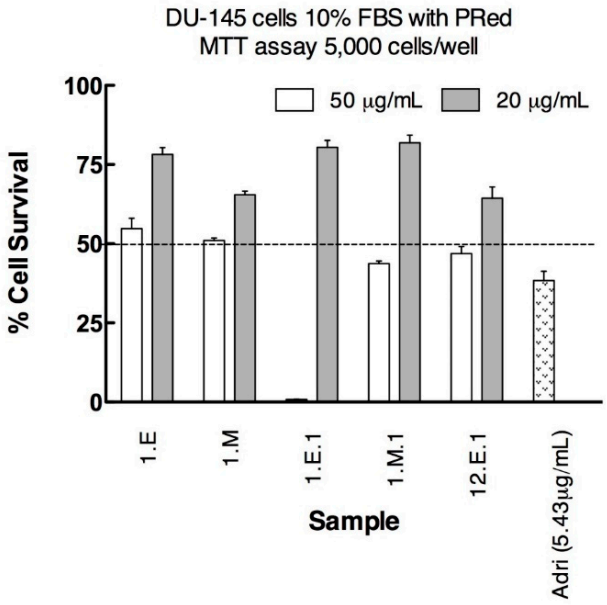


**Figure 2.** Cell survival of a cytotoxic assay against DU-145 prostate cancer cell line of different samples tested at two concentrations (20 µg/mL and 50 µg/mL). Adriamycin at 5.43 µg/mL was used as reference.

For the T47D breast cancer cell line, nineteen samples stood out showing values close or lower than 50% cell survival at the highest concentration tested (Figure 3). The strongest samples, 1.M.1 and 2.M.1, with the lowest cell survival values, corresponded to a cell growth inhibition ranging from 74 to 98% (Figure 3), although this cell line was also more resistant to the anticancer drug adriamycin employed as positive control, when compared with the results obtained with this drug in the other cell lines employed in this study.

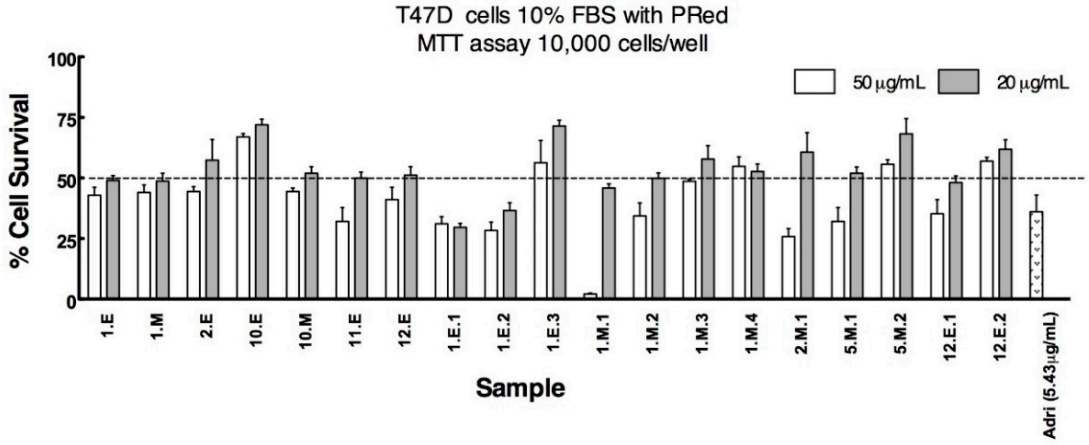


**Figure 3.** Cell survival of a cytotoxic assay against the T47D breast cancer cell line of different samples tested at two concentrations (20 µg/mL and 50 µg/mL). Adriamycin at 5.43 µg/mL was used as reference.

Regarding the $IC_{50}$ values, as seen in Table 2, five samples displayed values lower than of very close to 20 µg/mL, while three samples exhibited moderate activity ($IC_{50}$ values of 21.90 µg/mL to 23.11 µg/mL). These results provide evidence of the attractive moderate to high antitumor capacity of the samples against the T47D cell line. The SI recorded for the samples on T47D breast cancer cells showed that for eleven samples the SI was larger than 2, with the best results of 4.99 and 4.28 (1.M and 1.E.2), indicating an attractive selectivity of these samples for the target cancer cells (Table 3).

Fucoidans, sulfated polysaccharides, extracted from the seaweeds *Undaria pinnatifida* and *Saccharina japonica* (Ochrophyta, Phaeophyceae) have been evaluated on the T-47D breast cancer cell line, reporting

a 46% inhibition; unlike the present study, they were assayed at a rather high concentration of 200 μg/mL of pure polysaccharide [47]. Another study, employing brown alga collected in the Persian Gulf, showed a weak antiproliferative effect on the T47D cell line ($IC_{50}$ 99.9 μg/mL) and on the T47D tamoxifen-resistant cell line ($IC_{50}$ 143 μg/mL)[48], which may indicate differences in metabolites profiles of these species and in extraction methods compared with those evaluated in this study.

The cytotoxic activity of the samples against the HT29 cancer colon cell line was also studied. Some growth inhibition activity at 20 μg/mL and 50 μg/mL was recorded for just five samples (1.E; 1.M; 1.E.1; 1.E.2 and 13.E.1), with cell survival data varying from 65% to 2%, corresponding to a cell growth inhibition of 35% to 98% (Figure 4). The best sample was 1.M.1, which displayed an $IC_{50}$ value of 24.4 μg/mL and a selectivity index of 2.43 (Tables 2 and 3).

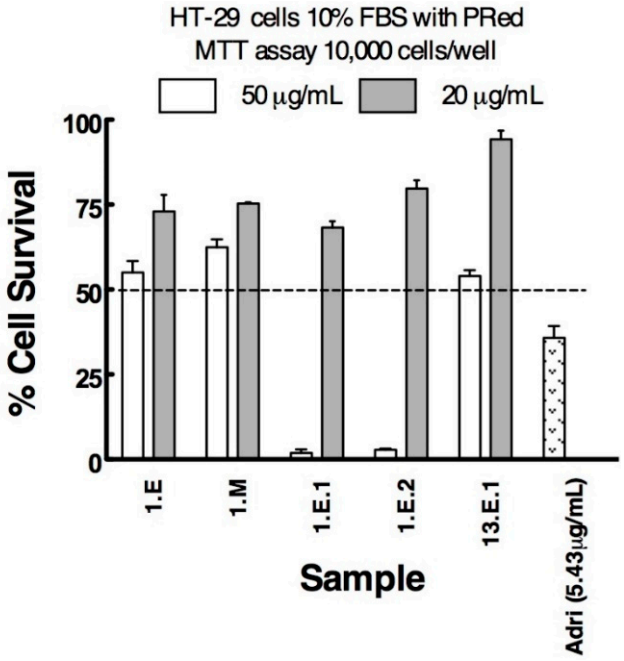


**Figure 4.** Cell survival of a cytotoxic assay against the HT29 colon cancer cell line of different samples tested at two concentrations (20 μg/mL and 50 μg/mL). Adriamycin at 5.43 μg/mL was used as reference.

Extracts of the tropical seaweed *Pylaiella littoralis* exhibited, in a dose-dependent manner, a clear antiproliferative activity against HT29 cells, with 55% inhibition when tested at 50 μg/mL, a result which in the present work achieved much better results for samples 1.E.1 and 1.E.2, and similar for samples 1.E and 13.E.1 at the same concentration; the $IC_{50}$ value of 28 μg/mL reported by those authors is between the results reported herein for samples 1.M.1 and 1.E.2. [41].

The in vitro results against the tumor cell lines studied were quite interesting, not only because 38% of the tested samples exhibit $IC_{50}$ values below 25 μg/mL, which means very promising samples with high to moderate cytotoxic activity, but also because of their high activity against several cancer cell lines. For example, samples 1.M.1 and 1.E.1 exhibited cytotoxic activity against at least three tumor cell lines with $IC_{50}$ values lower than 25 μg/mL. The best results were against NCI H1299 lung cancer, with four samples presenting $IC_{50}$ values lower than 14 μg/mL (1.M.1, 1.E.2, 11.E and 12.E), followed by T47D breast cancer with two samples with $IC_{50}$ lower than 16.5 μg/mL (1.E.2 and 1.M), all of them with SI values higher than 4.2. For the other two cancer cell lines (DU-145 prostate and HT29 colon cancer cell lines), the lowest $IC_{50}$ values were around 23–25 μg/mL. It should be noted that of the 38 SI values calculated, 65.8% were higher than 2, which indicated samples with a highly selective cytotoxic action, being much more active against the tumor cell lines than against the nontumor cell line employed.

These results demonstrated that macroalgae can be attractive sources of potential anticancer compounds, with many references also supporting this claim [49], such as the 22-membered cyclic lactone lobophorolide, isolated from *Lobophora variegata*, one of the main components of the beach-cast samples with highest activity (beach-casts 1, 11 and 12), which was extremely active at low concentration against human the HCT-116 colon cell line ($IC_{50}$ = 0.03 μg/mL) [50]. Furthermore, the different beach-casts evaluated here also showed large seaweed species composition variability within beach-casts, although those identified as *L. variegata* mono-specific, which occurred with a certain degree of frequency, displayed very attractive results.

### 3.3. Anticholinesterasic Activity

Decreased levels of neuromediators acetylcholine (ACh) and butyrylcholine (BCh) have been observed in the brains of patients with Alzheimer's disease (AD). For that reason, the inhibition of AChE and BuChE, responsible for the hydrolysis of ACh and BCh has become a treatment option of AD [51].

The extracts prepared were tested to assay their capacity to inhibit acetylcholinesterase and butyrylcholinesterase, two enzymes related with the regulation of acetylcholine (ACh) level in the synaptic cleft, a very relevant factor in promoting cognitive function. The results obtained showed that the tested samples cannot be seen as promising sources of anticholinesterase products, since the most active sample at 150 μg/mL inhibited only 51.3% of AChE activity, although 14 more (1.E, 1.M, 4.E, 5.E, 5.M, 6.M, 7.E, 8.E, 9.E, 10.E, 11.E, 11.M, 12.E, 12.M) inhibited the enzyme by values ranging from 30.8–49.5% at the same maximum tested concentration. These uninteresting results led to the decision that no fraction of these extracts should be tested for this activity.

It should be noted that in the present work the choice was to test only up to 150 μg/mL, whereas some results of anticholinesterasic activity reported in the literature correspond to extract concentrations more than 2.8-fold higher than the one reported herein, such as the crude methanol extract of *Ecklonia maxima* (Ochrophyta, Phaeophyceae) which inhibits 50% of acetylcholinesterase activity at 385.50 μg/mL [52]. Another example, by Olasehinde et al. [53], who tested the hydroethanolic extracts of *Ecklonia maxima*, *Gelidium pristoides*, *Gracilaria gracilis* (Rhodophyta) and *Ulva lactuca* (Chlorophyta), obtained 50% of anticholinesterasic effects with concentration ranging from 1.66–2.42 mg/mL. The review by Rathnayake et al. [54] also presents some of these high $IC_{50}$ values, going up to 10 mg/mL for AChE and BuChE. These values are too high to be of interest, and that is why 150 μg/mL was established in the present work as the highest sample concentration to test for anticholinesterasic effect.

To the most active sample, ethanol extract of the beach-cast 6 (6.E), the $IC_{50}$ value was determined, exhibiting an AChE inhibition of 137 ± 1.06 μg/mL and 138 ± 4.41 μg/mL to BuChE, with roughly 45% *Asparagopsis armata* (Rhodophyta) and the rest Phaeophyceae (25% *Lobophora variegata*). Although being a dual AChE/BuChE inhibitor, which is interesting when considering applications related with Alzheimer's disease therapy, this sample showed $IC_{50}$ value more than 13,000-fold higher the value for the standard (donepezil) in the case of AChE ($IC_{50}$ = 0.010 ± 0.003 μg/mL), being more favorable in the case of BuChE ($IC_{50}$ = 28.94 ± 1.76 μg/mL), where it is only 4.75-fold higher. These results reinforce the low expectation about these beach-cast extracts as a source of compounds for the treatment of Alzheimer's disease, although several macroalgae and their constituents are described in the literature as promising pharmacological principles for this pathology [55,56].

### 3.4. Antiaging Activity

The search for extracts and compounds with antiaging activity is a hot topic in the natural products field, especially concerning the protection and conservation of the skin and extracellular matrix (ECM) structure [57]. Repeated exposure to UV radiation leads to the formation of reactive oxygen species (ROS), which activate a series of processes involved in photoaging [58]. Once photoaging is initiated, degradation of collagen and elastin fibers occurs, causing the skin to become looser with wrinkles [59]. Excessive pigmentation also occurs on the skin due to abnormal proliferation of melanocytes and

activation of melanin production. In addition, increasing matrix metalloproteinase (MMP) content leads to intracellular matrix degradation and inflammatory infiltrates [60]. These consequences are related to an increase in the expression levels of certain enzymes, like tyrosinase (key enzyme on melanin production), elastase, collagenase and hyaluronidase. So, these enzymes are often the preferred targets of antiaging agents. A wide array of activities contributes to this antiaging action, such as protection against deleterious oxidations (reduction of ROS formation), chelating activity, and the inhibition of ECM and skin-degrading enzymes, i.e., collagenase, elastase, hyaluronidase and tyrosinase [61].

#### 3.4.1. Chelating and Antioxidant Activities

None of the extracts exhibited ferric chelating or β-carotene bleaching activity at the maximum concentration tested (250 μg/mL).

Regarding the antioxidant activity, five extracts (1.E, 1.M, 2.E, 2.M and 11.E) scavenged the DPPH radical by more than 50% at 250 μg/mL, with two of them (1.E and 2E) surpassing 80%. For these five samples, the $IC_{50}$ values were calculated (Figure 5 and Table S3 Supplementary Material). The most active extract (2.E) was 9.57-fold less active than Trolox ($IC_{50}$ values of 69.4 μg/mL vs. 7.25 μg/mL), followed by 1E with $IC_{50}$ of 81.57 (11.2-fold less active than Trolox), while the other exhibit very low activity. The fractionation of the extracts revealed seven fractions with $IC_{50}$ values below 229.06 μg/mL, four of which were the most promising with $IC_{50}$ values between 50.25 μg/mL and 71.70 μg/mL (Figure 5 and Table S3 Supplementary Material). It should be noted that the 2.M extract being only the third most active, it gave rise to the two most active fractions, about 2.5 times more active than the original extract.

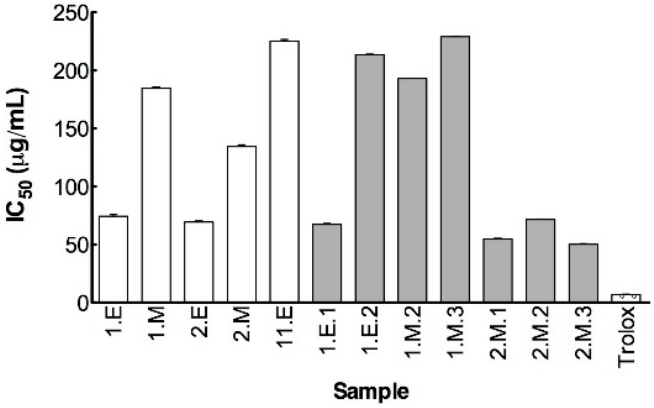


**Figure 5.** $IC_{50}$ values of DPPH scavenging activity by the extracts (light bars) and fractions thereof (dark bars). Trolox was used as reference.

The ABTS test also reflects the antioxidant capacity of the samples, being a more sensitive method than the DPPH method, since the neutralization of the ABTS radical can occur by transferring a hydrogen atom or an electron [62]. The ABTS test yielded more interesting results, with eight extracts capable of inhibiting more than 50% of the radicals at 250 μg/mL. To these extracts the $IC_{50}$ values ranging from 6.21 μg/mL to 242.78 μg/mL (Figure 6, Table S3 Supplementary Material). The most active extracts were, like in the DPPH method, 1.E and 2.E, with $IC_{50}$ values 2.31 and 2.68-fold higher than the activity of Trolox (2.68 μg/mL for the ABTS assay), respectively. The fractionation of the extracts gave rise to 12 active fractions ($IC_{50}$ values ranging from 8.89 μg/mL to 185.04 μg/mL) (Figure 6, Table S3 Supplementary Material) but none of the fractions exhibited higher activity than the reference compound Trolox. Additionally, with only one exception, no fraction was more active than the extract that gave rise to it, which seems to suggest a synergistic effect between the constituents of the extract with antioxidant action, which is lost when fractioned. The exception mentioned above is 2.M.4 ($IC_{50}$ = 9.57 μg/mL) a fraction 2.6 times more active than the original extract 2.M ($IC_{50}$ = 24.66 μg/mL).

From the antioxidant activity point of view, three extracts (1.E, 1.M, 2.E) and three fractions (1.E.3, 1.M.2 and 2.M.4) seem very good antioxidants products, which is not completely surprising considering the species compositions of most of the beach-casts, mainly composed of brown and red algae, rich in antioxidant molecules, such as pigments [63], phlorotannins [64], sulfated polysaccharides [65] and sterols [66], among others.

The antioxidant samples found here can act as reactive oxygen species (ROS) scavengers and in this way contributing to the protection of cellular structures such as proteins and lipids, and to a lower activation of extracellular matrix (ECM) degrading enzymes, since the ROS play an important role on the activation of matrix metalloproteinases that degrade ECM, namely collagenases [61].

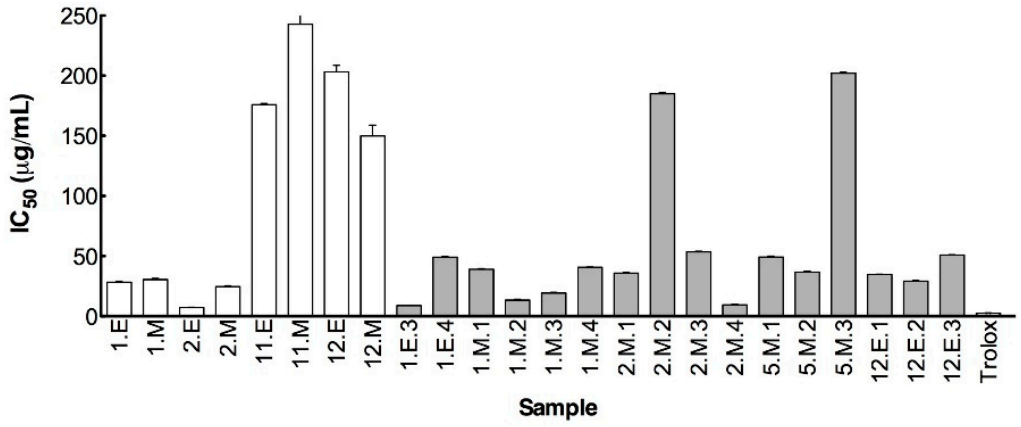


**Figure 6.** $IC_{50}$ values of ABTS radical scavenging activity by extracts (light bars) and fractions thereof (dark bars). Trolox was used as reference.

### 3.4.2. UV-Protection

All the extracts tested at 250 µg/mL provided UV protection comparable to the SPF 30 commercial sunblock standard Darphin Soleil at the same concentration, i.e., between 31.12 SPF and 34.37 SPF (Table 4).

**Table 4.** UV protection of samples at a concentration of 250 µg/mL.

| Sample | SPF | Sample | SPF |
|---|---|---|---|
| 3.E | 32.95 ± 0.49 | 8.M | 32.88 ± 1.30 |
| 3.M | 33.93 ± 1.83 | 9.E | 33.37 ± 1.25 |
| 4.E | 31.27 ± 0.52 | 9.M | 33.54 ± 2.23 |
| 4.M | 33.15 ± 1.95 | 10.E | 31.34 ± 0.48 |
| 5.E | 34.03 ± 1.03 | 10.M | 30.48 ± 0.59 |
| 5.M | 33.38 ± 1.68 | 11.E | 33.19 ± 0.52 |
| 6.E | 32.41 ± 1.34 | 11.M | 34.37 ± 3.32 |
| 6.M | 34.37 ± 0.95 | 12.E | 31.31 ± 0.88 |
| 7.E | 31.12 ± 0.78 | 12.M | 32.48 ± 1.51 |
| 7.M | 32.82 ± 0.34 | Standard | 32.86 ± 1.46 |
| 8.E | 33.62 ± 1.67 | | |

Standard—Standard Darphin Soleil SPF30 sunblock.

The exhibited activity seems to be independent of the beach-cast composition. This is an excellent feature in the context of beach casts biomass valorization, converting it into products with antiaging properties, since many photoaging changes in skin are due to action of the UV component of sunlight [67]. Therefore, in addition to the antioxidant activities expressed above, the protection against UV also helps to protect ECM structures against further degradation.

### 3.4.3. Inhibition of ECM and Skin-Degrading Enzymes

#### Hyaluronidase Inhibition

The tested samples presented little to no inhibition against hyaluronidase (percentage of inhibition between 19.00 ± 1.08 and 25.06 ± 2.65 at 250 μg/mL), therefore this enzyme is not a good target for the ethanol and methanol extracts analyzed. There are several examples of macroalgal extracts which inhibit hyaluronidase, namely the work of Fayad et al. [68] with Phaeophyceae *Padina pavonica*, although these authors used supercritical fluid extraction (SFE) and also pressurized liquid extraction (PLE), obtaining $IC_{50}$ value of 35.1 μg/mL. SFE maximizes the extraction yield, and the use of water greatly increases the extraction of sulfated polysaccharides, which are known for their hyaluronidase inhibition capability [69]. Seaweed compounds presented also hyaluronidase inhibition, such as the phlorotannin 8,8′-bieckol from *Eisenia bicyclis* brown alga, ($IC_{50}$ of 30 μg/mL)[70].

#### Tyrosinase Inhibition

Of the 30 extracts assayed, 14 samples inhibited the tyrosinase enzyme by 50% or more at the highest concentration tested (250 μg/mL), including eight (~1/4 of the extracts tested), which inhibited tyrosinase by at least 90% at same concentration.

The $IC_{50}$ values were calculated for these 14 active extracts (Figure 7 and Table S4, Supplementary Material). The $IC_{50}$ values obtained range from 15.18 μg/mL to 239.31 μg/mL, the three most active extracts being 11.E, 11.M, and 12.E ($IC_{50}$ values between 15.18 μg/mL to 32.85 μg/mL). None of the extracts were as active as control inhibitor kojic acid ($IC_{50}$ = 1.82 ± 0.13 μg/mL). The most active extracts were fractionated, and 12 fractions exhibited significant activity ($IC_{50}$ values from 9.01 μg/mL to 95.21 μg/mL) 50% of which presented no loss of activity in relation to the original extracts. On the contrary, each extract produced at least two fractions 1.2- to 3.6-fold more active than the original extract. The best fraction (12.E.1) presented an $IC_{50}$ that was only about five times higher than the $IC_{50}$ of kojic acid (Figure 7), which is quite impressive considering that fractions still contain an array of different compounds.

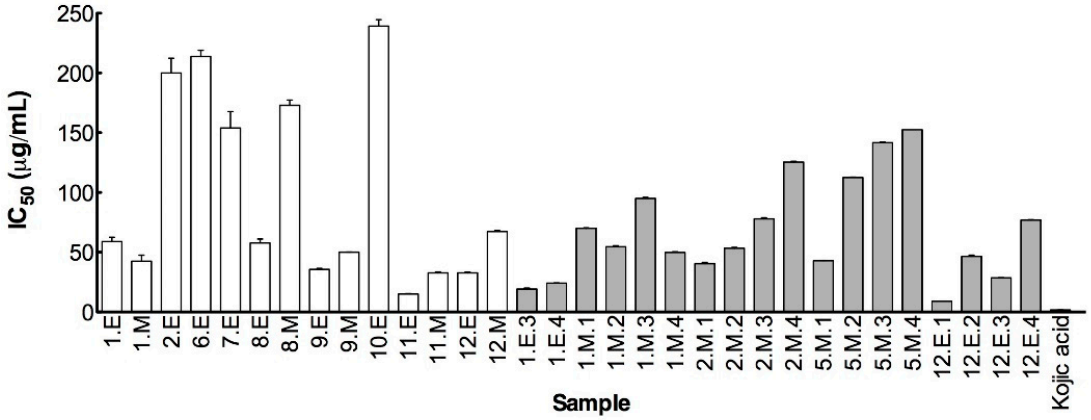


**Figure 7.** Tyrosinase inhibition activity ($IC_{50}$ values) by the extracts (light bars) and fractions thereof (dark bars). Reference: Kojic acid.

Comparing the tyrosinase inhibitory activity of extracts and fractions with the activity reported in the literature, it is concluded that the extracts and fractions of beach-casts seaweeds biomass exhibit excellent antityrosinase activity. In fact, Nurrochmad et al. [71] reported $IC_{50}$ values of 295.21 μg/mL and 436.89 μg/mL for *Turbinaria decurrens* brown alga extracts and fucoxanthin-enriched extracts, respectively, although these results should be analyzed with caution, since the $IC_{50}$ value reported for kojic acid standard by those authors was 479.95 μg/mL, compared with 1.82 μg/mL in the present work. It appears too high to be credible (see also, e.g., Neeley et al., [72]). Mahomoodally et al. [73] reported

$IC_{50}$ values on the order of 3.5–4.5 mg/mL for methanolic extracts of *Halimeda* spp., *Valoniopsis pachynema* (Chlorophyta), *Gracilaria debilis* (formerly *Gracilaria fergusonii) Spyridia hypnoides* and *Amphiroa anceps* (Rhodophyta) while the ethanol extract of *Turbinaria conoides* (Ochrophyta, Phaeophyceae) exhibits $IC_{50}$ value of 188.85 µg/mL [74]. All these activities are much lower than the ones presented here.

The compounds responsible for this activity can be very diverse, since several metabolites isolated from seaweed have been reported [75]. Phlorotannins are one of the chemical groups reported to be active against this enzyme, although their activity can be quite variable, depending on their chemical structure (e.g., from 2.6 µg/mL to 177 µg/mL as reported by Kang et al. [76], or even 730 µg/mL [77]. Brown algae, present in these beach-casts, are known to be rich in these molecules [64,78]. Therefore, it is to be expected to find tyrosinase inhibition properties in extracts containing a high percentage of brown macroalgae.

### Elastase Inhibition

Of the 30 extracts assayed, only six samples (20%) presented at least 40% inhibition of elastase enzyme at 250 µg/mL and at this concentration none of the samples exhibited more than 60% elastase inhibition. Although the antielastase activity reported here is lower, it is much higher than that reported by Jesumani et al. [79] using crude polysaccharide preparations purified from three *Sargassum* species (i.e., approximately 30% of inhibition with concentrations as high as 800 µg/mL). This makes it evident how fluctuating the level of antielastase activity displayed by the samples is, depending on their chemical composition.

The $IC_{50}$ value calculation for the six more active extracts (Table S4 Supplementary Material) showed values between 209.56 µg/mL and 249.58 µg/mL which demonstrated that elastase is not a good target for the compounds present in the extracts.

### Collagenase Inhibition

Eight out of 30 extracts presented 50% inhibition or more at 250 µg/mL, one reaching 81.7% (5.E).

The $IC_{50}$ values of these extracts were calculated (Figure 8, Table S4, Supplementary Material), six of them ranging from 163.06 µg/mL to 248.76 µg/mL (1.E, 1.M, 8.M, 11.E, 11.M and 12.M), while the other two (5.E and 5.M) exhibited potent collagenase inhibitory activity ($IC_{50}$ values of 41.04 µg/mL and 59.34 µg/mL, respectively). These two extracts are, respectively, better and similar collagenase inhibitors than the positive control EDTA ($IC_{50}$ = 59.26 µg/mL).

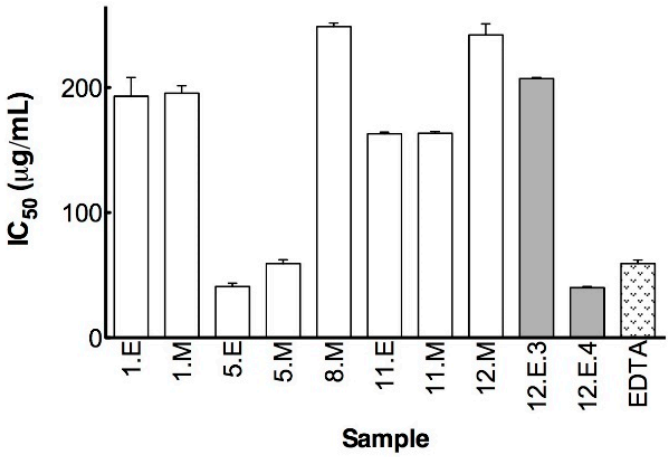


**Figure 8.** Collagenase inhibition activity ($IC_{50}$ values) by extracts. EDTA was used as reference.

Unfortunately, the fractionation of the most active extracts resulted in fractions with an $IC_{50}$ higher than 250 µg/mL (Table S4, Supplementary Material), which were considered inactive.

Surprisingly, extract 12.E which exhibited low anticollagenase activity but was fractionated due to its antityrosinase activity, gave rise to one very active fraction (12.E.4) ($IC_{50}$ = 40.11 ± 0.88 µg/mL),

more active than EDTA. Considering the solvent used to obtain this fraction (methanol: water (1:1, v/v)), it is expected that in its chemical composition it will have mostly polar compounds such as phlorotannins and fragments of polysaccharides.

There are several reports of collagenase inhibition by seaweed extracts and/or compounds extracted thereof. Kim et al. [80] obtained complete inhibition of bacterial collagenase-1 activity at 20 µg/mL of *Ecklonia cava* (Ochrophyta, Phaeophyceae) phlorotannins extract using a gelatin digestion assay. Mansauda et al. [81] report 54.5% inhibition of the enzyme by 50 µg/mL ethanol: water extracts of *Sargassum plagiophylloides* (Phaeophyceae), a value which the authors attributed to the high concentration of extracted phloroglucinols. That level of activity is comparable to the results reported herein, namely of extracts 5.M, 5.E and fraction 12.E.4 (50% of inhibition by 40.11–59.34 µg/mL). Ethanol extracts from *Turbinaria decurrens* brown alga, and a fucoxanthin-enriched fraction of this extract with an amount of fucoxanthin threefold higher than the found in the extract, almost completely inhibited collagenase activity at 40 µg/mL and 80 µg/mL [71].

Other authors reported results which were much less significant than the one obtained in the present work. For example, Thring et al. [82] reported a 24.5% inhibition of this enzyme by *Fucus vesiculosus* aqueous extracts at a concentration of approximately 166.7 µg/mL; and Kim et al. [83] reported 29.6% inhibition at 1 mg/mL of *Myagropsis myagroides* (Phaeophyceae) extract. Compound Sargachromaol I, isolated from *M. myagroides* brown alga, exhibit an $IC_{50}$ of 54.01 µg/mL [83], a value of the same order as the $IC_{50}$ values reported here for extracts 5.M, 5.E and fraction 12.E.4, which are mixtures containing a wide array of compounds.

## 4. Conclusions

The present work reports the attractive results of algal samples obtained from beach-casts with the potential to be converted into bioproducts with various properties, mainly anticancer, antioxidant and antiaging. It should be taken in consideration that species composition of beach-casts varies according to season and to specific conditions, which would alter or affect metabolites present in the extracted samples. However, this study showed that a large number of the samples, regardless of the beach-cast composition from which they were prepared, exhibited a significant cytotoxic effect against the NCI-H1299 cell line with high selectivity index, acted as tyrosinase inhibitors, as antioxidant agents and in UV protection.

These results confirm the high value of algal biomass of beach-casts often considered useless, establishing their applications and potential to be used in different fields, such as cosmetics and as drug natural sources. These potential applications should be considered to stop dumping them in landfills but to try instead to establish parallel industries in locations receiving frequent beach-casts. The data reported herein contribute to continue with future comprehensive studies for determining the nature of the bioactive compounds present in the active samples.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2076-3417/10/17/5831/s1, Table S1: Beach-cast composition, solvent extraction and sample codes of the samples studied, Table S2: Fractionation of the most active extracts, Table S3: Antiradical scavenging activity of samples ($IC_{50}$) in µg/mL, Table S4: Antiaging activity of samples ($IC_{50}$) in µg/mL.

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## Abbreviations

| | |
|---|---|
| **ABTS** | 2:2′ Azinobis-(3 ethylbenzothiazoline 6 sulfonic acid) |
| **AChE** | acetylcholinesterase |
| **ATCI** | acetylthiocholine |
| **BuChE** | butyrylcholinesterase |
| **BuTCI** | butyrythiocholine |
| **DMAB** | 4-dimethyaminobenzaldehyde |
| **DMSO** | dimethyl sulfoxide |
| **DPPH** | 1,1-diphenyl-2-picryl-hydrazyl |
| **DTNB** | 5,5′-dithiobis-[2-nitrobenzoic acid] |
| **ECM** | Extracellular Matrix |
| **FALGPA** | N-[3-(2-Furyl)acryloyl]-Leu-Gly-Pro-Ala |
| **MAAPVCK** | N-Methoxysucinil-Ala-Ala-Pro-Val-chloromethylketone |
| **MTT** | 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide |
| **PLE** | pressurized liquid extraction |
| **ROS** | reactive oxygen species |
| **SFE** | supercritical fluid extraction |
| **SI** | selectivity index |
| **SPF** | sun protection factor |
| **TES** | N-[Tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid |
| **TSB** | tryptic soy broth |
| **YPD** | yeast extract peptone dextrose |

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