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Antiaging and Antioxidant Activities of Upcycled Brazilian Coastal *Sargassum* sp. Beach-cast for Dermocosmetic Applications

Atividades Antienvelhecimento e Antioxidantes de *Sargassum* sp. Proveniente de Arribação: Valorização de Biomassa Sustentável da Costa Brasileira para Uso Dermocosmético

Johana Marcela Concha Obando,[a,b] Joana Silva,[c] Celso Alves,[c] Regildo Márcio Gonçalves da Silva,[d] Maria Vitória Raupp Sebastião,[a,e] Levi Pompermayer Machado,[b,e,f] Guilherme Wolff Bueno,[b,e,g] Rui Pedrosa,[c] Diana Negrão Cavalcanti,[h] Thalisia Cunha dos Santos[a,c,e,i,*]

[a]Seallg: Soluções, Inovação e Produtos, Laboratório Vivo de Bioeconomia e Incubadoras de Startups Baseadas em Ciência e Tecnologia no Vale da Ribeira (Aquário de Ideias), Zip Code 11900-000, Registro-SP, Brazil

[b]Instituto Nacional de Ciência e Tecnologia (INCT) em Nanotecnologia para Agricultura Sustentável (INCTNanoAgro), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Zip Code 70070-600, Brasília-DF, Brazil

[c]MARE: Centro de Ciências do Mar e do Ambiente & ARNET: Rede de Investigação Aquática, ESTM, Instituto Politécnico de Leiria, Zip Code 2520-630, Peniche, Portugal

[d]Universidade Estadual Paulista (UNESP), Faculdade de Ciências e Letras, Laboratório de Fitoterapia e Produtos Naturais, Zip Code 19806-900, Assis-SP, Brazil

[e]Universidade Estadual Paulista (UNESP), Faculdade de Ciências Agrárias, Departamento de Recursos Pesqueiros e Aquicultura, Instituto de Estudos Avançados em Ciências do Mar (IEAMar), Zip Code 11900-000, Registro-SP, Brazil

[f] Universidade Estadual Paulista (UNESP), Instituto de Farmácia, Programa de Pós-Graduação em Biomateriais e Engenharia de Bioprocessos, Zip Code 14800-903, Araraquara-SP, Brazil

[g]Centro de Aquicultura da UNESP (Caunesp), Zip Code 14884-900, Jaboticabal-SP, Brazil

[h]Universidade Federal Fluminense, Instituto de Biologia, Laboratório de Produtos Naturais de Algas Marinhas (ALGAMAR), CEP 24210–130, Niterói-RJ, Brazil

[i]Universidade Federal do Rio de Janeiro, Instituto de Pesquisas de Produtos Naturais Walter Mors, Programa de Pós-Graduação em Química de Produtos Naturais, Zip Code 21941-599, Rio de Janeiro-RJ, Brazil

***E-mail:** thalisia@seallg.com

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Seaweed *Sargassum* beach-casts are a seasonal phenomenon that regularly deposits tons of algae biomass on beaches, including along the Brazilian coast, where they can have significant environmental and economic impacts. This biomass is often discarded in landfills, contributing to waste accumulation. The present work aims to contribute to the valorization of this biomass and evaluate the dermocosmetic potential of conventional *Sargassum* extract (CES) and ultrasound-assisted Sargassum extract (UES). *In vitro* assays evaluated cytotoxicity, antioxidant capacity (DPPH -2,2-diphenyl–1-picrylhydrazyl radical scavenging assay, FRAP - Ferric Reducing Antioxidant Power, and ABTS- 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay), Total Phenolic Content (TPC), anti-enzymatic activity (inhibition of collagenase, elastase, and hyaluronidase), and photoprotective effects against UVB-induced reactive oxygen species (ROS) in human keratinocytes (HaCaT cells). CES showed minimal cytotoxicity (<5%) in HaCaT keratinocytes suggesting its potential safety for cosmetic use. Pre-treatment with *Sargassum* extracts significantly reduced ROS production in HaCaT cells, reaching levels similar to ascorbic acid and demonstrating notable photoprotective potential. CES exhibited the highest antioxidant activity in DPPH (30.87% of reduction at 100 μg mL^{-1}) and FRAP (53.17 μmol TE/g) assays, and total phenolic content (TPC, 142.80 ± 24.63 mg GAE/g), indicating strong reducing potential. CES exhibited hyaluronidase inhibition (52.53 ± 1.03% of inhibition at 100 μg mL^{-1}), a noteworthy result given the growing interest in hyaluronidase inhibitors in the cosmetics industry in anti-aging formulations. Overall, these bioactivities, associated with fatty acids, steroids, and phenolic compounds identified in chemical analyses, highlight the dermatological potential of beach-cast algae.

Keywords: *Sargassum*; bioproducts; antioxidant; photoprotective; anti-enzymatic; dermocosmetics.

1. Introduction

The skin is the body's largest organ responsible for covering and protecting it from physical damage.[1] Skin aging is a complex, multifaceted process characterized by various structural and physiological changes in the skin, driven by both intrinsic and extrinsic mechanisms.[2] Intrinsic aging is the natural, genetically driven process characterized by

programmed cellular changes and senescence. In contrast, extrinsic aging results from environmental influences and lifestyle factors that accelerate the visible signs of skin aging.[3]

Environmental stressors, including ultraviolet (UV) radiation, exposure to air pollutants, and chemicals, play a significant role in skin aging. UV radiation, in particular, induces pronounced degradative changes that result in photo-damaged skin (photoaging), which is recognized as the primary cause of environmentally induced skin aging and promotes the generation of reactive oxygen species (ROS).[3] This process leads to DNA damage, collagen degradation, wrinkle formation, and overall deterioration of skin health.[4] Natural products, particularly bioactives, such as antioxidants and anti-inflammatories have been identified as effective agents to counteract these effects. These bioactives act as free radical scavengers, antioxidants, and moisturizers, helping to repair skin damage and protect against environmental stressors.[5]

There is a continual demand for new cosmetic formulations by the market, with ingredients constantly evolving.[6] As the cosmetics market is highly dynamic and new products are being constantly launched at an extremely fast rate, new concepts have also been continuously emerging, and new terms have been coined.[7] Thus, there is growing interest and consumer demand for the use of natural sources in antioxidant cosmetics, such as extracts derived from a wide range of plants, grains, and fruits, due to their ability to reduce oxidative stress on the skin and provide anti-aging effects.[8,9] Notably, a significant wave of products derived from marine sources has emerged, demonstrating their potential and effectiveness in skincare-related activities.[10,11]

Marine organisms are rich sources of biologically active substances that can be extracted and used in a variety of applications. These molecules have great potential for the development of nutritional supplements, pharmaceuticals, cosmeceuticals, cosmetics, and fine chemicals.[12] Among the most valuable resources of the ocean are algae, encompassing both macroalgae and microalgae. Their remarkable ability to adapt to the most adverse conditions allows the production of a wide range of natural compounds including polysaccharides, terpenes, phenolic compounds, among others, which possess unique biological properties and capable of acting on different biological targets.[13] Algae have long been recognized for their skincare benefits, earning a reputation as a versatile ingredient in cosmetics. They help moisturize the skin, stimulate blood circulation, improve cell turnover and metabolism, regulate sebaceous gland activity, promote tissue drainage, provide anti-inflammatory effects, and increase skin resilience.[14,15] Different species of algae, both fractions and extracts, have been explored for various biological activities, including antioxidant properties and skincare applications, increasingly demonstrating their potential to be used as high-value dermocosmetic products beneficial for the skin.[16–19]

Sargassum species, a diverse group of brown algae, have gained attention for their biologically active metabolites, particularly for their antioxidant and photoprotective properties. Recently, 17 *Sargassum* species (2020-2024) have been studied, highlighting the importance of green extraction methods such as ultrasound, enzyme-assisted, and microwave-assisted extractions. The studies examine the bioprospecting potential of *Sargassum*, address challenges related to raw material quality, and explore the role of biorefineries in cosmetics development.[20] Additionally, the evaluation of bioactive compounds with dermocosmetic potential from beach-cast biomass of *Sargassum* algae has aroused considerable academic interest, attributed to its sustainable characteristics, as it does not depend on the exploitation of natural resources.[21,22] It is important to emphasize that *Sargassum* sp. species, considered invasive and rapidly spreading across various regions worldwide, including Brazil, have raised concerns among both the general public and the scientific community, generating environmental and economic impacts.[20]

However, their abundance also presents an opportunity, as these macroalgae are rich sources of bioactive compounds, making them attractive raw materials for several sectors, including cosmetics. Nevertheless, for cosmetic applications, this biomass must undergo rigorous quality control assessments and scientific validation of its bioactive potential, including cytotoxicity testing, validation of biological activities, and characterization of the chemical profiles associated with the biomass. These represent some of the initial steps required to unlock its potential and to guide the development of innovative products based on this resource. Therefore, this study aimed to investigate the potential of two *Sargassum* sp. extracts through *in vitro* evaluation of their antioxidant, anti-enzymatic, and photoprotective properties, with a view to their application in skincare products.

2. Experimental

2.1. Reagents

P.A. grade solvent ethanol (EtOH) Tedia (Fairfield, OH, USA) was used for extraction. For sample preparation and GC-MS analysis, HPLC-grade dichloromethane (DCM) from Tedia (Fairfield, OH, USA) was used. Fetal bovine serum (FBS) and antibiotic–antimycotic (penicillin/streptomycin/fungizone) solution were obtained from Hyclone (Logan, UT, USA). Dulbecco's Modified Eagle's

Medium (DMEM) from Sigma-Aldrich (St. Louis, MO, USA) was used for cell culture. All remaining chemicals and reagents were acquired from Sigma-Aldrich (Steinheim, Germany).

2.2. Seaweed sampling, biochemical characterization, and extraction

Sargassum sp. samples were collected in November 2023 at Prainha Beach in Arraial do Cabo (22° 57' 39.84" S, 42° 1' 12.60" W; Rio de Janeiro State, Brazil). Algal blooms and drift events have long been a regular occurrence in the region between November and January, and have been recorded over the past four years by our research group. The algal samples were obtained from drift algae along the coastline. Through a detailed visual assessment of their coloration and overall health, only thalli exhibiting a robust and intact morphology, free from signs of decay or extensive epiphytic overgrowth, were selected for collection.

Once the samples were selected, the algal biomass was cleaned in trays with seawater and stored in a thermal box for transport to the laboratory of the Fluminense Federal University. Upon arrival, the algae were rinsed with seawater and distilled water to remove residual epiphytes, sand, and debris. The biomass was then air-dried at room temperature (approximately 28–30 °C) for 7–10 days until complete dehydration. After drying, the material was stored in a freezer until later extraction. The algal morphotype was identified by Prof. Maria Teresa Menezes de Széchy and deposited in the Herbarium of the Department of Botany (RFA), Institute of Biology, Federal University of Rio de Janeiro (UFRJ), as *Sargassum cf. vulgare* C. Agardh sensu Taylor (1960). Although identified as *Sargassum cf. vulgare*, the material is hereafter referred to as *Sargassum* sp. throughout the manuscript to avoid taxonomic ambiguity.

In a mechanical grinder, a dried algae sample was ground into powder. The dried seaweed powder was extracted. Two hydroethanolic extracts containing *Sargassum* sp. biomass (10 g per extract) were prepared using an EtOH:H_2O solution (500 mL, 70% v/v). One extract underwent maceration at room temperature (25 °C) for seven days and was designated as the conventional *Sargassum* extract (CES). The other extract was subjected to ultrasound-assisted extraction (UAE) for one hour, referred to as the ultrasound *Sargassum* extract (UES). After the extraction periods, the solvents were evaporated under reduced pressure using a rotary evaporator maintained at 40 °C (Büchi® R–114, Switzerland). The extraction yield was calculated from the mass ratio of the dried extract (after complete solvent removal) to the initial dry biomass of *Sargassum* sp., expressed as a percentage. The dry extracts and evaporated extracts were stored in the refrigerator for subsequent analyses. For bioassays, samples were dissolved in dimethyl sulfoxide (DMSO), at a concentration of 10 mg mL^{-1}.

2.3. The cytotoxic and photoprotective activities of *Sargassum* sp. in vitro cellular models

2.3.1. Maintenance of cell culture

HaCaT (300493) cells were acquired from Cell Lines Services Germany (CLS) biobanks. The HaCaT cells were cultured in DMEM medium supplemented with 10% FBS, 100 IU of penicillin G (sodium salt) and 100 µg of streptomycin sulfate, and 2 mmol L^{-1} L-glutamine. Cells were kept in a 95% moisture and 5% CO_2 at 37 °C. Subculture was performed according to biobank instructions whenever cultures reached 80–85% confluence.

2.3.2. Evaluation of cytotoxicity

The cytotoxic activities of seaweed extracts were evaluated on HaCaT cells (4×10^4 cells $well^{-1}$) after seeding in 96-well plates and incubating overnight. Cells were then treated with UES and CES (100 µg mL^{-1}) for 24 h. Untreated cells were used as a control. The control situation was always treated with the highest concentration of DMSO as the vehicle. Saponin (Sigma, 47036-50G-F) was used as a cellular death positive control (85% of cell death). After that, the effects were estimated using the 3-[4–dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) colorimetric assay, as described by Freitas *et al*., 2020.[16] The intracellular formazan crystals were then extracted and solubilized with dimethyl sulfoxide (DMSO) and the absorbance was measured at 570 nm using a microplate reader (Synergy H1 Multi-Mode Microplate Reader, BioTek® Instruments, Vermont, VT, USA). The results were expressed as a percentage of control untreated cells.

2.3.3. Photoprotective capacity

The photoprotective effect of the extracts was determined according to Marto *et al.* 2018, with slight modifications.[23] This assay was evaluated through reactive oxygen species (ROS) production measurements on HaCaT cells exposed to ultraviolet radiation (UVA and UVB) using the fluorophore 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA) (Thermo FischerScientific, Eugene, OR, USA).[16] The HaCaT sub-confluent cells were grown in 96-well plates and were treated with the extracts at non-cytotoxic concentrations (10, 30 and 100 µg mL^{-1}) for 1 h in the dark. Ascorbic Acid was used as a positive control at 10 µM.

Subsequently, the medium was removed, and the cells were washed with fresh medium and then exposed to UVA–B radiation using a simulator (UVA Cube 400,

Hönle Technology, Gräfelfing, Germany) with an intensity of 7.9 mW cm^{-2} for 15 min. Cells were irradiated with a UV lamp equipped with an H2 filter (UVA + UVB) with a spectral output of 280–400 nm, and the intensity of UV radiation was measured using a sensor (UV-Metro, Hönle Technology, Gräfelfing, Germany). After the UVA–B exposure, the medium was removed and the cells were treated in the dark for 30 min with H_2DCFDA (20 µM) at 37 °C. Then, ROS levels were determined by fluorescence measurement (Multimodal Synergy H1, BioTek® Instruments, Winooski, VT, USA) at an excitation of 495 nm and emission of 527 nm wavelengths recorded each minute for 10 min. The results were expressed as a percentage of the negative control, which contained only the vehicle at the same concentrations as the extracts. The experimental design of the photoprotection assay using HaCaT cells is illustrated in Figure 1.

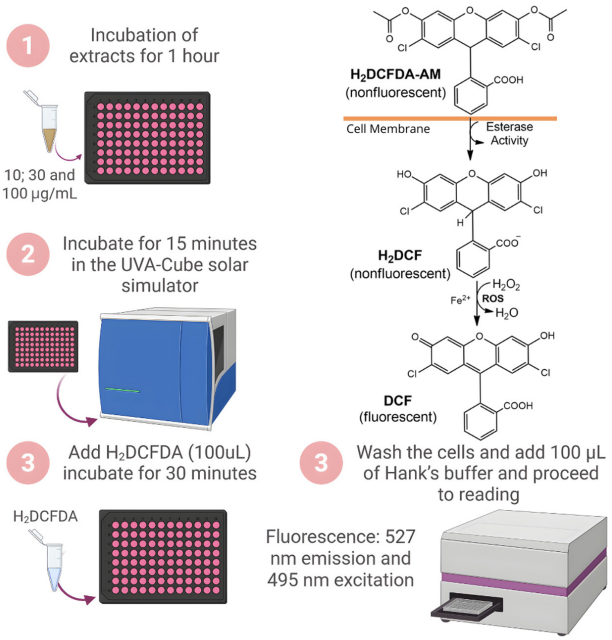


Figure 1. Schematic representation of the methodology used to evaluate the photoprotective potential of *Sargassum* extracts. Figure created with BioRender and ChemDraw v.12.0

2.4. Antioxidant capacity

2.4.1. 2,2-diphenyl–1-picrylhydrazyl (DPPH) radical scavenging activity

Antioxidant activity was measured by the DPPH method described in a previous study.[24] Briefly, the reaction mixture contained 1 mL of acetate buffer (100 mM, pH 5.5), 1.25 mL of absolute ethanol, 250 µL of 500 µM DPPH solution, and 50 µL of extracts at various concentrations. The mixture was shaken and incubated in low light for 30 min. Absorbance was measured at 517 nm using a UV-visible spectrophotometer. All measurements were conducted in triplicate for statistical analysis, with DPPH scavenging activity expressed as a percentage, calculated using the formula: Antioxidant activity (%) = [(Acontrol – Asample)/Acontrol] × 100, where Acontrol represents the absorbance of the control (containing all components except the sample), and Asample is the absorbance of the sample after 30 min. Trolox was used as a positive control. The ability of *Sargassum* sp. extracts to reduce DPPH radicals were evaluated at 100, 400 and 1000 µg mL^{-1}.

2.4.2. Ferric reducing antioxidant power (FRAP)

The FRAP test was performed according to a previous study.[25] The FRAP reagent was prepared immediately before analysis by mixing 25 mL of acetate buffer (300 mM, pH 3.6), 2.5 mL of TPTZ solution (10 mM TPTZ in 40 mM HCl), and 2.5 mL of $FeCl_3$ (20 mM) in aqueous solution. A 100 µL aliquot of the phenolic compound solution was added to 3 mL of the FRAP reagent and incubated at 37 °C in a water bath for 30 min. Absorbance was measured after incubation, with the spectrophotometer zeroed using the FRAP solution. The calibration curve was generated using $FeSO_4$ (100-2000 µM), and results were expressed as µmol Fe^{2+} mg^{-1} sample.

2.4.3. Quantification of total phenolic content (TPC)

The quantification of total phenolic content in hydroethanolic extracts of *Sargassum* sp. was performed using the colorimetric method based on the Folin–Ciocalteu reagent, as described by Martins *et al.*, 2024.[17] This colourimetric method is based on the reaction of phenolic compounds with the Folin-Ciocalteu reagent. Following a 1 h reaction in the dark, the absorbance was measured at 750 nm. Gallic acid (GA) was employed as the standard for the polyphenolic curve (0.01 mg mL^{-1}, 0.03 mg mL^{-1}, 0.1 mg mL^{-1}, 0.3 mg mL^{-1}, and 1 mg mL^{-1}) and TPC was expressed as mg GA Eq. g^{-1} of extract.

2.5. Anti-enzymatic activity of *Sargassum* sp. extracts

2.5.1. Anti-hyaluronidase activity

Anti-hyaluronidase activity was evaluated according to the procedure described by Susano *et al.*, 2022 and Freitas *et al.*, 2020.[26,16] In summary, *Sargassum* sp. extracts were mixed with 5 µL of hyaluronidase (7 U mL^{-1}) and 67 µL of enzyme diluent (20 mM sodium phosphate, 77 mM sodium chloride, and 0.01% bovine serum albumin (BSA)). This mixture was initially incubated at 37 °C for 10 minutes. Subsequently, a hyaluronic acid (HA) solution (25 µL; 0.03% in 300 mM sodium phosphate) was added and allowed to further incubate at 37 °C for 45 min. The hyaluronic acid was then precipitated using 200 µL of acidic albumin solution (24 mM sodium acetate, 79 mM acetic acid, and 0.1% BSA).

After a 10 minutes incubation time at room temperature, the absorbance was measured at 600 nm. The hyaluronidase inhibitory activity of each extract was quantified as a percentage relative to the control (% of control). Schematic representation of the anti-hyaluronidase assay is provided in the Supplementary Information (Figure S1).

2.5.2. Anti-elastase and anti-collagenase activities

The EnzChekTM Elastase Assay Kit (#E12056, InvitrogenTM, ThermoFisher Scientific, Waltham, MA, USA) was used to determine the anti-elastase activity. EnzChekTM Gelatinase/Collagenase Assay Kit (#E12055, InvitrogenTM, ThermoFisher Scientific, Waltham, MA, USA) was used to determine the anti-collagenase activity. Both protocols were performed as per the manufacturer's instructions. The results were expressed as arbitrary fluorescence units per minute (Δ fluorescence (a.u.) min^{-1}) as a percentage of the control.

2.5.3. Anti-tyrosinase activity

The inhibition of tyrosinase activity was performed as described by Luz *et al.,* 2025.[27] Initially, each extract was mixed with potassium phosphate buffer (0.5 mM, pH = 6.8) and L-DOPA (1 mM) and pre-incubated at 37 °C for 5 min, in the dark. After the pre-incubation time, tyrosinase (100 U mL^{-1}) was added, and the absorbance was measured using a microplate reader (Epoch Microplate Reader, BioTek® Instruments, Winooski, VT, USA) at 475 nm, every minute for 15 min. Kojic acid was used as a positive control and results were presented as tyrosinase activity in % of control.

2.6. Chemical characterization

2.6.1. CG-MS analysis

Gas Chromatography–Mass Spectrometry (GC–MS) analysis was performed using an Agilent 7890A gas chromatograph coupled to an Agilent 5973C quadrupole mass spectrometer (electron impact ionization, 70 eV) equipped with an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm). The injector and detector temperatures were set at 260 °C and 290 °C, respectively. The temperature program began at 40 °C (1 min), increased to 100 °C at 60 °C min^{-1}, then to 290 °C at 4 °C min^{-1}, holding for 2 minutes. The interface temperature was 300 °C. Helium was used as the carrier gas (1 mL min^{-1}), and 2 µL of the sample was injected in splitless mode. Mass spectrum data were obtained using Scan analysis mode (40-350 *m/z*), and compound identification was based on spectral matching with literature.[28] The chromatograms and mass spectra obtained from the experiments were analyzed using the open-source software OpenChrom v. 1.5.11.

2.6.2. RMN ^{1}H analysis

Nuclear Magnetic Resonance (NMR) spectra were recorded on a Varian® VNMRS spectrometer operating at 500 MHz for ^{1}H. $CDCl_3$ was used as the solvent for the samples, and tetramethylsilane (TMS) served as the internal reference. Chemical shifts (δ) are reported in parts per million (ppm), and coupling constants (*J*) are given in Hertz (Hz). Relative signal integrals were obtained by electronic integration, and spectral calibration was performed based on the TMS signal. Signals corresponding to fatty acids, phenolic compounds, pigments, and sterol classes were monitored. Spectral processing was carried out using MestReNova software, version 6.0.2.

2.6.3. FTIR analysis

Moreover, the Fourier-Transform Infrared Spectroscopy (FT-IR) spectra of the solid samples, prepared as KBr pellets, were recorded using an FTIR-IRTracer 100 Shimadzu spectrometer. Data acquisition was performed with a DTGS detector and a KBr beam-splitter. The spectra were collected within the 4000–400 cm^{-1} range at a resolution of 4 cm^{-1}.

2.6.4. Data and statistical analysis

The results are presented as mean plus standard error of the mean (SEM). A paired Student's *t*-test was used to compare two groups. For comparisons involving three or more groups, a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test to assess significant differences relative to the control treatment was performed. All data were checked for normality using the Shapiro–Wilk test. Comparisons concerning variables, which did not meet variance or distributional assumptions, were carried out with the Kruskal–Wallis non-parametric test. Differences were considered significant at the level of 0.05 ($p < 0.05$).

3. Results and Discussion

3.1. Cytotoxicity and photoprotective capacity

HaCaT cells were used as a cellular model to assess the cytotoxicity of seaweed extracts. The data presented in Figure 2 suggest that the viability of HaCaT cells was not significantly affected by the extracts. Cytotoxicity measurements indicated a maximum reduction in cell viability of 5% for CES (Figure 2). Regarding the photoprotective activity, the pre-treatment with *Sargassum* sp. extracts led to a significant reduction in ROS production in H_2O_2-stimulated HaCaT cells exposed to UV radiation, bringing the levels down to those observed with the positive control, ascorbic acid. Specifically, CES at 100 µg mL^{-1} showed a significant decrease in ROS

levels compared to the vehicle control. Similarly, UES at concentrations of 30 and 100 μg mL^{-1} also significantly reduced ROS production induced by UV exposure, showing decreases of approximately 15–25% 15–25% (30–100 μg mL^{-1}) compared with the vehicle (Figure 2). It is important to highlight that excessive UVB exposure induces the production of ROS in skin cells, leading to oxidative stress, inflammation, and cellular damage. Although ROS play essential roles in cell signaling, their overproduction can overwhelm antioxidant defenses, contributing to skin disorders and premature aging. Marine natural products with antioxidant properties, such as seaweed extracts, may represent a promising strategy to mitigate oxidative stress and enhance skin protection.[24]

Initial toxicity assessments were conducted using HaCaT cells, a well-established cellular model for evaluating cytotoxicity in skin-related products due to their human keratinocyte origin and ability to mimic cutaneous responses.[29,16] These results are important as they provide preliminary insights into the cytocompatibility of our extracts, corroborating their potential safe application in skin-related formulations. These assays constitute the first validation steps required for the development of new products. Moreover, the results here described are considered the first scientific baseline data obtained from *Sargassum* sp. biomass collected from beaches along Brazil's southeastern coast affected by seaweed stranding events.

Regarding the *Sargassum* genus, recent studies have advanced the investigation of the photoprotective potential of fractions with different polarities and bioactive compounds.[30] For example, the photoprotective effect of the fucoidan fraction (SHC4) was evaluated in human keratinocytes exposed to oxidative stress induced by UVB radiation. The purified fucoidan presented a structure mainly composed of fucose units linked by α-L-Fucp-(1→4) bonds. The SHC4 (25–100 μg mL^{-1}) promoted a dose-dependent reduction in intracellular levels of reactive oxygen species (ROS) and restored cell viability in keratinocytes exposed to UVB radiation. Another study evaluated the photoprotective effect of ultrasound-assisted ethanolic extract (USHE) of *Sargassum horneri* at concentrations of 15.6, 31.3 and 62.5 μg mL^{-1}, demonstrating that pretreatment with USHE significantly reduced the intracellular production of reactive oxygen species (ROS) in HaCaT keratinocytes exposed to UVB radiation, reaching ROS levels between 15-35%, similar to those obtained in our study.[24]

Several fractions of extracts obtained from *Sargassum* species have also been investigated for their antioxidant potential. For example, Piao *et al.* evaluated *Sargassum muticum*, which was extracted with 80% ethanol, and subsequently fractionated into *n*-hexane, dichloromethane, ethyl acetate, butanol, and aqueous fractions.[31] The intracellular ROS scavenging activity induced by UVB irradiation was assessed for the ethyl acetate fraction (SME), resulting in scavenging rates of 7% at 12.5 μg mL^{-1}, 20% at 25 μg mL^{-1}, 22% at 50 μg mL^{-1}, and 23% at 100 μg mL^{-1}. Thus, SME exhibited photoprotective activity.

Beyond the *Sargassum* genus, various green and red macroalgae have also exhibited photoprotective properties, highlighting the capacity of diverse marine species to synthesize a broad spectrum of bioactive compounds applicable to photoprotection. Some studies,[17–18] using the same cell model and hydroethanolic extracts, observed concentration ranges similar to those obtained in our study, already considered promising for photoprotection. Hydroethanolic extracts of *Codium s*p. also exhibited photoprotective activity, reducing UV-induced ROS production by 15–25% (Cd1, 30–100 μg mL^{-1}) and 30% (Cd2, 200 μg mL^{-1}) compared to the vehicle.[17] The photoprotective capacity of *G. corneum* fractions on HaCaT cells exposed to UVA-B radiation was evaluated. Results

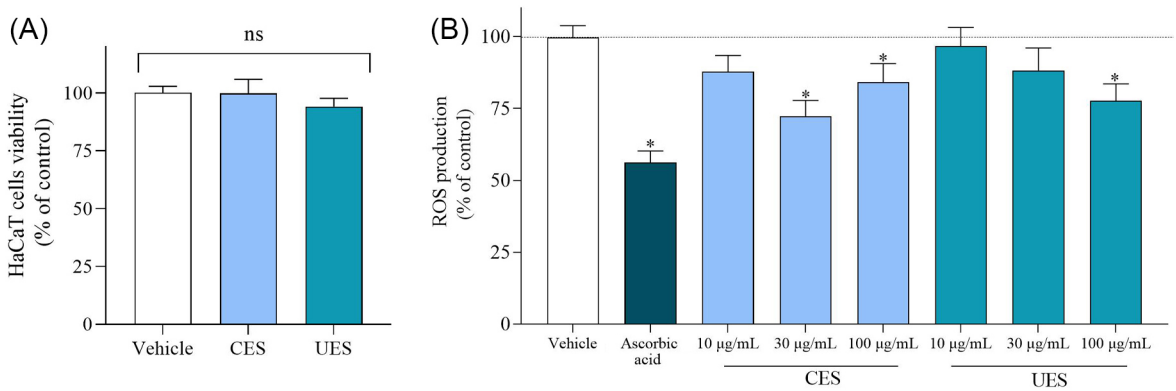


Figure 2. Cytotoxicity and photoprotective assays of *Sargassum* sp. extracts. (A) HaCaT cells' viability following 24 h of exposure to *Sargassum* sp. extracts (100 μg mL^{-1}). (B) Evaluation of ROS production by HaCaT cells exposed to UV radiation (12.5 mJ cm^{-2}) for 15 min, in the presence/absence of Sargassum sp. extracts (10-100 μg mL^{-1}) and ascorbic acid (10 μM). The values in each column represent the mean ± standard error of the mean (SEM) of 3 or 4 independent experiments. The symbol (*) represents significant differences ($p < 0.05$; Paired Student's t-test) when compared to the vehicle

showed that only the crude extract F1 (1000 μg mL^{-1}) and the water-insoluble fraction F2 (600 μg mL^{-1}) reduced ROS production compared to the vehicle, with F1 showing the best result (27.52 ± 5.84%).[18]

Photoprotective effects are increasingly crucial in skincare products, given the rising incidence of skin cancer, which is primarily associated with UV radiation exposure. As lifestyle trends promote greater sun exposure and artificial tanning, effective photoprotection has become essential to prevent skin damage, including photoaging and photocarcinogenesis.[32] The findings presented previously, both regarding marine algae in general and specifically species of *Sargassum*, reinforce that the results obtained in this study can be integrated into the body of evidence supporting the use of marine macroalgae as potential sources of photoprotective agents. Moreover, these discoveries, combined with the fact that they were derived from an alga considered marine waste and stranded biomass, underscore the valuable potential of this algal matrix, which is both available and sustainable. This foundation can enable the development of bioproducts and additives with crucial properties, such as photoprotection.

3.2. Anti-enzymatic activities

3.2.1. Hyaluronidase activity inhibition

The hydroethanolic extracts of *Sargassum* sp., obtained by UES and CES extraction, were evaluated for their enzyme inhibitory activity at a concentration of 100 μg mL^{-1}. It is noteworthy that at a concentration of 100 μg mL^{-1} the extracts did not show cytotoxicity when compared to untreated cells. The results were compared with the reference compounds, epigallocatechin gallate (EGCG) at 120 μg mL^{-1} and kojic acid (18 μg mL^{-1}), in relation to the inhibition of several enzymes. The CES extracts showed inhibition of 6.24 ± 1.40% for collagenase, 52.53 ± 1.03% for hyaluronidase and 8.44 ± 3.87% for tyrosinase, and no inhibition for elastase at the concentrations tested. The UES extracts demonstrated inhibition of 9.08 ± 3.06% for collagenase and 25.42 ± 2.31% for hyaluronidase. These results indicate that the extracts of *Sargassum* sp. exhibited variable anti-enzymatic activity, with emphasis on the inhibition of hyaluronidase activity by extracts obtained by ultrasound (UES), although this effect is moderate compared to the reference compounds (Table 1).

Our results indicate that *Sargassum* sp. extracts exhibited promising anti-enzymatic activities against hyaluronidase at specific concentrations in both extracts analyzed. In the CES extract, a broader range of bioactivities was observed, probably due to the time and extraction method, which allowed the recovery of molecules associated with a broader metabolic profile. These molecules contributed not only to anti-collagenase and anti-hyaluronidase activities, but also exhibited tyrosinase inhibitory effects. It is important to emphasize that, although the percentages observed for collagenase activity are relatively low compared to the ideal reference values, they could be enhanced by evaluating concentrations other than those tested in this work or also by using more purified fractions of the extracts. Furthermore, such activities may be optimized within cosmetic formulations, thereby enhancing the multifunctional potential of the extracts in targeting multiple pathways related to skin maintenance and protection. The HA is prevalent throughout the human organism, being found in dermal layers, synovial fluids, vitreous humor, and connective tissues. It undertakes vital roles within the dermis, including the cohesion and safeguarding of cells, the synthesis of dermal structures, the conservation of hydration within the skin, and the preservation of the skin's elasticity. With aging, the skin becomes dehydrated and loses firmness, resulting in wrinkles and sagging.[33] HA is degraded by the enzyme hyaluronidase. In this way, extracts or molecules of natural origin that inhibit this enzyme may be effective in preserving HA levels in the skin, thereby promoting its hydration and elasticity. [34]

The conventional extract exhibited hyaluronidase inhibitory activity, reaching 52.53 ± 1.03% inhibition. In comparison, the UES extract of *Sargassum* sp. showed a lower inhibition rate of 25.42 ± 2.31%. Both extracts showed significant differences when compared to the vehicle ($p < 0.05$). However, no significant differences were observed between either extract and the positive

Table 1. Enzymatic inhibitory activity of *Sargassum* sp. hydroethanolic extracts UES and CES (100 μg mL^{-1}) and of the reference compounds, epigallocatechin gallate (ECGC) and kojic acid (% control)

Sample	Collagenase (%)[a]	Elastase	Hyaluronidase (%)	Tyrosinase (%)
UES	9.08 ± 3.06	NI	25.42 ± 2.31	NI
CES	6.24 ± 1.40	NI	52.53 ± 1.03	8.44 ± 3.87
EGCG[a]	98.63 ± 1.36	62.62 ± 1.94%	38.08 ± 8.02	-
Kojic Acid[b]	-	-	-	81.12 ± 3.32

The values represent the mean ± standard error of the mean (mean ± SEM, n = 3). [a] Positive control, EGCG: 120 μg mL^{-1}; [b] Positive control, Kojic Acid: 18 μg mL^{-1}; (mean ± SEM - Standard Error of the Mean, n = 3) NI: no inhibition. (%) All percentages refer to the inhibition percentage of the enzymes tested.

control EGCG. It is important to note that evaluating higher concentrations could potentially enhance the inhibitory effect, as dose-response analysis indicates that greater concentrations lead to increased activity. Nevertheless, it is also essential to perform cytotoxicity assays to assess the safety and relevance of these results.

Marine algae have been studied for their anti-hyaluronidase activity properties,[25] with particular emphasis on brown algae.[36] Both extracts showed statistically significant differences (way ANOVA, $p < 0.01$) when compared to the ascorbic acid.[21] On the other hand, the chloroform:methanol (1:1 v/v) extract from the *S. tenerrimum* blade contained significantly higher phlorotannin content than other extracts and exhibited the strongest anti-hyaluronidase activity, with 37.67 ± 2.3% inhibition. Several *Sargassum* species were evaluated in this study, and the inhibition activity followed the order: *S. tenerrimum* (blade) > *S. vulgare* (blade) > *E. arborea* > *S. tenerrimum* (stipe) > *S. vulgare*.[36] Other brown algae have also been studied, for example, aqueous fractions and other brown algae-derived products have been explored for their bioactive potential. For instance, a study on a polysaccharide from *Porphyridium purpureum* reported significant hyaluronidase activity inhibition, with an IC_{50} of 0.210 mg mL^{-1}, demonstrating a 50% reduction in enzyme activity at this concentration.[37]

Hyaluronidase activity inhibition levels vary among algae species and are influenced by the type of extract and its chemical composition. As highlighted in the reported studies, inhibition rates above 35% indicate potential for use in skincare applications. To maximize this activity, it is essential to investigate the effects of extraction parameters, such as solvent composition, concentration, extraction time, and processing conditions. Refining these factors could enhance inhibition efficacy, positioning the UES as a promising additive for dermatological applications.

More specific studies involving isolated compounds, such as phlorotannins from brown algae, have not yet evaluated how these compounds hyaluronidase activity. For example, in the alga *Eisenia arborea*, five phlorotannins, except for eckol, exhibited stronger inhibitory activity than the standard inhibitors EGCG and disodium cromoglycate (DSCG). Inhibitory effects of eckol (IC_{50}=1036 µmol L^{-1}) were similar to these standard inhibitors. Among the others compounds, 6,8'-bieckol (IC_{50}=256 µmol L^{-1}) and PFF-A (IC_{50} =221 µmol L^{-1}) stood out with very strong inhibitory activity. In contrast, 8,8'-bieckol (IC_{50} = 419 µmol L^{-1}) and PFF-B (IC_{50} = 42 µmol L^{-1}) showed moderate activity, while 6,6'-bieckol (IC_{50} = 869 µmol L^{-1}) demonstrated the weakest inhibitory effect among the phlorotannins tested.[38] Isolated compounds like fucoxanthin have shown strong anti-hyaluronidase activity, highlighting its potential application in skincare products. Studies conducted on *Sargassum* species have revealed significant fucoxanthin yields, particularly in *S. wightii*, along with notable enzymatic inhibition, with *S. vulgare* exhibiting over 93% inhibition. The latest studies suggest that focusing on refining compounds such as phlorotannins and pigments may be a promising path for developing targeted and effective dermocosmetic productss.[39] The results obtained in this study are comparable with those reported in previous research. Furthermore, to our knowledge, there are no existing reports on hyaluronidase inhibitory activity in seaweed biomass collected from Brazilian beaches, which significantly enhances the novelty and potential impact of this study. This relevance is further underscored by the likelihood that the observed enzymatic inhibition is closely linked to the metabolic profile of the algae, influenced by the specific environmental conditions of the collection sites.

3.3. Antioxidant capacity

The evaluation of antioxidant activity using the DPPH method at the tested concentrations of 100, 400, and 1000 µg mL^{-1} indicated greater efficiency of CES compared to UES. CES showed a reduction of 30.8%, while UES showed a reduction of 24.4% at the minimum concentration tested (Table 2). This data suggests that CES may be more effective in donating electrons or hydrogen atoms to neutralize free radicals than ethanol extract. When antioxidants scavenge DPPH radical, its color changes from purple to yellow. The extent of this color change, reflected in lower absorbance values, depends on the concentration of the tested sample and its antioxidative capacity.[40]

Table 2. Antioxidant capacity of *Sargassum* sp. hydroethanolic extracts obtained through ultrasound-assisted extraction (UES) and conventional extraction (CES) at a single representative concentration

Sample	DPPH[a]	FRAP[b]	ABTS[b]	TPC[c]
UES	24.41 ± 3.37%	41.09 ± 2.55	27.48 ± 1.93	ND
CES	30.87 ± 4.82%	53.17 ± 1.41	17.69 ± 2.36	142.80 ± 24.63

* Results are expressed as mean and standard deviation. DPPH: 2,2-diphenyl–1-picrylhydrazyl; FRAP: ferric ion antioxidant power; ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); TPC: Total Phenolic Content; a % antioxidant potential at 100 µg mL^{-1}; [b]µmol of Trolox equivalents g^{-1} extract (µmol TE g^{-1}). [c]mg Gallic acid Equivalent g^{-1} extract. ND: not determined. The values represent the mean ± standard error of the mean (SEM) of 3 independent experiments.

Furthermore, it is important to highlight that a considerable amount of the antioxidant compounds in these seaweeds are polar in nature, as evidenced by the effectiveness of ethanol and water in extracting them. This polarity enhances their ability to donate electrons or hydrogen atoms, thereby neutralizing free radicals.[41]

Some studies have evaluated the antioxidant capacity of the holopelagic species *S. fluitans*, *S. natans* I and *S. natans* VIII during their arrival at the Mexican Caribbean coast, using water-ethanol extracts (2:1). The DPPH method indicated low antioxidant activity in all *Sargassum* species during the sampling period (20.2% on average), with a notable increase in August 2018. *S. fluitan*s presented values between 9.6% and 23.0%, with an exceptional peak of 63% in August 2018.[42] Sumandiarsa *et al.*, 2022, evaluated the DPPH antioxidant activity of *S. polycystum* and observed that effects were highest in the apical part of the thallus, exhibiting an IC_{50} value of 38.49 µg/mL.[43] The DPPH radical scavenging capacity of the ethanolic extract of brown seaweed, *Sargassum pallidum*, was reported to be 30.5% (2 mg mL^{-1}).[40] This data indicates an antioxidant capacity, as a compound is categorized as powerful if the IC_{50} value is below 50 µg mL^{-1}. In comparison with these findings, the results of this study show that the EUS and ECS extracts are positioning themselves above these averages, demonstrating antioxidant potential.

The antioxidant activity of the seaweed extracts by the FRAP method, which evaluates the capacity of molecules in reducing ferric ions (Fe^{3+} to Fe^{2+}), the UES extract exhibited values ranging from 39.51 ± 2.23 to 49.99 ± 3.09 µM TE g^{-1}, whereas the CES extract showed higher values, ranging from 31.41 ± 0.98 to 59.33 ± 1.27 µM TE g^{-1} (Table 2). This indicates that the two types of extracts evaluated, along with the varying concentrations, may possess different capacities to reduce ferric ions, underscoring their potential as significant sources of antioxidant compounds for cosmetic applications. The FRAP value for *S. vulgare* was determined to be 62.45 mg TE g^{-1} dry weight (dw), indicating antioxidant capacity.[44] On the other hand, the hot-water extract (HES) of *S. macrocarpum* demonstrated an IC_{50} value of 18.8 ± 0.4 mg mL^{-1}, reflecting its effective radical scavenging capacity.[45] These findings support our results, as the antioxidant capacities of *Sargassum* species have been increasingly evaluated in recent years, further highlighting the relevance of our findings.

The antioxidant activity determined by the ABTS assay (µM TE g^{-1} extract) varied between the *Sargassum* sp. extracts. The UES extract exhibited antioxidant values ranging from 14.23 ± 1.94 to 33.89 ± 2.11 µM TE g^{-1}, whereas the CES extract showed lower values, ranging from 11.43 ± 2.17 to 23.11 ± 1.49 µM TE g^{-1} (Table 2). These results demonstrate that UES exhibited higher antioxidant activity for this method and are supported by recent studies that evaluated the antioxidant potential of hydroethanolic extracts. For example, Silva *et al.*, 2024,[46] employed microwave-assisted extraction (MAE) to optimize the extraction of bioactive compounds from *S. muticum*, determining that an ethanol:water solvent mixture, with a time of 14 min, a pressure of 11.03 bar, and an ethanol concentration of 33.31%, maximizing the yield of bioactive compounds extraction, directly increasing the antioxidant activity measured by the ABTS assay. Furthermore, the antioxidant activity of *S. vulgare*, determined by the ABTS method, was 19.88 mg of TE g^{-1} of dry weight (dw).[44]

The quantification of phenolic compounds was used as a complementary chemical characterization technique, since it is well established that phenolic compounds constitute a class of compounds capable of inhibiting the oxidation of biological materials. Their antioxidant capacity is closely related to their chemical structure, especially the number and position of hydroxyl groups. Compounds with a greater number of hydroxyl groups generally have higher antioxidant activity, due to their ability to donate hydrogen atoms to neutralize free radicals.[47] Among these compounds, the so-called phlorotannins, found in brown algae, stand out. Phlorotannins are recognized as the most relevant algal polyphenols due to their biochemical properties. These molecules play important biological roles in all phases of algal development, from the initial stages to maturity.[48,49] According to Lopes *et al.*, 2024, phlorotannins also perform essential structural functions in the algal cell wall, contributing to oxidative stress response and providing protection against various external abiotic factors, including light, nutrient fluctuations, salinity, ultraviolet radiation, dehydration, and herbivory.[50]

Chemically, phlorotannins are polymers composed mainly of monomeric units of phloroglucinol (1,3,5-benzenetriol), linked together by different types of bonds.[51] The results obtained for the total phenolic content in *Sargassum* species indicated significant variability, reflecting the influence of extraction conditions. For CES, the content was 142.80 ± 24.63 mg GAE g^{-1} of extract, while for UES, no detectable levels of phenolic compounds were observed under the conditions employed. It is important to note that conventional methods based on the Folin–Ciocalteu reagent, although widely used, are nonspecific and may overestimate phenolic content due to interference from other reducing substances.[52] Therefore, the use of alternative colorimetric assays is recommended, such as those developed for the specific quantification of phlorotannins, which are the main class of phenolics in brown algae.[53] Among these, the DMBA (2,4-dimethoxybenzaldehyde) method has gained prominence due to its higher specificity for phloroglucinol units.[54]

Compared to the literature, Baek *et al*. 2021,[55] reported values ranging from 20.57 to 88.97 mg GAE g^{-1} for various *Sargassum* species, with *S. miyabei* exhibiting the highest levels. Dharmawan *et al*. 2023,[52] found 149.04 ± 5.14 mg GAE g^{-1} for *Sargassum* sp. collected from Pameungpeuk Beach, indicating a similar amount to our results, which may be attributed to differences in collection and extraction conditions. In another study, Arguelles *et al.*, 2020[56] reported 30.34 mg GAE g^{-1} for *S. siliquosum*, a considerably lower value compared to ours, although the authors still considered it representative of the phenolic content. These findings together with the present study confirm the potential of *Sargassum* species as rich sources of phenolic compounds and bioactive molecules, with variations depending on the species and extraction methods used. It is important to emphasize that extraction methods should be continually refined and standardized to achieve more efficient and consistent yields of bioactive compounds, thereby maximizing their potential for various industrial and cosmetic applications.

All things considered, the anti-hyaluronidase activity and antioxidant assays demonstrated relevant differences between the extraction methods. The CES presented a hyaluronidase inhibitory activity of 52.53 ± 1.03%, a value more than double that observed for UES, which was 25.42 ± 2.31%. This difference suggests that the longer conventional extraction time favored the extraction of compounds with anti-hyaluronidase action, which is particularly interesting for anti-aging cosmetic applications, where preservation of the extracellular matrix is desired. In the antioxidant tests, CES also showed higher values of DPPH (30.87 ± 4.82%), FRAP (53.17 ± 1.41 µmol TE g^{-1}), and TPC (79.6 ± 18.2 mg GAE g–1) compared to UES (DPPH: 24.41 ± 3.37%; FRAP: 41.09 ± 2.55 µmol TE g^{-1}; TPC: 17.4 ± 7.0 mg GAE g^{-1}).

Interestingly, the ultrasonic extract showed higher performance in the ABTS assay (27.48 ± 1.93%), surpassing that of the conventional extract (17.69 ± 2.36%), which may reflect a selective extraction of more sensitive or soluble antioxidant compounds under cavitation. It is important to highlight that the ultrasonic extract was obtained with a significantly shorter extraction time compared to the conventional method (1 hour). Nevertheless, the results have already proven to be representative, indicating that the technique has promising potential. This leads us to believe that longer ultrasonic extraction times could substantially increase the yield and diversity of bioactive compounds extracted, making the process even more attractive for industrial applications that demand efficiency, speed, and selectivity. Future studies will include more repetitions and different ultrasonic extraction times, with the aim of optimizing and standardizing the process, seeking a balance between yield, biological activity and industrial viability. A deeper understanding of the influence of these parameters will allow the development of extracts targeted at specific applications, especially in the cosmetic, functional and pharmaceutical areas.

3.4. Chemical analysis

After extraction, CES and UES yielded 1.2% and 0.8%, respectively. Both extracts were analyzed using FT-IR spectroscopy to identify functional groups of *Sargassum* sp. extracts (Table 3). A strong broad band at 3375.40 cm^{-1} (UES) and 3391.04 cm^{-1} (CES) corresponds to O–H stretching vibrations, indicating the presence of phenolic compounds. The bands observed around 2929.83 cm^{-1} and 2937.49 cm^{-1} are attributed to aliphatic C–H stretching of alkanes. Notably, the absorption band at 1735.76 cm^{-1} observed in the UES extract suggests the presence of carbonyl containing compounds, particularly esters. A secondary band with lower absorption intensity was also detected at a slightly lower wavenumber, which could be associated with carboxylic acids present in the sample. The carbonyl (C=O) stretching of esters typically appears at higher wave numbers than that of carboxylic acids, due to differences in electron density and the extent of hydrogen bonding within these functional groups. The extracts exhibit bands around 1636–1644 cm^{-1} and 1456–1457 cm^{-1}, confirming the presence of alkenes and methylene groups.

Table 3. FT-IR absorption bands observed in hydroethanolic extracts of *Sargassum* sp. obtained by conventional extraction (CES) and ultrasound-assisted extraction (UES) in Kbr (n_{max}, cm^{-1})

Functional Group	CES	UES	Observations
O–H (phenolic groups)	3271.87 and 3391.04	3375.40	Strong and broad bands indicating the presence of free hydroxyl groups, slight shift suggests altered hydrogen bonding environment
C–H (alkanes)	2937.49 and 2854.40	2929.83 and 2853.43	Consistent with aliphatic chains, similarly extracted by both methods
C=O (carbonyl groups)	ND	1735.76	Clear enhancement of carbonyl band
C=C, CH_2 (alkenes, methylene)	1644.18 and 1457.49	1456.13	Indicates unsaturated compounds and methylene bending
C–O (esters, ethers)	1231.38 and 1157.30	1234.91 and 1151.11	Bands characteristic of esters

In the 1200–1000 cm^{-1} region, absorptions at 1157.30 cm^{-1} (CES) and 1151.11 cm^{-1} (UES) correspond to ester C–O–C stretching vibrations.[57]

The ^{1}H NMR spectra of both extracts were analyzed based on chemical shift assignments reported in the literature for compounds commonly found in brown algae. Several specific regions of chemical shifts were considered in each spectrum (Figure 3, zones A–F), allowing preliminary identification of different classes of metabolites. Signals in the 0.68–2.36 ppm region (Figure 3, zones A and C) can suggest the presence of shielding protons, commonly attributed to aliphatic protons (–CH_3 and –CH_2–) which can comprise various classes, including hydrocarbon fragments of fatty acids, sterols, pigments, and other lipophilic metabolites.[59] These signals represent shielded hydrogen atoms in aliphatic environments, but do not directly indicate the overall polarity of the molecule.

In addition, the presence of olefinic signals between 5.2–5.5 ppm (Figure 3, zone E) suggests the existence of double bonds. Signals observed in the 5.50–6.13 ppm range (Figure 3, zones E and F) can be attributed to aromatic protons. It is noteworthy that brown algae are known producers of phlorotannins, a group of phenolic compounds that include structural units such as phloroglucinol, fucol, and fucophlorethol.[60] However, confirming the presence of these compounds in the analyzed extracts would require more robust analytical approaches, such as HPLC using standards, as well as 1D and 2D NMR experiments on isolated metabolites to enable unambiguous identification. Finally, signals between 3.4–3.8 ppm (Figure 3, zone D) indicate protons attached to hydroxylated carbons, which are commonly found in oxygenated compounds, including phlorotannins from brown algae. Nevertheless, as mentioned above, further targeted analyses would be necessary to corroborate these preliminary assignments.[61]

Additionally, signals at 5.33 ppm (doublet) and 3.50 ppm (multiplet) were detected, corresponding to olefinic and oxygenated methine protons, respectively. These resonances are within the ranges commonly reported for H-6 and H-3 in sterol-type compounds from brown algae.[62,63] However, to confirm the presence of steroids, and complementary spectroscopic analyzes would be required for definitive identification. Future efforts will be directed toward fractionation and isolation of individual constituents, followed by spectroscopic characterization to enable the structural elucidation of the metabolites and comparison with literature data.

The lipophilic profile of *Sargassum* sp. was characterized by gas GC-MS analysis based on mass spectrometric data obtained by the research group and comparisons with literature reports, as presented in Table 4 (The corresponding mass spectra are provided in the Supplementary Information, Figure S2).

Evaluation of the CES and UES revealed the presence of

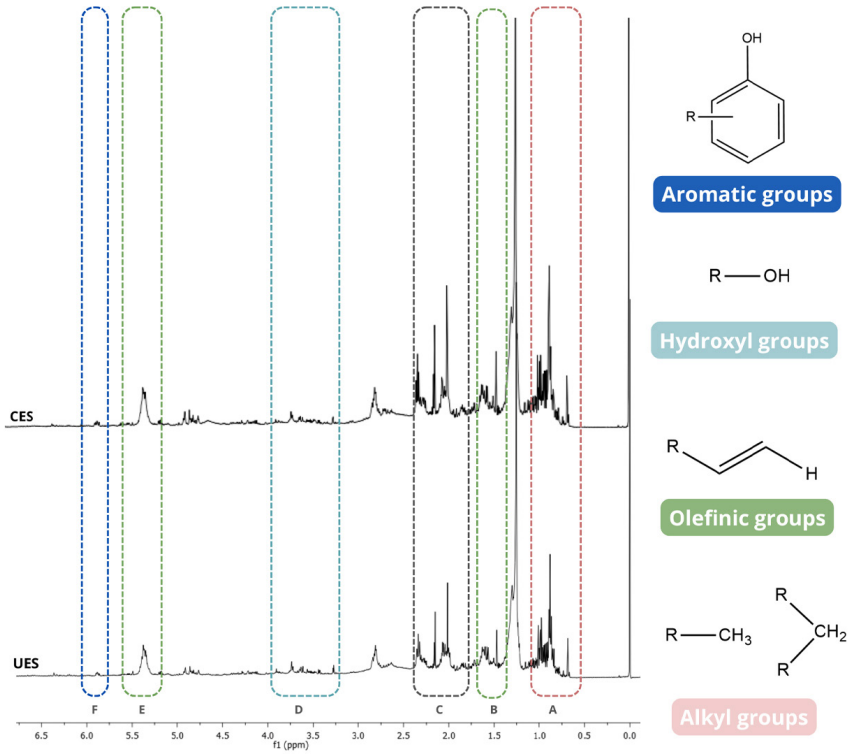
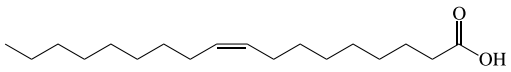
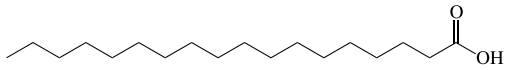
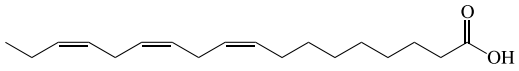
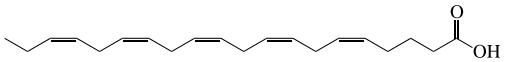
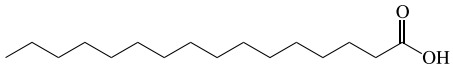
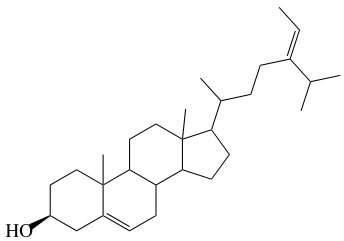


Figure 3. ^{1}H NMR spectra of *Sargassum* sp. extracts exhibited signals in zones A–F: lipids (A–C: 0.6–2.3 ppm), methylene/methyl groups (B: ~1.5 ppm), hydroxylated groups (D: 3.4–3.8 ppm), phlorotannins (E–F: 5.5–6.1 ppm), and olefinic protons (E: 5.2–5.5 ppm)

Table 4. Compounds identified by GC-MS analysis on the hydroethanolic extracts of *Sargassum* sp. obtained by conventional extraction (CES) and ultrasound-assisted extraction (UES). Retention time (Rt, min); molecular ion $[M]^+$; fatty acids (FA)

Compound	Class	RT (min)	$[M^+]$	Molecular Formula	Structure
Oleic acid	FA	23.935	282	$C_{18}H_{34}O_2$	
Stearic acid	FA	25.473	284	$C_{18}H_{36}O_2$	
Hexadecanoic acid, ethyl ester	FA	25.772	284	$C_{16}H_{32}O_2$	
α-Linoleic acid ethyl ester	FA	29.494	308	$C_{18}H_{30}O_2$	
Eicosapentaenoic acid	FA	32.991	302	$C_{20}H_{30}O_2$	
Fucosterol	Sterol	49.938	412	$C_{29}H_{48}O$	

several fatty acids, including oleic acid (RT = 23.935 min), stearic acid (RT = 25.473 min), hexadecanoic acid, ethyl ester (RT = 25.772 min), linoleic acid ethyl ester (RT = 29.494 min), and eicosapentaenoic acid (RT = 32.991 min).[64] Additionally, the detection of fucosterol (RT = 49.938 min), a compound associated with skin barrier restoration which presents anti-inflammatory and antioxidant effects.[65-67] It is noteworthy that other signals observed in the chromatograms were not uncharacterized and warrant further investigation, including targeted isolation strategies and structural elucidation to enable precise compound identification. These results could suggest the applicability of *Sargassum* sp. extracts in cosmeceutical formulations and in the development of innovative cosmetic products. However, complementary analytical techniques are required to comprehensively characterize more polar constituents, such as phlorotannins, which are also known to contribute to the biological activity of the extract.[68] Figure 4 provides a schematic overview of the valorization pathway of *Sargassum* biomass derived from beach-cast events, emphasizing its potential for sustainable applications in the dermocosmetic sector and outlining the key findings of this study.

In summary, the valorization of *Sargassum* sp. derived components represents an environmentally responsible strategy to mitigate the ecological and socio-economic impacts of beach-cast events, while simultaneously offering a promising avenue for diversifying the cosmetic industry. This approach aligns with the principles of sustainability and the goals of the circular and blue economies, as it promotes the efficient use of marine biomass through low-impact technologies. Furthermore, it fosters innovation in the development of natural products and opens strategic opportunities for sectoral transformation, encouraging a transition toward eco-conscious formulations and the integration of underutilized marine resources as renewable, high-value raw materials.

4. Conclusion

Sargassum sp. extracts did not exhibit cytotoxicity in HaCaT cells at the concentrations tested, indicating a promising safety profile for topical use until 100 μg mL^{-1}. Furthermore, the extracts showed antioxidant activity in cellular models of oxidative stress. Pretreatment with the extracts effectively reduced the production of ROS induced by UVA-B exposure, reaching levels comparable to the positive control (ascorbic acid). The inhibition of the hyaluronidase enzyme activity observed in *Sargassum* extracts, especially in the conventional extract (CES), which showed an inhibitory activity (>50%), highlights the potential of these bioactives as anti-aging agents.

The antioxidant capacity of *Sargassum* sp. extracts, evaluated through DPPH, FRAP, ABTS and TPC, revealed distinct activity profiles. The conventional extract (CES) exhibited greater efficacy in the DPPH, FRAP and TPC

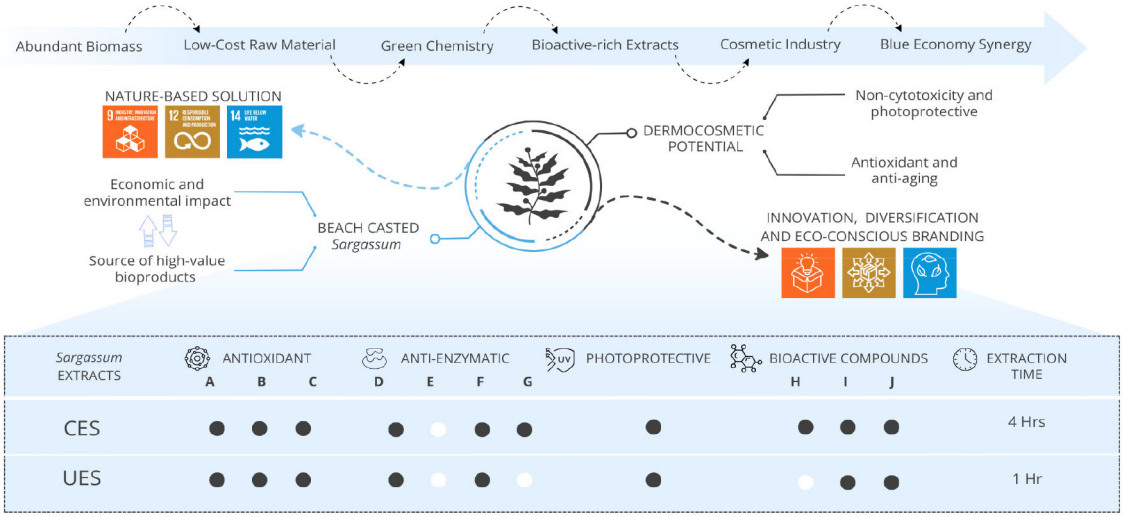


Figure 4. Schematic representation of the valorization pathway of *Sargassum* sp. biomass from beach-cast events. The comparative panel summarizes the biological activities and compound profiles of *Sargassum* sp. extracts obtained by conventional (CES) and ultrasound-assisted (UES) methods. Black circles: presence of activity or compound detection; white circles: absence or undetectable levels of compounds. Antioxidant activity: DPPH (A), FRAP (B), and ABTS (C) assays; anti-enzymatic activities: elastase (D), collagenase (E), tyrosinase (F), and hyaluronidase (G); photoprotective effects (G); bioactive compounds: phenolics (H), fatty acids (I), and sterols (J)

tests, whereas the ultrasonic extract (UES) showed superior performance in the ABTS. These results highlight the potential of *Sargassum* sp. extracts as a source of antioxidant molecules, with promising application in the cosmetic industry, especially in products aimed at combating oxidative stress and skin aging.

This study is the first to demonstrate the antioxidant and anti-aging potential of *Sargassum* sp. extracts from Brazilian beach-cast biomass, underscoring their value for sustainable bioprospecting. These eco-friendly extracts offer promising applications in skincare, providing protection against oxidative stress and premature aging while promoting environmental sustainability. Future studies should focus on the identification and characterization of the compounds responsible for the activities observed, as well as in the study of their efficacy in more complex models and final cosmetic formulations.

Supplementary Information

The schematic representations of the enzymatic assays, FT-IR spectra, and mass spectra of the compounds identified in the *Sargassum* extracts are provided in the Supplementary Information, Figures S1–S3, and are available for free at https://rvq.sbq.org.br/pdf/AR2026-5005-MS

Credit Authorship Contribution Statement

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