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Brazilian Beach-Cast Seaweeds: Antioxidant, Photoprotection and Cytotoxicity Properties

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Abstract

The potential of seaweed extracts in cosmetic applications have increased with novel development and market. Beneficial biological properties include the ability to struggle aging and anticancer agents. This research aimed to evaluate antioxidant, photoprotective and cytotoxic properties of extracts from beach-cast seaweed species from the Brazilian coast in order to propose an appropriate use of this sustainable resource. Analysis of antioxidant capacity, UV/VIS absorption, quantification of UV photoprotectors, total content of C, N and S, development of cosmetic creams and evaluation of the cytotoxicity activity against tumoral cells lines were performed. The highest antioxidant capacity was found in extracts of brown seaweeds, followed by red seaweeds, with the lowest activity detected in the green seaweed. Same pattern was observed for phenolic compounds. The extracts did not show cytotoxicity activity against healthy human cells. Other forms of extraction and incorporation of the extract into the base creams should be evaluated, the isolation and purification of substances could increase the efficiency of the photoprotective capacity. The species were highly promising and proved to be natural sources of antioxidants and substances with cytotoxicity activity against tumoral cells; therefore, they could be exploited as functional ingredients with specific applications for different types of industries. The application of seaweed extracts in combination with other natural ingredients can help in the design of new cosmetics against the negative effects of UV radiation, in addition to having the great advantage of not presenting toxicity to health or the environment because they are biodegradable.

Graphical Abstract



Keywords Antitumor · Functional properties · Natural products · Phenolic compounds · Seaweed · Sulfated polysaccharides

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Statement of Novelty

Beach-cast seaweeds are a renewable resource with high biotechnological potential. This study is a pioneer in the evaluation of the chemical composition, antioxidant, cytotoxic, and photoprotection properties of seaweed extracts from the Brazilian coast. Several properties were found in this study, allowing the valorization of this biomass usually considered as waste, and its potential brings countless benefits in the scientific, social, economic, ecological and public policy development, also contemplating the interests of companies and farmers. The present research represents a significant scientific advance on the field of beach-cast seaweeds because is the first report for the beach-cast species selected evaluating the cytotoxicity potential against tumoral cells and photoprotection.

Introduction

In recent years, marine macroalgae or seaweeds have attracted attention around the world in the search for bioactive substances to develop new drugs and functional bioproducts due to their low toxicity and high bioactivity [1]. As fundamental components of the marine ecosystem, seaweeds constitute one of the most diverse groups among photosynthetic organisms [2], representing a strategic natural resource for biotechnological development. In Brazil, attention has increased considerably due to its large coastline and wide biodiversity.

Seaweeds are known to produce a great diversity of natural products. A review of marine natural products for 2018 shows the discovery of 76 new isolated compounds with relevant biological activities, three from green seaweeds, 28 from brown seaweeds and 45 from red seaweeds [3], evidencing the increasing interest in the study of these organisms. The strategical investment in research, development and innovation (RD&I), both cosmeceutical, evaluating antioxidant and photoprotection [4, 5] and pharmaceutical areas, investigating cytotoxic and antitumoral activities [6] have an up-and-coming for an encouraging future for multifunctional bioactive compounds.

The development and marketing of seaweed-based products for cosmetic healthcare can be improved from natural secondary metabolites with antioxidant and photoprotection properties, as well as health-promoting food ingredients like functional supplements [7]. Studies have delineated the potential of seaweed extracts in cosmetic applications, highlighting photoprotective, moisturizing, whitening and anti-cellulite properties and activities, which have been attributed to a variety of seaweed components [8]. Besides, skincare and cosmetic company trials have been reported several seaweed compounds as containing promising properties, such as anti-inflammatory and skin hydration, as well as protection and enhancement of skin repair processes [9]. These properties have been used in a broad spectrum of market segments, such as food and feed ingredients, skincare, cosmetics, fertilizer and biofuels [10]. Several species of seaweeds have shown biological properties with positive benefits, such as the ability to reduce the photoaging

(oxidative stress) and cancer, improve the immune system, protect against UV radiation and contribute to the treatment of diseases [11].

One of the major limitations in the search for new extracts, fractions or natural products with biological action is the reduced availability of biomass for studies and bioprospection. Nowadays, there is a limitation of sustainable biomass that includes population management practices and few seaweed species are commercially cultivated [12]. Besides that, about 80% of the seaweed mass correspond to water, significantly reducing the amount of material for the studies [13]. In this context, the high availability of beach-cast seaweed biomass can be a sustainable and highly productive alternative.

The beach-cast seaweeds are those organisms detached from the natural substrate or drifted seaweeds that accumulate on the beaches, which are detached by sea turbulence caused by currents, winds and tides [14]. The literature of beach-cast seaweeds in Brazil is still scarce; most studies have evaluated only the taxonomy and abundance of this biomass on different beaches of the country [14-18]. These studies show that Brazil has a high potential for this waste and underused biomass and many of them evaluate their potential use as fertilizer [19, 20]. Recently studies showed the potential of beach-cast seaweeds as a raw material for several applications, combined with the large quantity available on the beaches of certain regions along the Brazilian coast with suitable application proposed [21-24]. From other countries, studies of beach-cast seaweeds are not numerous, as shown in the reecent review by [23]. In the last 5 years, special attention has been increased on pelagic Sargassum golden tides due to the massive coastal influx [25-27]. However, scientific subsidies are still lacked to clearly establish its potential and application in the field of photoprotection and cytotoxicity.

Thus, this study aimed to evaluate biochemical characteristics, antioxidant activity and cytotoxicity potential from extracts of beach-cast seaweeds collected on the Brazilian coast, besides evaluate properties for cosmetic use and test formulation of creams with photoprotection properties. The use of beach-cast seaweed extracts in cosmetics and cytotoxicity activity from extracts is discussed.

Materials and Methods

Biological Material and Extraction Procedure

Seven materials of beach-cast seaweeds were collected in the northeast (Paraíba State) and southeast (Espírito Santo State) regions of Brazil as described in Table 1, four brown seaweeds, two red seaweeds and one green seaweed. These species were selected as they were abundant in the respective

 Table 1
 List of selected species of beach-cast seaweeds harvested from the northeast (Paraíba, PB) and southeast (Espírito Santo, ES) Brazilian coast

Taxonomical group species	Beach (State)	Localization	N° Voucher (herbarium)	Collection date	
Ochrophyta/phaeophyceae(Brown seaweeds)					
Dictyopteris jolyana E.C. Oliveira & R.P. Furtado	Pontal (ES)	20°58'22.5"S40°48'38.6"W	SPF58249	06/09/2017	
Dictyopteris jolyana	Coqueirinho (PB)	07°17′58″S34°47′54″W	SPF58249	02/25/2012	
<i>Dictyopteris polypodioides</i> (A.P. De Candolle) J.V. Lamouroux	Ponta do Cabo Branco (PB)	07°08'43.6"S34°48'20.7"W	SPF58249	07/18/2016	
Zonaria tournefortii (J.V. Lamouroux) Montagne	Pontal (ES)	20°58'22.5"S40°48'38.6"W	SPF58252	06/09/2017	
Rhodophyta(Red seaweeds)					
Alsidium seaforthii (Turner) Kützing	Piúma (ES)	20°50'31.5"S40°43'46.0"W	SPF58253	06/11/2017	
Osmundaria obtusiloba (C. Agardh) R.E. Norris	Ponta do Cabo Branco (PB)	7°08'43.6"S4°48'20.7"W	SPF58082	07/18/2016	
Chlorophyta(Green seaweed)					
Codium isthmocladum Vickers	Itaoca (ES)	20°54′18.0″S40°46′42.3″W	SPF470207	04/30/2018	

collection areas and they were easily separable, reducing the risk of inclusion of not desired material together with algal biomass. The samples consisted of pooled complete thallus separated by species and local, therefore, they correspond to pooled sample, recently stranded by the low tide and non-decomposition evidence. The biomass was collected by systematic sampling, in which only healthy appearance individuals and non-fragmented material were sorted. The material was cleaned of macroepiphytes, washed three times in abundant tap water and then shadow air-drying, the shadedried material was packaged and transported to the laboratories of the University of São Paulo. In the laboratory, the air-dried samples were oven-dried at 40 °C with air circulation until constant mass and then pulverized by a ball mill until a fine powder was obtained. Vouchers were deposited in the SPF Herbarium of the Institute of Bioscience at the University of Sao Paulo, Brazil (Table 1).

Carbon, Nitrogen, Sulfur and Total Protein Analysis

Elemental analysis of carbon (C), nitrogen (N) and sulfur (S) was performed from 50 mg of pulverized samples (n=1) by using an elemental analyzer (LECO, CHNS 932, USA) in the Central Research Support Services (SCAI) of the University of Malaga. The complete and instantaneous oxidation of the sample was reached by combustion with pure oxygen at approximately 1000 °C. The different combustion of CO₂, N₂ and SO₂ products were selectively separated into specific columns and thermal conductivity detected. EDTA (ethyl-enediaminetetraacetic acid) and sulfamethazine were used as standard references, which has well known C, N and S contents (%). The total crude protein content was estimated using the conversion factor of 4.92 on the percentage of N

according to [28]; All measured parameters were expressed in percentage of dry matter (DM).

Extraction

Extracts from the seven materials were prepared in the proportion of 100 mg mL⁻¹ with addition of two different solvents, distilled water and hydro-ethanol (1:1 v/v; 50% EtOH). The proper mixing was achieved with utilization of an ultra-turrax homogenizer (T10, Ika Works Inc., USA) for 5 min. Then, for the aqueous extraction, an alkaline extraction was carried out at 85 ± 2 °C for 1.5 h and adding potassium carbonate (for brown seaweed) or sodium carbonate (for green and red seaweeds) in the proportion of 1% according to the percentage of carbon determined for each species multiplied by the extracted biomass. Alkaline extraction was used to weaken the cell walls of seaweed biomass for better extract the substances [29, 30]. For hydroethanolic extraction, the samples (n=3) were extracted at 45 ± 2 °C for 6 h in a thermostatic bath (Grant Instruments Ltd SS40-2, USA). After that time, all extracts were filtered through a 50 µm mesh and subsequently centrifuged at 10,000 rpm (Beckman GS-15 R, USA) for 10 min at room temperature. Each extraction was performed with three technical repetitions that were considered for statistical procedure analysis (n=3).

Supernatants from alkaline aqueous extracts were evaluated for phenolic compounds, antioxidant capacity by ABTS assay and cytotoxicity. Supernatants from hydroethanolic extracts were assessed for phenolic compounds, ABTS assay, effective solar absorption radiation (ESAR) and extract photoprotection index (EPI) and cytotoxicity activity according to [31].

After evaluating the results obtained from the previous above extraction at a proportion of 100 mg mL⁻¹, a second

extraction procedure was carried out only for *Dictyopteris jolyana* (ES), *Zonaria tournefortii* (Phaeophyceae), *Alsidium seaforthii* (Rhodophyta) and *Codium isthmocladum* (Chlorophyta) at the same conditions described previously for alkaline and hydroethanolic extracts (n = 3) but using a higher extraction proportion of 500 mg mL⁻¹, as proposed by [32]. For these extracts, UV-VIS absorbance spectra, phenolic compounds, ABTS assay, ESAR and EPI were analyzed.

Analysis of UV-Visible Absorption Spectrum

The UV-VIS absorption spectrum of algal extracts was assessed by a spectral scan from 280 to 720 nm, with an interval resolution of 1 nm, using an UVMini-1240 spectrophotometer (Shimadzu, Columbia, USA) and a quartz cuvette, optical length 10 mm. The absorbance data were standardized, and the results were expressed as relative absorbance (normalized data with the absorbance value of the longest wavelength for UV at 400 nm and visible at 750 nm). Three measurement technical replicates were performed.

Phenolic Compounds

The determination of phenolic compounds was carried out by the photo-colorimetric method of Folin-Ciocalteu [33], in which 250 µL of the extract was added to 1250 µL of distilled water and 125 µL of the Folin-Ciocalteu phenol reagent 2 N (Sigma-Aldrich, USA) and left to react for 2 h in the dark at 4 °C. Then, aliquots of 1000 µL of the reaction mixture were read at 760 nm, in a quartz cuvette and by using an UVMini-1240 spectrophotometer. The assay was performed with three technical replicates (n=3). A standard reference curve was performed with phloroglucinol at concentrations of 1–25 µg mL⁻¹ and the equation y=0.0757x – 0.021 (R² = 0.99) was obtained. Results were expressed as mg of PGE (phloroglucinol equivalent) per g of algal DM.

Antioxidant Capacity by ABTS Assay

The antioxidant capacity was determined by the ABTS radical cation assay [34] and the assay was performed with three technical replicates. Aliquot of 50 μ L of crude extract was added to 940 μ L of sodium phosphate buffer (50 mM, pH 7) and 10 μ L of ABTS solution (7 mM ABTS and 2.45 mM potassium persulfate, kept in dark at room temperature for at least 12–16 h) and the absorbance read after 7–8 min of reaction at 727 nm using an UVMini-1240 spectrophotometer.

As reference antioxidant, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used [35] at

concentrations of standard curve from $1-5 \ \mu g \ mL^{-1}$, with the equation $y = 13.593x + 0.8717 \ (R^2 = 0.99)$. Antioxidant capacity was expressed as μmol of TE (Trolox equivalent) per g of algal DM.

Preparation of Cosmetic Products

An Unguator 2100 mixing machine (GAKO International GmbH, Germany) was used for allowing a perfect homogenization of the formulation to manufacture the cosmetic cream. All components (Table 2) were mixed until homogeneity was achieved (oil phase, water and algal extract). All formulations were mixed at 750 rpm for 2 min. The ideal pH was reached with dissolved lactic acid. The liquid extract corresponds to 25% of the cream composition, which was gradually added until the weight reached the corresponding to 25%. From this cream base formulation, the following analysis were performed and ESAR was calculated.

Effective Solar Absorption Radiation (ESAR) and Extract Photoprotection Index (EPI)

Photoprotective capacity was evaluated by calculating the percentage of effective solar absorption radiation (%ESAR) and extract photoprotection index (EPI) for the extracts according to [31]. Both %ESAR and EPI are analogous to

 Table 2
 Components of the cream base formulation. The steps represent the order of addition until homogeneity was achieved

Base cream formulation (1000 g)	
Step 1	Quantity (g)
Olivem 1000	40
Protelan	40
Stearic acid	10
Cetyl alcohol	10
Cetyl Palmitate	20
Sesame oil	30
Grape oil	20
Coconut fractioned. oil	30
Mango oil	20
Isopropyl	20
Vitamin E	10
Step 2	
Distilled water	460
60% Sorbitol	30
Step 3	
Preservative	10
Step 4	
Algal liquid extract (pH 5.5)	250

the sun protection factor (SPF) allowing the utilization of other action spectra different than erythematic spectrum (which is usually applied to calculate FPS) in the calculation. %ESAR was calculated using the solar radiation retained and/or absorbed by algal compounds and other components from the cream formulation.

ESAR was calculated by applying action spectra (Act.Sp.) to four different biological responses driven by UV (Fig. 1) by the following formula:

$$\mathrm{ESAR}(\mathrm{W}.\mathrm{m}^{-2}) = \mathrm{SA}(\lambda) \times \mathrm{Act.Sp.}(\lambda),$$

where SA(λ) stands for the values of solar absorptance (SA) in each wavelength (λ) and Act.Sp.(λ) represents the values of action spectra in each wavelength.

The proportion of %ESAR to solar radiation available was calculated as:

$$%ESAR = \frac{\Sigma t(290 - 420 \text{ nm})ESAR(\lambda)}{\Sigma t(290 - 420 \text{ nm})eSS(\lambda)}$$

where $\Sigma tESAR(\lambda)$ stands for the sum of transmittance for ESAR in the respective wavelengths; $\Sigma teSS(\lambda)$ stands for the sum of transmittance for effective solar radiation in the respective wavelengths for the cream formulation.

EPI was calculated using the radiation transmitted through algal extracts [31] by applying 500 μ L of the extracts on the rough side of polymethylmethacrylate (PMMA) plates. The plates used in this study had a rough side, imitating the skin's surface, as recommended in [40] method. The extract was evenly distributed on the plates with the tips of the fingers covered by nitrile gloves, following the recommendations of the method. The plates were incubated in darkness at room temperature for 15 min. After this period,



Fig. 1 Biological response rate based on action spectra driven by UV radiation. Erythema [36], persistent pigment darkening [37], elastosis [38] and photoaging [39] absorption radiation (%ESAR) from the creams formulations and EPI from algal extracts according to their influence in different spectral regions, UVB (290–320 nm), UVA-I (320–340 nm) and UVA-II (340–400 nm)

they were positioned in the trajectory of the radiation emitted by the solar simulator (Spectra-Physics Model 66,902, USA) equipped with a mercury xenon lamp (Lamp Power 50–500 W). Below the plate, the transmittance was recorded by a spectroradiometer (Sphere Optics SMS-500, Contoocook, USA) between 200 and 800 nm (resolution of 1 nm). Each measurement was repeated four times (different points of the plate) and the average was calculated. The hydroethanolic extracts at 100 mg mL was tested with a concentration of 2 mg cm² of cream on the plate. The aqueous alkaline and hydroethanolic extracts at 500 mg mL² were tested with a concentration of 10 mg.cm² of cream on the plate.

Transmittance values were converted to absorptance values $[A(\lambda)]$ as:

$$A(\lambda) = 1 - (Tt(\lambda)/To(\lambda)),$$

where $Tt(\lambda)$ is the transmittance by each sample at wavelength λ and $To(\lambda)$ is the blank transmittance at wavelength λ . The blank was a PMMA plate containing water instead of algal extract.

Absorptance was utilized to calculate solar absorptance (SA) values for each nm from a solar spectrum (SS) as:

$$SA(\lambda) = A(\lambda) \times SS(\lambda),$$

where $A(\lambda)$ stands for the values of absorptance at each wavelength (λ) and SS (λ) stands for the solar spectrum intensities at each wavelength (λ) . The unit of this parameter was expressed as W m⁻².

With the same solar spectrum, it was also calculated the effective solar radiation (eSS) of each action spectrum analyzed (Act.Sp.) as:

 $eSS(\lambda) = SS(\lambda) \times Act.Sp.(\lambda)$

The calculation of EPI was done with transmittance $To(\lambda)$ values by using the effective solar radiation (eSS), obtained as:

$$EPI = \frac{\Sigma t(290 - 420nm)eSS(\lambda)}{\Sigma t(290 - 420nm)[eSS(\lambda)*To(\lambda)]}$$

Cell Cultures and Cytotoxicity Assay

Cytotoxicity was studied in four cell lines: human colon cancer (HCT116), human leukemia (HL60), human gingival fibroblasts (HGF1) and human keratinocyte (HaCat), all obtained from American Type Culture Collection (ATCC, USA). HTC116 is commonly used in therapeutic research and drug screening, HL60 is used for research on blood cell formation and physiology, HGF1 is a non-tumor connective tissue cells and used as negative cytotoxic cell viability, and HaCat is a non-tumor cell line widely used for its high capacity to differentiate and proliferate in vitro [41].

HCT116, HGF1 and HaCat cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) (Biowset, Spain) supplemented with 10% fetal bovine serum (Biowset, Spain), 1% penicillin-streptomycin solution 100× (Biowset, Spain) and 0.5% amphotericin B (Biowset, Spain). HL60 cells were cultured in RPMI-1640 medium (Biowset, Spain) supplemented with 10% fetal bovine serum (Biowset, Spain), 1% penicillin-streptomycin solution 100× (Biowset, Spain), 1% penicillin-streptomycin solution 100× (Biowset, Spain) and 0.5% amphotericin B (Biowset, Spain). Cells were subconfluent maintained at 37 °C in humidified air containing 5% CO₂. Cultured cells were collected by gentle scraping when confluence reached 75% for HTC-116, HGF-1 and adherent cells were obtained for HaCaT. HL60 cells were collected by centrifugation at 1500 rpm for 5 min due to the suspension trait.

The proliferation of the cell lines was estimated by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay according to [42]. A simple method widely used to determine cell viability (survival) and the biochemical reaction occurs in the mitochondria, in which MTT (yellow color) is reduced by the mitochondrial succinate dehydrogenase to its formazan insoluble form (purple color) that is quantified spectrophotometrically. The ability of cells to reduce MTT constitutes a bioindicator of mito-chondrial integrity and its functional activity is interpreted as cell viability [43].

A first previous screening of cytotoxicity was evaluated from 20 mg of DM of the seven beach-cast seaweeds extracted in 1 mL of DMEM medium and different concentrations of supernatant extract (0.019, 0.039, 0.078, 0.156, 0.312, 0.625, 1.25, 2.5, 5 and 10 mg mL⁻¹) with the cell lines, HCT116, HL60 and HaCat.

A second previous screening of cytotoxicity was performed with hydroethanolic and alkaline aqueous extracts at 100 mg mL⁻¹ from the beach-cast species *Z. tournefortii*, *A. seaforthii* and *C. isthmocladum* and tested against HCT116, HL60 and HGF1. Positive control (cell suspension without algal extract) and blank (culture medium) were performed. All tests were performed with four technical replications (n=4).

The cell suspension was prepared from 6×10^4 mL⁻¹ cells in a culture medium, checked with a Neubauer chamber by the exclusion of non-viable cells with trypan blue. The number of cells per well for the cytotoxic assay was approximately 6000–9000 cells (depend on the cell line used) in a 96-well microplate, containing 100 µL of algal extract and 50 µL of cell suspension medium. For HCT116, HGF1 and HaCat it was used DMEM as suspension medium and RPMI for HL60.

Cytotoxic responses were evaluated after the microplate incubation at 37 $^{\circ}$ C in a humid atmosphere with 5% CO₂ for

72 h. After this time, the microplate was centrifuged, and the medium was removed from the wells. Then, 100 μ L of medium (DMEM or RPMI) and 10 μ L of sterile MTT solution (5 mg mL⁻¹ in phosphate-buffered saline autoclaved) were added to the plate and incubated at 37 °C with 5% CO₂ for 4 h. After this last incubation time, the crystals formed were dissolved by adding 150 μ L of acidic isopropanol solution (0.04 N HCL–2 propanol) and the absorbance measured spectrophotometrically at 550 nm (Micro Plate Reader 2001, Whittaker Bioproducts, USA). The results were expressed as percentage (%) of living cells, according to the following formula:

Cell viability(CV%) = $(Abs^{\text{treated cell}} \times Abs^{\text{control}}) \times 100$

where: Abs^{treated cell} is the absorbance of the cells treated with algal extracts and Abs^{control} is the absorbance of the control cells. The cell viability was expressed as the mean percentage of viable cells compared with untreated cells. Additionally, the IC₅₀ values were calculated by linear regression and expressed as mg mL⁻¹.

Data Analysis

Statistical analyses were performed using "Statistica" 10 software. Data were tested for normality (Kolmogorov-Smirnov test) and homoscedasticity (Bartlett's test). Oneway or two-way analysis of variance (ANOVA) was used to compare the results (p < 0.05) (see Supplementary material, Table S1). When differences were detected, Newman-Keuls *post-hoc* multiple comparison test was applied.

Results

Elemental cell contents of carbon, nitrogen and sulfur, elemental stoichiometry and crude proteins of the beachcast species are presented in Table 3. Carbon percentage was similar for most species, ranging from 30.12 to 39.84%, except for *C. isthmocladum* with 14.77%. Nitrogen and sulfur varied from 0.82 to 3.04% and 0.65 to 3.59%, respectively. The green alga *C. isthmocladum* had the lowest contents of carbon and nitrogen but showed the highest percentage of sulfur among the species.

Among the species, the ratios of C:N ranged from 9.91 to 43.35, C:S ranged from 4.11 to 60.36 and C:N:S ranged from 5.01 to 65.68. In general, the beach-cast *D. jolyana* (PB) showed the highest ratios among the species analyzed. On the other hand, *C. isthmocladum* and *A. seaforthii* showed the lowest ratios.

Crude protein, calculated from the N percentage, ranged from 4.52 to 14.94%, with the highest protein level registered

Taxonomical group species	Carbon	Nitrogen	Sulfur	C:N	C:S	C:N:S	Crude protein	
Ochrophyta/ phaeophyceae(Brown seaweeds)								
D. jolyana (ES)	38.57	1.85	1.60	20.80	24.11	13.00	9.12	
D. jolyana (PB)	39.84	0.92	0.66	43.35	60.36	65.68	4.52	
D. polypodioides	34.22	1.68	0.91	20.36	37.60	22.37	8.27	
Z. tournefortii	34.59	1.78	0.95	19.49	36.41	20.52	8.73	
Rhodophyta(Red seaweeds)								
A. seaforthii	30.12	3.04	1.50	9.91	20.08	6.61	14.95	
O. obtusiloba (PB)	34.06	2.39	0.65	14.23	52.40	21.89	11.78	
Chlorophyta(Green seaweed)								
C. isthmocladum	14.77	0.82	3.59	17.98	4.11	5.01	4.04	



Fig. 2 Relative absorption spectra (normalized data with the absorbance value of the longest wavelength for UV at 400 nm and visible at 750 nm) for hydroethanolic and aqueous alkaline extracts from four selected beach-cast species for further concentration procedures. **a** UV relative absorption spectra and **b** visible relative absorption spectra

in A. seaforthii (14.95%), O. obtusiloba (11.78%) and D. jolyana (ES) (9.12%).

The relative UV-VIS absorption spectra did not register absorption band peaks for the hydroethanolic and aqueous alkaline extracts (extract proportion of 500 mg mL⁻¹; Fig. 2), although some patterns are relevant to highlight. The relative absorption spectra were more intense at 280 and 300 nm (UV-B spectrum region) (Fig. 2a) and 400–440 nm



Fig. 3 Level of phenolic compounds (mg PGE g⁻¹) in hydroethanolic and aqueous alkaline extracts at extraction proportion of **a** 100 mg mL⁻¹ and **b** 500 mg mL⁻¹ from beach-cast seaweed species collected in the northeast (Paraíba, PB) and southeast (Espírito Santo, ES) from Brazilian coast (Mean \pm SD; n=3). Letters indicate significant differences (p < 0.05) according to two-way ANOVA and Newman-Keuls *post-hoc* test

(short-blue visible spectrum) and small absorption bands at 640–700 nm (visible spectrum; Fig. 2b).

The hydroethanolic and aqueous extracts (at 100 mg mL⁻¹ extraction proportion) from brown species (*D. jolyana, Dictyopteris polypodioides* and *Z. tournefortii*) showed higher phenolic contents than red (*O. obtusiloba* and *A. seaforthii*) and green (*C. isthmocladum*) species (Fig. 3a). Additionally, phenolic compounds in the aqueous



Fig. 4 Antioxidant capacity (Mean ± SD; n=3) for the ABTS assay of hydroethanolic and aqueous alkaline extracts at **a** 100 mg mL⁻¹ and **b** the comparison of 100 mg mL⁻¹ and 500 mg mL⁻¹ from beach-cast seaweed species collected in the northeast (Paraíba, PB) and southeast (Espírito Santo, ES) from the Brazilian coast. Letters indicate significant differences (p < 0.05) according to two-way ANOVA and Newman-Keuls *post-hoc* test. For figure **b**, the bifactorial ANOVA was performed independently for each species

alkaline extracts were higher than hydroethanolic extracts (Fig. 3a), except for the extracts from the red seaweeds. Higher amounts of phenolic compounds were detected for both extracts at 500 mg mL⁻¹ extraction proportion from *Z. tournefortii*, *D. jolyana* (ES), *A. seaforthii* and *C. isthmocladum* (Fig. 3b) when compared to the 100 mg mL⁻¹ extraction proportion.

Antioxidant capacity obtained by the ABTS method was higher in both extracts from brown and red beachcast seaweeds at an extraction proportion of 100 mg mL⁻¹ compared to green alga extracts (Fig. 4a). Comparing the antioxidant capacity between the extracts at 100 mg mL⁻¹ versus 500 mg mL⁻¹ extraction proportion similar levels were recorded (Fig. 4b), except for the extracts from *C*. *isthmocladum* and *Z. tournefortii*, in which 500 mg mL⁻¹ showed lower antioxidant power.

The percentage of ESAR (effective solar absorption radiation) for hydroethanolic extracts at 100 mg mL⁻¹ extraction proportion (Fig. 5a) and for both extracts at 500 mg mL⁻¹ (Fig. 5b) were analyzed. From the extracts at 100 mg mL⁻¹, the ESAR percentages were lower than 25% (Fig. 5a). The extracts at 500 mg mL⁻¹ enhanced ESAR values were registered (Fig. 5b), with hydroethanolic extracts from *Z. tournefortii* and *A. seaforthii* and both extracts from *D. jolyana* exhibiting the highest values for all radiationinduced responses, greater than 50%. In general, %ESAR results showed a higher tendency response for the erythema's spectrum than persistent pigment darkening, elastosis and photoaging.

The EPI (extract photoprotection index; Fig. 6) was higher at 10 mg.cm⁻² at extract proportion of 500 mg mL⁻¹ of hydroethanolic extracts from *D. jolyana* and *A. seaforthii*, reaching values of 47 and 17, respectively.

Figure 7 evidenced a pattern of relationship between EPI versus %ESAR responses with the erythema action spectra. For hydroethanolic extracts at 2 mg.cm⁻² at extract proportion of 100 mg mL⁻¹ a linear correlation among the species was observed, with the tendency of low ESAR and EPI and high ESAR and EPI results (Fig. 7a). Extract proportion of 500 mg mL⁻¹ and 2 mg.cm⁻² (Fig. 8B) the highest %ESAR and EPI was the hydroethanolic from *D. jolyana* (ES). Other species showed the tendency of high ESAR and medium EPI (*A. seaforthii* and *Z. tournefortii*) and *C. isthmocladum* showed low values for both parameters (Fig. 7b). The aqueous alkaline extracts with a concentration of 500 mg mL⁻¹ showed species with low and medium values of ESAR and EPI and *D. jolyana* showed the best response values for both analyses (Fig. 7c).

Cytotoxicity activity was evaluated for two tumoral cell lines, leukemia (HL60) and colon (HTC116), and nontumoral cell lines, keratinocytes (HaCat), with crude extracts in DMEM (20 mg mL⁻¹) for all the seven species of beachcast seaweeds (see Supplementary material, Fig. S1). It is important to point that a lower IC₅₀ represents higher activity. The extracts from *C. isthmocladum*, *Z. tournefortii* and *D. jolyana* (ES) showed the best results against HL60 with IC₅₀ values lower than 1 mg mL⁻¹ (Fig. 8a). Regarding the cytotoxicity activity against HTC116, all extracts showed IC₅₀ values lower than 1 mg mL⁻¹, except for *D. polypodioides* that had 4.15 mg mL⁻¹ (Fig. 8b). All the crude extracts from the beach-cast species (at 0.019 to 10 mg mL⁻¹) did not show cytotoxicity activity with the non-tumoral cell lines HGF1 (see Supplementary material, Fig. S2).

After the evaluation of the above results, only one species was chosen from each seaweed group: *Z. tournefortti* (brown), *A. seaforthii* (red) and *C. isthmocladum* (green) and the cytotoxicity activity was evaluated for hydroethanolic and aqueous alkaline extracts at extract proportion of 100 mg mL⁻¹ (Fig. 9). Both extracts showed low IC₅₀ values against leukemia cell lines (HL60) ranging from 0.06 mg mL⁻¹ to 5.58 mg mL⁻¹ (Fig. 9a). For the HL60 strain, *C. isthmocladum* showed better cytotoxicity activity for DMEM and aqueous alkaline extracts, while for the brown beachcast *Z. tournefortii* the hydroethanolic extract showed the lowest IC₅₀ (0.06 mgmL⁻¹). The extracts from the red seaweed *A. seaforthii* showed the less satisfactory result among the three species, but for this species the best result was

Fig. 5 Percentage of effective solar absorption radiation (ESAR) (Mean \pm SD; n=3) evaluated for erythema, persistent pigment darkening, elastosis and photoaging action spectra of **a** hydroethanolic extracts at 100 mg mL⁻¹ extraction proportion and b hydroethanolic and aqueous alkaline extracts at 500 mg mL⁻¹ extraction proportion from beach-cast seaweed species collected in the northeast (Paraíba, PB) and southeast (Espírito Santo, ES) from the Brazilian coast. Letters indicate significant differences (p < 0.05) according to one-way ANOVA performed independently for each species and Newman-Keuls post-hoc test





Fig.6 Extract photoprotection index (EPI) of hydroethanolic and aqueous alkaline extracts from four beach-cast seaweed species collected on the Brazilian coast (Espírito Santo, ES). Letters indicate significant differences (p < 0.05) according to two-way ANOVA and Newman-Keuls *post-hoc* test

achieved with the crude extract in DMEM (1.76 mg mL⁻¹) (Fig. 9a).

For colon cell lines (HTC116), all extracts showed low IC_{50} ranging from 0.01 mg mL⁻¹ to 1.41 mg mL⁻¹ (Fig. 9b), the crude extract in DMEM of *C. isthmocladum* showed the

highest cytotoxicity activity (0.01 mg mL⁻¹). Regarding Z. *tournefortii*, the aqueous alkaline extract was more efficient (0.05 mg mL⁻¹) and for A. *seaforthii* the crude extract in DMEM showed the best response (0.06 mg mL⁻¹) (Fig. 9b). In general, all the extracts of beach-cast seaweeds had a stronger cytotoxicity activity against HTC116 when compared to HL60 cell lines inhibition.

Discussion

Seaweeds can be a sustainable source of natural metabolites due to their huge biodiversity and nutritional and chemical composition, these organisms are sessile and inhabit areas under constant stressing environmental factors; consequently, many species of seaweeds have developed the biosynthesis of compounds as such proteins, carbohydrates, fatty acids and vitamins and secondary metabolites such as phenolic compounds, terpenoids and pigments, which them represent great candidates for industrial applications [44, 45].

There is a growing interest in the search for new ingredients for cosmetic industry with biological actions and



Fig. 7 Extract photoprotection index (EPI) *versus* effective solar absorption radiation (%ESAR) for **a** hydroethanolic extracts at 2 mg cm⁻² from extract proportion of 100 mg mL⁻¹, **b** hydroethanolic extracts at 10 mg cm⁻² from extract proportion of 500 mg mL⁻¹ and **c** aqueous alkaline extract at 10 mg cm⁻² from extract proportion of 500 mg mL⁻¹ of beach-cast seaweed species collected in the northeast (Paraíba, PB) and southeast (Espírito Santo, ES) from the Brazilian coast. The action spectra of biological responses applied was erythema. Data were plotted considering three technical replicates per each extract concentration of 2 or 10 mg.cm⁻²

multifunctional properties from seaweeds, where one of the limitations is the availability of biomass [46]. Currently, most of the biomass is destined for food and phycocolloid markets. In Brazil, one of the main challenges in the exploitation of seaweeds as a resource is the absence of cultivation on a commercial scale to supply the market. In this way, the Brazilian coast with an extension of more than 7000 Km, offers great potential for the use of different beach-cast seaweeds species with tones of biomass available all over the year [23].

Chemical Composition

Most of the species evaluated in the present study showed a high content of total carbon (> 30%), similar to results for C content describe in literature for non-beach-cast seaweeds [47]. The N content obtained in this study varied



Fig.8 Cytotoxicity capacity expressed as IC_{50} (mg mL⁻¹) of the crude extract in DMEM for the tumoral lines **a** leukemia HL60 and **b** colon HTC116 from beach-cast seaweed species collected in the northeast (Paraíba, PB) and southeast (Espírito Santo, ES) from the Brazilian coast

between 0.82 and 3.04% in the beach-cast seaweeds, lower than results obtained by other authors for non-beach-cast seaweeds, which ranged from 2.50 to 4.50% [47, 48]. This study found that brown beach-cast seaweeds had high C:N ratios values, and optimal C:N values are considered close to 10 [49]. Most of the species studied here showed a C:N ratio higher than 10, indicating species high-producing secondary metabolites, especially carbohydrates. [50] proposed that the high C:N ratio is a good indicator of increased production of secondary metabolites. The red beach-cast A. seaforthii showed low C:N content, suggesting the elevate presence of nitrogen compounds (e.g. proteins and amino acids). Metabolites such as carbohydrates and proteins impact C:N ratio, where a higher accumulation of carbohydrates is commonly correlated with high C:N ratios and low C:N ratios is correlated with high amino acids and proteins in seaweeds [51–53]. Total protein content based in the internal nitrogen pool was lower than that in fresh algae [54] but protein level remained higher in red seaweeds (11-14%) compared to brown (4.5-9.12%) and green (4.04%) beach-cast seaweeds. In this study we have used the N-protein conversion

Fig. 9 Cytotoxicity capacity expressed as IC_{50} (mg mL⁻¹) of crude extracts in DMEM and hydroethanolic and aqueous alkaline extracts at 100 mg mL^{-1} for the tumoral cell lines a leukemia HL60 and b colon HTC116 from beach-cast seaweed species collected in the southeast (Espírito Santo, ES) from the Brazilian coast. Letters indicate significant differences (p < 0.05) according to one-way ANOVA performed independently for each species and Newman-Keuls post-hoc test



factor of 4.92 as average of seaweeds according to [28]. Almost 52% of studies on seaweeds determined protein by applying an indirect nitrogen-to-protein conversion factor of 6.25 according to [55] method based in food proteins [56]. [57] according to the review study reported a median nitrogen-to-protein conversion factor of 4.97 and an overall mean nitrogen-to-protein conversion factor of 4.76. [57] suggested the use a value of 5 be as the most accurate universal seaweed nitrogen-to-protein (SNP) conversion factor, a value very close to that used in this study.

Secondary metabolites are bioactive compounds that could be exploited as functional ingredients for cosmetic applications. In the field of skin moisturizing, seaweeds rich in amino acids are of particular interest for these purpose [58]. A patent filed in 2009 by founder of the Bioderma-Esthederm group, proposes the use of an aqueous extract of the green seaweed *Blidingia minima* rich in polysaccharides with moisturizing properties in order to improve the state of the skin [59]. Sulfated polysaccharide composition in cosmetic and skincare products has often been associated to antioxidant, tonifying, cleaning, hydrating and revitalizing bioactivities [60]. An aqueous extract of the brown seaweed *Macrocystis pyrifera* is available on the market and seems to stimulate the synthesis of hyaluronic acid [59], which is the principal component of the extracellular matrix of the skin [61]. In addition, seaweeds have been marketed as functional foods and nutraceuticals, which include foods that contain bioactive compounds or phytochemicals that could benefit human health [62]. In this context, our studied extracts of beach-cast seaweeds could be a valuable source of active ingredients for moisturizing and antiaging products, functional foods, nutraceuticals and improving nutritional supplementation.

Antioxidant Capacity and Phenolic Compounds

The antioxidant capacity is one of the most studied biofunctional properties, driven by the search for natural antioxidants that have minimal side effects, in order to replacement the synthetic antioxidants that can be harmful to health [63]. Natural matrices with high antioxidant properties are of great interest because many diseases, infections, agricultural pests and food damage can occur due to oxidative stress conditions [64]. One of the first steps to evaluate bioactive properties is the screening of phenolic compounds and antioxidant activity, due to its practical, fast and low-cost methods. This can lead to effective investment in species or extracts with cosmeceutical and antitumoral properties.

In the last decades, phenolic compounds present in different marine seaweed species have been used in cosmetic and nutraceutical products (oral or topically). Brown seaweeds are the main producers of these compounds, which phlorotannins are the most relevant substances in these seaweeds; however, green and red seaweeds can also produce phenolic compounds in lower concentrations, and bromophenols are the main molecules for the last group [47, 65]. Purified phlorotannins are included in cosmetic formulations that can prevent and decrease the skin aging process, mainly associated with free radical damage and reduction of hyaluronic acid concentration [66]. The levels of phenolic compounds registered for the extracts of the red beach-cast seaweeds could be attributed to the presence of bromophenols, commonly found in Rhodophyta species. [67], studying the red seaweed Pyropia columbina (formerly Porphyra columbina), pointed out phenolic compounds as the main responsible for the antioxidant capacity in Rhodophyta species.

A large number of seaweed extracts have been screened in an effort to find new ingredients or extracts suitable for use as skin lighteners and proven to be good candidates, this effect was linked to its antioxidant capacity [59]. Nonetheless, there are few studies with prospection and antioxidant screening for beach-cast seaweeds [21]. In this study, beachcast seaweeds with high amounts of phenolic compounds also showed higher antioxidant activity by the ABTS assay. Similarly, a positive correlation between ABTS assay and phenolic compounds was described for aqueous extracts from non-beach-cast seaweeds [47].

Our findings are very promising because indicate the potential of beach-cast seaweed extracts in formulations in the field of antiaging thanks to its remarkable antioxidant properties. Moreover, the extracts analyzed did not show cytotoxicity activity against human fibroblast cells (HGF1), therefore could be used in cosmetic formulations for skin care, sun protection, hair care, emollient, refreshing or regenerate care products and antiaging creams with guarantee safe.

Photoprotection

Seaweeds have developed throughout evolution natural chemical protection mechanisms, as UV screen substances with antioxidant capacity, anti-inflammatory properties among others i.e., as mycosporine-like amino acids (MAAs) and polyphenols [45, 68, 69]. Some commercial applications for MAAs have become interested in research because they are used as a standard by the industry in the production of pure compounds or as ingredients in sunscreens and cosmetics [70–72]. On the other hand, polyphenols are high diversity molecules presented mainly in brown seaweeds but also present normally a lower amount in both green and red seaweeds [47, 73]. Antidiabetic, neuroprotective, anti-obesity, antitumoral, neuroprotective and antimicrobial activities have been reported [11, 67]. In a previous report, [21] showed antioxidant activity and chemical composition of methanolic and aqueous extracts from fifteen beach-cast seaweeds from the Brazilian coast. In general, the highest antioxidant activities were found in extracts from brown seaweeds followed by the extracts of red seaweeds, and the lowest activities were detected in the green beach-cast seaweed. The concentrations of phenolic compounds exhibited a positive correlation with the antioxidant activities of the tested extracts [21].

Our results of UV absorption spectra, no prominent peaks of MAAs were observed, even for the red beachcast seaweed species in which some species show elevated concentrations. These findings are in accordance with [73] which evaluated the content of MAAs in red seaweed species in the coastal regions of Brazil and showed low MAAs contents for *O. obstusiloba* and *A. seaforthii.*

EPI and ESAR followed a direct relationship with increasing values for both parameters. The responses of extracts did not reach a saturation pattern; except for *D. jolyana* hydroethanolic extracts conferring the highest values of %ESAR and EPI, indicating that the other species could still be more concentrated to confer a higher response of %ESAR and EPI. [31] observed a clear pattern of hyperbolic responses between %ESAR and EPI with two species from seaweeds, suggesting that other species could still be studied, and higher concentrations can be tested to reach the same pattern of hyperbolic saturation responses.

The search for photoprotection properties is not limited to applications in formulations of sunscreens or creams for cosmeceutical applications. Biomaterials with photoprotection properties can be used in civil construction, in automobiles and eyeglasses, as they represent the interface between the skin and sun radiation [74]. However, most research with seaweeds and photoprotection has been focused on cosmeceutical applications. Our study evaluated the properties of the extracts for different action spectra, whereas ESAR is indicative of effective solar absorption radiation and EPI is indicative of extract photoprotection index, both useful parameters for cosmeceutical photoprotective properties with skin biological responses.

Cytotoxicity Activity

Other compounds with high antioxidant capacity, cytotoxicity activity and photoprotection are the sulfated polysaccharides, specific metabolites from seaweeds. These metabolites are not present in land plants and are commonly found in the three groups of seaweeds, brown (Ochrophyta, Phaeophyceae), red (Rhodophyta) and green seaweeds (Chlorophyta) [75]. Sulfate is a typical substitution of seaweed's polysaccharides [76]. This anion is linked to the polysaccharide through an ester bond (O-SO3⁻) and plays a very important role from the point of view of the biological properties of seaweeds. Several studies showed a positive correlation between sulfate content and biological properties such as antioxidants, anticoagulants, anticancer, antiviral, antiallergic and antiinflammatory [54, 77, 78]. The findings reported by [79] also supports this idea, the study found extracts rich in carbohydrates, with low sulfation and low antioxidant activity, indicating that bioactivity is related to a high content of sulfated carbohydrates and not only to carbohydrates.

The extracts of *C. isthmocladum* showed low antioxidant capacity and phenolic compounds; however, they showed the highest cytotoxicity activity against the cell lines of leukemia (HL60) and colon (HTC116), possibly associated to the higher percentage of sulfate groups. Other studies with *Codium* species also observed low content of phenolic compounds, low antioxidant potential and high bioactivity [80]. The bioactivity of aqueous extracts from non-beach-cast *C. ishtmocladum* was also observed by [79], with high antiviral and cytotoxicity potential, which was related to the sulfated polysaccharides according to the author. These sulfated polysaccharides have been considered safe additives for many commercial products [1].

Recently, [81] showed antitumor activities of a sulfated polysaccharide from *C. isthmocladum* in the murine melanoma cell line. The polysaccharide did not induce cytotoxicity; however, it was able to reduce solid tumor growth and metastasis, while not inducing side effects in mice. Therefore, the sulfated polysaccharides have been showed promising antitumor activities without the commonly collateral effects of the disease. Despite the unclear action mechanism of these metabolites from seaweeds, it has been reported tumor cells inhibition by suppressing their expression and consequently showing anticancer property [82].

The extracts of the brown and red beach-cast seaweeds analyzed exhibited also antitumoral activity against leukemia and colon cancer. [83] showed that bromophenols from the red alga *Vidalia colensoi* (formerly *Osmundaria colensoi*) exhibited moderate cytotoxic activity against leukemia cells. Similar to our results, eight of the most active samples from 15 materials of beach-cast seaweeds exhibit antitumoral activity against lung cancer and breast cancer with low IC_{50} values [84]. These findings reinforce the importance of this underused waste biomass.

Extracts with promising biological activities should have an IC₅₀ value below to 0.1 mg mL⁻¹ [85]. In this context, the most promising species with cytotoxic activity for HL60 was the hydroethanolic extract of *Z. tournefortii* (IC₅₀=0.06 mg mL⁻¹). Regarding HTC116, the extracts with promising activity were crude extracts in DMEM and hydroethanolic extracts from *C. isthmoclaum* (IC₅₀=0.01 mg mL⁻¹ and 0.07 mg mL⁻¹, respectively) and *A. seaforthii* (IC₅₀=0.06 mg mL⁻¹ and 0.09 mg mL⁻¹, respectively). The aqueous alkaline extract from *Z. tournefortii* also showed promising activity with IC₅₀ of 0.05 mg mL⁻¹.

The evaluation of cytotoxicity activity against healthy cells, such as fibroblast and keratinocytes cells, is an important parameter in cosmeceutical evaluation, as it indicates non-toxicity and safety use. In the present study, the extracts of *Z. tournefortii*, *A. seaforthii* and *C. isthmocladum* did not show any cytotoxicity activity against human fibroblast cells (HGF1), therefore the use in relation to health and safety can be supposed.

The isolation and purification of the substances responsible for the antitumor and antioxidant activities present in the aqueous and hydroethanolic extracts of the beach-cast seaweeds open perspectives for industrial purposes, given its capacity to increase the shelf life of cosmetics, besides the use as a nutraceutical in the prevention of diseases, such as cancer.

The set of chemical parameters, photoprotection and cytotoxicity potential exposed here provide important subsidies for the increased knowledge of Brazilian beach-cast seaweeds. Thus, this underused waste biomass becomes an interesting matrix to supply the market demand for these purposes.

The screening for bioactive properties and the potential use of beach-cast seaweeds are strategic tools and practices, which could be implemented to reduce environmental pollution at the beaches, public health risks and promoting also an economic activity for local communities. The practice combines the market and society's demands for natural products and cosmetic products with photoprotection, antioxidant and cytotoxicity properties. The use of beach-cast seaweeds allows the development of new functional products, fortifying their nutritional composition, quality and health beneficial properties.

Conclusion

Extracts from brown and red beach-cast seaweeds were highly promising as cosmeceutical formulation, which evidenced high antioxidant properties and effective solar absorption radiation. As the extracts were obtained as crude extracts, improved application could be explored by using biorefinery concept and semi-purification of the extract in order to concentrate the bioactive compounds. The extracts of beach-cast seaweeds had antitumoral potential and nontoxicity, therefore extracts of beach-cast seaweeds may become valuable for the development of cosmetic products.

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Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code Availability Not applicable.

Declarations

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

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent for publication The authors hereby confirm that all authors mutually agree for submitting their manuscript and that the manuscript is original work of the authors.

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