Talissa Barroco Harb

Chemical characterization, antioxidant potential and biological activity of beach-cast macroalgae from the Brazilian coast

Caracterização química, potencial antioxidante e atividade biológica de macroalgas arribadas do litoral brasileiro

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Versão Revisada

Thesis presented to the Institute of Bioscience of the University of São Paulo, to obtain the PhD Degree in Biological Sciences, in the area of Botany.

Advisor: Prof[a]. Dr[a]. Fungyi Chow

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For the loves of my life

Sandra and Geraldo

Diego (*in memoriam*), Marcelo and Victor

“It's a new dawn
It's a new day
It's a new life
For me
And I'm feeling good”

Nina Simone - Feeling Good

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GENERAL ABSTRACT

A wide variety of metabolites extracted from macroalgae is currently used as functional food ingredients, natural nutraceuticals, biomedical materials, cosmetic formulations, as well as in other products that promote human health. One of the major limitations in the search for new extracts or natural products with biological action is the lack of available biomass for studies and even for their bioprospection. In this context, the availability of beach-cast marine algae is a sustainable and highly productive alternative. The main objective of this study was to evaluate the chemical and bioactive potential of extracts from 15 materials of beach-cast marine algae collected from the Brazilian coast to provide subsidies that allow identifying the potentialities and its uses as functional product. The findings showed that the highest antioxidant activities were found in extracts from brown beach-cast algae followed by the extracts of red beach-cast algae, and the lowest activities were detected in the green beach-cast species. The most efficient extracts of beach-cast species with antioxidant properties were from *Dictyopteris jolyana, Zonaria tournefortii,* and *Osmundaria obtusiloba.* Regarding antiviral activity, the aqueous extract of *Codium isthmocladum* and methanolic extracts from *D. jolyana*, *Z. tournefortii Alsidium seaforthii*, *Spiridia clavata* and *O. obtusiloba* were highly promising, reaching inhibition percentage above 90%. The extracts of *Z. tournefortii*, *A*, *seaforthii* and *C. isthmocladum* showed high cytotoxicity activity against the tumoral cells strains HL60 (leukemia) and HTC116 (colon) and did not show any cytotoxicity activity against non-tumoral human cells (HGF1). Currently, the large amount of beach-cast seaweeds deposited on the beaches of Brazil is collected and treated as trash, being incinerated or disposed in landfills by the government. Instead of using public resources and making efforts for the removal of this biomass, it is possible to propose different uses for the Brazilian beach-cast seaweeds and transform it into applications for several sectors. Due to the antioxidant, antiviral, nutritional, photoprotective and cytotoxic properties reported for macroalgae, such applied features bring environmental benefits by mitigating coastal pollution generating a usable resource, in addition to ensuring sustainable social and economic development. The review data shown in the literature of Brazilian beach-cast algae shows the relevance and innovation of this study. This is the first report evaluating chemical composition, antioxidant potential, antiviral activity, cytotoxicity potential and photoprotection of extracts from beach-cast algae from the Brazilian coast. The potential of beach-cast seaweeds provides raw-material for several applications, combined with the large amount available on the beaches of certain regions at the Brazilian coast can be better exploited. The present study significantly contributes to increasing knowledge of Brazilian beach-cast algae and their potential uses as well as the worldwide scientific and applied knowledge.

Keywords: antitumoral; antiviral; bioprospection; functional properties; seaweeds.

RESUMO GERAL

Uma grande variedade de metabólitos extraídos de macroalgas é atualmente utilizado como ingredientes alimentares funcionais, nutracêuticos naturais, materiais biomédicos, formulações cosméticas, bem como em outros produtos que promovem a saúde humana. Uma das principais limitações na busca por novos extratos ou produtos naturais com ação biológica é a falta de biomassa disponível para estudos e até mesmo para sua bioprospecção. Nesse contexto, a disponibilidade de algas arribadas na praia é uma alternativa sustentável e altamente produtiva. O principal objetivo deste estudo foi avaliar o potencial químico e bioativo de extratos de 15 materiais de algas arribadas coletados na costa brasileira, a fim de fornecer subsídios que permitam identificar as potencialidades e seus usos como produto funcional. Os resultados mostraram que as maiores atividades antioxidantes foram encontradas em extratos de algas arribadas pardas, seguidos pelos extratos de algas arribadas vermelhas, e as atividades mais baixas foram detectadas na espécie de alga arribada verde. Os extratos mais eficientes com propriedades antioxidantes foram das arribadas *Dictyopteris jolyana*, *Zonaria tournefortii* e *Osmundaria obtusiloba*. Em relação à atividade antiviral, o extrato aquoso de *Codium isthmocladum* e os extratos metanólicos de *D. jolyana*, *Z. tournefortii Alsidium seaforthii*, *Spiridia clavata* e *O. obtusiloba* foram altamente promissores, atingindo percentuais de inibição acima de 90%. Os extratos de *Z. tournefortii*, *A, seaforthii* e *C. isthmocladum* apresentaram alta atividade citotóxica contra as cepas de células tumorais HL60 (leucemia) e HTC116 (cólon), e não apresentaram atividade citotóxica contra células humanas (HGF1) não tumorais. Atualmente, a grande quantidade de algas marinhas depositadas nas praias do Brasil é coletada e tratada como lixo, sendo incinerada ou depositada em aterros sanitários pelo governo. Ao invés de utilizar recursos públicos e empenhar-se na remoção dessa biomassa, seria possível propor diferentes usos para esse material e convertê-lo em aplicações para diversos setores. Devido às propriedades antioxidantes, antivirais, nutricionais, fotoprotetoras e citotóxicas registradas para macroalgas, tal característica aplicada traz benefícios ambientais ao mitigar a poluição costeira, gerando recursos utilizáveis, além de garantir o desenvolvimento socioeconômico sustentável. A revisão de dados da literatura sobre algas arribadas no Brasil mostra a relevância e a inovação deste estudo. Este é o primeiro relato que avalia a composição química, potencial antioxidante, atividade antiviral, potencial de citotoxicidade e fotoproteção de extratos de algas arribadas da costa brasileira. O potencial das macroalgas arribadas fornece matéria-prima para diversas aplicações, aliado à grande quantidade disponível nas praias de certas regiões do litoral brasileiro, pode ser melhor explorado. O presente estudo contribui significativamente com o aumento do conhecimento das algas arribadas nas praias brasileiras e seus potenciais usos, assim como com o conhecimento mundial científico e aplicado.

Palavras-chave: antitumoral; antiviral; bioprospecção; macroalgas arribadas; propriedades funcionais.

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LIST OF ABBREVIATIONS

ABTS = 2,2-azinobis (3-ethylbenzthiazoline-6-sulphonic acid)

AIDS = acquired immunodeficiency syndrome

BHA = butylated hydroxyanisole

BHT = butylated hydroxytoluene

C = carbon

DCM = dichloromethane

DEPC = diethyl pyrocarbonate

DM = dry material

DPPH = 2,2-diphenyl-1-picrylhydrazyl

EC_{50} = effective concentration to reduce maximum activity by 50%

EPI = extract photoprotection index

ESAR = effective solar absorption radiation

ETAC = ethyl acetate

FRAP = ferric reducing antioxidant power

GAE = gallic acid equivalent

GAL = galactose

HC = hydrochloric acid

Hex = hexane

HIV = human immunodeficiency virus

IC_{50} = half maximal inhibitory concentration

MAAs = mycosporine-like amino acids

MeOH = methanol

MTT = tetrazolium 3-(4,5-dimethyl-2-thiazole)-2,5-diphenyl-2-H-bromide

N = nitrogen

PGE = phloroglucinol equivalent

RNS = reactive nitrogen species

ROS = reactive oxygen species

HIV-RT = HIV-reverse transcriptase inhibition assay

S = sulfate

SPF = solar protection factor

TAC = total antioxidant capacity

TE = trolox equivalent

1. General introduction

In recent years, biological marine resources have been worldwide-attracted attention in the searching of natural bioactive products to develop new drugs and functional ingredients due to the demonstrated low toxicity and high bioactivity (Tanna and Mishra, 2019). As fundamental components of the aquatic ecosystem, marine macroalgae or seaweeds present unique and diverse chemical classes, potential to explore functional and biological properties as promising ingredients (*e.g.* excipients, additives, improving agents) and applications like food and feed (*e.g.* nutraceuticals), pharmaceuticals and treatments of diseases (*e.g.* drugs), cosmeceutical (*e.g.* antioxidants, antiaging, photoprotection), plant biofertilizers, biostimulators (*e.g.* immunostimulator) and biomaterials (*e.g.* biopolymer, composites, bioplastics) (Fig. 1) (Leandro et al., 2020; Lourenço-Lopes et al., 2020). Therefore, these organisms become a strategic natural resource to develop innovation of different products with biotechnological applications and can be used in a variety of ways in food and feed, medicines, agricultural, paper manufacturing and biogas industries, as well as bioremediation and integrated multitrophic systems and phycocolloid extraction.

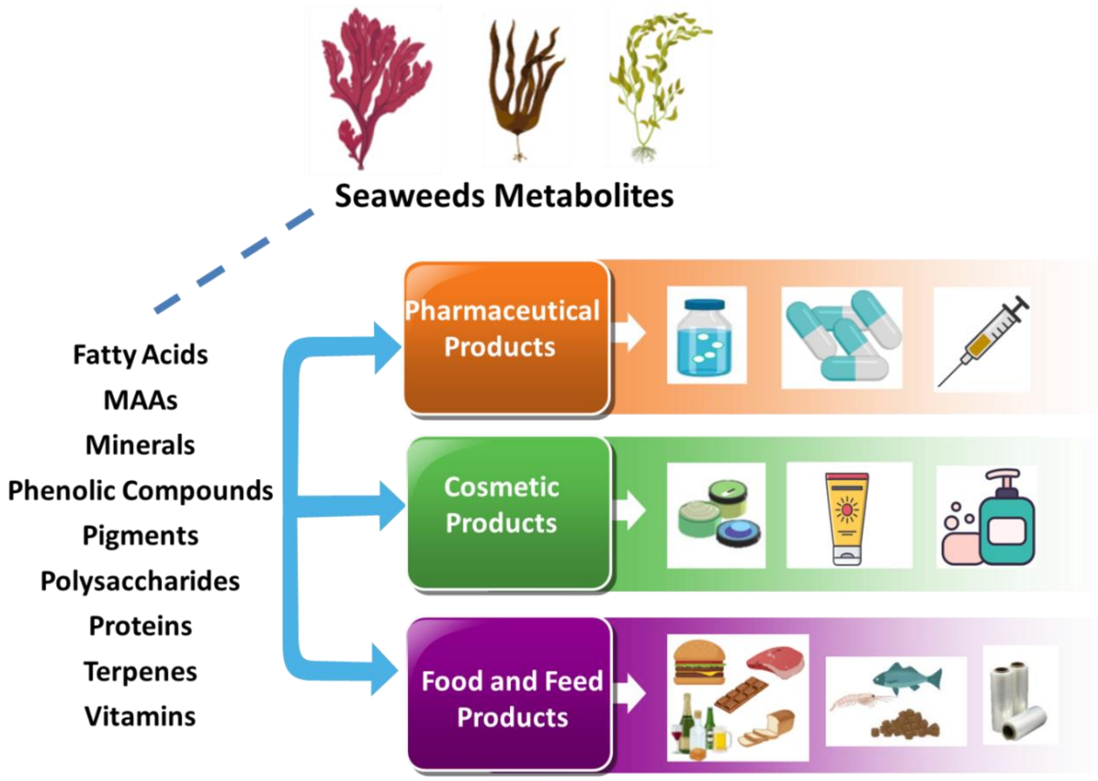


Figure 1. Potential use of macroalgae as a suitable source for obtaining primary and secondary metabolites promoting a variety of applications.

The uses of macroalgae in human food are based on its high nutritional value in minerals, vitamins, carbohydrates, proteins and fibers (Salehi et al., 2019; Ferrara, 2020; Peñalver et al., 2020). It has been widely recognized from ancient times of folk cultures, especially from Asian countries (*e.g.* Japan, Korea, Thailand, Malaysia, China) (Pérez-Lloréns et al., 2020) and Pre-Columbian civilizations and other ancient cultures (Dillehay et al., 2008; Pérez-Lloréns et al., 2020) in cooking and medicine preparations. Macroalgae can produce tonnes of proteins and are able to efficiently remove nitrogen from seawater, reducing the eutrophication effects and improving water quality (El Gammal, 2020), however, polysaccharides are one of the major abundant metabolites in seaweeds that contribute to the nutraceutical value (Fig. 2).

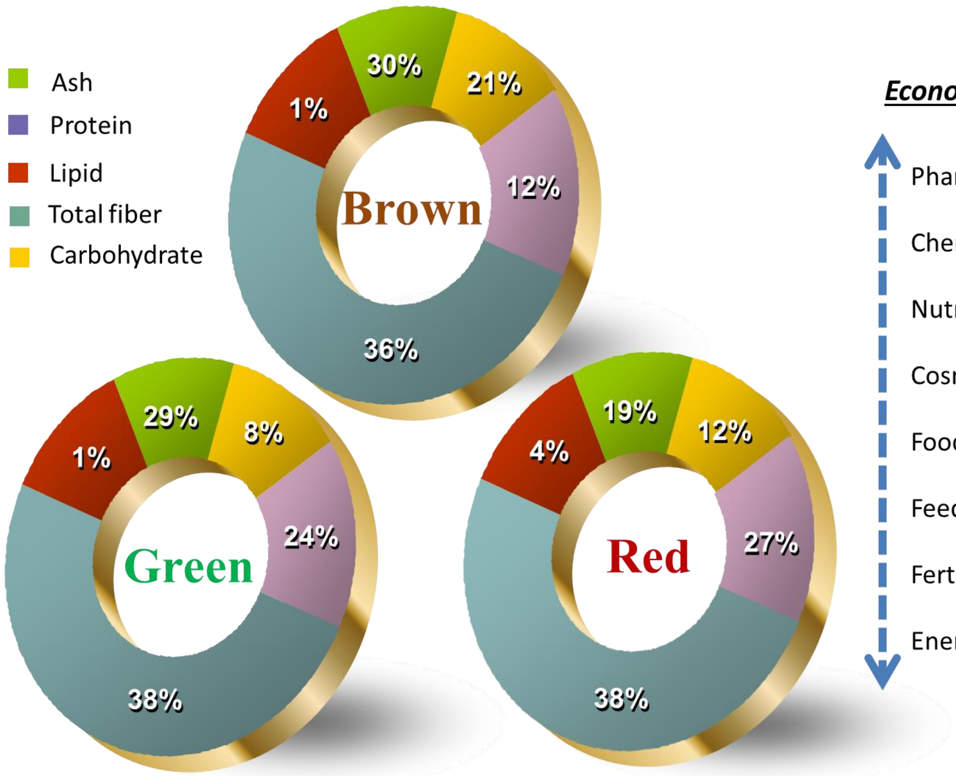


Figure 2. Major metabolites from brown, green and red marine macroalgae with main composition of total fiber, proteins and carbohydrates (based on Tenorio et al. (2018) and Torres et al. (2019a)).

In addition to the nutritional property, which can serve as functional ingredient for human healthcare, macroalgae contain several compounds with high biological activity (primary and secondary metabolites) capable of improving the immune system, protecting against radiation and contributing to the treatment of chronic diseases, such as cancer, cardiovascular, obesity and diabetes (Padam and Chye, 2020). Furthermore, several studies have provided insight into neuroprotective properties of seaweeds, which can be considered as candidates to prevent neurodegenerative diseases, such as autism, epilepsy, Alzheimer's and Parkinson's (Déléris et al., 2016). Beneficial effects have been also demonstrated including anti-inflammatory, anticoagulant, antiangiogenic and antitumor properties and can be used as pharmaceuticals, cosmeceuticals, nutraceuticals, or even in agriculture or feeding exploitation (Leandro et al., 2020).

The incorporation of seaweed as part of the normal daily diet has been correlated with a lower incidence and decreased risk of mortality for diseases such as hyperlipidemia, coronary heart disease and other cardiovascular diseases based on epidemiological studies of the Japanese diet (Nanri et al., 2017; Yokoyama et al., 2019).

Natural antioxidants from macroalgae, such as carotenoids and complex polyphenols, can reduce oxidative stress caused by toxic radicals and reactive species as reactive oxygen species (ROS) and reactive nitrogen species (RNS). These antioxidants can protect organisms against oxidative stress involved in diseases as cancer, UV radiation damage (*e.g.* aging, dermatitis and skin cancer), arteriosclerosis, neurodegeneration, diabetes, chronic inflammation and asthma (Souza et al., 2011). Natural antioxidants can also reduce the lipid peroxidation of food and protect other products sensitive to oxidation (Corsetto et al., 2020). Several macroalgae's antioxidant display a multiphase bioactive functionality with a confirmed potential of cytotoxic activities against cancer cells, besides antiviral, anti-fungal, antimicrobial, anti-tubercular, anti-bacterial and anti-inflammatory properties (Corsetto et al., 2020; Cotas et al., 2020).

As recently reviewed by Carrol et al. (2020), macroalgae are valuable resources of biomass and natural components known to produce more than 3000 natural products. Between 2014 and 2016, 203 new natural products were discovered from these organisms. Thus, the interest of the scientific community for study these organisms has been increased in recent years.

In 2018, world aquaculture production of macroalgae accounted for 32 million tonnes annual revenue reached USD 13.3 billion (FAO, 2020; Poblete-Castro et al., 2020) with few mariculture species and 1.1 million tonnes/year from wild harvest (Poblete-Castro et al., 2020) (Fig. 3A-B). Nowadays, about 221 species of seaweed have been exploited commercially, including few genera intensively worldwide cultivated and marketed primarily as human food (FAO, 2020). China is still the biggest producer of seaweed by aquaculture accounting for approximately 58% of global production, followed by Indonesia (29%) and Republic of Korea (5%) (FAO, 2020).

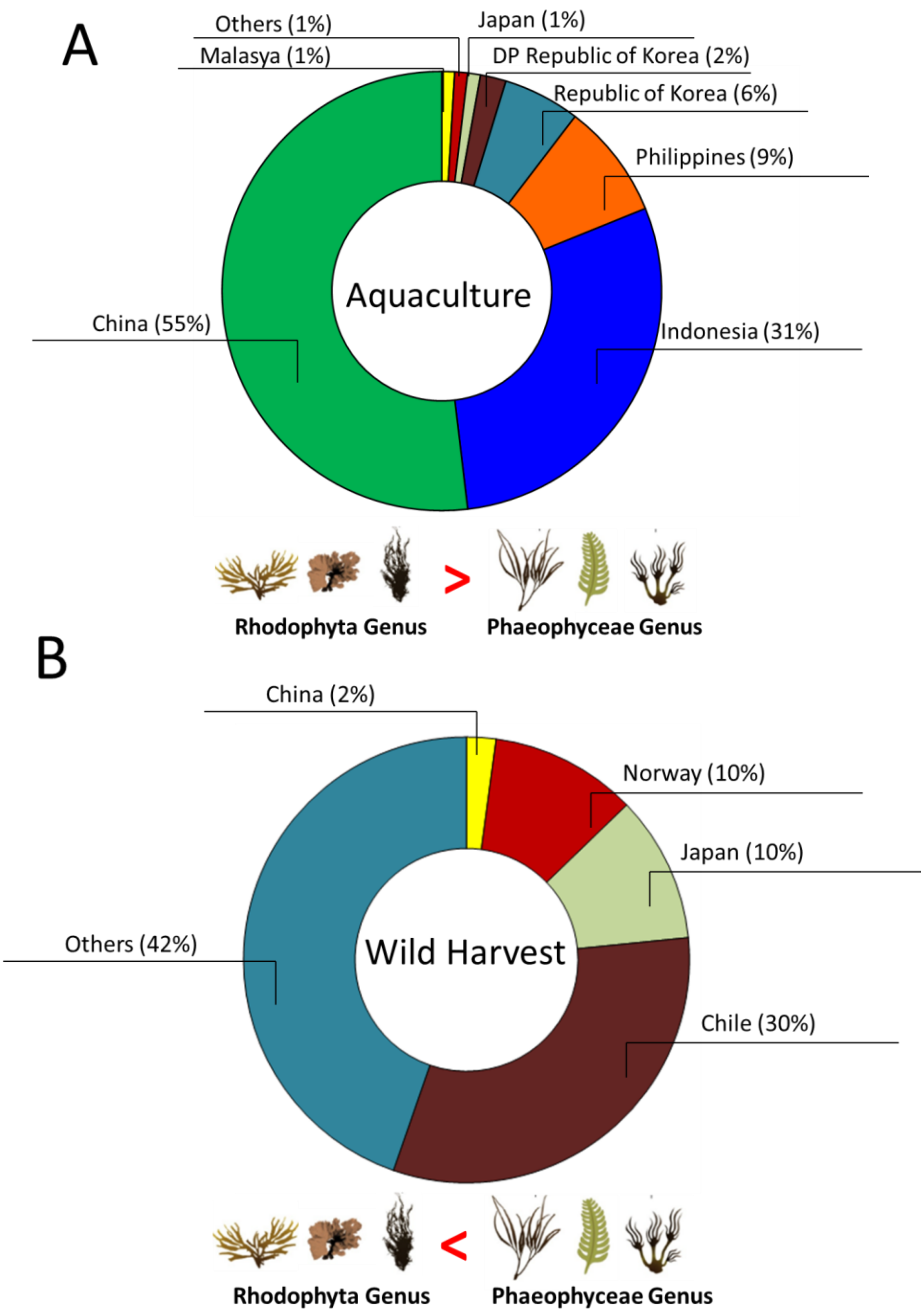


Figure 3. Worldwide (A) aquaculture and (B) wild harvest of commercial seaweeds markets including the main countries responsible for commercial production and the main groups of macroalgae accounting by the major volume (based on Poblete-Castro et al. (2020)).

Under this overview, there is an extensive demand for new sources of marine natural products, in which macroalgae are relevant components to supply this need and the high availability of algal biomass is critical to make this activity sustainable. Considering that aquaculture algal crops and field harvests from natural beds do not supply all market biomass for exploitation and the searching for potential new raw-material, the beach-cast seaweeds can represent an abundant and accessible biomass with potential of utilization for industries and local algal fishermen.

2. Justification

As previously presented, there is great potential for the discovery of new naturally occurring functional ingredients, in which macroalgae are important resources inhabiting the large Brazilian coast (about 7000 km coastline) with wide biodiversity. As a potential alternative to biomass utilization, beach-cast algae have the advantage of a renewable, economically viable and abundant resource (see Chapter I). Considering the existence of substances with biological properties, which are part of the composition of several products used by industry, researches with these underused waste marine macroalgae should be intensified. Many of the algae that have been launched on the beaches of certain regions along the Brazilian coast can be used in several commercial sectors, but are often burned or buried by local governments due to the bad smell caused by the deterioration of the organic matter, keeping away the users of coastal environments.

An intensive review of the literature about Brazilian beach-cast algae shows the relevance and innovation of this study (see Chapter I), with few worldwide studies and none evaluating the parameters analyzed in our research. Therefore, the present study will significantly contribute to the state of the art about the application of beach-cast macroalgae by increasing the knowledge of Brazilian and global beach-cast algae and their potential uses.

Thus, it is evident the need for basic knowledge, such as chemical characterization and biotechnological application, to know this natural patrimony and to prospect and propose future researches with beach-cast algae. In turn, the knowledge generation may contribute to public policies, heat the artisanal productive sector and consolidate companies to notice the bioeconomic and sustainable opportunity of the beach-cast macroalgae.

3. Thesis structure and objectives

The main objective of this thesis was to analyze the bioactive properties and chemical composition of Brazilian beach-cast macroalgae to provide subsidies that allow identifying the potentialities and uses as a functional product.

The present study proposed to perform a chemical characterization, in addition to evaluating the antioxidant and bioactive potential, regarding antiviral and antitumor/toxicity activities of crude extracts from abundant beach-cast macroalgae biomass from the Brazilian coast.

In Chapter I, the main aim was to contribute to the scientific and technological worldwide knowledge related to the literature of beach-cast seaweeds, through a compilation of the on-line available literature, focusing mainly on applied aspects related to the use of beach-cast seaweeds as biomass for different applications.

In Chapter II, the objective was to analyze the antioxidant potential and chemical composition of crude extracts of beach-cast marine algae from the Brazilian coast, to highlight potential new natural biomass that can be used as a matrix for several ingredients for the food industry and other applications. A manuscript from this chapter was accepted for publication in the *Journal of Applied Phycology*.

In Chapter III, the intention was to determinate the *in vitro* biotechnological potential of methanolic and aqueous extracts from different species of beach-cast seaweeds as an antiviral agent by the capacity to inhibit the enzyme reverse transcriptase of the HIV-1 virus.

In Chapter IV, the main purpose was to evaluate biochemical characteristics, antioxidant activity and cytotoxicity potential from extracts of beach-cast marine algae collected on the Brazilian coast, besides evaluate properties for cosmetic use and test formulation of creams with photoprotection properties. The analyses were carried out during the Sandwich Doctorate period in Málaga – Spain under the guidance of Prof. Dr. Felix Figueroa and Prof. Dr. Roberto Abdala as co-advisor of the University of Málaga.

4. General materials and methods

4.1 Collection and selection of species

The information of the selected species of brown, red and green beach-cast seaweeds harvested from the Brazilian coast is summarized in Table 1 and Figure 4. Our first collection of the beach-cast seaweeds was held in 2017 at Espírito Santo State (ES). Five species of macroalgae were collected: *Dictyopteris jolyana*, *Zonaria tournefortii*, *Alsidium seaforthii*, *Osmundaria obtusiloba* and *Spyridia clavata*. A second expedition for collection in Trairi – Ceará State (CE) was held in 2018 with five materials: *Alsidium triquetrum*, *Botryocladia occidentalis*, *Gracilaria domingensis* and two mix of beach-cast algae. Finally, the last collection was performed in Piúma – ES State in 2018, two species were collected: *Agardhiella ramosissima* and *Codium isthmocladum*. The species *Dictyopteris polypodioides* and *O. obstusiloba* were kindly collected in João Pessoa – Paraíba State (PB) and provided by Dra. Patrícia Guimarães Araújo and *D. jolyana* from PB was kindly provided by Dra. Mutue T. Fujii.

The material was collected by systematic sampling (authorization by Ministry of the Environment No. 17141-4/2016 and SisGen No. ACED12A), in which only visually healthy individuals were collected. The material was cleaned of macroepiphytes, washed three times in abundant tap water and then air-drying in the shadow. Fresh individuals for each species were separated for exsiccate (n = 3) and deposited in the SPF Herbarium (Phycological Section) at the University of São Paulo, except for *A. ramosissima* and *C. isthmocladum* that were deposited at the Herbarium of the Instituto de Botânica, São Paulo, Brazil (SP) (Table 1).

In the Laboratory of Marine Algae "Édison José de Paula" (LAM), the air-dried samples were oven-dried in an oven with air circulation at 40 °C (Marconi, M037, Brazil) until constant mass (approximately four to five days). The grinder process with the dry material was made in a ball mill (Marconi, MA 350, Brazil). The powder material was randomly divided into five equal parts (n = 5; technical replicates) and submitted to the extraction procedure.

Table 1. List of selected species of beach-cast marine algae harvested from the Brazilian coast at northeast (CE, Ceará and PB, Paraíba states) and (ES, Espírito Santo state).

Taxonomical group **Species**	**Beach (State)**	**Localization**	**Nº Voucher (herbarium)**	**Collection date**
Ochrophyta/Phaeophyceae (brown algae)				
Dictyopteris jolyana E.C. Oliveira & R.P. Furtado	Pontal Beach (ES)	20°58'22.5"S; 40°48'38.6"W	SPF58249	06/09/2017
Dictyopteris jolyana	Coqueirinho Beach (PB)	7°17'58"S; 34°47'54"W	SPF58249	02/25/2012
Dictyopteris polypodioides (A.P. De Candolle) J.V. Lamouroux	Ponta do Cabo Branco Beach (PB)	7°08'43.6"S; 34°48'20.7"W	SPF58249	07/18/2016
Zonaria tournefortii (J.V. Lamouroux) Montagne	Pontal Beach (ES)	20°58'22.5"S; 40°48'38.6"W	SPF58252	06/09/2017
Rhodophyta (red algae)				
Agardhiella ramosissima (Harvey) Kylin	Itaoca Beach (ES)	20°54'18.0"S; 40°46'42.3"W	SP470206	04/30/2018
Alsidium seaforthii (Turner) Kützing	Piúma Beach (ES)	20°50'31.5"S; 40°43'46.0"W	SPF58253	06/11/2017
Alsidium triquetrum (S.G. Gmelin) M. Howe	Emboaca Beach (CE)	3°12'23.5"S; 39°18'37.1"W	SPF58318	03/30/2018
Botryocladia occidentalis (Børgesen) Kylin	Emboaca Beach (CE)	3°12'23.5"S; 39°18'37.1"W	SPF58317	03/30/2018
Gracilaria domingensis (Kützing) Sonder ex Dickie	Emboaca Beach (CE)	3°12'23.5"S; 39°18'37.1"W	SPF58316	03/30/2018
Osmundaria obtusiloba (C. Agardh) R.E. Norris	Piúma Beach (ES)	20°50'31.5"S; 40°43'46.0"W	SPF58344	06/11/2017
Osmundaria obtusiloba	Ponta do Cabo Branco Beach (PB)	7°08'43.6"S; 34°48'20.7"W	SPF58082	07/18/2016
Spyridia clavata Kützing	Pontal Beach (ES)	20°58'22.5"S; 40°48'38.6"W	SPF58251	06/09/2017
Chlorophyta (green algae)				
Codium isthmocladum Vickers	Itaoca Beach (ES)	20°54'18.0"S; 40°46'42.3"W	SP470207	04/30/2018
Mix 1	Emboaca Beach (CE)	3°12'23.5"S; 39°18'37.1"W	-	03/30/2018
Mix 2	Emboaca Beach (CE)	3°12.443"S; 39° 18.537"W	-	03/31/2018

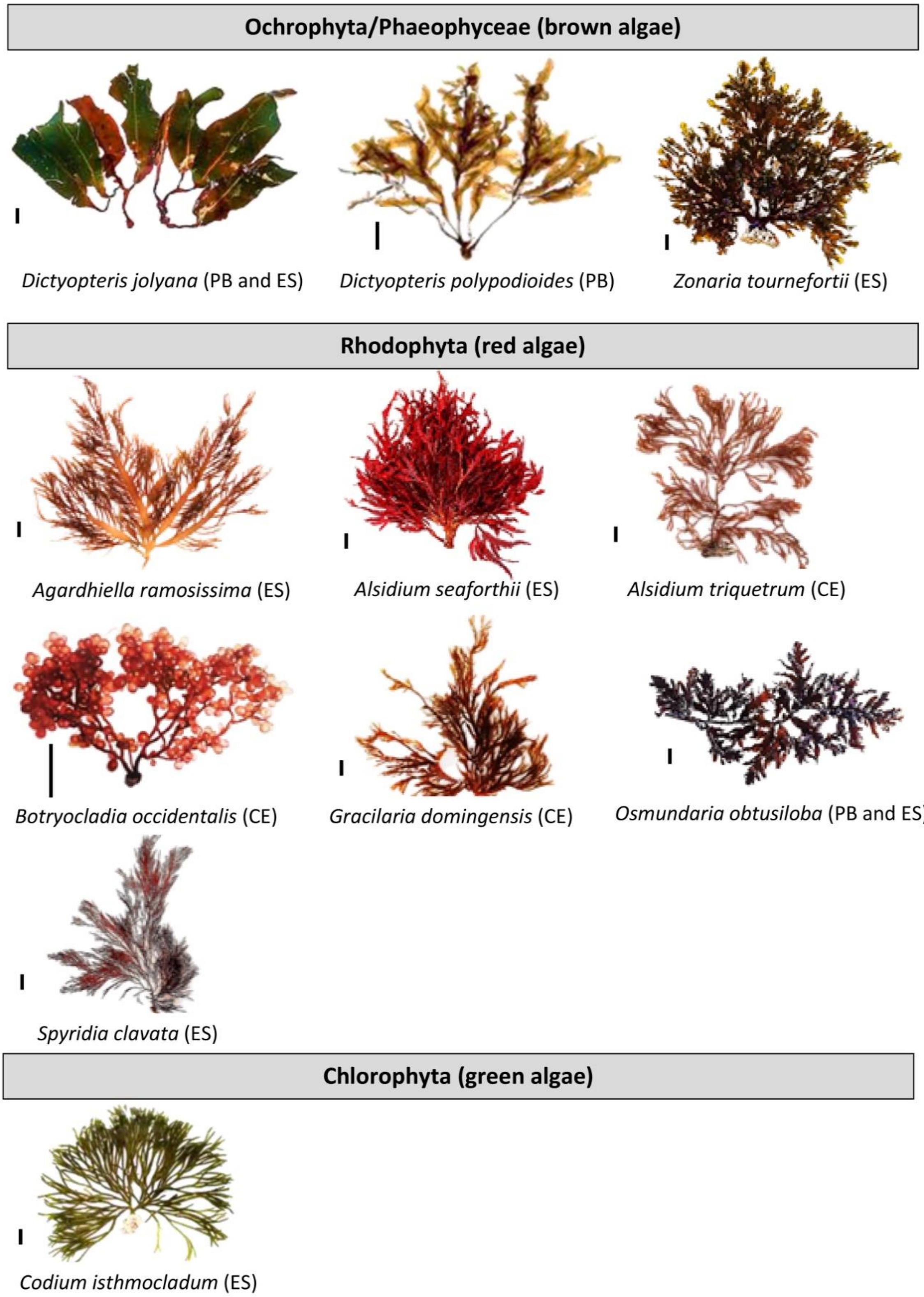


Figure 4. General habit of the selected species of beach-cast marine algae harvested from the Brazilian coast at northeast Ceará (CE) and Paraíba (PB) and southeast Espírito Santo (ES) states. Scale bar: 1 cm. Images: Cavalcanti, M.I.L.G; *Dictyopteris polypodioides*: Google images.

4.2 Climatological patterns of harvest regions

The climatic characterization of the northeast region in Brazil, including the CE and PB states, is influenced by four circulation systems: South, North, East and West disturbed current systems (Kousky, 1979). The south system, represented by the polar fronts that reach the region in the spring-summer in the coastal areas until the south of Bahia, brings frontal and postfrontal rains. The currents of the North cause rains from summer to autumn. In different circumstances, the eastern currents are more frequent in winter and usually cause abundant rains on the coast. Finally, the system of western currents, brought by the lines of Tropical Instability, occurs from the end of spring to the beginning of autumn (Kousky, 1979).

The trade winds are persistent and intense throughout the year (Varejão-Silva, 2001). Consequently, the intensity and duration of the rainy season of the region are also affected, giving rise to events of beach-cast algae.

The Intertropical Convergence Zone is the most important factor in determining how abundant or deficient the rains will be in northeast Brazil. This area is formed mainly by the union of the trade winds of the northern hemisphere with the trade winds of the southern hemisphere, which causes hot and humid air to rise and clouds are formed (Ferreira and Mello, 2005). Different rainfall regimes are identified in the region, but the main rainy season in the northeast is from April to July and the dry season occurs from September to December (Rao et al., 1993).

The southeast region, including the ES State, has a rather rugged topography and the influence of disturbed circulation systems are factors that lead to the climate of the region being very diverse concerning temperature. About the rainfall regime, there are two areas with the greatest precipitation: one, following the coast and the ‘Serra do Mar’, where the rains are brought by the south currents; and another, from the west of Minas Gerais to the Municipality of Rio de Janeiro, where the rains are brought by the Western system. The highest rainfall in the southeast region usually occurs in January and the minimum in July, while the dry period, usually centralized in winter, lasts from two to six months (Cavalcanti et al., 1982).

4.3 Extraction procedure

For Chapters II and III the powdered dry material (*n* = 5) was extracted using solvents of increasing polarity (serial gradient extraction with solvents) by simple maceration in hexane (Hex), dichloromethane (DCM), ethyl acetate (ETAC), methanol (MeOH) and hot water (80 °C) in a ratio of 1 g dry mass (DM) in 30 mL of solvent. The extraction with Hex, DCM, ETAC and MeOH was performed at room temperature and three-times by changing the respective solvent every 24 h and for hot water extraction, it was carried out three-times every 4 h each, in the meanwhile samples stayed in a water bath with constant temperature at 80 °C . The supernatant from each solvent was filtered separately and collected as a single sample, totaling five crude extracts for each beach-cast material: Hex, DCM, ETAC, MeOH and aqueous extracts. The extracts were concentrated and then lyophilized.

The values of dry mass and lyophilized extract were recorded to calculate the percent of yield. Only the MeOH and aqueous extracts were further study in their chemical composition, antioxidant potential and bioactivity, due to the higher yields in relation to the Hex, DCM and ETAC extracts.

As different extraction procedure was performed for Chapter IV, it will be described in the respective chapter.

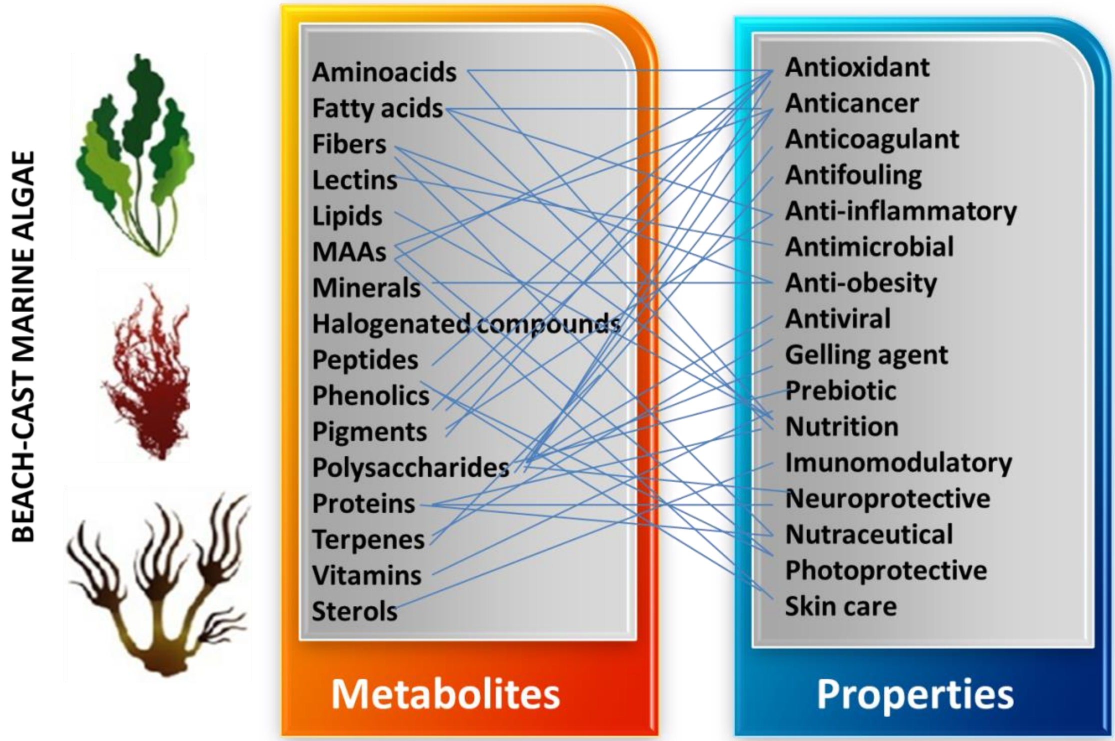
4.4 Data analysis

The entire chapter's analysis was carried out from the extraction of five subsamples from the same sample set (*n* = 5, technical replicates), except for Chapter III, which will be described in the respective chapter. Statistical analysis was performed using Statistica 10 software. Data were tested for normality (Kolmogorov-Smirnov test) and homoscedasticity (Bartlett's test). One-way analysis of variance (ANOVA) was used to compare the species or two-way ANOVA to compare species and extracts, with significant differences at $p < 0.05$. When differences were detected, Newman-Keuls *post-hoc* multiple comparison test was applied.

Chapter I

An overview of beach-cast seaweeds: potential and opportunities for the valorization of underused waste biomass

Graphical abstract



Abstract

There is an extensive demand for new sources of natural products with biofunctions, in which macroalgae can be relevant components to meet this need, due to the synthesis of metabolites with rich properties. Nowadays, there are no commercial-scale algae crops in Brazil to supply the biomass market for exploitation. The high availability of biomass, associated with the lack of use of fertilizers or pesticides, makes the beach-cast seaweeds an attractive and sustainable alternative to meet this demand. The beach-cast algae could constitute a potential biomass as a source of functional ingredients and new biotechnological applications. Currently, the excess of beach-cast seaweeds on the beaches causes an environmental problem, as its decomposition affects the use of the beach and keeps tourists and bathers away, impacting financial income in the region. Considering this negative impact on the environment and the possibility of using these organisms, some countries establish quotas that make it possible to reduce the volume and maintain the stock in the environment. This review aims to gather information on the state of the art on studies related to beach-cast marine algae in the world and Brazil, addressing ecological, economic and potential applications. The exploitation of beach-cast algae can be a socio-economic and environmentally sustainable solution, since it reduces financial costs, generates an alternative income for the local population and limits the impact of extraction on natural banks of macroalgae, threatened by over-exploitation. The data presented in this review show the relevance and richness of this material by gathering information on the prospecting of the biomass given the development of technology for algae matrices with a focus on the development of functional products and sustainable use of a waste unexplored biomass.

1. Introduction

The beach-cast algae are those naturally removed from the substrates or drifted from the local distribution (*e.g.* sargasso golden tide) or extraordinarily bloomed (*e.g.* ulvoid green tides) and then end up deposited in the sand of the beach, mainly driven by currents, winds and tides (Kersen and Martin, 2007; Santos et al., 2013; Suursaar et al., 2014). The accumulation of beach-cast seaweeds on the beaches cause environmental problems, after a while, the decomposition of these excessive biomass cause a strong odor, damaging the use of the beach and moving away tourists and bathers. The exacerbated agglomeration of seaweeds harms the tourism sector; besides promote the release of greenhouse gases and even the death of some organisms by anoxic habitat due to bacterial production (Arroyo and Bonsdorff, 2016). Tonnes of marine algae are worldwide removed per year by the government and dumped in landfills and dumps, but there is very few registers of abundance and composition of beach-cast marine algae around the world. Thus, new strategies are needed to reuse this biomass.

The accumulation of these beach-cast seaweeds in large quantities can constitute a potential biomass as source of functional ingredients and new biotechnological applications to support the claims for new natural products that can be used as matrix of diverse ingredients for the industry.

We would like to pay special attention to Brazil since there are no extensive natural beds of macroalgae for sustainable exploitation and the cultivation activities are extremely reduced. Nevertheless, there is an extensive shoreline and several informal reports of beach-cast seaweed occurrences in the newspapers. It is, therefore, important to understand the scientific knowledge about beach-cast seaweeds and the potential usage of this abundant underused biomass. Thereat, this overview aims to contribute to the scientific and technological worldwide knowledge related to the literature of beach-cast seaweeds, through a compilation of the on-line available literature on the Google Scholar and Google platforms, focusing mainly on applied aspects related to the use of beach-cast seaweeds as biomass for different applications. In turn, we intend to focus our efforts to verify the state of art in relation to the beach-cast seaweeds in Brazil, since the aquaculture is still extremely limited in the country and it does not have sustainable management and sustainable exploitation program for natural banks. At the same time, there is a great abundance of beach-cast seaweeds in Brazil, especially in the northeast region of the country. Therefore, by focusing and compiling the information, we intend to provide subsidies at the world and national levels for the exploitation and sustainable use of this renewable resource, which has not been benefited or valued. The potential uses and research applications of these organisms are discussed.

2. Categorization of the compiled literature on beach-cast seaweeds

An on-line literature review concerning beach-cast seaweeds and few other related biological materials as seagrasses, including scientific articles, monographs, dissertations and thesis, book chapters, books and technical reports, was performed until August 2020 based on Google Scholar and Google databases.

In Google Scholar, the literature review included English-records by the keywords (beach cast macroalgae), (beach cast algae), (beach cast seaweed), (beach cast seaweeds) and (wrack algae), Portuguese-records by the terms (alga arribada) and (algas arribadas) and Spanish-records by the term (algas arribazón). The search in these three languages was due to the fact to our knowledge about beach-cast seaweeds events from some countries and research groups working in this area. The advanced research was used on the database to find the exact phrase of keywords anywhere in the text.

After that, a new search with the same keywords was done in English and Portuguese on the Google website. All the results were analyzed and filtered based on studies in which beach-cast macroalgae were relevant or focus of interest, duplicates studies were removed. On the Google website, the results were all analyzed up to page 15. Additionally, the literature for the present review was expanded by new references that were cited in the found studies but did not appear in the systematic search by keywords on Google Scholar and Google databases.

When we consider the wide scientific literature on macroalgae/seaweed beach-cast researches and the filtered literature by checking repeat information, the available literature is limited to 132 reports (Table 2), distributed on 50 records from English literature, 32 records from Portuguese literature, nine records from Spanish literature and 41 records from other studies.

Table 2. Number of records from the total scientific literature concerning beach-cast seaweeds until August 2020 based on the compilation of Google Scholar and Google databases and checking repeat information.

Keywords	Total records	Filtered records
beach cast macroalgae	76	50 (English)
beach cast algae	43	
beach cast seaweed	205	
beach cast seaweeds	133	
wrack algae	52	
alga arribada	10	32 (Portuguese)
algas arribadas	159	
algas arribazón	296	9 (Spanish)
References from other studies	91	41 (others)

From the total of 132 scientific records about beach-cast seaweeds, we categorized the study approach into 13 levels: animal feed, bioactivity, bioenergy, biosorption of metals, carrageenan, chemical composition, ecology, fertilizer, human food, occurrence, physiology, review and taxonomy and abundance (Fig. 5). Of the 94 studies registered in other countries than Brazil, four were related to animal feed, three to bioactivity, eight to bioenergy, two to biosorption of metals, 10 to chemical composition, 49 to ecology, six to fertilizer, two to human food, six to reviews and four to with taxonomy and abundance (Fig. 5A). Some of the literature address more than one subject of our categories. It is important to note that the oldest available on-line literature was found in 1952, in which a scientific gap of studies is observed until 1970. The main subject of interest was registered for ecological approaches, with an increased scientific effort from 2011. We believe that there will be an increasing number of published articles in the coming years, since to our knowledge there are several research groups around the world studying beach-cast seaweeds, especially the golden *Sargassum* tide along the coastlines of Korea, Caribbean, Gulf of Mexico and West Africa and the proliferation of invasive species, probably related to global climate changes, concerning the ecological, physiological and potential use approaches.

Similarly, despite the information on the high historical occurrence of beach-cast seaweeds in Brazil, mainly by local newspapers and stories of the local population, the scientific literature is very small. Only 38 studies from the total 132 scientific records were found analyzing beach-cast seaweeds from Brazil (Table 2). The oldest available on-line literature corresponds to 1971 and deals with taxonomy (species identification) and abundance (amount of biomass), some other studies were also recorded until 2020 (Fig. 5B). Most of the Brazilian literature of beach-cast seaweeds concerns taxonomy and abundance with 12 records, while in other countries ecological approaches were the most studied. From the 38 studies registered in Brazil, four were related to bioactivity, one to bioenergy, seven to biosorption of metals, one to carrageenan, two to ecology, eight to fertilizers, one to occurrence, two to physiology and 12 to taxonomy and abundance (Fig. 5B). Some of the literature address more than one subject of our categories.

From these two categorized frameworks (Fig. 5A-B), we can identify some studies concerning the use of the beach-cast seaweeds mainly as fertilizer and metal bioabsorption, evidencing the knowledge hole in scientific research about beach-cast marine macroalgae.

Figure 5. Category distribution of the beach-cast macroalgae scientific records separated by year of the total 132 records including (A) other countries literature with 94 reports and (B) Brazilian literature with 38 reports.

3. Brazilian beach-cast seaweeds: distribution and abundance

In Brazil, beach-cast seaweeds are managed by Normative Instruction 46° IBAMA (2004), which understands beach-cast seaweeds as: “Algae that have been detached from the natural substrate and accumulate on the beaches, in the area between the low-tide and high-tide levels”. During the new and full moons, the moon and the sun are in the same line and then the pull gravitation reinforces are maximal and the tide’s range is increased. Therefore, a proven increase in fishing activities and beach-cast algae events are most probably in those two moon phases, associated with current hydrodynamic (Lira and Texeira, 2008).

The benthic seaweed flora of the Brazilian coast presents a great diversity, mainly due to the abundance of reef formations, rocky shores and rhodolite beds, which are favorable substrata to the fixation and establishment of these organisms, besides the high and constant temperature and salinity (Santana, 2005). The diversity of benthic flora is very similar to the flora of beach-cast algae, because there is an intrinsic relation between the richness of beach-cast macroalgae and the benthic ones since most of the beach-cast algae came from the benthic algal beds of near regions (Schreiber et al., 2020), excepted for the pelagic seaweed tides. Although not all macroalgae of the shoreline are suitable to occur as beach-cast macroalgae, significant contribution to the diversity of the flora of beach-cast algae is given by species that occur in the subtidal zone and larger sizes susceptible to detachment by the current forces. This last feature is a very interesting concern, because the subtidal species are the less studied groups of seaweeds due to the difficulty of collecting and requiring specialized diving effort.

Special analysis is detailed for Brazilian literature. The region with the highest number of published studies was the northeast region with Bahia, Pernambuco, Paraíba, Ceará, Rio Grande do Norte, Alagoas and Piauí states, followed by the South and southeast regions with Santa Catarina and Rio de Janeiro states (Fig. 6).

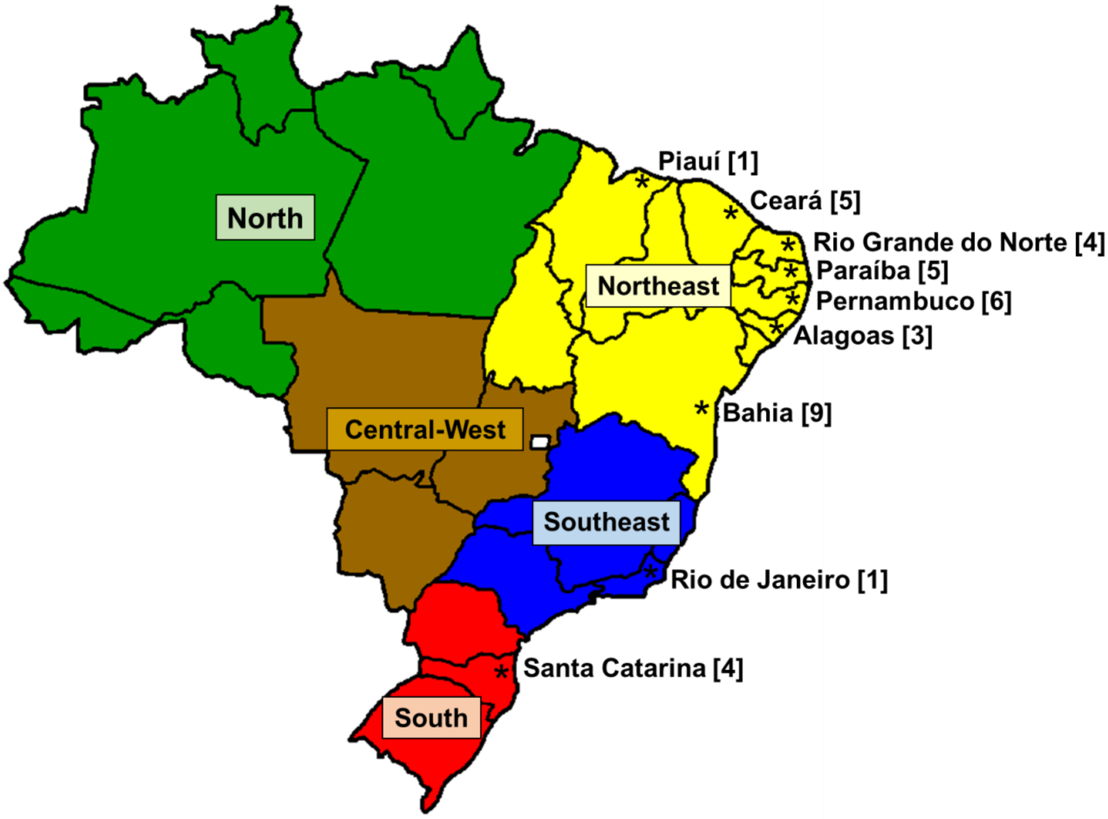


Figure 6. Distribution of Brazilian beach-cast seaweeds scientific records by region and state. The number within parenthesis represents the number of registers.

In the literature, it is possible to note that species from the order Dictyotales (brown seaweeds) and the genus *Sargassum* (brown seaweeds) and *Ulva* (green seaweeds) are the most seen as beach-cast macroalgae. Piriz et al. (2003) attribute the increase and detach of *Ulva* spp. as beach-cast material by the continuous presence of eutrophication, which can rapidly proliferate in a short-time and be detached to the environment. In turn, Pedreira and Nunes (2009) proposed that the functional morphotype exerts considerable influence on the occurrence of some beach-cast species, since larger macroalgae are more susceptible to the action of the marine currents and consequently to substrate detachment. Usually large-size macrophytes like corticated (*e.g.* cylindrical, compressed, blade-like, tubular thallus), siphonaceus, palmelloid or thick leathery are the most common morphofunctional types observed as component of beach-cast seaweeds.

The occurrence of beach-cast macroalgae have been related to almost all the Brazilian coast, except in the São Paulo State, which it was not found any report until our knowledge. The beach-cast macrophytes have a huge amount of biomass in the

Brazilian northeast, especially in the states of Ceará, Rio Grande do Norte, Paraíba, Pernambuco and Bahia (newspaper reports).

Câmara Neto et al. (1981) estimated the potential volume of beach-cast macroalgae in more than 300 tonnes in two different locations in Rio Grande do Norte (northeast region). Almost 50 years later, it still has these huge amounts of beach-cast algae occurring on the Brazilian coast. The "*Superintendência de Desenvolvimento do Nordeste*" (SUDENE, Northeast Development Superintendence) estimated a potential of 54,669.2 tonnes of fresh weight beach-cast seaweeds from studies performed on the macroalgal beds of the northeast Brazilian region, including the states of Rio Grande do Norte, Paraíba, Pernambuco and Alagoas, with an abundance of the species from the genus *Gracilaria*, *Hypnea*, *Cryptonemia* and *Sargassum* (Silva, 2005).

The beaches of the Bahia State (northeast region) registered also great diversity of beach-cast seaweed species. Vasconcelos and Nunes (2007) listed 27 species representing 16 Rhodophyta, six Phaeophyceae and five Chlorophyta for the Itapoã Beach, Salvador. Pedreira (2009) found 53 taxa, 24 of which were Rhodophyta, 18 Phaeophyceae and 11 Chlorophyta. In addition, Nascimento (2010) identified a total of 65 specific taxa, including 34 Rhodophyta, 17 Chlorophyta and 14 Phaeophyceae from Pituba Beach, Salvador. Rios (2010) found 71 specific taxa when studied two beaches of the Bahia coast (Stella Maris Beach, Salvador and Itacimirim Beach, Camaçari), 41 corresponding to Rhodophyta, 15 Phaeophyceae and 15 Chlorophyta. Santos et al. (2013) identified 123 specific taxa distributed in Rhodophyta (68), Phaeophyceae (28) and Chlorophyta (27) from four beaches of the Bahia State. In the same state, the study of Nobrega and Moura (2015) in Ponta da Ilha Beach revealed the occurrence of 113 beach-cast seaweeds, in which Rhodophyta represented the highest number of macroalgal taxa (70), followed by Phaeophyceae (22) and Chlorophyta (21). From the Brazilian coast, except for Bahia State, the other states with occurrences of beach-cast macroalgae have been scarcely studied despite the extensive knowledge of the occurrence of these beach-cast macroalgal events and future research perspectives must be intensified.

From the Ceará State (northeast region), recently, 18 species of beach-cast seaweeds at Praia do Pacheco in Fortaleza have been identified (de Souza Ferreira et al., 2020), including 14 red algae, two green algae and two brown algae. The species in greater abundance were *Hypnea pseudomusciformis* Nauer, Cassano & M.C. Oliveira, *Gracilaria cearensis* (A.B. Joly & Pinheiro) A.B. Joly & Pinheiro (Rhodophyta) and *Ulva fasciata* Delile (Chlorophyta).

4. Worldwide overview of beach-cast seaweeds

Studies performed outside Brazil have described some insight about the occurrence of beach-cast seaweeds. In other countries, studies focused on taxonomy (richness identification) or abundance of beach-cast macroalgae were not the most frequent and ecological approaches were the most reported in the literature. There are scientific records of beach-cast seaweeds with other topics studied in several countries such as New Zealand, Argentina, Kenya, Sweden, Peru, Japan, Germany and South Africa among others.

The biomass of detached algae is mainly influenced by the type of parent algal substratum (*e.g.* roughness and compaction) that both provide easier detachment or not, while current forces and air exposure depend on local tidal regime and periodicity (Biber, 2007). Other factors such as nutrient availability, temperature, turbidity and hydrography have also a substantial role in influencing the occurrence of beach-cast macroalgae in specific areas, interfering with the ability to predict impacts of biomass volume (Arroyo and Bonsdorff, 2016).

The occurrence of macroalgae tides or blooms has been associated with temperature changes, water salinity and nitrogen compounds excreted mainly by fish (El Gammal, 2020). Martins et al. (2020) carried out a review that showed the importance and risks of *Sargassum* blooms, as well as alternatives for the sustainable reuse of this biomass. The green, red or brown algae tides or blooms of pelagic seaweeds represent a potential raw material of underutilized biomass (Torres et al., 2019a).

Considering the studies with occurrence and abundance of beach-cast seaweeds, the research of Koop and Griffiths (1982) in South Africa estimated as much as 2,000 kg per year per cubic meter of shoreline with a higher amount of biomass in autumn and winter and decreased quantity in summer.

Moreira et al. (2006), comment briefly the literature about the beach-cast seaweed in the 90's in Cuba, especially concern to *Sargassum* genus. The authors studied the coverage biomass of beach-cast *Sargassum* spp. in different localities and estimated an annual average biomass ranging from 225 to 5,000 tonnes. The month of major beach-cast events was registered during anticyclonic currents, low air pressure, tropical storms, oceanic winds and wave height above 5 m. The beach-cast macroalgae events became to be larger and more frequent from the last 15 years and they have been associated with global climate changes and extreme climate events.

According to Santos et al. (2013), there is a predominance of *Ulva*, *Sargassum* and *Hypnea* among the algae often occurring as beach-cast in Bahia State, Brazil. The kelps *Durvillaea* and *Laminaria* are very common to accumulate in Australia and Scotland, respectively (Pirsa, 2014; Angus, 2018).

Changes in an unattached community of red macroalgae in the Sea of Japan from 1992 to 2005 were studied by Skriptsova et al. (2016), describing the current state at the studied date. The community was dominated by *Ahnfeltia tobuchiensis* (Kanno & Matsubara) Makienko for the first time and increasing species richness, significant changes in the stock dominant species and drastic decrease in the stock codominant species over time. This replacement of the unattached community has been associated with warmer waters. The knowledge of community dynamics associated with environmental influencing drivers is important for understanding the community functionality as well as an improved prediction on changes in commercial algal resources.

Lopez et al. (2019) showed a positive relation between the inputs of beach-cast *Durvillaea antarctica* (Chamisso) Hariot and local storm intensities at the beaches along the coast of Chile. Schreiber et al. (2020) showed that the majority of beach-cast *D. antarctica* strands relatively close to the population beds, confirming the origin from the adjacent rocky shores.

The knowledge of macroalgae diversity can support strategies for the conservation of aquatic ecosystems, as well as are essential for the development of environmental monitoring projects.

5. Ecological role

The beach-cast macroalgae have a significant ecological role including ecosystem services, especially when non-occurrence of large wrack deposition on the beaches. They are an important source of energy and nutrients for the community of sandy beach, in which the biomass deposited in the intertidal zone is incorporated into the trophic chain by invertebrates, important preys to other consumers (Kirkman and Kendrick 1997, Dugan et al., 2003, Smetacek and Zingone, 2013). The disaggregation and posterior deposition on the coastal bottom can supply macrophytic stands and nursery grounds for diverse marine organisms. In turn, beach-cast seaweeds, especially the drifting algae, serves as propagation vector for a variety of invertebrate species, increasing colonization of new areas and recovery of disturbed patches by opportunistic macrophytes and invertebrates (Koop and Griffiths 1982; Arroyo and Bonsdorff, 2016).

In Brazil, the beach-cast algae are collected by the government and dumped in landfills. Experiments carried out in the Paraíba State found that the decomposition of these algae is an important factor in the productivity of the Brazilian coastal zone (Sassi et al., 1998).

According to Lastra et al. (2014), during the degradation process, seaweeds release nutrients in the form of nitrate, nitrite, ammonia and phosphorus, which contribute to nutrient cycling when the tide rises, enhancing the surrounding growth of macroalgae, bacteria and invertebrates and promoting the fertilization of the entire coastal zone. The stay of beach-cast algae along the beach and subsequent decomposition is an attractive marine-derived resource available to terrestrial animals, such as birds, lizards, geckos and scorpions. Marine invertebrates associated with beach-cast seaweeds are also a significant source of food for these animals (Catenazzi and Donnelly, 2007).

The dynamics of nutrient flows depend on not only the distribution and amount of macroalgae launched on the beach, but also on species composition and deterioration rates (Mews et al., 2006). Species-rich in phenolic compounds are known to mitigate the deterioration of detritus and can change the microfauna of invertebrate’s preference to other brown algae with less phenolic content or more palatable species (Pennings et al., 2000).

Catenazzi et al. (2009) verified that two arachnid species of the genus *Chinchippus* use beach-cast algae as feed. In Australia, studies showed that the concentrations of compounds and population size of several fishes were highest from fishes inhabiting areas close to the drifting algae, which was related to the presence of the beach-cast algae (Lenanton et al., 1982; Lira and Teixeira, 2008; Santos et al., 2017). In British Columbia, a small-scale collection of the beach-cast *Macrocystis pyrifera* (Linnaeus) C. Agardh had a minimal impact on algae and local fish populations (Krumhansl et al., 2016).

Innocenti et al. (2018) showed that amounts of beach-cast *Sargassum* were able to mitigate coastal erosion by the force of waves, with 12% natural hydrodynamic wave attenuation, 46% reduction in shoring speed and 103% reduction in dunes erosion.

Despite the large ecological roles and ecosystem services from beach-cast macroalgae, adverse effects can also be identified. The environmental negative impact of beach-cast algae is largely associated to the extensive mass decomposition and depends greatly on the volume of detached algae and the air exposure time (Rodil et al., 2018). These huge amounts of beach-cast macroalgae potentiate the possibility of public health risks due to the increasing microbiological decomposition that generates a strong smell, damaging the use of the beach for lazer and local fishing, being hazardous for the local population (Lück, 2007; Risén et al., 2017; Fránzen et al., 2019). To improve the quality of beaches, millions of tonnes of beach-cast algae are removed by local city halls, spending millions of dollars per year for enhancing the landscape value and wellbeing for residents and tourists (Dessle, 2017; Risén et al., 2017; Fránzen et al., 2019).

The harvest of beach-cast seaweeds in Galicia, Spain, is mainly related to the cleaning of the beaches, which is governed by the regulations of treatment of organic waste, however, this biomass could be reused and employed for the production of agar (*Gelidium*) or as fertilizer for crops such as potatoes or corn (*Laminaria* and *Ulva*) (Tasende and Peteiro, 2015).

Normative that regulate the collection and cleaning of beach-cast seaweeds are important, as non-management practices may bring negative consequences. Informal harvest practices of beach-cast algae in Australia were considered harmful for some populations of birds and communities of invertebrates (Kirkman and Kendrick, 1997). The harvest of beach-cast algae can remove between 100-400 cubic meters of sand per season, accelerating the erosion process and changing the topography of the beach (Piriz et al., 2003). These collections would also affect the subtidal communities through the removal of reproductive structures as well as the organic matter and associated nutrients (Piriz et al., 2003; Martinsson 2015). Other scientists defend that the non-regulated removal of beach-cast algae causes a negative effect on sea turtles that visit the beaches for breeding at certain times of the year (Williams and Feagin, 2007). Zielinski et al. (2019) provided a review concerning beach cleaning and analyzed cleaning-related requirements on beach management and beach certification.

Despite the common practice of removing beach-cast algae, ecological impacts had not been registered according to several authors. In Texas, a study by Williams and Feagin (2007) did not confirm the concern that the removal of algal biomass would cause erosion on the beach sand by the use of tractors. Morton et al. (2015) do not registered impact on levels of total nitrogen as well as nitrate and bacteria, meiofauna and macrofauna abundances and only short-time physical impact was mentioned, with a natural recovery of the environment.

Angus (2017) reviewed the legal and ecological implications of beach-cast seaweeds harvesting in Scotland and described that small-scale harvesting is sustainable if certain guidance is followed. According to the author there is interest in commercial harvesting the beach-cast kelp *Laminaria hyperborean* (Gunnerus) Foslie, however, Orr (2013) reported considerable implications for the environment, as beach-cast seaweeds play an important role in the ecosystem function of the beaches of Uists Islands. Additionally, Angus (2017) proposes that leaving band areas of beach-cast algae on exposed beaches have the advantage of providing a growth medium for foreshore development and a source of feed for animals.

Dugan et al. (2003) also suggest the need to define cleaning beach areas, thus maintaining some ribbon areas where beach-cast algae remain and continue to provide food and habitat for a variety of species. On the coast of Costa Rica, a committee that gathers several stakeholders decides on the incidence related to the collection of beach-cast *Sargassum* spp. (Wischnat, 2013).

In countries such as Canada and Costa Rica, there is the collection and use of beach-cast seaweeds in large quantity, considering that they play an essential role in the dynamics of the ecosystem, these countries have established normative for their management and extractivism. In Canada, the licenses established by the ministry were in accordance with previous environmental protection indicators elaborated by McLaughlin et al. (2006), in which non-mechanical harvesting procedures are allowed and sand, sediment and substrate removal are forbidden.

It is known that the harvesting of beach-cast algae can cause positive and negative effects on the ecosystem. According to Blidberg and Gröndal (2012), the amount of biomass removed by cleaning or commercial harvest must be carefully monitoring and quotas could be established to exercise a sustainable use of this resource.

From the studies described above, we can highlight ecological researches in many countries such as Argentina (Piriz et al. 2003), Baltic Sea (Malm et al., 2004; Schultz-Zehden and Matczak, 2012), Canada (Glickman et al., 2012; Holden, 2016; Holden et al., 2016; Holden et al., 2018), Germany (Springer, 2014), Kenyan (Ochieng and Erftemeijer, 1999), Mexico (Milledge and Harvey, 2016), New Zealand (Hurd et al., 2004; Zemke-White et al. 2003; Zemke-White et al., 2005; Thomsen and Wernberg, 2009; Dufour, 2011; Dufour et al., 2012), Scotland (Angus, 2018), Sweden (Kathleen, 2013; Dessle, 2017; Risén et al., 2017) and USA (Young, 2003).

6. Economic feasibility of harvesting beach-cast macroalgae

The small- and large-scale commercial use of macroalgae has increased exponentially in recent years, currently as valuable raw material for several areas, such as human consumption, animal feed, fertilizers, biochemical production, gelling substances, biofuels and new biomaterials (Blidberg and Gröndal, 2012; Fitton et al., 2019).

According to Angus (2017), industrial-scale exploitation in Scotland began in Orkney Islands in 1720, reaching the Uists Islands in 1735, when the burned beach-cast *Laminaria* was used in several processes, including bleaching and the manufacture of soaps and glasses.

Beach-cast red algae have been exploited commercially in the Baltic Sea since the 1960s, since then continuous monitoring of the biological characteristics of the community has been carried out to enable the sustainable exploitation of this resource in the region. Annually 5.000 tonnes of wet weight were collected (Kersen, 2006; Kersen and Martin, 2007).

Historically in Ireland, the harvest of beach-cast macroalgae is used as an essential raw-material for soil treatment as a source of nitrates and phosphates, soap-making or food additive for humans and animals (O'Neill, 1970; Walsh and Watson, 2011; Guiry and Morrison, 2013).

Smaller industries also operated in countries such as New Zealand (Luxton and Courtney, 1987). In Namibia, commercially harvesting of beach-cast algae occur throughout the year, but quantities vary greatly, the biomass is manually loaded into vehicles and taken to the desert to be sun-dried. To use a large volume of beach-cast algae is needed to collect and dry the material as fresh and soon as possible to avoid decomposition (Critchley et al., 1991). In Australia, commercial beach-cast algae harvesting has been reported for alginate extraction (Kirkman and Kendrick, 1997) and after in south of Australia that was reported for the manufacture of low-value commodities such as fertilizer and animal feed, including modest industries producing hydrocolloid and agricultural products (Lorbeer et al., 2013).

The commercial harvest of beach-cast algae also occurred in South Africa (Zemke-White et al., 2005), Canada (Chopin and Ugarte, 2005) and Ireland (McLaughlin et al., 2006), as well as in Patagonia for human consumption, nutraceutical, cosmetic and fucoidan industries, with an incipient economical income (Rebours et al., 2014).

The commercial uses of beach-cast seaweed biomass will depend on the quality of the material, the species, the availability of the resource and the local market. To high value-added market products, such as human food, biochemicals and gelling substances, the biomass of beach-cast algae must be of superior quality, including fresh, undegraded, unmixed with other species of algae or contaminated with sand and stones (Blidberg and Gröndal, 2012). On the other hand, products with a lower market value, which do not require the same quality and homogeneous quantity of biomass can be also produced. The latter can include biofertilizer, bioenergy production, soil improvement, land restoration and animal feed (Blidberg and Gröndal, 2012).

As reported by Wischnat (2013), a modified potato digger was used to remove the beach-cast seaweeds from the beaches in Maui Hawaii, an automated collector that save time and labor. The same study, reports the use a customized rakes and blades that can gather trash and debris by the USA’s Beach Raker company specialized in harvesting biomass on beaches.

Some structures are attached to the bow of boats and canoes to minimize changes in habitat after harvest in Ireland (Ugarte et al., 2006). This type of harvest method improves socio-economic opportunities for the harvesters in the country, reducing the use of machines and promoting income and employment generation (Mac Monagail and Morrison, 2020).

Environmental problems due to the over-exploitation of beach-cast seaweeds restricted the amount of collections allowed in some countries, therefore, for commercial purposes it typically involves quotas and regulations that preclude the complete removal of wrack. The South Atlantic Fisheries Management Council had recommended a maximum sustainable yield limit for *Sargassum* spp. to 100,000 metric tonnes of wet weight per year (Wischnat, 2013). In Portugal, six different harvesting areas of beach-cast seaweeds were defined and annual licenses issued allow harvesting for commercial purposes (Santos and Duarte, 1991). In Australia, harvesters are permitted to collect up to 50% of available beach-cast kelp *Durvillaea potatorum* (Labillardière) Areschoug (Pirsa, 2014) and in the south there are licenses up to 75% of estimated biomass for others beach-cast macroalgae (McLaughlin et al., 2006). On the other hand, in New Zealand, a license is required for the collection of unattached, free-floating macroalgae. Only beach-cast red seaweeds can be taken for commercial purposes without a fishing license, as a result of the long-established and continuous use of red seaweeds for the production of agar (Hurd et al., 2004).

According to Milledge and Harvey (2016), tourism generated US$ 29.2 billion in the Caribbean in 2014 and contributed more than 80% to the regional financial income. Tourists are avoiding resorts affected by the golden tides of *Sargassum*. The inundation accommodates approximately 4–10 megatonnes of over 100 species of *Sargassum* seaweeds.

In addition, the prevention or removal of *Sargassum* from beaches can be very expensive. It is estimated that at least US$ 120 million would be needed to clean the *Sargassum* floods throughout the beaches in the Caribbean (Thompson et al., 2020). In Mexico, the restoration of the beaches on the Quintana Roo coast, expended US$ 9.1 million and approximately 5,000 people working (Schell et al., 2015).

Galveston Island spends about US$ 3.5 million a year to keep 32 miles of beach clean. Prevention of algae reaching the beaches can also be expensive with a floating 300 m algal bloom estimated at US$ 80,000 to protect some of the beaches of St. Vincent and the Grenadines (Milledge and Harvey 2016).

On the German Baltic coast, cleaning is done regularly by hand, the cost is € 38 per meter of “algal line” removed, the average amount of available biomass is 269 tonnes/km (Mossbauer et al., 2011). A beach association located on the southeast coast of Gotland has an annual member fee of 300 kroners for the payment of the cost with the machine used for cleaning the beach and harvest the beach-cast seaweeds (Dessle, 2017).

Charoensiddhi et al. (2018) carried out a case study for simulated an industrial-scale production of high-value functional food products from the beach-cast alga

Ecklonia radiata (C. Agardh) J. Agardh. The biomass used in the simulations was commercially available dried beach-cast seaweed for human consumption from Australia, the purchase price of the raw material was set at US$ 15 per kg, similar to the price charged for Australian commercial dried seaweed for human consumption. The authors reported that industrial process simulation and economic analysis demonstrated potential commercial viability and profitability for the industrial production of bioactive health supplements of the beach-cast seaweed *E. radiata*.

Beach-cast algae of the genus *Hypnea* from the Brazilian northeast have been exported as raw material or already processed to the carrageenan industry (Vidotti and Rollemberg, 2004). The company AgarGel, located in João Pessoa – PB, processes algae collected by “algueiras” along the coast of the northeast and also imports from Chile for the production of agar as its main production focus. The company pays around R$ 0.60 per kilogram of beach-cast algae, not counting the taxes that may be levied on the final product. However, it is suggested that this price can be improved, since such value is paid by a mixture of algae that are dried inadequately (Carvalho Filho, 2004).

The company Cia das Algas, located in Trairi – CE, produces a liquid fertilizer with a mix of beach-cast algae that occurs all over the year in a huge amount on the beaches in Trairi. Nowadays, the company processes more than 400 tonnes of the dry weight of beach-cast algae monthly (Cia das Algas, pers. comm.).

It is important to note that due to the great ecological importance of these organisms, the collection of beach-cast marine algae for commercial purposes requires appropriate control by licenses, environmental management and regulation by quotas in many countries, to maintain sustainable exploitation.

7. General aspect about uses and applications of beach-cast macroalgae

Macroalgae have diverse primary and secondary components suitable for several uses and applications. Therefore, the potential of beach-cast macroalgae as feedstock for these uses and applications should not be quite different from the evidence already presented for the attached macroalgae. Despite that the extensive literature about the uses and bioactive properties of macroalgae, few studies are exploring other alternative applications for beach-cast seaweeds and the few studies available are described in the following sections.

The use of beach-cast seaweeds offers several economic and ecological benefits. The high availability of biomass associated with the absence of fertilizer or pesticide use makes the beach-cast seaweeds an attractive and sustainable alternative. Thus, algae could be a biological material used to meet a demand for sustainable use (socially and economically) and an ecological strategy for public health.

7.1 Antioxidant potential

Macroalgae suffer from the action of environmental factors. Consequently, seaweeds have several defense mechanisms, such as antioxidant activity, which can change with season, irradiance, temperature, nutrients, salinity, desiccation and other factors (Maschek and Baker, 2008). Species displaying antioxidant activity could prevent several pathological effects such as DNA damage, carcinogenesis and cellular degeneration (Heo et al., 2005). Therefore, a screening of beach-cast algae antioxidant potential could be a useful tool for selecting species and extracts to obtain new compounds and prospects for biotechnological application.

In a study with the beach-cast *Caulerpa racemosa* (Forsskål) J. Agardh harvested in northeastern Brazil, the antioxidant test of DPPH revealed that benthic algae showed higher antioxidant activity with low values of half maximal inhibitory concentration than the beach-cast algae (Padilha, 2014). This is the only study to our knowledge with antioxidant activity of beach-cast seaweeds in Brazil. Despite the lower antioxidant potential for the beach-cast material, other studies show the potential of beach-cast as raw-material for natural antioxidants, therefore, the need for investment in further research is evident. The antioxidant activity of the red beach-cast *Gracilaria bursa-pastoris* (S.G. Gmelin) P.C. Silva was evaluated in Morocco by Ramdani et al. (2017). They investigated the antioxidant activity for the Folin-Ciocalteu and DPPH assays with ethanolic and aqueous extracts at temperatures of 20 °C and 40 °C and the beach-cast algae showed excellent antioxidant potential with values equivalent to the commercial standard ascorbic acid. The higher antioxidant activity of ethanolic extract was attributed to the presence of phenolic compounds. The results showed that the beach-cast *G. bursa-pastoris* can be a promising source of natural antioxidant compounds and may become valuable for the development of therapeutic products, food supplements and pharmaceutical applications.

The antioxidant properties in ethanolic and aqueous extract solutions from beach-cast algae in Japan were also investigated, the total phenolic compound content and the antioxidant potential by the DPPH and FRAP assays were higher in the brown beach-cast *Eisenia bicyclis* (Kjellman) Setchell (Takei et al., 2017). These findings suggest that the studied beach-cast algae could be used as natural resources of antioxidants and raw material for functional foods.

Although the extensive arsenal of information on the potential of macroalgae as antioxidant sources, few studies have addressed the beach-cast algae as object of study, making it of great relevance for further studies.

7.2 Bioenergy

In the increasing energy demand, macroalgae blooms, industrial waste and the beach-cast algae can be an alternative for the production of methane gas, being a source of essential raw material for the production of biogas and biofuels (Barbot et al., 2016; Fernand et al., 2017; Torres et al., 2019a). Methane production from beach-cast algae is comparable to the production from bovine manure and terrestrial energy crops, such as grass-clover (Milledge et al., 2014; Barbot et al., 2016). Beach-cast seaweeds have the advantages of being generated with continuous quantities, similar biomass composition and easily linked to the production process (Özçimen et al., 2015).

Biogas production partially produced from the beach-cast macroalgae has been investigated in the southern Baltic Sea (Bucholc et al., 2014) and Sweden (Yohannes, 2015; Dessle, 2017) but the residue contained high levels of heavy metals, reducing its use as fertilizer (Nkemka et al., 2014). A post-treated residue was suggested to increase biogas production, reducing the amount of heavy metal (Briand and Morian, 1997). The suggested assays for the removal of heavy metals in macroalgae were chemical removal, ion exchange and adsorption (Bergström, 2012).

In Brazil, one study evaluated the methane gas extraction from the biomass of beach-cast seaweeds harvested in Rio Grande do Norte. Bisanz et al. (1981) described that the gas obtained reached an average of 300 liters/kg of the raw material of dry beach-cast biomass, with a percentage of 60% – 70% of methane gas in the produced biogas.

Studies suggest that anaerobic digestion of beach-cast seaweeds would be an alternative to mitigate the negative impacts of decomposing biomass along the coast and at the same time produce high-value fuel, methane biogas. Co-digestion performed among a mixture of brown (20%) and red beach-cast algae (80%) with corn, grass, rye and chicken manure produced 500 kWh of energy (Ertem et al., 2017), with a reduction in global warming, acidification and eutrophication. Sustainable energy production was achievable with the co-digestion of chicken manure (40%) and beach-cast algae (60%). However, the production of bioenergy from beach-cast seaweeds still has some challenges: to optimize the pretreatment to improve substrate performance, to evaluate the toxicity caused by high levels of phenols, heavy metals, sulfides, salts and volatile acid compounds found in algae and to improve harvesting procedures to minimize environmental impacts and overall costs (Ertem et al., 2017).

The anaerobic digestion of 10,000 m^3 of beach-cast algae harvested on annual beaches in Trelleborg could generate a potential of 14.5 GWh of energy, with estimated annual potential of 101.5 GWh for the region (Li et al., 2013). However, the morphology of macroalgae may hinder conventional anaerobic digestion and pre-treatment is necessary to assist mechanical management and microbiological digestion. Species with a more rigid thallus and cell wall, such as *Fucus vesiculosus* Linnaeus, the final methane potential was increased by 100% with a mechanical pre-treatment, however, for red beach-cast filamentous algae, the final potential of methane was the same without pre-treatment (Li et al., 2013).

Sargassum biomass from the Caribbean coast, Gulf of Mexico, Florida and West Africa would be used as raw-material for the production of electricity. Co-digesting this biomass with municipal organic solid wastes proved to be economically advantageous, increasing the energy recovery up to 5 times (Thompson et al., 2020). The gas fraction can also be used in cooking as a substitute for natural gas derived from fossil fuels.

Macreadie et al. (2020) were the first to show the potential of *Sargassum* beach-cast for obtaining biochar (pyrogenic vegetal carbon or black carbon), a promising and favorable alternative to avoid the elimination at landfillsand part of the greenhouse gas emissions.

A sustainable circular bioeconomy can be supported by the biorefinery framework that converts biomass into a spectrum of multiple chemicals and bioproducts with value-added. Integrated biorefinery solutions are enough developed to allow economical fuel production from underutilized algae or industrial waste biomass (Tiwari and Troy, 2015). These findings show the potential of beach-cast algae for energy production, in which coastal communities could use this low-cost energy source on a small scale as a domestic fuel.

It is possible that the cultivation of macroalgae for the production of bioenergy is not economically feasible as the social and environmental benefits of the activity are not included in the economic system. When ecosystem services are included, the gain will be around € 2,220-2,640 per year for each ton of beach-cast algae sold as biogas in Sweden (Wersal and Madsen, 2012). For an improved product, such as vehicle fuels, profit ranges about € 2,410-2,750 per ton per year. These additional values may also include options such as extracting high-value substances from macroalgae before being used as a bioenergy substrate.

The beach cast seaweeds as biofuel can potentially reduce the reliance on fossil fuels and mitigate global warming while producing high-value renewable energy (Kaspersen et al., 2016). The demand for energy alternatives puts the beach-cast algae in a prominent position for a promising future; however more studies are needed to confirm the economic viability on a large scale of energy production through the biomass of beach-cast seaweeds.

7.3 Centesimal and chemical composition

Beach-cast macroalgae have a wide variation in biochemical composition, which changes according to the species. According to Nunes et al. (2020), this allows the targeting of bioresources for specific purposes, including functional foods. A viable alternative would be to use this biomass for the extraction of biocompounds for industry intents (Nunes et al., 2019).

The centesimal composition and minerals were determined for beach-cast seaweeds and different macroalgae raw-materials attached from Tahiti, French Polynesia (Zubia et al., 2003). Food fibers (38.1-42.8% DM) and ashes (30.6-39.8% DM) were the most abundant constituents in all samples, with similar patterns of amino acids and predominance of aspartic and glutamic acids. All samples had high mineral content, especially calcium, magnesium, potassium, iodine, iron and zinc, but beach-cast seaweeds usually accumulated more minerals than attached ones. Concentrations of heavy metals were below to the permissible French's limits (Zubia et al., 2003).

The content of minerals, soluble polysaccharides, total phenolic compounds and antioxidant properties were investigated in beach-cast macroalgae, aiming their use as food and in healthy diets (Kuda and Ikemori, 2009). The extracts showed high potassium content, viscosity and content of phenolic compounds, in addition to strong antioxidant activities, showing that the beach-cast seaweeds from Japan could be used as new natural resources for functional foods.

High levels of lipid content are indicative of possible applications of some beach-cast seaweeds on the Patagonian coasts as raw-material for lipid-based bioproducts for nutritional and nutraceutical purposes. Species that showed the highest amounts of lipids belong to the genus *Undaria*, *Ceramium* and *Codium* (Dellatorre et al., 2020).

Beach-cast seaweeds were also suggested as an abundant and low-cost promising source of natural pigments that could be used in the food industry. Iglesias and Del Rosario (2009) evaluated three beach-cast seaweeds in Cuba for nutraceuticals purposes. The notable presence of pigments such as carotenoids and chlorophylls and secondary metabolites such as polyphenols, flavonoids, saponins and alkaloids in the extracts value their use as raw-material for nutraceutical products. This would allow the recovery of this waste resource and its incorporation in the economic sector by the replacement of synthetic substances commonly used (Pardilhó et al., 2020).

Different from the bromatological composition that is desirable high levels of certain nutritional compounds, heavy metals are a particular concern due to their toxicity as nutritional element or fertilizers. Therefore, Greger et al. (2007) investigated the concentrations of heavy metals in beach-cast seaweeds from the Baltic Sea in a composite made from red and brown macroalgae and determined the transfer of these metals to food crops. The concentrations of cadmium in lettuce and oats grown directly with the composite of beach-cast seaweeds exceeded the official UE limit values, while the concentrations in tubers and legumes were lower. Regardless of growing cultivar foods directly into composite algae increased productivity, it would not be recommended without determining the heavy metal levels. Instead, the beach-cast seaweed could be used in smaller amounts in agricultural soils as a valuable source of nutrients for growing non-food crops.

Absorption of cadmium is affected by several factors that can be controlled in beach-cast algae to provide nutrients and improve soil structure without contaminating it. Recent studies also indicate that food crops can be safely fertilized with harvested beach-cast algae without cadmium transfer if biomass and specific sites with low metal content are chosen (Dessle, 2017). In addition, if biomass of harvested beach-cast algae is pre-composted with other substrates it is possible to limit the transfer of cadmium to food crops. By following the established norms and maximum limits for heavy metals, the use of beach-cast algae can efficiently supply the nutrients to food crops and other products with high added value.

Chemical analysis was performed with beach-cast algae and sea wrack from Gotland, in the Baltic Sea by Franzén et al. (2019), showing variations in the concentration of cadmium, depending on the sampling site. From the samples analyzed, one had a cadmium content above the Swedish limit for biofertilizer (2 mg Cd/kg DM) and four samples had values above the limit suggested by the Swedish Environmental Protection Agency for 2030 (0.8 mg/kg DM). The species-specific analysis showed that the sea wrack *Zostera marina* Linnaeus contained significantly higher cadmium concentrations than beach-cast algae species of the genus *Ceramium* and *Polysiphonia*.

Despite the records of high levels of heavy metals for some samples of beach-cast seaweeds, the safe use of any bioresource as the beach-cast seaweeds must be guaranteed by assessing potential toxic components and only after this evaluation the application can be thought depending on the safety limits. Therefore, beach-cast seaweed can be an important eco-friendly biomass source.

7.4 Food and Feed

The world population is expected to reach 9.3 billion by 2050 (Merino et al., 2012). In order to sustain this large number of people, food production has to grow by 70% over what currently is produced (Lemos, 2011). Due to the world population growth, the search for new feedstock for human and animal feeding has been determinant to assure the global supply of food. Therefore, studies show that the use of beach-cast algae can be an efficient alternative to attenuate this demand.

The number of countries that consume macroalgae as food has been increased, such as Japan, China, Hong Kong, Taiwan, Philippines, France, Scotland, Peru, Chile, Jamaica (Pérez-Lloréns et al., 2020). Nowadays it is possible to find *Ulva* snacks being sold in Portugal, Ireland, Germany, Brazil, among others.

Traditionally, macroalgae industrial waste from agar extraction have been used as “macroalgae meal” (Ferrera-Lorenzo et al., 2015). According to Alonso et al. (2020), the beach-cast *Codium tomentosum* Stackhouse from Ria de Vigo in Galicia, Spain, can be used as food.

Beach-cast algae can also be used as a supplement to animal nutrition. There are currently companies such as Acadian Seaplants Ltd. (Canada) and AlgeaFeed (Norway) which market nutritional supplements of the brown seaweed *Ascophyllum nodosum* (Linnaeus) Le Jolis in the form of flour that can be incorporated into diets for cattle and poultry (Wischnat, 2013). Nutrient-rich macroalgae can be excellent supplementary diets for aquatic organisms like shrimp and fish farming, reducing the feed cost. Silva (2005) evaluated the effects of the beach-cast seaweeds *Hypnea cervicornis* J. Agardh and *Cryptonemia crenulata* (J. Agardh) J. Agardh as protein sources for the shrimp *Litopenaeus vannamei* (Boone) and concluded that there was no decline in growth rates, confirming that beach-cast algae could be used as an ingredient for animal feed.

The potential of beach-cast *Sargassum* from Costa Rica was evaluated as prebiotic, demonstrating positive effects on egg's production and quality, including low cholesterol and high iodine contents from chickens fed with diets containing *Sargassum* (Wischnat, 2013). The study proposed an income activity for the local communities; however, the effort to process the fresh *Sargassum* biomass by the local cooperatives still needs to be improved. Nevertheless, the author emphasizes that *Sargassum* can be used for animal nutrition in conventional or organic production systems and emphasize that more studies are needed to evaluate the seasonal variations of nutrient composition.

The inclusion of 15-20% of beach-cast seaweeds in the diet feed of carps also showed benefits, despite the decreasing growth of the fish, the algal supplementation contributed with positive health effects probably favored by the lipolytic and antioxidant activity described for the algae (Barrios Lorenzo, 2020).

The invasive beach-cast *Undaria pinnatifida* (Harvey) Suringar had similar nutritional and biomechanical properties than three native macroalgal species (the kelps *M. pyrifera* and *D. antarctica*, and the green alga *Ulva* spp.); therefore, Suárez Jiménez et al. (2017) proposed the use of these invasive beach-cast as an alternative feed source for invertebrates.

To our knowledge, the use of beach-cast seaweeds has been most studied for animal feed than human food. This scenario may be due to the fact of food safety and novel food legislation that imposes hard restrictions on the use of some non-traditional food sources such as macroalgae, beyond the controversy over the safety contamination of algae by heavy metals.

7.5 Fertilizer and biostimulants

Products based on some species of macroalgae used in dry form or extracts have been marketed as biostimulants in countries of Europe and the United States, such as Folical E®, Phycarine®, Roots®, Selecal® and Tonialg® and are generally used to increase plant resistance to diseases (Stadnik, 2003). Traditionally, seaweeds have been also used for centuries as fertilizer in soils. Studies suggest that the practice was common in coastal regions of some European countries, such as England, Scotland and France (Greger et al., 2007). In the 1900s the use of beach-cast seaweeds was so popular that authorities had to regulate its use. Nowadays, the use of beach-cast macroalgae can be in the form of flours or commercial liquid extracts.

The use of beach-cast algae as fertilizers is due to the chemical properties of some nutrient-rich species containing nitrogen, phosphorus, potassium, magnesium, manganese, zinc, boron, phytonutrients, soluble polysaccharides and phenolic compounds which synergistically can improve soil health and plant cultivation (Blidberg and Gröndal, 2012).

Dantas et al. (1998) showed that coriander and lettuce plants fertilized with flour of *Sargassum vulgare* C. Agardh showed a huge growth and intense coloration, probably related to the presence of phytohormones and nutritive substances such as nitrogen, phosphorus and potassium. Similar results were obtained by Coelho et al. (2018), confirming the potential of beach-cast *S. vulgare* as fertilizer in sunflower growth. Kuda and Ikemori (2009) suggested the use of *Sargassum* golden-tides blooms around the world as fertilizers. Other examples of agricultural practices based on beach-cast macroalgae were reported for *Laminaria* used by smallholders to fertilize fodder grain in Scotland (Angus, 2018) and for a long history by farmers and landowners, in the country most of the biomass is utilized for soil improvement (Dessle, 2017).

The use of organic waste from beach-cast algae can be an alternative to fertilizing plant cultivation, besides to value a sustainable destination (Britto et al., 2018). The use of beach-cast macroalgae as alternative fertilizer has been also demonstrated to be better than commercial fertilizers in lettuce *Lactuca sativa* L. (Sacramento et al., 2013) and seedlings of *Moringa oleifera* Lam (Nova et al., 2014). In

Brazilian northeast, beach-cast algae are traditionally used *in natura* as coconut plant fertilizer (Sacramento et al., 2013).

Other studies also evaluated the use of the biomass of beach-cast algae to produce a compost, a subproduct made by decomposing organic material rich in plant nutrients an commonly used to improve the soil fertility. Illera-Vives et al. (2011) tested drift seaweed compost (mainly comprising *Laminaria* spp. and *Cystoseira* spp.) with possitive results for tomato's yield (diameter and weight) and lettuce's yield, which was recommended by the authors for improving horticultural crop yields. In Argentina, noticeably improved physical soil properties were observed with lower density and higher infiltration rates when beach-cast seaweeds based compost was added (Eyras and Rostagno, 1995).

Systematic harvest and compost derived seaweed wrack provide an environmentally sound alternative to land disposal, obtaining a product of agricultural value and controlling the dispersal of the invasive macroalga *U. pinnatifida* by reducing the load of fertile sporophylls on beaches (Eyras and Sar, 2003). The use of beach-cast algae is an ecologically correct alternative, making them an agricultural input that can improve soil structure and increase humus content (Weber-Qvartfort, 2016).

Composting has been also successfully used to manage waste algae removed from eutrophied aquatic environments (Han et al., 2014) and could be used as an environmental education tool (Bastos et al., 2019). In Brazil, it has also been recommended as an environmentally friendly practice that can help the local economy income. The production of compost using beach-cast seaweeds to grow gardens in a community of fishermen located in Praia da Penha, in João Pessoa, Brazil, was evaluated by Araújo (2016) with positive results regarding the quality of the compost, promoting environmental education through composting activities and contributing to local sustainable development.

Villares et al. (2016) alert to the fact of temporal concentration variability and possible unappropriated levels of heavy metals, which must be assessed carefully. One of the major concerns for the use of the beach-cast macroalga biomass as fertilizer is the presence of cadmium, harmful to human consumption, which can cause endocrine malfunctions in humans and is related to certain types of cancer (Pan et al., 2010; Dessle, 2017). To reduce the accumulation of cadmium in European soils, it was suggested to regulate maximum permissible cadmium concentration per kg of phosphorus in all mineral fertilizers and monitoring the cadmium concentrations from the beach-cast algae (Franzén et al., 2019).

Summarizing, the use of beach-cast algae as fertilizer and biostimulator seems to be one of the most viable ecologically and sustainable correct practices and widely possible in different countries around the world. However, it is necessary to keep in mind some important concerns such the quantification of heavy metals and salinity, the latter because salt excess may alter the soil health (Sacramento et al., 2013). In addition, the use of beach-cast seaweeds as fertilizer also provides important services by replacing the use of finite resources for a renewable resource, by mitigating eutrophication and increasing the quality of coastal zones.

7.6 Phycocolloids

The commercial exploitation of macroalgae-derived compounds is mainly related to phycocolloids (carrageenan, agars and alginates) for their gelling, emulsifying and thickening properties (Rosales-Mendoza et al., 2020). In Brazilian northeast, the beach-cast *Hypnea musciformis* (Wulfen) J.V. Lamouroux (now *Hypnea pseudomusciformis*), has been exploited and exported as raw material or processed for the production of kappa-carrageenan for years (Oliveira et al., 2002).

The carrageenan yield extracted from the beach-cast *H. musciformis* harvested in Piauí (Brazil) was evaluated by Oliveira Farias (2014) in order to use this phycocolloid in future food products. The beach-cast algae used for carrageenan extraction showed values of yield varying depending on the form of drying and bleaching step. However, those submitted to bleaching obtained lower yield in relation to those that did not undergo this process. In this way, the beach-cast algae dried in natural environment and not bleached concentrated greater carrageenan content.

Nowadays, the seaweed-derived substances that have received most attention from the scientific community are sulfated polysaccharides. These bioactive compounds are extracted from red algae (*e.g.* galactans, carrageenan, agar), brown algae (*e.g.* fucans, fucoidans) and green algae (*e.g.* ulvans, heterofucans) (Klongklaew et al., 2020). Their high-value is attributed to the expressive biological activity demonstrated, which is related to the number and location of sulfate groups in their structure (Besednova et al., 2019).

As sulfated polysaccharides are a complex metabolite composition, some studies improved the application by obtaining specific polysaccharides extracts. Obluchinskaya and Minina (2004) developed more effective methods for obtaining rich fucoidan-containing extracts of the beach-cast *Fucus vesiculosus* Linnaeus by serial extraction that yielded 88.15% of extract with aqueous:ethanol and then water extraction, leaving a residue of only 10%. Sodium alginate from fresh brown beach-cast seaweeds from Cuba was extracted for evaluating its performance and quality to be used in preparing a dental impression material in the form of paste. The physical-mechanical tests carried out in the paste had satisfactory results within established limits (García et al., 2000). Similarly, Abraham et al. (2019) extracted valuable bioproducts, such as alginate, fucoidan and laminarin from the beach-cast *D. potatorum* in a biorefinery process. Both studies show the possibility of improving the obtaining saccharides-rich extracts, which can be useful for assessing bioactivity properties.

The increasing demand for natural products has promoted great interest in the food, pharmaceutical and cosmetic industries, which make beach-cast seaweeds an ideal natural matrix to extract bioactive compounds for purposes of industrial application. The application of these rich-compounds (*e.g.* phycocolloids and sulfated polysaccharides) extracted from beach-cast algae as functional ingredients could offer opportunities for the development of new products, which have beneficial effects on human health.

7.7 Biological activity

Beach-cast seaweeds contain several substances, including polysaccharides, that make it possible to be used in several applications. Considering the presence of these metabolites mainly in macroalgae, it is not surprising that most bioactivity studies with beach-cast seaweeds are focused on these metabolites, especially in sulfated polysaccharides, since multiphase bioactivity is widely known.

The sulfated polysaccharides isolated containing fucose from the beach-cast *Sargassum wightii* Greville ex J. Agardh were able to provide bioactive actions, such as anticancer and immunomodulatory properties against the cell line HCT116 (Human Cell Tumor Colon) (Deepika, 2017).

The few studies with bioactive properties of sulfated polysaccharides from beach-cast macroalgae in Brazil were mainly performed with beach-cast algae harvested from the northeast of Brazil. Rodrigues et al. (2016) found that sulfated polysaccharides extracted from the Brazilian beach-cast *Halymenia pseudofloresia* (Clemente) C. Agardh presented stress-reducing effects on juvenile *Litopenaeus vanname* shrimps. Daily administration of small amounts of sulfated polysaccharides in the freshwater culture of adult shrimps was able to improve the survival rate after the induction of stress situations in the culture.

Anticoagulant activity from the genus *Halymenia* showed activity almost three times higher than commercial standard (Rodrigues et al., 2010) and antimicrobial activity from *C. racemosa* was efficient against *Enterococcus faecalis* and resistant strains of *Enterococcus* (Padilha, 2014). The latter study included a phytochemical screening in which saponins, alkaloids, chlorophyll, steroids and caulerpicin (a mixture of ceramides) were confirmed.

Similar to attached macroalgae, beach-cast seaweeds can be raw-material for extracting different bioactive polysaccharides depending on the taxonomic group. Agar, carrageenan, agaropectin, cellulose, xylans and mannans polysaccharides with D-galactose, D-fructose, 3,6-anhydro-D-galactose and glucose oligosaccharides are obtaining from red algae; fucoidan, laminaran, alginates, cellulose polysaccharides and mannitol, glucose, guluronate, mannuronate, glucoronate and sulphated fucose oligosaccharides from brown algae; ulvan, starch, xylopyranose, glucopyranose, xyloglucan, glucuronan, cellulose and hemicellulose polysaccharides and glucose, xylose, uronic acids, rhamnose and galactose olisaccharides from green algae (Mannein et al., 2018).

7.8 Bioremediation and bioadsorption properties

Macroalgae are used as biological indicators of heavy metal pollution and some studies have been carried out with beach-cast seaweeds to verify their potential to bind and remove heavy metals from liquid industrial residues, indicating the potential beach-cast algae biomass as excellent metal biosorbent.

An example performed in the northeast of Brazil studied the possibility to use beach-cast seaweeds to remove lead from battery factories industrial wastewater. The harvested beach-cast seaweeds removed up to 99% of lead from a synthetic solution with 4 g of dried and ground algae into 100 mL of solution, with average removal of 96% and 89% for dry samples (Duarte et al., 2001). The adsorptive capacity for the retention of lead in liquid media with beach-cast algae of the genus *Sargassum* on the coast of Pernambuco (Brazil) was evaluated, showing 85% lead adsorptive capacity in 30 min (Calado et al., 2003).

Cadmium is another toxic metal commonly present in industrial wastes. Beach-cast algae from Brazil showed a fast increase in cadmium adsorptive ability reaching equilibrium in 30 min (Silva, 2010). Aluminum removal was investigated in synthetic and natural wastewaters using beach-cast algae as adsorption material in Spain, including the influence of pH, metal concentration and aluminum elimination time (Lodeiro et al., 2010). Maximum aluminum adsorption was 14 mg g^{-1} for the harvested algae against 1.6 mg g^{-1} from activated carbon (Lodeiro et al., 2010). These studies evidence the possibility to apply beach-cast biomass in the treatment of industrial waste

In Brazil, pioneer studies have been carried out with beach-cast algae containing predominantly *Sargassum* and compared two adsorbent materials based on coconut shell active charcoal and sawdust as suitable adsorbent for dye removal in tinctures effluents (Koroishi et al. 2000). The adsorbent material choice will depend on the availability and costs of the available material. Experiences with methylene blue dye beach-cast seaweeds from Brazil were able to remove 97% of the dye (Silva Filho, 2020).

Another possibility as bioremediator is the adsorption of petroleum-derived contaminants. Experiments to purify aquatic effluents with petroleum-derived contaminants were performed in Brazil with beach-cast *Sargassum* biomass (Medeiros, 2017). Concentrations of 3% and 6% of *Sargassum* resulted in 100% adsorptive capacity in the first minutes, while 9% of *Sargassum* removed around 80% of the contaminant. Up to our knowledge, this is the first report describing efficient adsorption of petroleum-derived, which significantly expand the usefulness of beach-cast algae.

For being a low-cost, abundant and naturally occurring renewable source, the removal of heavy metals through beach-cast seaweeds may be interesting for industrial applications on a large scale (Wersal and Madsen, 2012). In summary, the bioremediation of heavy metals, industrial dyes, petroleum-derived and eutrophication from industrial waste effluents by beach-cast macroalgae, related to human activities or natural disasters, open the possibility to use this renewable natural resource with a large amount of available biomass for generating added-value and product processing outcomes of an underused waste feedstock. The exploitation of beach-cast seaweeds as bioadsorbents can enhance the productive chain because it is a low-cost alternative, economically competitive, low aggression potential to the environment (ecofriendly), public policy solution for algae blooms and beach cleaning and socially sustainable by generating additional income for coastal communities.

8. Socio-economic benefits

Outcomes from added-value and product processing of an underused waste feedstock as the beach-cast algae biomass can be an additional source of income for the local population. During cold periods as early winter in Norway and Canada, the harvesting of beach-cast algae is reduced and the algae collectors return to fishing and processing lobster activities (Rebours et al., 2014), enabling the complementary activities between beach-cast algae harvesting and fishing extractivism operations.

Similarly, in France and Spain, the harvest of beach-cast seaweeds can be carried out throughout the year by local communities, supplementing their incomes to the fishing (Alban and Boncoeur, 2004). In Brazil, the same situation of additional incomes is reported from the "*algueiras*" (women dedicated to algiculture and algae collection), but there are still no regulations on limitation of area or quotas for beach-cast algae biomass and the harvests are mostly handmade.

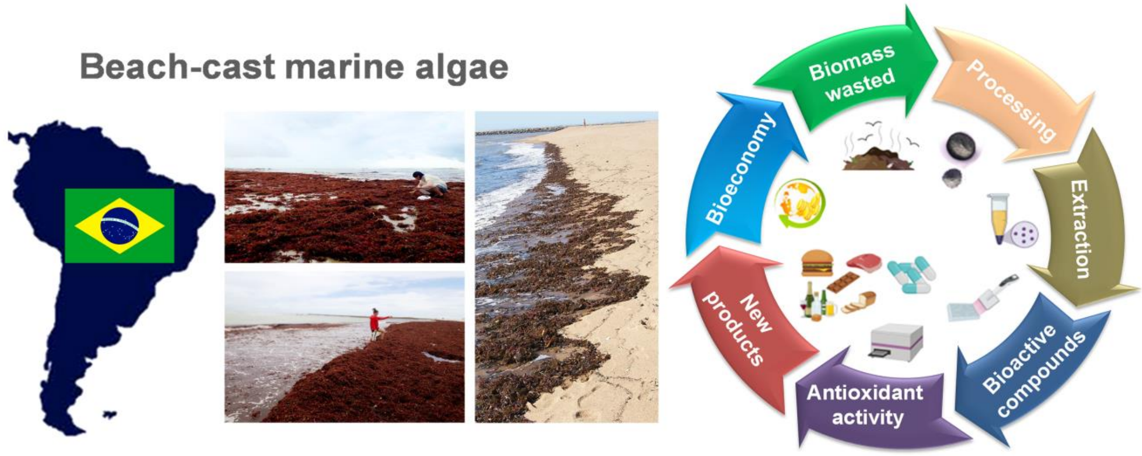
On a small scale, the beach-cast algae harvested can contribute to generate livelihoods for the local population if used in small food processing cooperatives, where it could be incorporated into feed mixtures or marketed as meal according to the seasonal availability (Wischnat, 2013).

Excluding the socio-economic impacts associated with big companies, which allow the cleaning of coastal areas and employ a large amount of labor force, at small- and medium-scale for local coastline communities the activity can enhance their income by collecting the excessive biomass of beach-cast macroalgae. Thereby they can sell the algae as raw-material for different companies or even improve the added-value through the generation of algae-based products or their production benefits. The integration of the local population of fishermen and algimen not only leads to higher well-being of local residents but could also generate more income to the region from tourism (Malm et al., 2004). In turn, the harvest of beach-cast seaweeds proves to be an important activity to not only mitigate problems of eutrophication, disable use of beaches and public health, but also to stimulate the economy and provide job opportunities.

Chapter II

Antioxidant activity and related chemical quantification of extracts from Brazilian beach-cast marine algae: a potential sustainable valorization of underused waste biomass

Graphical abstract



Abstract

Beach-cast marine algae are a potential biomass for several biofunctional products and present in large volumes in some coastal regions due to natural current processes or drifted algae like pelagic *Sargassum*. Antioxidant activity and chemical composition of methanolic and aqueous extracts from fifteen materials of beach-cast marine algae from the Brazilian Coast were evaluated. In general, the highest antioxidant activities were found in extracts from brown macroalgae followed by the extracts of red algae, and the lowest activities were detected in the green beach-cast algae. The concentrations of phenolic compounds and carbohydrates exhibited a positive correlation with the antioxidant activities of the tested extracts. To the best of our knowledge, this is one of the few worldwide studies concerning beach-cast seaweeds and antioxidant activity and related antioxidant metabolites. This study suggests that these algae are potential sources for obtaining extracts with antioxidant properties, rich in phenolic compounds and sulfated carbohydrates. Beach-cast macroalgae are unused biomass and the beneficial utilization of this biomass for prospection of natural products and functional foods is suggest.

1. Introduction

Seaweeds have been used in many countries as food and traditional medicine since ancient times (Pérez-Lloréns et al., 2020). Macroalgae are classified into three groups, Phaeophyceae (brown algae), Rhodophyta (red algae) and Chlorophyta (green algae), mainly inhabiting the marine environment under continuous abiotic factor changes. These stressing conditions can result in the synthesis and accumulation of active metabolites against oxidative stress (Maschek and Baker, 2008), some of them with high-value antioxidants for a broad variety of applications, especially in food.

The incorporation of macroalgae in the human diet with antioxidant properties have attracted the interest of food industries and other commercial sectors due to the substantial benefit to prevent oxidation processes (*e.g.* oxidation food damage) and oxidative stress-related diseases (*e.g.* neurodegenerative, inflammatory, cardiovascular diseases and aging) (Hrelia and Angeloni, 2020; Peñalver et al., 2020).

In addition, macroalgae are low in calories and can be added to meat products, such as burgers, frankfurters, sausages and steaks, among others (Peñalver et al., 2020). The incorporation of macroalgae or byproducts can acts as antioxidants preserving the quality of products and as fat substitutes developing low-fat products (Gullón, et al., 2020).

According to Ferdouse et al. (2018), in 2017 30.4 million tonnes of seaweed were collect, being 29.4 million from aquaculture and 1.1 million harvested from the field. Brazil has short participation in this amount; however, the Brazilian littoral has great potential for collecting beach-cast marine algae.

The beach-cast marine alga are often those organisms that naturally come off the substrate, arrive and accumulate at the beaches driven by the turbulence of the sea by currents, winds and tides (do Santos Nascimento et al., 2013). In recent years, several studies have been carried out in floating seaweed clumps, especially on ecological approaches.

Tonnes of macroalgae are launched on the beaches of certain regions along the Brazilian coast and could be used in several commercial sectors, but are often burned or buried by local governments due to the bad smell caused by the deterioration of the organic matter, which affect the tourism and keep away the users of coastal environments. By contrast, beach-cast seaweeds can be reused due to their high nutritional quality and chemical diversity and converted into applications for several sectors, production of packaging, supplementation, fertilizers, food, feed, nutraceuticals, cosmetics and even for the discovery of new drugs, among others.

Nowadays, beach-cast algae are already sold to supplement nutrition and as fertilizers, some companies elaborate nutritional supplements as brown seaweed flour that can be incorporated into diets for cattle and poultry (Wischnat, 2013).

Beach-cast algae have the advantage of being a renewable, economically viable and abundant resource. The use of this resource is an eco-efficient production that favors society and the environment, generating usable resources that were previously seen as limited and "garbage", in addition to mitigating pollution in coastal regions and sustainably encouraging socio-economic development.

The worldwide perspective of population growth indicates an accelerated and continuous increase in the next decades, which should enhance the general demand for food. The United Nations (2019) expects an increase of 2 billion people by 2050, especially in developed countries, promoting, consequently, the increase of marketable supplies and demands in the search for expansion alternatives of several industrial sectors, especially food industry. Regarding this scenario, beach-cast seaweeds represent a potential raw material that can contribute to biofunctional and bioactive properties with wide application in food (Peñalver et al., 2020).

Therefore, the existence of compounds with antioxidant activity, which are part of the composition of many products used in the food industry, the main objective was to analyze the antioxidant potential and chemical composition of crude extracts of beach-cast marine algae from the Brazilian coast, to highlight potential new natural biomass that can be used as a matrix for several ingredients for the food industry and other applications.

2. Material and methods

2.1 Biological material and extraction procedure

The procedures for collecting and extraction of the biological material are described in section 4. GENERAL MATERIAL AND METHODS, item 4.1. *Collection and selection of species* and item 4.3. *Extraction procedure*.

2.2 Antioxidant potential

Antioxidant activities were assessed based on simple spectrophotometric and colorimetric *in vitro* methods: DPPH (1,1-diphenyl-2-picrylhydrazyl) free-radical scavenger activity (Pires et al., 2017a; Santos et al., 2019), capture of the ABTS (2,2'-azinobis-(3-ethylbenzthiazolin-6-sulfonic acid) radical (Torres et al., 2017; Santos et al., 2019), metal chelator activity (Harb et al., 2016; Santos et al., 2019) and ferric reducing antioxidant power (FRAP) (Urrea-Victoria et al., 2016; Santos et al., 2019). Lyophilized methanolic and aqueous extracts were dissolved in DMSO 10% solution (n = 5), antioxidant tested in 96-well microplates with final volume of 300 μL and the respective absorbance of each assay read in a UV-vis microplate spectrophotometer (Epoch Biotek, USA).

Standard curves of gallic acid, Trolox and phloroglucinol were assessed as there are different equivalent units in the literature and conversion factors were calculated according to gallic acid equivalent (GAE) (Table 3). GAE was used as reference substance and the results were expressed in mg.GAE.g^{-1} of lyophilized algal extract. One concentration of crude extract (600 µg.mL^{-1} or 1000 µg.mL^{-1}) was initially tested for each antioxidant assay and material, depending on the response of antioxidant activity, other concentrations above or below were chosen in order to evaluate the EC_{50} (half maximal effective concentration). The EC_{50} was calculated using a sigmoidal dose-response fit model curves (Chen et al., 2013) with the software GraphPad Prism v. 6.01 and the results were expressed as 1 divided by the value of EC_{50} (1/EC_{50}; mg.mL^{-1}). Therefore, a higher 1/EC_{50} indicates better antioxidant activity.

For an overview of the total antioxidant activities of methanolic and aqueous extracts, the Total Antioxidant Capacity (TAC) was assessed as described in Seeram et al. (2008) based on results of 1/EC_{50} (mg.mL^{-1}) for DPPH, ABTS and chelator assays and antioxidant level (mg.GAE.g^{-1}) for FRAP assay. Zero values were attribute to species that was not able to calculate 1/EC_{50}. The highest value of 1/EC_{50} or mg.GAE.g⁻

[1] per extract and assay was assigned as 100% and the following percentages calculated according to this ratio.

DPPH

The measurement of the DPPH radical sequestering activity of the extracts was performed according to Pires et al. (2017a) and Santos et al. (2019). The method is based on the capture of the DPPH radical by antioxidants, producing a decrease in absorbance. In the assay, an oxy-reduction reaction occurs, where the DPPH radical, which presents violet coloration, is reduced, becoming the reaction yellow and the formation of reduced and stable DPPH-H is made.

Aliquots of 20 µL of samples (extracts, standards, or negative controls) and 280 µL of a methanolic solution of DPPH (Sigma-Aldrich, Brazil) 32 µg.mL^{-1} (80 µM; MW = 394.32 g.mol^{-1}) were added to each well. The microplate was incubated at 25 °C for 20 min at room temperature and then the absorbance was measured at 517 nm.

ABTS

The ABTS free radical capture assay was performed according to the method of Torres et al. (2017) and Santos et al. (2019). The total antioxidant method by the free radical capture ABTS is one of the most used and it is based on the capture of the ABTS radical. With this methodology, it is possible to measure the antioxidant activity of hydrophilic and lipophilic substances (Rufino et al., 2007).

The solution of the ABTS was prepared by mixing 1 mL of ABTS (7 mM) with 17.6 µL of potassium persulfate (140 mM). The solution reacted for 16 h at room temperature. The absorbance of the solution was adjusted to 0.8 at 734 nm by diluting in methanol (1:60 v/v). In each well of the microplate, 20 µL of samples was added (extracts, standards, or negative controls) and 280 µL of ABTS solution (pH 6.7). Absorbance was measured at 734 nm after 20 min at room temperature.

Chelator

Metals are also capable of generating free radicals. In the literature, there are several methods to estimate antioxidant activity, among them the ability to chelate transition metals. The chelating activity of iron ions II (Fe^{2+}) was performed according to the methodology of Harb et al. (2016) and Santos et al. (2019). Ferrozine is a compound frequently used in the determination of Fe^{2+}. In the presence of this ion, ferrozine forms a pink complex whose absorbance can be determined. When there is a presence of chelating agents, less Fe^{2+} ions will be available for complex formation with ferrozine, which will induce a decrease in absorbance.

For the metal chelating assay, aliquots of 20 µL of samples (extracts, standards, or negative controls), 250 µL of 10% ammonium acetate, and 15 µL of ammonium sulfate solution 1 mM were added into each well. After 5 min, 15 µL of ferrozine 6.1 mM was added and the reactive mixture was incubated for 10 min at room temperature under stirring and then the absorbance readings were taken at 562 nm.

FRAP

The antioxidant activity through the FRAP (Ferric Reducing Antioxidant Power) reduction was based on the method of Urrea-Victoria et al. (2016) and Santos et al. (2019). The FRAP method emerged as an alternative to determine the iron reduction in biological fluids and aqueous solutions of pure compounds. At acid pH, the ferric tripyridyl hydrazine complex (Fe^{3+} TPTZ) is reduced, producing an intense blue color, which can be monitored by measuring the absorbance. The change in absorbance is directly related to the total reducing power of the electron-donating antioxidants present in the reaction mixture (Tandon et al., 2008).

The FRAP reagent solution was prepared by mixing 25 mL of acetate buffer (0.3 M; pH 3.6), 2.5 mL of TPTZ (10 mM in 40 mM hydrochloric acid) and 2.5 mL of ferric chloride 20 mM. Aliquots of 20 µL of samples (extracts, standards, or negative controls), 15 µL de ultrapure water and 265 µL of FRAP reagent solution were added to each well. The microplate was incubated at 37 °C for 30 min and the absorbance readings were measured at 595 nm.

2.3 Total phenolic compound, total carbohydrate and sulfur content

The evaluation of phenolic compounds of the algal extracts was performed by the Folin-Ciocalteu method, following Pires et al. (2017b) and Santos et al. (2019). This test is a simple spectrophotometric method and is based on the reducing property of some substances having a reducing power, such as sugars and phenolic compounds. Folin-Ciocalteu reagent, a phosphotungstic-phosphomolybdic acid mixture, react with phenolic compounds or reducing substances, under alkaline conditions achieved with the addition of sodium carbonate (Na_2CO_3), the dissociation of a proton leads to the formation of the phenolate anion. The anion is more willing to donate electrons and can reduce the reactant, in other words, has a higher reducing capacity, thus forming a molybdenum blue complex (Huang et al., 2005).

For this assay, aliquots of 20 µL of samples (extracts, standards, or negative controls), 200 µL ultrapure water, 20 µL of Folin-Ciocalteu reagent and 60 µL of a saturated solution of sodium carbonate were added to each well. Absorbance was measured at 760 nm after 30 min at 25 °C.

Standards curves of phloroglucinol, gallic acid and Trolox were performed and conversion factors were calculated according to phloroglucinol equivalent (PGE) (Table 3). PGE was used as a reference substance and the results were expressed in mg.PGE.g^{-1} of lyophilized algal extract.

For the quantification of total soluble carbohydrates, the phenol-sulfuric acid colorimetric method of Masuko et al. (2005) was performed. In this method, the reaction of carbohydrates with sulfuric acid produces furfural derivatives, which when reacted with the phenol develops a yellow color.

The methanolic and aqueous extracts were solubilized in 10% DMSO at an initial concentration of 5 mg.mL^{-1}. Quantification of total carbohydrates was performed for a final concentration of 200 µg.mL^{-1} of extract. The assay was performed with three technical replicates (n = 3).

Aliquots of 50 µL of samples (extracts, standards, or negative controls), 150 µL of sulfuric acid 98% and 30 µL of phenol 5% were added to each well. The microplate was incubated at 90 °C for 5 min and the absorbance readings were measured at 490 nm.

Standard curves of galactose (GAL), glucose and fucose were performed and conversion factors were calculated according to GAL equivalent (Table 3). GAL was used as a reference substance and the results were expressed in µg equivalent of GAL per mass of lyophilized algal extract (µg.GAL.mg^{-1}).

Analysis of the sulfur content were performed according to Torres et al. (2018) adapted from Dodgson and Price (1962). This procedure was chosen as a method to estimate the sulfation of hydrolyzed (that confer the amount of total sulfur) and non-hydrolyzed (free sulfur content) samples that could be related to the content of sulfated polysaccharides. Lyophilized methanolic and aqueous extracts were dissolved in HCl

0.5 N at the concentration of 10 $mg.mL^{-1}$ (n = 5) and placed in a dry bath at 100 °C for 2 h (hydrolyzed samples). Another part of the extracts was solubilized at the same concentration (10 $mg.mL^{-1}$) in 958 µL of ultrapure water, without heating, and just before the analysis 42 µL of HCl 0.5 N was added (non-hydrolyzed samples).

All samples were centrifuged and the supernatant was analyzed. Aliquots of 50 µL of the turbidimetric reagent (5 $mg.mL^{-1}$ animal gelatin, 10 $mg.mL^{-1}$ barium chloride, 10 $mg.mL^{-1}$ sodium chloride), 125 µL of ultrapure water and 25 µL of the hydrolyzed or non-hydrolyzed sample were added to each well. The microplate was placed under agitation for 5 min at 200 rpm, after resting for 15 min, the absorbance readings were measured at 405 nm.

Standard curve of sodium sulfate was performed in concentrations from 200 to 600 $µg.mL^{-1}$ (y = 0.0052x + 0.0703; R^2 =0.97) and data were expressed in percentage of esterified sulfur, obtain by the difference between hydrolyzed sample and non-hydrolyzed sample.

$$\text{ES}\ (\%) = \text{H}\ (\%) - \text{NHS}\ (\%)$$

where: ES - esterified sulfur; NHS - non-hydrolyzed sample; HS – hydrolyzed sample,

Table 3. Summary of the respective standard curve for the different antioxidant assays, phenolic compounds and carbohydrates specifying standard concentration range (μg.mL^{-1}), linear equation (y = ax + b), regression coefficient (r^2). Conversion factors (CF) were calculated in relation to gallic acid for antioxidant assays, phloroglucinol for phenolic compounds and galactose for carbohydrates.

Assay	Gallic acid	Trolox	Phloroglucinol
DPPH*	0.5 – 3.0 (μg.mL^{-1}) y = -0.1707x + 0.7533 R^2 = 0.9883 CF = 1	2 – 10 (μg.mL^{-1}) y = -0.0445x + 0.7808 R^2 = 0.9907 CF = 3.83	20 – 100 (μg.mL^{-1}) y = -0.006x + 0.7122 R^2 = 0.9954 CF = 28.45
ABTS*	0.5 – 2.0 (μg.mL^{-1}) y = -0.3411x + 0.7657 R^2 = 0.9900 CF = 1	1 – 5 (μg.mL^{-1}) y = -0.0995x + 0.7835 R^2 = 0.9801 CF = 3.42	0.25 – 1.75 (μg.mL^{-1}) y = -0.3595x + 0.704 R^2 = 0.9830 CF = 0.95
Chelator*	2 – 10 (μg.mL^{-1}) y = -0.0686x + 0.8592 R^2 = 0.9808	**	**
FRAP*	0.5 – 3.5 (μg.mL^{-1}) y = 0.193x + 0.086 R^2 = 0.994 CF = 1	1.5 – 8.0 (μg.mL^{-1}) y = 0.0869x + 0.0572 R^2 = 0.9819 CF = 2.22	20 – 100 (μg.mL^{-1}) y = 0.0123x - 0.0198 R^2 = 0.9803 CF = 15.69
Phenolic compounds[1]	2 – 12 (μg.mL^{-1}) y = 0.0763x + 0.0534 R^2 = 0.9926 CF = 0.16	20 – 100 (μg.mL^{-1}) y = 0.0109x + 0.0697 R^2 = 0.9900 CF = 1.15	20 – 60 (μg.mL^{-1}) y = 0.0125x + 0.029 R^2 = 0.9901 CF = 1
Assay	**Galactose**	**Glucose**	**Fucose**
Carbohydrates[2]	15 – 75 (μg.mL^{-1}) y = 0.0439x – 0.0258 R^2 = 0.985 CF = 1	15 – 75 (μg.mL^{-1}) y = 0.0324x + 0.0611 R^2 = 0.995 CF = 1.26	15 – 195 (μg.mL^{-1}) y = 0.0085x - 0.0217 R^2 = 0.977 CF = 5.22

* For antioxidant activity, to convert a value of GAE (gallic acid equivalent) to Trolox or phloroglucinol equivalent multiply the value by the conversion factor (CF) and from Trolox or phloroglucinol into GAE divided by CF.

** Not detected

[1] For phenolic compounds, to convert a value of phloroglucinol equivalent to Trolox or GAE multiply the value by the CF and from Trolox or GAE into phloroglucinol divided by CF.

[2] For carbohydrates, to convert a value of galactose equivalent to glucose or fucose multiply the value by the CF and from glucose or fucose into galactose divided by CF.

2.4 Data analysis

Statistical analysis was carried out from the extraction of five subsamples, considered as technical replication with Statistica 10 software. Data were tested for normality (Kolmogorov-Smirnov test) and homoscedasticity (Bartlett's test) previous one-way analysis of variance (ANOVA) to compare the species ($p < 0.05$). When differences were detected, Newman-Keuls *post-hoc* multiple comparison test was applied.

Additionally, a clustered correlation matrix (Pearson correlation coefficient, r) by Euclidean distance was associated with a heatmap graphic for assessing the multiple paired comparisons for species and dependent variables (antioxidant potential and chemical composition). The Pearson correlation coefficient values were categorized based on Callegari-Jacques (2003): r = 0.10 to 0.30, weak; r = 0.40 to 0.6, moderate; r = 0.70 to 0.90, strong; r = 0.90 to 1, very strong.

3. Results

The fifteen samples of beach-cast algae collected from the northeast and southeast of the Brazilian coast were identified as four representatives of brown macroalgae (Ochrophyta, Phaeophyceae), eight of red macroalgae (Rhodophyta), one of green macroalga (Chlorophyta) and two Mix samples including a mixture of different species of macroalgae.

Results of one-way ANOVA analysis for extract yield values from methanolic and aqueous extracts showed significant p values < 0.005 (Table 4). The highest extract yields were registered in the methanolic extracts, which ranged from 2.55 ± 0.13% to 29.00 ± 1.22% and in the aqueous extracts, which ranged from 9.51 ± 0.42% to 58.50 ± 6.11% (Table 5). The percentage of crude extract yield with other solvents, like hexane, dichloromethane and ethyl acetate, was lower than 1.90%. Therefore, only methanolic and aqueous extracts were evaluated for antioxidant activity and chemical quantification.

Table 4. Statistical ANOVA results for all evaluated parameters for extract yields (%), antioxidant activity (DPPH, ABTS, Chelator and FRAP assays; mg.GAE.g^{-1}), 1/EC$_{50}$ (mg.mL^{-1}), phenolic compounds (mg.PGE.g^{-1}), carbohydrates (mg.GAL.g^{-1}) and sulfation degree (%) of methanolic and aqueous extracts. SS – square sum, DF – degree freedom, MS – medium square, *F* – statistic index, *p* – probability. Bold *p* values represent statistical differences by one-way ANOVA ($p < 0.05$).

	SS	DF	MS	*F*	*p*	SS	DF	MS	*F*	*p*
	Yield (Methanolic extracts)					Yield (Aqueous extracts)				
Intercept	10365.97	1	10365.97	690.03	**<0.005**	41207.08	1	41207.38	1182.47	**<0.005**
Species	4355.23	14	311.09	207.07	**<0.005**	9755.92	14	696.85	19.99	**<0.005**
Error	90.14	60	1.50			1916.66	55	34.85		
	DPPH GAE (Methanolic extracts)					DPPH GAE (Aqueous extracts)				
Intercept	242.05	1	242.05	7779.18	**<0.005**	217.58	1	217.58	14183.61	**<0.005**
Species	110.21	14	7.87	253.014	**<0.005**	154.50	14	11.03	719.39	**<0.005**
Error	1.27	41	0.03			0.62	41	0.01		
	ABTS GAE (Methanolic extracts)					ABTS GAE (Aqueous extracts)				
Intercept	244.50	1	244.50	21593.36	**<0.005**	394.31	1	394.31	33033.54	**<0.005**
Species	54.58	14	3.89	344.31	**<0.005**	120.19	14	8.58	719.26	**<0.005**
Error	0.49	44	0.01			0.56	47	0.01		
	Chelator GAE (Methanolic extracts)					Chelator GAE (Aqueous extracts)				
Intercept	1920.27	1	1920.27	6367.74	**<0.005**	3535.67	1	3535.67	4373.09	**<0.005**
Species	777.40	14	55.52	184	**<0.005**	1947.15	14	139.08	172.02	**<0.005**
Error	15.07	50	0.30			38.00	47	0.80		
	FRAP GAE (Methanolic extracts)					FRAP GAE (Aqueous extracts)				
Intercept	402.83	1	402.83	26576.88	**<0.005**	476.41	1	476.41	27964.65	**<0.005**
Species	123.22	14	8.80	580.71	**<0.005**	161.59	14	11.54	677.54	**<0.005**
Error	0.63	42	0.01			0.71	42	0.01		
	DPPH 1/EC$_{50}$ (Methanolic extracts)					DPPH 1/EC$_{50}$ (Aqueous extracts)				
Intercept	43.83	1	43.83	38139.72	**<0.005**	80.04	1	80.04	8510.24	**<0.005**
Species	0.35	7	0.05	44.07	**<0.005**	2.47	7	0.35	37.62	**<0.005**
Error	0.01	16	0.00			0.15	16	0.00		
	ABTS 1/EC$_{50}$ (Methanolic extracts)					ABTS 1/EC$_{50}$ (Aqueous extracts)				
Intercept	159.90	1	159.90	5045.85	**<0.005**	145.46	1	145.46	6312.812	**<0.005**
Species	19.47	11	1.77	55.85	**<0.005**	8.44	9	0.93	40.70	**<0.005**
Error	0.76	24	0.03			0.46	20	0.02		
	Chelator 1/EC$_{50}$ (Methanolic extracts)					Chelator 1/EC$_{50}$ (Aqueous extracts)				
Intercept	47.01	1	47.01	7567.89	**<0.005**	164.16	1	164.16	1530.99	**<0.005**
Species	1.20	5	0.24	38.68	**<0.005**	11.03	6	1.83	17.14	**<0.005**
Error	0.07	12	0.00			1.50	14	0.10		
	Phenolic compounds (Methanolic extracts)					Phenolic compounds (Aqueous extracts)				
Intercept	1530.30	1	15328.30	2982.25	**<0.005**	163205.10	1	163205.1	16241.98	**<0.005**
Species	771.0	14	5507.40	1071.68	**<0.005**	71933.00	14	5138.1	511.34	**<0.005**
Error	241.5	47	5.1			401.9	40	10.00		
	Carbohydrates (Methanolic extracts)					Carbohydrates (Aqueous extracts)				
Intercept	69052.10	1	69052.10	330.92	**<0.005**	875483.10	1	875483.10	6766.13	**<0.005**
Species	49520.48	14	3537.17	169.19	**<0.005**	605796.50	14	43271.20	334.41	**<0.005**
Error	689.90	33	20.90			4528.70	35	129.40		
	Sulfation degree (Methanolic extracts)					Sulfation degree (Aqueous extracts)				
Intercept	73.79	1	73.79	1967.58	**<0.005**	554.41	1	554.41	1970.82	**<0.005**
Species	90.08	14	6.43	116.00	**<0.005**	177.80	14	12.70	45	**<0.005**
Error	3.32	35	0.09			10.40	37	0.28		

Table 5. Extract yields (%) in hexane (Hex), dichloromethane (DCM), ethyl acetate (ETAC), methanol (MeOH) and aqueous (80 °C hot water) of lyophilized crude extracts of the selected species of beach-cast marine algae harvested from Ceará (CE), Espírito Santo (ES) and Paraíba (PB) states (Mean ± SD; $n = 5$). Statistical analysis was applied for methanolic and aqueous extracts with letters indicating significant differences ($p < 0.05$) among the species according to one-way ANOVA and Newman-Keuls *post-hoc* test.

	Yield (%)				
Species	**Hex** (- polar)	**DCM**	**ETAC**	**MeOH**	**80 °C Water** (+ polar)
Ochrophyta (brown algae)					
Dictyopteris jolyana (ES)	0.86 ± 0.02	1.41 ± 0.14	1.00 ± 0.31	4.49 ± 0.07^{f}	30.04 ± 4.81^{bc}
Dictyopteris jolyana (PB)	0.81 ± 0.09	0.35 ± 0.08	0.21 ± 0.01	2.66 ± 0.40^{g}	29.67 ± 1.80^{bc}
Dictyopteris polypodioides (PB)	0.74 ± 0.08	0.77 ± 0.04	0.31 ± 0.03	2.55 ± 0.13^{g}	9.51 ± 0.42^{f}
Zonaria tournefortii (ES)	0.96 ± 0.21	1.90 ± 0.40	0.86 ± 0.05	4.12 ± 0.89f	12.02 ± 1.25^{f}
Rhodophyta (red algae)					
Agardhiella ramosissima (ES)	1.05 ± 0.03	0.79 ± 0.05	0.46 ± 0.01	24.72 ± 2.86^{b}	34.87 ± 2.97^{bc}
Alsidium seaforthii (ES)	0.93 ± 0.08	1.63 ± 0.29	0.78 ± 0.09	13.06 ± 0.74^{d}	11.75 ± 4.16^{f}
Alsidium triquetrum (CE)	0.47 ± 0.05	0.57 ± 0.12	0.50 ± 0.03	14.85 ± 2.46^{c}	34.71 ± 2.61^{c}
Botryocladia occidentalis (CE)	0.22± 0.03	0.48 ± 0.01	0.45 ± 0.03	29.00 ± 1.22^{a}	15.20 ± 3.75^{ef}
Gracilaria domingensis (CE)	0.18 ± 0.04	0.49 ± 0.03	0.43 ± 0.01	11.79 ± 0.44^{d}	58.50 ± 6.11^{a}
Osmundaria obtusiloba (ES)	0.46 ± 0.03	1.06 ± 0.10	0.64 ± 0.03	15.02 ± 0.39^{c}	24.12 ± 8.75^{de}
Osmundaria obtusiloba (PB)	0.63 ± 0.12	0.64 ± 0.10	0.24 ± 0.28	12.56± 0.28^{d}	10.40 ± 3.05^{f}
Spyridia clavata (ES)	0.38 ± 0.04	0.59 ± 0.15	0.57 ± 0.13	5.68 ± 0.20^{f}	21.34 ± 2.21^{de}
Chlorophyta (green algae)					
Codium isthmocladum (ES)	0.38 ± 0.12	0.22 ± 0.07	0.45 ± 0.07	15.96 ± 2.04^{c}	36.92 ± 0.21^{bc}
Mix 1 (CE)	0.80 ± 0.15	0.81 ± 0.08	0.48 ± 0.05	10.46 ± 0.86^{e}	20.73 ± 0.71^{de}
Mix 2 (CE)	0.46 ± 0.07	1.04 ± 0.05	0.48 ± 0.01	11.80 ± 0.46^{de}	19.91 ± 1.05^{de}

The antioxidant potential of methanolic and aqueous extracts by DPPH, ABTS, Chelator and FRAP *in vitro* assays is shown in Table 6. Regarding methanolic extracts, *O. obtusiloba* (ES), *S. clavata* (ES), *D. jolyana* (ES and PB), *Z. tournefortii* (ES) and *A. seaforthii* (ES) showed the best results for DPPH, which ranged from 3.17 ± 0.08 mg.GAE.g^{-1} to 4.10 ± 0.02 mg.GAE.g^{-1}. In the ABTS assay, *Z. tournefortii* (ES), *D. jolyana* (ES and PB), *D. polypodioides* (PB) and *O. obtusiloba* (ES and PB) had the best responses between 2.72 ± 0.07 mg.GAE.g^{-1} to 3.16 ± 0.03 mg.GAE.g^{-1}. For the chelator assay, the best activities were observed for *D. jolyana* (PB and ES), *O. obtusiloba* (ES), *Z. tournefortii* (ES), *O. obtusiloba* (PB) and *D. polypodioides* (PB), which ranged from 8.63 ± 0.25 mg.GAE.g^{-1} to 10.80 ± 0.53 mg.GAE.g^{-1}. The brown alga *D. jolyana* (PB and ES) had a better response for the FRAP assay, followed by *O. obtusiloba* (ES), *D. polypodioides* (PB) and *Z. tournefortii* (ES) with values from 3.85 ± 0.16 mg.GAE.g^{-1} to 6.99 ± 0.10 mg.GAE.g^{-1}.

For the aqueous extracts (Table 6), *O. obtusiloba* (PB), *Z. tournefortii* (ES), *S. clavata* (ES), *O. obtusiloba* (ES), *D. jolyana* (PB and ES) showed the best activities in DPPH assay, with a range from 3.59 ± 0.12 mg.GAE.g^{-1} to 4.09 ± 0.14 mg.GAE.g^{-1}. For ABTS, *D. jolyana* (PB) and *O. obtusiloba* (PB) were highlighted followed by *D. jolyana* (ES), *Z. tournefortii* (ES), *D. polypodioides* (PB) and *O. obtusiloba* (ES) with results from 3.25 ± 0.11 mg.GAE.g^{-1} to 4.97 ± 0.04 mg.GAE.g^{-1}. Regarding chelator assay, *O. obtusiloba* (PB), *D. jolyana* (PB), *Z. tournefortii* (ES), *D. jolyana* (ES) and *O. obtusiloba* (ES) showed the highest activities with a range from 12.27 ± 0.49 mg.GAE.g^{-1} to 16.79 ± 2.09 mg.GAE.g^{-1}. For FRAP assay, *O. obtusiloba* (PB), *D. jolyana* (PB) and *Z. tournefortii* (ES) showed the best responses which ranged from 4.63 ± 0.35 mg.GAE.g^{-1} to 5.71 ± 0.19 mg.GAE.g^{-1}. All antioxidant activities showed significant differences among the species for the respective antioxidant assay for methanolic and aqueous extracts (Table 4).

The results of 1/EC_{50} for the different antioxidant assays per extract and species are described in Table 7. For methanolic extracts, 1/EC_{50} values for the DPPH assay ranged from 1.18 ± 0.01 mg.mL^{-1} to 1.56 ± 0.02 mg.mL^{-1}. For the ABTS test, the 1/EC_{50} results ranged from 1.08 ± 0.02 mg.mL^{-1} to 3.57 ± 0.02 mg.mL^{-1}. Finally, for chelator assay the values ranged between 1.18 ± 0.01 mg.mL^{-1} to 1.86 ± 0.03 mg.mL^{-1}. Regarding aqueous extract (Table 7), the 1/EC_{50} for the DPPH assay ranged from 1.13 ± 0.02 mg.mL^{-1} to 2.22 ± 0.02 mg.mL^{-1}. For the ABTS test, the 1/EC_{50} results ranged from 1.16 ± 0.06 mg.mL^{-1} to 3.20 ± 0.03 mg.mL^{-1}. Finally, for chelator assay, the values ranged between 2.16 ± 0.03 mg.mL^{-1} to 4.31 ± 0.04 mg.mL^{-1}. For FRAP assay and some species, the 1/EC_{50} could not be calculated because the antioxidant activity was lower than 50% for both extracts. All estimated 1/EC_{50} showed significant differences among the species for the respective antioxidant assay for methanolic and aqueous extracts (Table 4).

Table 6. Antioxidant activity (mg.GAE.g^{-1}) for DPPH, ABTS, Chelator and FRAP *in vitro* assays of beach-cast marine algae from Ceará (CE), Espírito Santo (ES) and Paraíba (PB) states for methanolic and aqueous extracts (Mean ± SD; *n* = 5). Letters indicate significant statistical differences ($p < 0.05$) among the species according to one-way ANOVA and Newman-Keuls *post-hoc* test per extract and assay separately.

Species / Assay	DPPH	ABTS	Chelator	FRAP
Methanolic extracts				
Ochrophyta (brown algae)				
Dictyopteris jolyana (ES)	3.61 ± 0.26[bc]	3.08 ± 0.06[ab]	9.33 ± 0.48[b]	5.22 ± 0.16[b]
Dictyopteris jolyana (PB)	3.45 ± 0.11[cd]	2.93 ± 0.15[bc]	10.80 ± 0.53[a]	6.99 ± 0.10[a]
Dictyopteris polypodioides (PB)	2.86 ± 0.15[e]	2.92 ± 0.10[bc]	8.63 ± 0.25[b]	3.93 ± 0.20[cd]
Zonaria tournefortii (ES)	3.37 ± 0.14[cd]	3.16 ± 0.03[a]	8.94 ± 0.51[b]	3.85 ± 0.16[d]
Rhodophyta (red algae)				
Agardhiella ramosissima (ES)	0.52 ± 0.02[g]	0.37 ± 0.15[g]	1.99 ± 0.34[e]	0.85 ± 0.10[k]
Alsidium seaforthii (ES)	3.17 ± 0.08[d]	1.87 ± 0.14[f]	7.01 ± 0.03[c]	3.26 ± 0.11[e]
Alsidium triquetrum (CE)	0.50 ± 0.04[g]	1.88 ± 0.16[f]	4.29 ± 0.04[d]	2.30 ± 0.18[i]
Botryocladia occidentalis (CE)	0.59 ± 0.08[g]	0.53 ± 0.06[g]	1.31 ± 0.04[e]	0.32 ± 0.01[l]
Gracilaria domingensis (CE)	0.58 ± 0.01[g]	2.36 ± 0.05[e]	1.21 ± 0.06[e]	1.29 ± 0.17[j]
Osmundaria obtusiloba (ES)	4.10 ± 0.02[a]	2.83 ± 0.09[c]	9.17 ± 0.45[b]	4.09 ± 0.02[c]
Osmundaria obtusiloba (PB)	2.82 ± 0.09[e]	2.72 ± 0.07[d]	8.80 ± 0.22[b]	3.17 ± 0.05[ef]
Spyridia clavata (ES)	3.86 ± 0.42[ab]	1.98 ± 0.03[f]	6.83 ± 0.53[c]	2.69 ± 0.13[h]
Chlorophyta (green algae)				
Codium isthmocladum (ES)	0.35 ± 0.02[g]	0.40 ± 0.01[g]	2.07 ± 0.34[e]	0.98 ± 0.04[k]
Mix 1 (CE)	1.09 ± 0.14[f]	2.46 ± 0.15[e]	1.37 ± 0.04[e]	2.81 ± 0.03[gh]
Mix 2 (CE)	1.22 ± 0.05[f]	2.05 ± 0.07[f]	1.80 ± 0.04[e]	2.99 ± 0.01[fg]
Aqueous extracts				
Ochrophyta (brown algae)				
Dictyopteris jolyana (ES)	3.59 ± 0.12[b]	3.91 ± 0.11[c]	13.33 ± 0.80[bc]	3.94 ± 0.20[cd]
Dictyopteris jolyana (PB)	3.71 ± 0.18[b]	4.97 ± 0.04[a]	16.69 ± 1.29[a]	5.48 ± 0.06[a]
Dictyopteris polypodioides (PB)	2.61 ± 0.15[d]	3.40 ± 0.08[d]	10.95 ± 0.20[d]	3.49 ± 0.04[d]
Zonaria tournefortii (ES)	3.82 ± 0.21[b]	3.77 ± 0.19[c]	14.24 ± 0.30[b]	4.63 ± 0.35[b]
Rhodophyta (red algae)				
Agardhiella ramosissima (ES)	1.09 ± 0.01[e]	1.97 ± 0.19[f]	4.09 ± 0.84[g]	2.20 ± 0.45[g]
Botryocladia occidentalis (CE)	0.06 ± 0.01[f]	0.81 ± 0.14[i]	2.79 ± 0.69[ghi]	2.25 ± 0.09[g]
Alsidium seaforthii (ES)	3.15 ± 0.16[c]	3.05 ± 0.07[e]	5.90 ± 0.29[f]	2.59 ± 0.06[f]
Alsidium triquetrum (CE)	0.07 ± 0.03[f]	0.55 ± 0.06[j]	1.15 ± 0.21[i]	3.94 ± 0.29[c]
Gracilaria domingensis (CE)	0.25 ± 0.09[f]	2.95 ± 0.12[e]	1.99 ± 0.03[hi]	0.59 ± 0.03[h]
Osmundaria obtusiloba (ES)	3.71 ± 0.07[b]	3.25 ± 0.11[d]	12.27 ± 0.49[c]	3.94 ± 0.23[c]
Osmundaria obtusiloba (PB)	4.09 ± 0.14[a]	4.22 ± 0.11[b]	16.79 ± 2.09[a]	5.71± 0.19[a]
Spyridia clavata (ES)	3.81 ± 0.11[b]	2.91 ± 0.11[e]	8.92 ± 1.02[e]	2.75 ± 0.20[f]
Chlorophyta (green algae)				
Codium isthmocladum (ES)	0.15 ± 0.06[f]	0.14 ± 0.04[k]	1.27 ± 0.05[i]	2.19 ± 0.17[g]
Mix 1 (CE)	0.07 ± 0.09[f]	1.22 ± 0.05[h]	2.12 ± 0.04[hi]	3.03 ± 0.13[e]
Mix 2 (CE)	0.21 ± 0.03[f]	1.71 ± 0.07[g]	3.64 ± 0.03[gh]	3.88 ± 0.04[cd]

Table 7. Antioxidant activity expressed as $1/EC_{50}$ ($mg.mL^{-1}$) of beach-cast seaweeds from Ceará (CE), Espírito Santo (ES) and Paraíba (PB) states for methanolic and aqueous extracts (Mean ± SD; *n* = 5). Letters indicate significant statistical differences ($p < 0.05$) among the species according to one-way ANOVA and Newman-Keuls *post-hoc* test. *$1/EC_{50}$ not detected due to activity lower than 50%.

Species / Assay	DPPH	ABTS	Chelator
Methanolic extracts			
Ochrophyta (brown algae)			
Dictyopteris jolyana (ES)	1.56 ± 0.02[a]	1.90 ± 0.02[c]	1.85 ± 0.04[a]
Dictyopteris jolyana (PB)	1.32 ± 0.01[b]	1.76 ± 0.06[c]	1.78 ± 0.01[a]
Dictyopteris polypodioides (PB)	1.18 ± 0.01[c]	1.99 ± 0.01[c]	1.18 ± 0.01[d]
Zonaria tournefortii (ES)	1.35 ± 0.03[b]	1.94 ± 0.03[c]	1.61 ± 0.04[b]
Rhodophyta (red algae)			
Agardhiella ramosissima (ES)	*	*	*
Alsidium seaforthii (ES)	1.51 ± 0.03[a]	1.08 ± 0.05[d]	*
Alsidium triquetrum (CE)	*	1.72 ± 0.03[c]	*
Botryocladia occidentalis (CE)	*	*	*
Gracilaria domingensis (CE)	*	2.94 ± 0.04[b]	*
Osmundaria obtusiloba (ES)	1.27 ± 0.01[b]	1.62 ± 0.01[c]	1.86 ± 0.03[a]
Osmundaria obtusiloba (PB)	1.29 ± 0.01[b]	2.79 ± 0.02[b]	1.37 ± 0.02[c]
Spyridia clavata (ES)	1.30 ± 0.01[b]	1.08 ± 0.02[d]	*
Chlorophyta (green algae)			
Codium isthmocladum (ES)	*	*	*
Mix 1 (CE)	*	3.22 ± 0.02[b]	*
Mix 2 (CE)	*	3.57 ± 0.02[a]	*
Aqueous extract			
Ochrophyta (brown algae)			
Dictyopteris jolyana (ES)	2.07 ± 0.01[ab]	3.20 ± 0.03[a]	3.16 ± 0.01[b]
Dictyopteris jolyana (PB)	2.06 ± 0.03[ab]	2.41 ± 0.20[b]	2.57 ± 0.01[bc]
Dictyopteris polypodioides (PB)	1.13 ± 0.02[e]	2.21 ± 0.20[b]	2.16 ± 0.03[c]
Zonaria tournefortii (ES)	1.79 ± 0.02[cd]	2.50 ± 0.02[b]	4.31 ± 0.04[a]
Rhodophyta (red algae)			
Agardhiella ramosissima (ES)	*	*	*
Botryocladia occidentalis (CE)	*	*	*
Alsidium seaforthii (ES)	1.59 ± 0.04[d]	2.23 ± 0.30[b]	*
Alsidium triquetrum (CE)	*	*	*
Gracilaria domingensis (CE)	*	*	*
Osmundaria obtusiloba (ES)	1.76 ± 0.02[cd]	2.20 ± 0.20[b]	2.43 ± 0.03[bc]
Osmundaria obtusiloba (PB)	2.22 ± 0.02[a]	2.54 ± 0.10[b]	2.57 ± 0.01[bc]
Spyridia clavata (ES)	1.92 ± 0.04[bc]	1.88 ± 0.03[c]	2.20 ± 0.06[c]
Chlorophyta (green algae)			
Codium isthmocladum (ES)	*	*	*
Mix 1 (CE)	*	1.16 ± 0.06[e]	*
Mix 2 (CE)	*	1.49 ± 0.11[d]	*

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Table 7. Continued from previous page

Standard references			
Gallic acid[1]	460.38	611.11	179.52
Trolox[1]	119.15	264.92	
Phloroglucinol[1]	18.16		
Alfa-tocopherol[2]	3.22		
BHT[2]	6.25		
Ascorbic acid[2]	11.11		
BHA[2]	16.66		

[1]Present study; [2]Zubia et al. (2007).

Total antioxidant capacity (TAC) represents the global antioxidant activity covering all antioxidant assays, which is shown in Figure 7. For methanolic extracts (Fig. 7A), the top-five most efficient beach-cast algae with antioxidant properties were *D. jolyana* (PB – 62.82%), *D. jolyana* (ES – 60.46%), *O. obtusiloba* (PB – 53.39%), *Z. tournefortii* (ES – 52.15%) and *O. obtusiloba* (ES – 51.28%), whereas the lowest TAC index (< 5%) were registered for the red algae *B. occidentalis* (CE – 1.14%) and *A. ramosissima* (ES – 3.04%) and the green alga *C. isthmocladum* (ES – 3.51%) (Fig. 7A). For the aqueous extract (Fig. 7B), the top-five best species with the highest TAC index values (> 60%) were *Z. tournefortii* (ES – 79.46%), *D. jolyana* (ES – 78.48%), *O. obtusiloba* (PB – 78.36%), *D. jolyana* (PB – 74.81%) and *O. obtusiloba* (ES – 63.70%), whereas the lowest index was for *G. domingensis* (CE – 2.11%) (Fig. 7B).

The percentages of calculated TAC index for antioxidant assays in methanolic and aqueous extracts are shown in Table 8. For methanolic extracts, the antioxidant activity considering all species was better evaluated by the ABTS (48.21%) and FRAP (42.67%) assays. Regarding aqueous extracts, the best activities were obtained by the methods FRAP (48.27%), DPPH (43.73%) and ABTS (41.26%). Considering the average of the total TAC of all species and assays, it was possible to observe that aqueous extracts were more efficient than methanolic, reaching 40.82% and 34.57% of TAC, respectively.

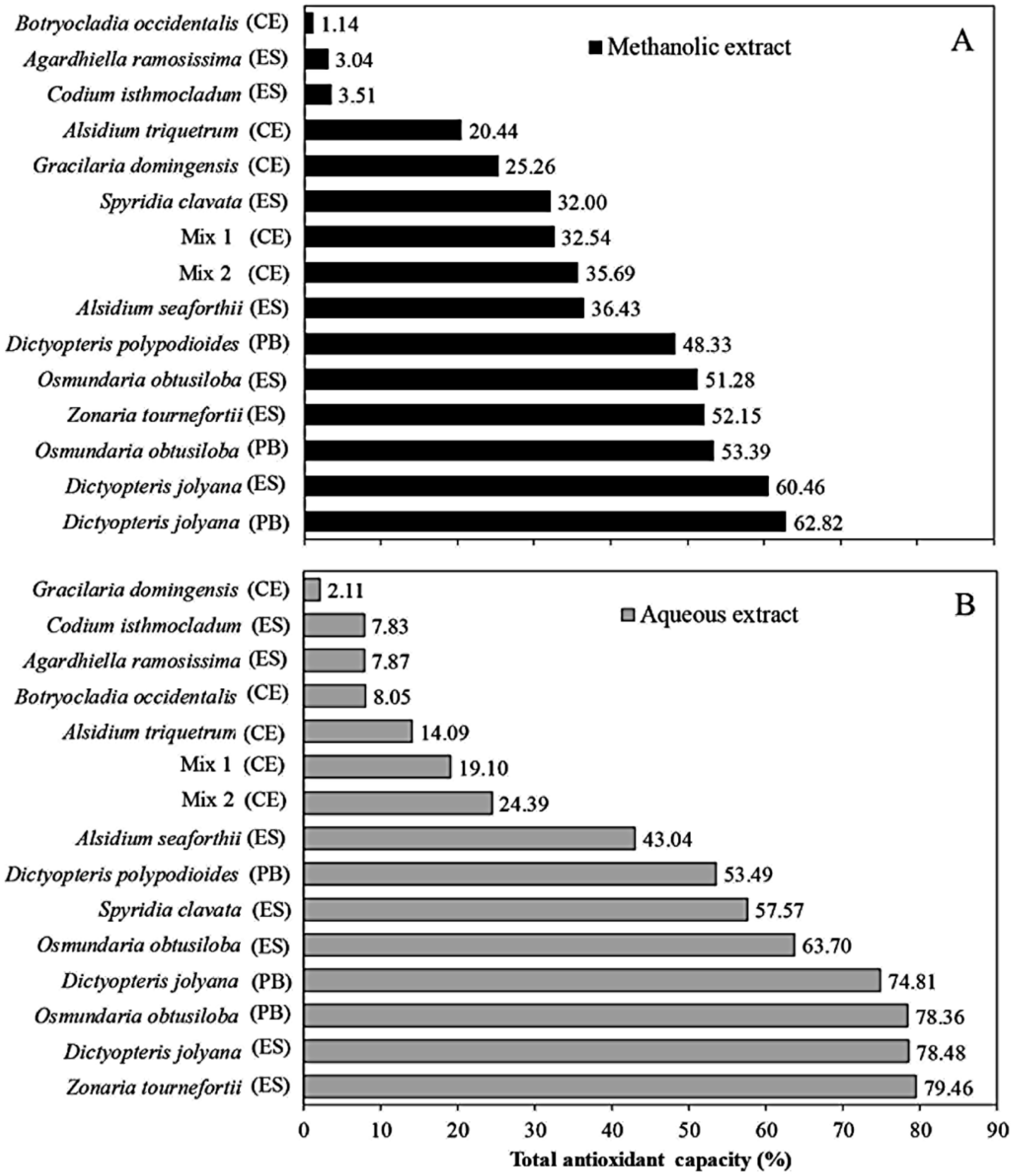

Figure 7. Ranking of total antioxidant capacity (TAC) percent of the selected beach-cast marine algae for (A) methanolic and (B) aqueous extracts (Mean; *n* = 5) from Ceará (CE), Espírito Santo (ES) and Paraíba (PB) states, considering the $1/EC_{50}$ ($mg.mL^{-1}$) for DPPH, ABTS and chelator and FRAP ($mg.GAE.g^{-1}$) *in vitro* assays.

Table 8. Summary of the percentage of the total capacity antioxidant (TAC, %) of beach-cast seaweed extracts (methanolic and aqueous) from Ceará (CE), Espírito Santo (ES) and Paraíba (PB) states based on the antioxidant activity results expressed in $1/EC_{50}$ ($mg.mL^{-1}$) for DPPH, ABTS and Chelator assays and gallic acid equivalent ($mg.GAE.g^{-1}$) for FRAP assay.

	TAC by assay (%)				
Species / Assay	**DPPH**	**ABTS**	**Chelator**	**FRAP**	**TAC by species (%)**
Methanolic extracts					
Ochrophyta (brown algae)					
Dictyopteris jolyana (ES)	70.45	53.80	42.91	74.68	60.46
Dictyopteris jolyana (PB)	59.75	50.02	41.52	100.00	62.82
Dictyopteris polypodioides (PB)	53.21	56.44	27.43	56.22	48.33
Zonaria tournefortii (ES)	60.94	55.09	37.49	55.08	52.15
Rhodophyta (red algae)					
Agardhiella ramosissima (ES)	0.00	0.00	0.00	12.16	3.04
Alsidium seaforthii (ES)	68.36	30.72	0.00	46.64	20.44
Alsidium triquetrum (CE)	0.00	48.86	0.00	32.90	36.43
Botryocladia occidentalis (CE)	0.00	0.00	0.00	4.58	1.14
Gracilaria domingensis (CE)	0.00	82.60	0.00	18.45	25.26
Osmundaria obtusiloba (ES)	57.22	46.04	43.35	58.51	51.28
Osmundaria obtusiloba (PB)	57.37	79.00	31.83	45.35	53.39
Spyridia clavata (ES)	58.81	30.69	0.00	38.48	32.00
Mix 1 (CE)	0.00	89.95	0.00	40.21	32.54
Mix 2 (CE)	0.00	100.00	0.00	42.78	35.69
Chlorophyta (green algae)					
Codium isthmocladum (ES)	0.00	0.00	0.00	14.02	3.51
Mean	32.41	48.21	14.97	42.67	34.57
Aqueous extracts					
Ochrophyta (brown algae)					
Dictyopteris jolyana (ES)	93.38	90.74	73.45	53.37	78.48
Dictyopteris jolyana (PB)	92.85	68.36	59.64	78.40	62.82
Dictyopteris polypodioides (PB)	51.00	62.77	50.24	49.93	48.33
Zonaria tournefortii (ES)	80.86	70.75	100.00	66.24	52.15
Rhodophyta (red algae)					
Agardhiella ramosissima (ES)	0.00	0.00	0.00	31.47	3.04
Alsidium seaforthii (ES)	71.90	63.22	0.00	37.05	20.44
Alsidium triquetrum (CE)	0.00	0.00	0.00	56.37	36.43
Botryocladia occidentalis (CE)	0.00	0.00	0.00	32.19	1.14

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Table 8. Continued from previous page

Gracilaria domingensis (CE)	0.00	0.00	0.00	8.44	25.26
Osmundaria obtusiloba (ES)	79.53	62.37	56.54	56.37	63.70
Osmundaria obtusiloba (PB)	100.00	72.11	59.64	81.69	78.36
Spyridia clavata (ES)	86.44	53.43	51.08	39.34	57.57
Mix 1 (CE)	0.00	33.04	0.00	43.35	19.10
Mix 2 (CE)	0.00	42.07	0.00	55.51	24.39
Chlorophyta (green algae)					
Codium isthmocladum (ES)	0.00	0.00	0.00	31.33	7.83
Mean	43.73	41.26	30.04	48.27	40.82

The contents of total phenolic compounds, soluble carbohydrates and sulfation degree are shown in Table 9. The top-five highest content of phenolic compounds for methanolic extracts was registered in the beach-cast seaweeds *D. jolyana* (PB), *O. obtusiloba* (PB), *Z. tournefortii* (ES), *O. obtusiloba* (ES) and *D. jolyana* (ES), ranging between 77.30 ± 0.99 mg.PGE.g^{-1} to 141.55 ± 6.35 mg.PGE.g^{-1}. Regarding aqueous extract, the top-five phenolic compounds contents were higher for *D. jolyana* (PB) and *O. obtusiloba* (PB), followed by *Z. tournefortii* (ES), *D. polypodioides* (PB) and *D. jolyana* (ES), with values ranged between 69.51 ± 2.35 mg.PGE.g^{-1} to 118.51 ± 1.20 mg.PGE.g^{-1}.

Regarding carbohydrate content (Table 9), the species that showed better results in methanolic extracts were *D. jolyana* (ES and PB), *D. polypodioides* (PB), *O. obtusiloba* (PB), *A. seaforthii* (ES), *O. obtusiloba* (ES) and *Z. tournefortii* (ES), with values varying among 49.48 ± 4.06 µg.GAL.mg^{-1} to 95.19 ± 9.73 µg.GAL.mg^{-1}. For aqueous extracts, *D. jolyana* (ES), *Z. tournefortii* (ES), *D. jolyana* (PB), *S. clavata* (ES) and *O. obtusiloba* (ES) showed the highest carbohydrate content, with values of 123.89 ± 15.32 µg.GAL.mg^{-1} to 416.91 ± 27.07 µg.GAL.mg^{-1}. All carbohydrate contents showed differences among the species for methanolic and aqueous extracts (Table 4).

The sulfation degree (Table 9) of methanolic extracts was highlighted for the red alga *O. obtusiloba* (ES and PB) and the brown algae *D. jolyana* (ES) with results among 2.64 ± 0.91% to 5.10 ± 0.08%. In respect of aqueous extract, sulfation degree showed high values for *S. clavata* (ES), *G. domingensis* (CE), *A. ramosissima* (ES), *O. obtusiloba* (ES) and *D. polypodioides* (PB), with data ranging from 4.39 ± 0.97% to 7.88 ± 1.00%. All sulfation degree values showed significant differences among the species for methanolic and aqueous extracts (Table 4).

Table 9. Phenolic compounds (mg.PGE.g^{-1}), carbohydrates (µg.GAL.mg^{-1}) and sulfation degree (%) of the beach-cast seaweeds from Ceará (CE), Espírito Santo (ES) and Paraíba (PB) states for methanolic and aqueous extracts (Mean ± SD; *n* = 5). Letters indicate significant statistical differences ($p < 0.05$) among the species according to one-way ANOVA and Newman-Keuls *post-hoc* test per extract and assay separately.

Species / Parameter	Phenolic compounds	Carbohydrates	Sulfation degree
Methanolic extracts			
Ochrophyta (brown algae)			
Dictyopteris jolyana (ES)	77.30 ± 0.99[e]	95.19 ± 9.73[a]	2.64 ± 0.91[c]
Dictyopteris jolyana (PB)	141.55 ± 6.35[a]	93.80 ± 13.38[a]	1.00 ± 0.25[def]
Dictyopteris polypodioides (PB)	58.59 ± 0.64[f]	74.36 ± 4.55[b]	0.05 ± 0.11[h]
Zonaria tournefortii (ES)	89.85 ± 1.66[c]	49.48 ± 4.06[d]	1.54 ± 0.25[d]
Rhodophyta (red algae)			
Agardhiella ramosissima (ES)	36.52 ± 3.49[h]	7.60 ± 0.86[f]	0.49 ± 0.08[fgh]
Alsidium seaforthii (ES)	46.69 ± 3.56[g]	57.86 ± 2.49[cd]	1.39 ± 0.27[de]
Alsidium triquetrum (CE)	20,49 ± 0.35[j]	11.27 ± 0.64[f]	0.87 ± 0.14[efg]
Botryocladia occidentalis (CE)	12.30 ± 0.29[k]	3.95 ± 0.37[f]	0.13 ± 0.02[gh]
Gracilaria domingensis (CE)	28.15 ± 4.39[i]	9.23 ± 0.88[f]	0.10 ± 0.05[gh]
Osmundaria obtusiloba (ES)	82.20 ± 3.33[d]	53.81 ± 1.88[d]	5.10 ± 0.08[a]
Osmundaria obtusiloba (PB)	117.56 ± 3.48[b]	63.75 ± 3.41[c]	3.34 ± 0.25[b]
Spyridia clavata (ES)	62.15 ± 2.15[f]	34.92 ± 2.29[e]	0.39 ± 0.19[fgh]
Chlorophyta (green algae)			
Codium isthmocladum (ES)	19.17 ± 1.60[j]	6.02 ± 1.20[f]	0.42 ± 0.05[fgh]
Mix 1 (CE)	22.97 ± 0.49[ij]	5.35 ± 1.24[f]	0.40 ± 0.10[fgh]
Mix 2 (CE)	25.53 ± 0.56[ij]	6.13 ± 0.44[f]	0.54 ± 0.16[fgh]
Aqueous extracts			
Ochrophyta (brown algae)			
Dictyopteris jolyana (ES)	69.51 ± 2.35[e]	416.91 ± 27.07[a]	3.33 ± 0.11[d]
Dictyopteris jolyana (PB)	118.51 ± 1.20[a]	258.70 ± 9.57[c]	1.21 ± 0.21[f]
Dictyopteris polypodioides (PB)	79.82 ± 2.95[d]	84.25 ± 13.72[gf]	4.39 ± 0.97[bc]
Zonaria tournefortii (ES)	98.42 ± 2.42[c]	354.66 ± 8.59[b]	1.15 ± 0.43[f]
Rhodophyta (red algae)			
Agardhiella ramosissima (ES)	8.82 ± 2.64[m]	99.27 ± 6.86[f]	4.85 ± 0.49[b]
Alsidium seaforthii (ES)	55.89 ± 3.01[g]	100.41 ± 12.32[f]	2.09 ± 0.32[ef]
Alsidium triquetrum (CE)	20.84 ± 0.79[k]	74.45 ± 10.69[g]	3.14 ± 0.97[de]
Botryocladia occidentalis (CE)	6.46 ± 0.83[m]	64.82 ± 7.27[gh]	1.66 ± 0.77[f]
Gracilaria domingensis (CE)	14.71 ± 1.35[l]	47.58 ± 3.30[h]	5.17 ± 1.03[b]
Osmundaria obtusiloba (ES)	59.60 ± 2.90[f]	123.89 ± 15.32[e]	4.51 ± 0.26[bc]
Osmundaria obtusiloba (PB)	105.90 ± 2.85[b]	81.99 ± 9.89[gf]	2.22 ± 0.12[ef]
Spyridia clavata (ES)	45.96 ± 2.79[h]	157.91 ± 14.08[d]	7.88 ± 1.00[a]
Chlorophyta (green algae)			
Codium isthmocladum (ES)	5.36 ± 1.15[m]	49.28 ± 5.75[h]	1.32 ± 0.22[f]
Mix 1 (CE)	35.39 ± 2.79[j]	18.81 ± 2.14[i]	3.68 ± 0.22[cd]
Mix 2 (CE)	39.67 ± 1.32[i]	70.23 ± 3.15[hg]	2.88 ± 0.35[de]

The hierarchical clustering Euclidean distance was performed for similarity comparison of antioxidant potential and chemical composition for the methanolic and aqueous extracts (separately) (Fig. 8). The species with the best response for the parameters tested in methanolic extracts were grouped by the brown beach-cast seaweeds and the red species *O. obtusiloba* (PB and ES), *A. seaforthii* (ES) and *S. clavata* (ES) (Fig. 8A). They were arranged according to higher results in most of the parameters analyzed, except for *S. clavata* and *D. polypodioides* for sulfation degree and *S. clavata* and *A. seaforthii* for ABTS assay. The sulfation degree was the most distant descriptor.

According to the values of Pearson's correlation coefficient described in Figure 9, for methanolic extracts, the data show a positive strong–very strong correlation (between r-0.78–0.96; $p<0.05$) for most of the antioxidant assays, amount of carbohydrates and phenolic compounds for the methanolic extracts, except for some pair-comparison for DPPH, ABTS and chelator assays and carbohydrates. Sulfation degree showed a moderate correlation with the other parameter and r-values from 0.35 to 0.55.

The hierarchical clustering Euclidean distance for the aqueous extracts (Fig. 9B), the same species grouped for methanolic extracts were arranged together for aqueous extracts and these species showed the best results in the analyzed parameters, except for some species in FRAP assay and sulfation degree. The sulfation degree was again the most distant descriptor.

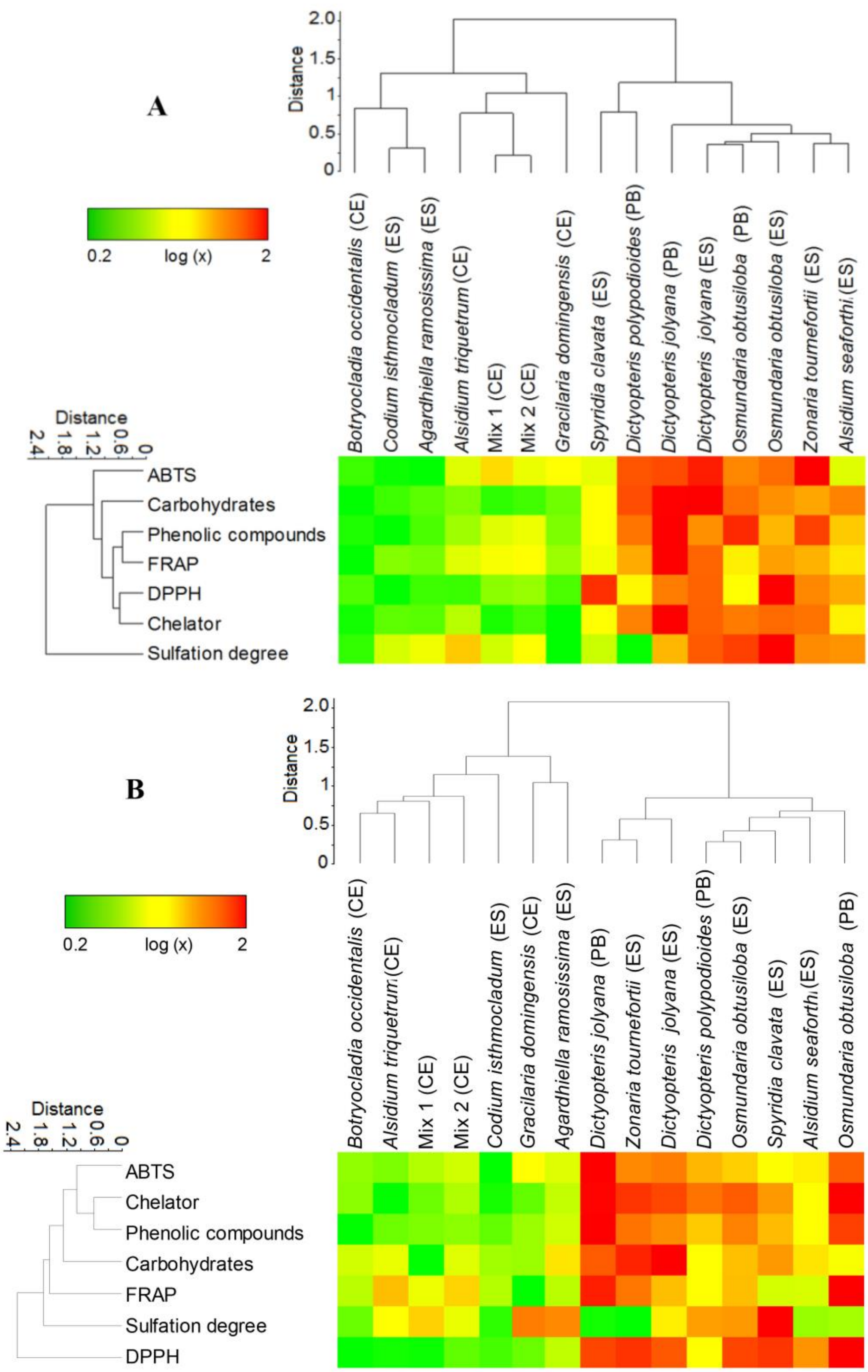


Figure 8. Hierarchical analysis of cluster Euclidean distance associated to the heatmap representation with the responses of the (A) methanolic and (B) aqueous extracts of all parameters analyzed with beach-cast macroalgae species from Ceará (CE), Espírito Santo (ES) and Paraíba (PB) states of the Brazilian coast.

	ABTS	Chelator	FRAP	Phenolics	Carbohydrates	Sulfation
DPPH	r = 0.73 R^2= 0.539 *p* = 0.002	r = 0.85 R^2= 0.716 *p*< 0.05	r = 0.79 R^2= 0.622 *p*< 0.05	r = 0.91 R^2= 0.821 *p*< 0.05	r = 0.88 R^2= 0.778 *p*< 0.05	r = 0.53 R^2= 0.286 *p* = 0.040
ABTS		r = 0.59 R^2= 0.344 *p* = 0.022	r = 0.85 R^2= 0.720 *p*< 0.05	r = 0.88 R^2= 0.775 *p*< 0.05	r = 0.66 R^2= 0.438 *p* =0.007	r = 0.35 R^2 = 0.126 *p* > 0.05
Chelator			r = 0.76 R^2= 0.582 *p* = 0.001	r = 0.81 R^2= 0.655 *p*< 0.05	r = 0.96 R^2= 0.914 *p*< 0.05	r = 0.63 R^2= 0.398 *p* = 0.0120
FRAP				r = 0.91 R^2= 0.837 *p*< 0.05	r = 0.78 R^2= 0.615 *p* =0.001	r = 0.56 R^2= 0.318 *p* = 0.028
Phenolics					r = 0.84 R^2= 0.714 *p*< 0.05	r = 0.53 R^2= 0.283 *p* = 0.041
Carbohydrates						r = 0.55 R^2= 0.298 *p* = 0.035

No correlation
Weak (0.10 to 0.30)
Moderate (0.40 to 0.60)
Strong (0.70 to 0.90)
Very strong (0.90 to 1)

Figure 9. Statistical parameters for the Pearson's correlation coefficient (p < 0.05) between antioxidant activities from the different assays and chemical quantification for methanolic extracts of the beach-cast seaweeds. Correlations ranges: r = 0.10 to 0.30 (weak); r = 0.40 to 0.60 (moderate); r = 0.70 to 0.90 (strong); r = 0.90 to 1 (very strong).

Regarding aqueous extract and Pearson's correlation coefficients, a positive strong–very strong correlation (between r-0.70–0.93, p < 0.05) for most of the antioxidant assays (DPPH and Chelator), phenolic compounds and carbohydrates (Fig. 10). Correlation from FRAP assay showed weak (r-0.24) and moderate correlations (r-0.42-0.57). A positive strong correlation was also observed between DPPH (r-0.74) or chelator (r-0.72) or phenolic compounds (r-0.70) and carbohydrate content. The sulfation degree showed no correlation or weak correlation with the other parameters.

	ABTS	Chelator	FRAP	Phenolics	Carbohydrates	Sulfation
DPPH	r = 0.77 R^2 = 0.592 *p* = 0.001	r = 0.91 R^2 = 0.835 *p* < 0.05	r = 0.42 R^2 = 0.172 *p* >0.05	r = 0.93 R^2 = 0.857 *p* < 0.05	r = 0.74 R^2 = 0.553 *p* = 0.001	r = 0.05 R^2 = 0.002 *p* >0.05
ABTS		r = 0.82 R^2 = 0.672 *p* < 0.05	r = 0.24 R^2 = 0.057 *p* >0.05	r = 0.78 R^2 = 0.608 *p* = 0.010	r = 0.54 R^2 = 0.291 *p* = 0.037	r = 0.22 R^2 = 0.048 *p* >0.05
Chelator			r = 0.57 R^2 = 0.324 *p* = 0.024	r = 0.92 R^2 = 0.846 *p* < 0.05	r = 0.72 R^2 = 0.518 *p* = 0.002	r = -0.06 R^2 = 0.003 *p* >0.05
FRAP				r = 0.54 R^2 = 0.291 *p* = 0.034	r = 0.44 R^2 = 0.193 *p* >0.05	r = -0.36 R^2 = 0.1296 *p* >0.05
Phenolics					r = 0.70 R^2 = 0.490 *p* = 0.003	r = -0.05 R^2 = 0.002 *p* >0.05
Carbohydrates						r = -0.20 R^2 = 0.040 *p* >0.05

No correlation
Weak (0.10 to 0.30)
Moderate (0.40 to 0.60)
Strong (0.70 to 0.90)
Very strong (0.90 to 1)

Figure 10. Statistical parameters for the Pearson's correlation coefficient ($p < 0.05$) between antioxidant activities from the different assays and chemical quantification for aqueous extracts of the beach-cast seaweeds. Correlations ranges: r = 0.10 to 0.30 (weak); r = 0.40 to 0.60 (moderate); r = 0.70 to 0.90 (strong); r = 0.90 to 1 (very strong).

4. Discussion

Algal secondary metabolites of polar and non-polar nature have great interest due to their bioactive properties. Among the prominent polar metabolites from methanolic extracts in marine algae, there are monosaccharides, heterosides, amino acids, sulfonic, dicarboxylic and tricarboxylic acids, phenolic compounds, polar terpenoids, polyketides like acetogenins and others (Esquivel-Hernández et al., 2017). Some compounds such as mycosporine-like amino acids (MAAs) are found mainly in red algae and phlorotannins are generally rich in brown macroalgae (Shibata et al., 2008). Aqueous crude extracts present as major components sulfated polysaccharides (Dobrinčić et al., 2020) that differ depending the group of algae (Phaeophyceae, Rhodophyta and Chlorophyta). Metabolites of non-polar nature include fatty acids, glycolipids, terpenes and steroids, among others. Although these metabolites have wide bioactivity describe as antiviral, antitumor, antifungal and other activities (Mayer et al., 2013), there is a major limitation due to low yield, which makes prospection or more in-depth studies difficult.

The extract yields obtained in this study with hexane, dichloromethane and ethyl acetate were low (lower than 1%) when compared to methanolic and aqueous extracts, which justifies our targeting with these last one's extracts. Similar results were obtained by Araújo et al. (2020) studying the red macroalga *Kappaphycus alvarezii* (Doty) Doty ex P.C. Silva. Additionally, the highest extract yields obtained for methanolic and aqueous extracts for our selected species of beach-cast marine algae suggest that the main matrix of secondary metabolites is composed of polar components. These compounds may be involved in the defense processes in macroalgae, suggesting that these metabolites, or at least some of them, may have an important role in the antioxidant results observed for the beach-cast seaweeds.

Sulfated polysaccharides, with polar nature, have been widely studied in macroalgae due to their bioactivity and also as a source of antioxidants that can be used as a functional ingredient in many applications to obtain functional foods (Porse and Rudolph, 2017). Brown algae synthesize fucans and fucoidans as sulfated polysaccharides, while red algae synthesize carrageenan and agar sulfated galactans and green algae are characterized by high levels of heterofucans (de Jesus Raposo et al., 2015). The fact that most of these polysaccharides are water-soluble compounds and are present in the external matrix of the cell wall makes extraction with high temperature a necessary procedure. Besides, the increase of temperature in the extraction methods allows the development of faster and more efficient extraction of target compounds of interest.

Therefore, the highest yield percentages obtained from our aqueous beach-cast extracts than methanolic extracts can be explained by the cell wall polysaccharides composition, which can constitute at least 50% of dry weight in macroalgae. Additionally, the increased amount of polyssacahrides can be favored by molecule accelation diffusion and higher solubility of solutes at high temperature, characteristic for phycocoloid extraction. It has been previously demonstrated in the literature that the use of water at high temperatures is a valuable tool for the extraction of metabolites in macroalgae, such as polysaccharides and phenolic compounds. Extraction yield, total phenols, total phlorotannins and antioxidant activity have been optimized with increasing temperature in macroalgae extracts by del Pilar Sánchez-Camargo et al. (2020), in which polyphenols are attached to cell wall polysaccharides.

In the present study, all the beach-cast species showed a content of phenolic compounds in both extracts; these results are in agreement with other studies, that confirms the presence of these metabolites in cold, warm aqueous and hydroalcoholic extracts (Esquivel-Hernández et al., 2017). The antioxidant activity is attributed to these compounds due to the -OH group of phenolic compounds be capable of transferring an electron and also donating hydrogen (Fernando et al., 2016). Phenolic compounds, such as polyphenols, phenolic acids and bromophenols are reported as excellent antioxidants in red algae (Mayer et al., 2013).

High antioxidant activities were corroborated for the extracts of the brown beach-cast *D. jolyana*, *D. polypodioides* and *Z. tournefortii* probably related to the presence of phenolic compounds in the extracts analyzed, as positive Pearson's correlation was attributed. High antioxidant activity in brown algae has been also attributed to the presence of high levels of phenolic compounds, especially phlorotannins for other species (Shibata et al., 2008). The presence of phenolic compounds suggests great potential for functional foods. Nowadays, phenolic acid salts are used as industrial food preservatives (Cotas et al., 2020) and could be used in other forms in the preservation of food products, such as biofilms for fruits and vegetables, which are highly perishable or even in the composition of packaging to avoid product degradation.

Then, as phlorotannins are active antioxidants in our studied brown beach-cast seaweeds, these compounds could be used in food products to limit or prevent the oxidation process as a potential substitute for synthetic antioxidants in the food industry. It is important to note that the material analyzed are crude extracts of beach-cast algae, therefore substances such as carbohydrates, phenolic compounds, amino acids or even the synergy among them can contribute to the reported antioxidant activity.

The evaluation of EC_{50} is very useful tool because it is a typically employed parameter to express the antioxidant capacity and to compare the activity of different compounds in the literature (Chen et al., 2013), but the negative aspect is that requires a greater amount of extract. A higher $1/EC_{50}$ indicates better antioxidant activity. The major antioxidant activities in our study were registered for the aqueous extracts, indicating, once again, the great potential of the polysaccharides as natural antioxidants.

The beach-cast seaweeds are considered unusable waste materials, our results of $1/EC_{50}$ show that the extracts analyzed have similar or better antioxidant activity than the reported by Zubia et al. (2007) and Bianco et al. (2015) for some attached macroalgae species. Also, the antioxidant activity from methanolic and aqueous extracts of the beach-cast seaweeds showed values of $1/EC_{50}$ close to those reported for the commercial standards α-tocopherol and BHT described by Zubia et al. (2007).

The antioxidant tests performed present distinct mechanisms of action and reaction systems with different conditions, which can affect the reactivity of the substances present in the raw extracts. These reinforce the practice of evaluating more than one assay to better estimate the antioxidant activity in macroalgae. All beach-cast species from this study showed antioxidant activity; however, some species exhibited better antioxidant levels than others by the global evaluation regarding the TAC index and the cluster analysis. The antioxidant test by ABTS method was the most sensitive to detect antioxidant activity for the methanolic extracts and DPPH assay for aqueous extracts, indicating that the transfer of electrons may be the main mechanism of antioxidant action of the substances present in the extracts analyzed. *Dictyopteris jolyana* (PB and ES), *D. polypodioides* (PB), *Z. tournefortii* (ES), *O. obtusiloba* (PB and ES) and *A. seaforthii* (ES) were the species that showed the best results.

However, the values of extracts yield need to be considered for application. All the studied species proved to be more efficient with aqueous extraction and reinforce the differences in chemical composition between the extracts, which can be attributed to a large amount of sulfated polysaccharides, especially in the aqueous extract. In addition, the high yields of aqueous extracts associated with the low cost of the green solvent and simple chemical composition, mainly sulfated polysaccharides with promising biological activities, are characteristics that make it interesting for commercial applications. Regarding all, *D. jolyana* (PB and ES) becomes more interesting for commercial applications, due to the high yield and antioxidant results showed with both extracts.

Particularly, we would like to comment about the beach-cast *G. domingensis*, because gracilarioids species have great commercial importance as a source of agar, an exclusive phycocolloid produced for some red algal species (Porse and Rudolph, 2017) and commonly used in salads and beverages with increasing commercial input as animal feed ingredients (Ferdouse et al., 2018). Despite the low antioxidant activity, *Gracilaria* species have shown high biological activity, such as antiviral and antiinflammatory (Porse and Rudolph, 2017). Beach-cast seaweeds with low antioxidant activity, such as *G. domingensis*, may have high biological activity. Polar metabolites are extremely promising in their bioactivity, which is why it is suggested that future studies should also invest in the evaluation of these fractions in beach-cast seaweeds.

Beach-cast seaweeds analyzed proved to be a good source of antioxidants, which makes it an excellent option to incorporate into a heart-healthy diet. The supplementation with beach-cast seaweeds to obtain functional low-fat foods could be interest in countries with a high prevalence of the cardiovascular disease, for instance. The potential use of beach-cast seaweeds offers several economic and ecological benefits, whereas there is limited biomass to supply the industrial sectors. The fast and large availability of underuse waste biomass, usually discarded in landfills, makes overplus beach-cast algae biomass an attractive and sustainable alternative as raw material. The appropriate use of this biological material can meet growing social, economic and public health requests sustainably, being able to supply several social and commercial needs.

With the expected population growth scenario, beach-cast seaweeds will be highlighted in the next years to answer the growing market and its high demand for foods and nutraceuticals. Food enrichment or natural nutraceutical stimulant is a technically and economically viable alternative that can be developed in a regulated manner on the Brazilian coast. The present research is the first report of the antioxidant potential and chemical composition for the beach-cast algae species collected in the Brazilian coast, where the vast majority of studies in the country evaluate only the taxonomy and abundance of these organisms (see Chapter I).

Our results showed the potential of this unexplored and wasted biomass. The beach-cast seaweeds studied may become valuable for the development of natural products and food supplementation, presenting potential as a rich source of antioxidant substances. Their use allows the development of new functional food products, fortifying their nutritional composition, quality and health beneficial properties.

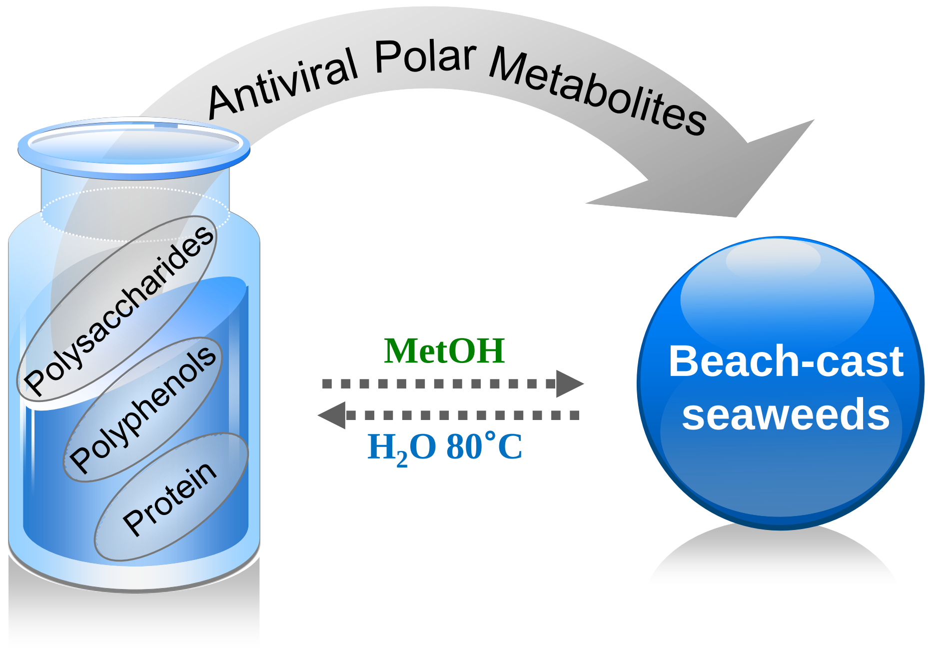
5. Conclusions

The antioxidant substances present different mechanisms of action, so the use of different *in vitro* assays is recommended for a reliable analysis of the antioxidant potential of extracts. Phenolic compounds and sulfated polysaccharides were probably responsible for antioxidant efficiency in the methanolic and aqueous extracts, respectively. However, the synergies of other substances are not discarded. The beach-cast seaweeds are a renewable and abundant resource of natural antioxidants for the prospection of novel functional foods and eco-efficient production with social and environmental benefits.

Chapter III

Anti-HIV activity of methanolic and aqueous extracts of fifteen materials of beach-cast macroalgae: valorization of underused waste biomass

Graphical abstract



Abstract

The discovery of new drugs based on seaweeds with therapeutic properties is an eternal demand for health care and well-being. Beach-cast seaweeds are unexplored raw material and important source of natural products. This study aims to evaluate the biotechnological potential of beach cast seaweeds as an antiviral agent by the ability to inhibit the reverse transcriptase enzyme of the HIV-1 virus. Assays assessing biological properties are usually time and cost-consuming. Then, preliminary general screening is an effective tool for reducing investment effort and select suitable materials for in-depth studies. The first screenings were performed at a concentration of 400 $\mu g.mL^{-1}$ for methanolic extracts and 200 $\mu g.mL^{-1}$ for aqueous extracts. The extracts from *Alsidium seaforthii*, *Spiridia clavata*, *Osmundaria obtusiloba*, *Dictyopteris jolyana* and *Zonaria tournefortii* were highly promising, reaching percentages of inhibition above 90%. The aqueous extract of *Codium isthmocladum* also showed high potential. Macroalgae are characterized by high levels of sulfated polysaccharides, such as the sulfated galactans carrageenan and agar in red algae, fucans and fucoidans in brown algae and heterofucans in green algae. These polysaccharides have been related to biological activity with antiviral effect in literature. Besides, polyphenols and tannins have been reported as the main substances responsible for high antiviral activity in methanol extracts, large amounts of phenolic compounds such as catechins, flavonoids and glycosylated flavonoids have been also identified in methanolic extracts of red and brown algae. Those substances or maybe the synergy of them could explain our findings. Nowadays, there is a demand in the industrial and pharmaceutical sectors for natural products with potential bioactivity and beach-cast seaweeds could be a renewable, economically viable and abundant resource for biotechnological approaches.

1. Introduction

The number of human HIV (human immunodeficiency virus) infected in the world is growing exponentially. World health organization (WHO, 2019) estimated that 38 million people were living with HIV in 2019, being acquired immunodeficiency syndrome (AIDS) one of the greatest contemporary public health diseases.

HIV-1 and HIV-2 are two different strains of viral particles consisting of similar kinds of structure and symptoms, but the latter confined to Africa, whereas the former was dispersed to the rest of the world (Artan et al., 2010). More than 25 million people infected with HIV live in African countries.

The discovery of new drugs based on seaweeds with therapeutic properties is an eternal demand for health care and well-being. The indiscriminate use of antibiotics in the past years has led to the resistance of pathogens; therefore, studies have sought new sources of substances with antiviral activity (Zaman et al., 2017). Marine macroalgae are an important source of natural products with bioactive properties, including antivirals (Torres et al., 2019b). In addition, natural products of macroalgae exhibit low toxicity when compared to synthetic drugs (Wang et al., 2008). Macroalgae have antiviral properties that provide a protective effect against several virus species by obstructing the spread of HIV and other sexually transmitted viruses, such as herpes simplex virus (HSV) and genital warts (Mendis and Kim, 2011).

Several bioactive metabolites derived from seaweeds including terpenes, lipids, proteins, polyphenols and especially a variety of algal polysaccharides have shown antiviral activity for the viral infections caused by HIV and others, such as HSV, human papillomavirus, dengue virus, as well as coronavirus (SARS-CoV2) (Wittine et al., 2019; Sangtani et al., 2020). All these compounds evidenced the inhibition of different stages of the virus life cycle. Polysaccharides and phenolic compounds from seaweeds have better antiviral activity than other bioactive substances via preventing viral adsorption (simultaneous-treatment assay) and replication (post-treatment assay) (Kwon et al., 2013).

A carrageenan-based vaginal microbicide called Carraguard has been shown to block HIV and other sexually transmitted diseases *in vitro* (Zeitlin et al., 1997). Clinical trials have been carried out on women in South Africa to investigate the effectiveness of the gel as a topical microbicide for the prevention of HIV infections (Spieler, 2002).

The safety of Carraguard was demonstrated, although protective action against HIV was not confirmed into phase III of clinical trials (Turville et al., 2008).

The mechanism of macroalgal polysaccharides against viral diseases is focusing on the viral attachment phase by 1) attaching immediately with virions, 2) connecting to the protein to bind the respective receptors and/or 3) immunomodulating that activate natural killer cells or prompt immune reactions (Shi et al., 2017). However, there is still the need for more research to comprehensively understand the antiviral action mechanisms of algae compounds and to benefit from their use as functional ingredients in the pharmaceutical and food industries (Pina-Pérez et al., 2017).

Recent COVID-19 pandemic event demonstrated the need for further efforts to invest in the search for new marine natural products with antiviral activity and recent research is promising (Gentile et al., 2020; Sangtani et al., 2020).

In this context, there is an extensive demand for new sources of bioactive products, in which beach-cast macroalgae can be considered emerging and promising sources of novel natural marine bioactive, where the high availability of biomass is critical to make this activity sustainable. Based on these facts and considering that there are no algal cultures on a commercial scale in Brazil to supply the biomass market for exploitation, the beach-cast algae present in large quantities in the northeast coast and some localities in the southeast and south of Brazil could constitute a potential biomass as a source of functional ingredients and new biotechnological applications to meet the demands for new natural products that can be used as a matrix for various ingredients for biotechnological purposes and diverse industries.

This study aims to evaluate the *in vitro* biotechnological potential of methanolic and aqueous extracts from different species of beach-cast seaweeds as an antiviral agent by the capacity to inhibit the enzyme reverse transcriptase of the HIV-1 virus (HIV-RT). Beach-cast seaweeds are underexplored raw material and could be a renewable, economically viable and abundant resource for biotechnological approaches.

2. Material and Methods

2.1 Biological material and extraction procedure of beach-cast seaweeds

The procedures for collecting and extraction of the biological material are described in section 4. GENERAL MATERIAL AND METHODS, item 4.1. *Collection and selection of species* and item 4.3. *Extraction procedure.*

2.2 HIV-reverse transcriptase (HIV-RT) inhibition assay

For analysis of the antiviral activity of the samples, the methanolic and aqueous extracts were dissolved in 10% DMSO and different concentrations were tested (*n* = 3; technical replicates).

The antiviral activity of the extracts was analyzed according to their ability to inhibit the activity of the HIV-1 virus reverse transcriptase enzyme. A colorimetric method was performed with the reverse transcriptase test kit (Roche, Germany).

The dried extracts were solubilized in 10% DMSO prepared with DEPC (diethylpyrocarbonate) water at a stock concentration of 2 $mg.mL^{-1}$. Based on previous studies by the research group, a preliminary screening of the antiviral activity was performed at the concentration of 400 $\mu g.mL^{-1}$ for methanolic extracts and 200 $\mu g.mL^{-1}$ for aqueous extracts of all species in order to choose others algal concentrations to be tested and estimate the IC_{50} (50% inhibition concentration of the HIV-RT activity). The extracts were analyzed in triplicate at different concentrations ranging from 0 to 600 $\mu g.mL^{-1}$.

The negative control was done with DMSO 10% with DEPC water and Foscarnet standard solution (sodium phosphonoformate tribasic hexahydrate) was used as a positive control. The Foscarnet solution was prepared in DMSO 10% at the stock concentration of 1 $mg.mL^{-1}$. From this solution, a standard curve with different concentrations between 0-1 $\mu g.mL^{-1}$ was constructed.

Preparation of the reaction followed the manufacturer's instructions. For this purpose, 20 µL of sample or standard or negative control were mixed in 200 µL microtubes; 20 µL of template/primer containing solution poly(A)+oligo(dT) and nucleotides labeled with biotin and digoxigenin (DIG); 19.5 µL of lysis buffer; and 0.5 µL of HIV-RT enzyme. The reaction was incubated under a shaker for 1 h at 37 °C.

After this step, the reaction mixture was transferred to a 96 well microplate treated with streptavidin, a tetrameric protein that has a strong affinity with biotin. The reaction mixture was incubated once again in a shaker for 1 h at 37 °C, binding the RNA/DNA molecules to the wells of the microplate. After the incubation period, the microplate was washed five times with 200 µL of wash buffer. After complete removal of the wash buffer, 200 µL of the solution containing incubation buffer and anti-digoxigenin-peroxidase antibody (anti-DIG-POD) was added to each well, this antibody binds strongly to the digoxigenin present in the labeled nucleotides of RNA/DNA molecule.

In the final step for this assay, the microplate was incubated again under a shaker for 1 h at 37 °C and after that period was washed five times with 200 μL wash buffer. The buffer was withdrawn completely and, in each well, 200 μL of ABTS solution dissolved in substrate buffer (sodium perborate and citric acid/phosphate buffer) was added. The enzyme peroxidase (bound to the antibody) catalyzes the breakdown of the substrate, releasing hydroxyl radical (OH●), which reacts with ABTS (green color), forming the ABTS+ radical, which presents intense green coloration. The microplate was incubated for 30 min at room temperature; then the absorbance readings were performed at 405 nm and 490 nm in the UV-vis microplate spectrophotometer (Epoch Biotek, USA). The lower the absorbance, the greater the activity of inhibition of HIV-RT.

The results were expressed as a percentage of inhibition, according to Woradulayapinij et al. (2005):

$$\%\text{inhibition} = \left[\frac{(\text{Abs405NC} - \text{Abs490NC}) - (\text{Abs405S} - \text{Abs490S})}{(\text{Abs405NC} - \text{Abs490NC})}\right] \text{X } 100$$

where: Abs405NC – absorbance of the negative control at 405 nm; Abs490NC – absorbance of the negative control at 490 nm; Abs405S – absorbance of the sample at 405 nm; Abs490S – absorbance of the sample at 490 nm.

With the percentage of inhibition at different concentrations, the IC_{50} of the samples and Foscarnet were calculated with the software GraphPad Prism®6, using a sigmoidal fit model.

For methanolic extract, the species *D. jolyana* (ES and PB), *Z. tournefortii*, *A. seaforthii* and *O. obtusiloba* (ES and PB) had antiviral activity tested in more than one concentration and for the aqueous extract, the species tested in more than one concentration were *D. jolyana* (ES and PB), *Z. tournefortii*, *A. seaforthii*, *O. obtusiloba* (ES) and *S. clavata*.

2.3 Data analysis

All the analytical analyses were carried out from the extraction of five subsamples (*n* = 3, technical replicates) and considered for statistical analysis. Statistical analyses were performed using Statistica 10 software, previously tested for normality (Kolmogorov-Smirnov test) and homoscedasticity (Bartlett's test). The percentage values of antiviral activity were transformed into square root arcsine in proportion to the percentage, according to Snedecor (1966). One-way analysis of variance (ANOVA) was used to observe significant differences ($p < 0.05$). When differences were detected, Newman-Keuls *post-hoc* multiple comparison test was applied.

3. Results

Only the methanolic and aqueous extracts were studied, due to the higher yield in relation to the other solvents (hexane extract, dichloromethane and ethyl acetate; see Chapter II, Table 5). The methanolic extracts with the highest percentage of yield were from the beach-cast species *B. occidentalis*, *A. ramosissima*, *A. triquetrum*, *O. obtusiloba* (ES) and *C. isthmocladum*. Regarding aqueous extract, the best yields were from *G. domingensis*, *A. ramosissima*, *D. jolyana* (ES and PB), *A. triquetrum* and *C. isthmocladum*.

Based on results from other species studied in our laboratory, a preliminary screening was conducted at concentration of 400 $\mu g.mL^{-1}$ for methanolic extracts (Fig. 11A) and 200 $\mu g.mL^{-1}$ for aqueous extracts (Fig. 11B) of all species in order to choose the others algal concentrations to be tested and estimate the IC_{50}. From the preliminary test, both methanolic and aqueous extracts of *D. jolyana* (ES) and (PB), *Z. tournefortii* (ES), *A. seafortii* (ES) and *O. obtusiloba* (ES) showed an inhibitory percentage of HIV-RT equal or over than 45%, as well as the methanolic extract of *D. polypodioide*s and *O. obtusiloba* (PB) and aqueous extract of *S. clavata* (ES). Therefore, further tests for other extract concentrations were performed with some of these species and extracts. The other species displayed low antiviral activity (< 45%) for methanolic extract and for aqueous extracts.

Unfortunately, it was not possible to test other concentrations for the species *D. polypodioide*s, *A. triquetrum*, *B. occidentalis*, *G. domingensis*, Mix 1, Mix 2, *A. ramosissima* and *C. isthmocladum* because the kit was no longer available. The species *D. polypodioide*s for methanolic extracts (Fig. 11A) and *A. triquetrum*, *B. occidentalis*, Mix 2 and *C. isthmocladum* were highly promising, reaching percentages of inhibition equal or above 45% for aqueous extracts in the concentration tested (Fig. 11B).

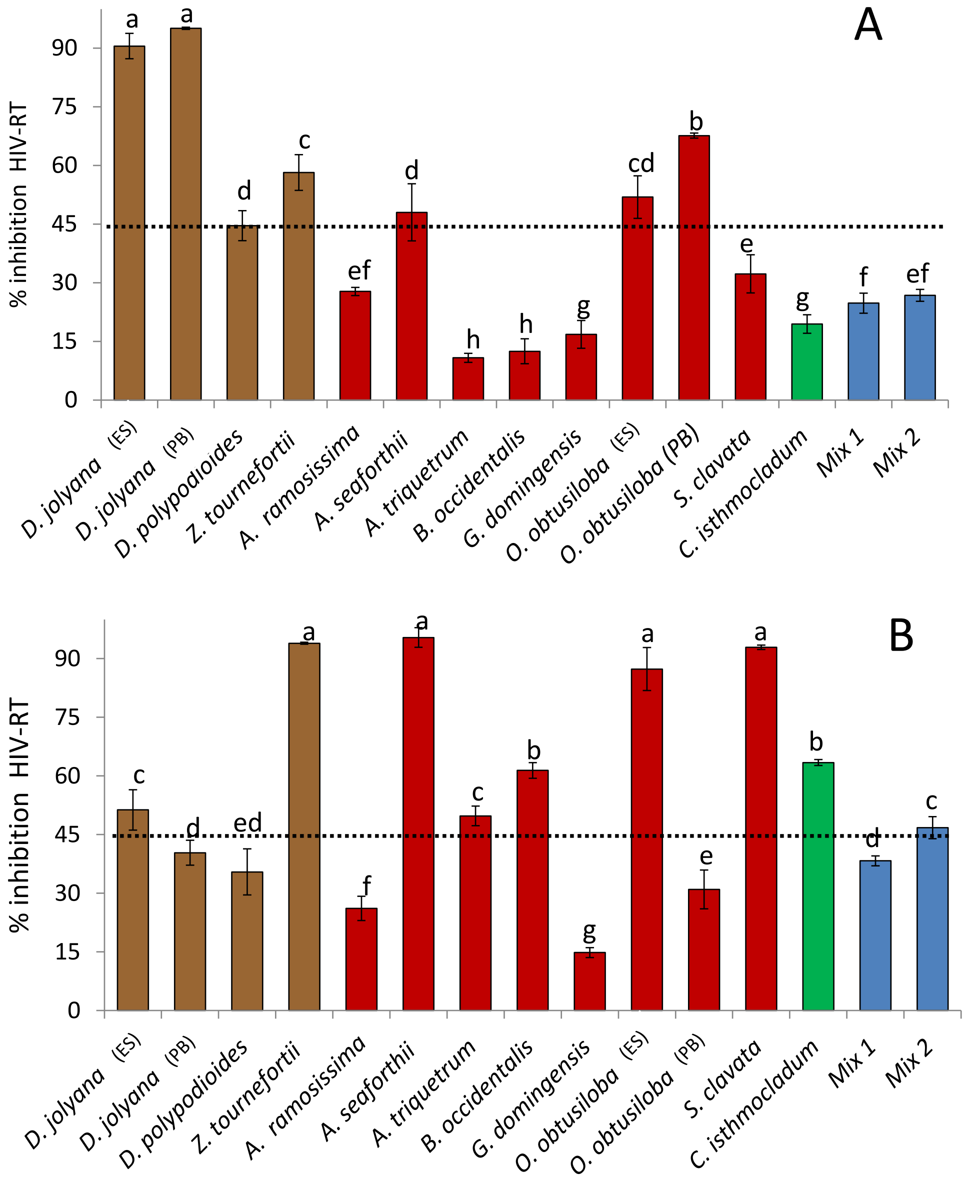


Figure 11. Preliminary screening of inhibition of the enzyme HIV-RT (Mean ± SD; n = 3) of beach-cast seaweeds for (A) methanolic extracts at the concentration of 400 µg.mL^{-1} and (B) aqueous extracts at the concentration of 200 µg.mL^{-1}. Letters indicate significant differences ($p < 0.05$) according to one-way ANOVA and Newman-Keuls *post-hoc* test. Brown bars: Phaeophyceae's species; red bars: Rhodophyta's species; green bars: Chlorophyta's species and blue bars: mixtures of species. The dotted line corresponds to 50% of antiviral activity.

Methanolic and aqueous extracts with higher inhibitory activity (above 45%) were tested in different concentrations and are presented in Figures 12 and 13, respectively. Both extracts showed significant differences among concentrations of algal crude extracts with a increasing tendency between concentration and activity, excepted for the methanolic extract of *D. jolyana* (PB) (50-400 µg.mL^{-1} – 90% of HIV-RT inhibition; Fig. 12B) and aqueous extract of *Z. tournefortii* (ES) (5-100 µg.mL^{-1} – 80-85% of HIV-RT inhibition; Fig. 13C). Both exceptions showed the greatest efficiency with inhibitory values close to 100% at the minimum algal crude extract tested and few significant differences were observed among concentrations of these algal extracts; therefore, IC_{50} was not calculated. The standard curve of Foscarnet (positive control) obtained an IC_{50} of 0.061 µg.mL^{-1} (*data not shown*).

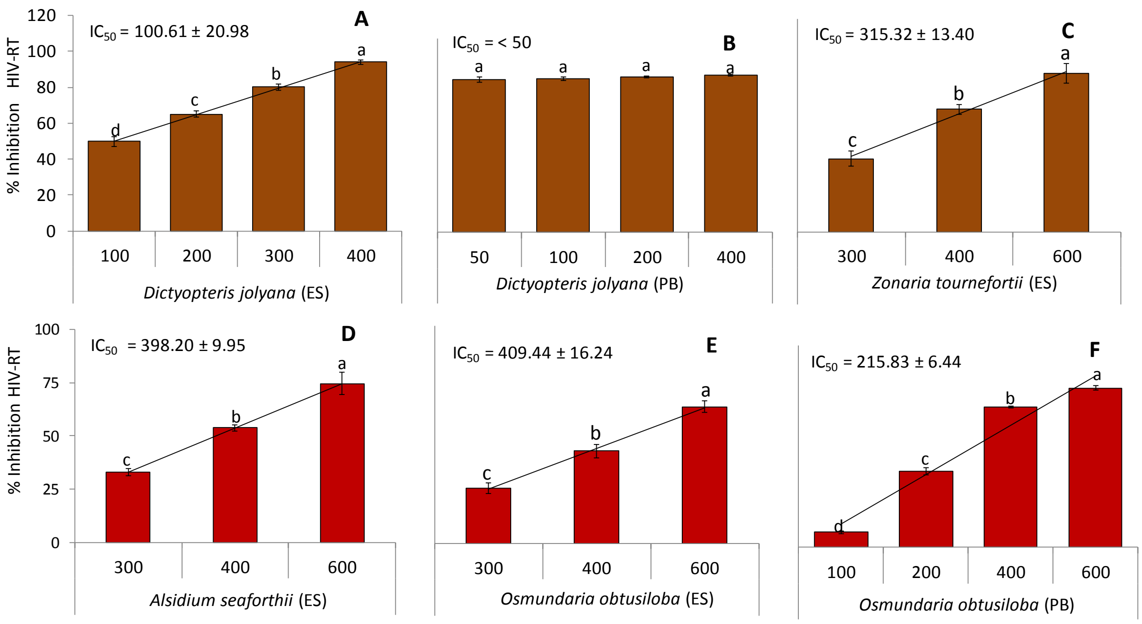


Figure 12. (A-F) Percentage of inhibition of the enzyme HIV-RT (Mean ± SD; *n* = 3) of beach-cast seaweeds at different concentrations of methanolic extracts (µg.mL^{-1}). The calculated values of IC_{50} (half-maximal inhibitory concentration, µg.mL^{-1}) were included for each species. Letters indicate significant differences ($p < 0.05$) according to one-way ANOVA and Newman-Keuls *post-hoc* test. Brown bars: Phaeophyceae's species; red bars: Rhodophyta's species.

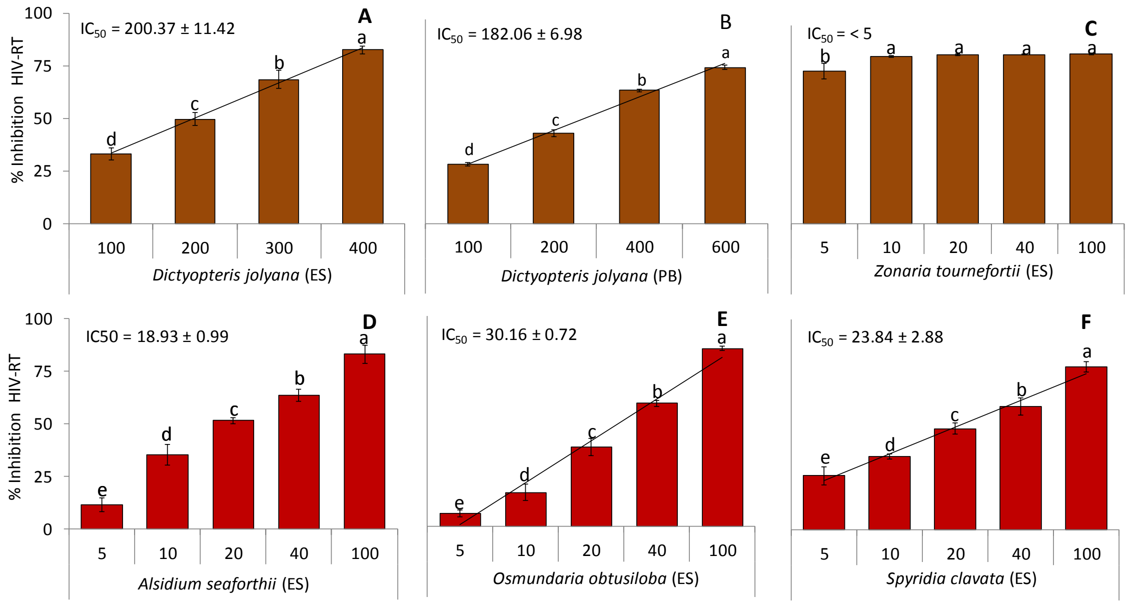


Figure 13. (A-F) Percentage of inhibition of the enzyme HIV-RT (Mean ± SD; *n* = 3) of beach-cast seaweeds at different concentrations of aqueous extracts (µg.mL^{-1}). The calculated values of IC_{50} (half-maximal inhibitory concentration, µg.mL^{-1}) were included for each species. Letters indicate significant differences ($p < 0.05$) according to one-way ANOVA and Newman-Keuls *post-hoc* test. Brown bars: Phaophyceae's species; red bars: Rhodophytas's species.

Table 10 shows the comparison of antiviral activity (HIV-RT) from extracts and isolated compounds from macroalgae present in literature with the results of the present study. The isolated polysaccharides and diterpenes metabolites showed antiviral activity with low IC_{50} values (0.50-1.00 µg.mL^{-1}). The IC_{50} values for the crude aqueous and methanolic extracts of macroalgae showed activity between 12.39 to 300 µg.mL^{-1}, the IC_{50} response of beach-cast seaweeds from the present study ranged from 5 to 409.44 µg.mL^{-1}.

Table 10. Studies that evaluated antiviral activity (HIV-RT) in macroalgae.

Reference	Species	Metabolite/Extract	IC_{50} (µg.mL^{-1})
Queiroz et al. (2008)	*Fucus vesicularis*	Polysaccharides	0.50 – 1.00
Trinchero et al. (2009)	*Adenocystis utricularis*	Polysaccharides	0.60 – 70.00
Cavalcanti et al. (2011)	*Dictyota menstrualis*	Diterpens	1.00
Pires (2016)	*Sargassum vulgare*	Aqueous	12.39 (dry season) 28.47 (rainy season)
Amorim (2018)	*Sargassum cymosum* *Padina gymnospora* *Codium isthmoclaum* *Chnoospora minima* *Dictyopteris plagiogramma*	Methanolic	70 100 118 225 300
Amorim (2018)	*Sargassum cymosum* *Codium isthmoclaum* *Padina gymnospora* *Chnoospora minima* *Dictyopteris plagiogramma*	Aqueous	50 < 60 70 100 100
Present study	*Dictyopteris jolyana* (PB) *Dictyopteris jolyana* (ES) *Osmundaria obtusiloba* (PB) *Zonaria tournefortii* (ES) *Alsidium seaforthii* (ES) *Osmundaria obtusiloba* (ES)	Methanolic	< 50.00 100.61 215.83 315.32 398.20 409.44
Present study	*Zonaria tournefortii* (ES) *Alsidium seaforthii* (ES) *Spyridia clavata* (ES) *Osmundaria obtusiloba* (ES) *Dictyopteris jolyana* (PB) *Dictyopteris jolyana* (ES)	Aqueous	< 5.00 18.93 23.84 30.16 182.06 200.37

4. Discussion

HIV infection is caused by a virus belonging to the subfamily of retroviruses, also known as lentiviruses or slow viruses. This means that from the moment of infection to the appearance of the first signs of the disease, a long time can pass (Besednova et al., 2019). Over the last years, numerous nucleoside analogs have been applied in antiviral therapy and play an important role in combatting HIV, herpes viruses, hepatitis B and hepatitis C viral infections (Jordheim et al., 2013). The targets of these nucleoside analog drugs are the virus-encoded DNA- or RNA-polymerases, such as reverse transcriptase, a central step in viral infection (El Safadi et al., 2007; Sarafianos et al., 2009).

The process of RT occurs after the penetration of HIV genetic material into the cell, with the transfer of information from viral RNA to host DNA. The RNA of the virus using RT begins to synthesize the DNA that is inserted into the genetic apparatus of the host cell, having the form of a provirus. The virus begins to multiply in dividing T-lymphocytes, although it can function in a non-dividing cell. This is the main reason why HIV infection is difficult to treat (Besednova et al., 2019).

In the results obtained from our preliminary test, the species of the aqueous extracts obtained better responses than the methanolic ones, there was a highlight for the aqueous extracts of *B. occidentalis*, *A. triquetrum, C. isthmocladum* and Mix 2; therefore it would be very interesting to test other concentrations with these species. Moreover, the species *D. jolyana* (ES and PB), *A. triquetrum* and *C. isthmocladum* showed the highest percentage yields for the aqueous extracts, species with high activity and high extraction yield are promising for bioprospecting purposes.

Over the last decade, red and brown algae have attracted much interest in the search of anti-HIV action and fewer reports are found for green algae (Vlietinck et al., 2008, Rodrigues et al., 2009; Yasuhara-Bell and Lu, 2010; Mattos et al., 2011). The antiviral activity described in the present study can be attributed to bioactive polar compounds that are methanolic- and water-soluble. Some seaweed's polar metabolites such as sulfated polysaccharides, proteins and polyphenols have been described with high antiviral activity for HIV (Besednova et al., 2020).

Sulfated polysaccharides are the most important metabolites contained in large amounts in aqueous extracts of seaweeds (Shi et al., 2017). The type of sulfated polysaccharides in macroalgae, such as fucoidan, agar, carrageenan, porphyran, laminarin, galactan and ulvan differs depending on the taxonomic group (Klongklaew et al., 2020).

Among several kinds of algal polysaccharides, carrageenans from red algae are the most studied and considered safe for human use (Weiner, 2016). An isolated polysaccharide from *Agardhiella tenera* (J. Agardh) F. Schmitz showed activity against HIV, with IC_{50} values of 0.5 $\mu g.mL^{-1}$ (Witvrouw et al., 1994). A study conducted on the anti-HIV activity of a mixture of carrageenans from red algae, the antiviral activity of polysaccharides were strong selective inhibitors of HIV-1 replication in human T-cell leukemia (MT4) cells (Besednova et al., 2019). Moreover, the antiviral activity increased with an increase in the molecular weight of the compounds and the degree of their sulfation (Besednova et al., 2019). The results of aqueous extracts showed high activity for the red beach-cast algae *A. seaforthii*, *O. obtusiloba* (ES) and *S. clavata*, these findings might result from the action of sulfated polysaccharides, especially carrageenans.

Anti-HIV activity is also well reported for brown algae polysaccharides (Ahn et al., 2002; 2004; 2006; Artan et al., 2008; Queiroz et al., 2008; Kim and Karadeniz, 2011). In the study of Thuy et al. (2015), fucoidan isolated from three species of brown algae possessed anti-HIV activity in the cell lines. Trinchero et al. (2009) showed that galactofucan fractions from brown algae were active against HIV-1 *in vitro*. It was proved that the inhibitory effect is not due to the inactivation of the virus, but by blocking the early stages of virus replication and, therefore, the authors recommend these substances as good candidates as preventive and therapeutic drugs against HIV infection. Studies reported that laminaran, a water-soluble polysaccharide from brown algae, potently prevents the replication and proliferation of HIV via suppressing the viral binding with lymphocytes (Ahmadi et al., 2015).

Ulvan is a water-soluble sulfated polysaccharide isolated from cell walls of Ulvales green seaweeds (Lahaye and Robic, 2007). Ulvans and fucoidans have the same action mechanism through antiviral attachment. The synergistic effect can occur with combined usage (Rosales-Mendoza et al., 2020). Among the beach-cast seaweeds from the area of collection, green algae species are the least frequent, this directed our study because did not have other Chlorophyta species in abundance. In addition, *C. isthmocladum* collected was trapped in rhodoliths, which is also an indication of the abundance of this species in rhodolith banks in the region.

For extracts of species of the genus *Codium* there are few studies that evaluated anti-HIV potential. Ahn et al. (2002) studied the anti-HIV potential of two species of this genus and the methanolic extracts did not show activity to inhibit HIV-RT. In contrast, the results from the present study obtained for the aqueous extracts of beach-cast *C. isthmocladum* in inhibiting HIV-RT were promising. These results are in accordance with Amorim (2018) for the aqueous extract of non-beach-cast *C. isthmocladum* that also showed high antiviral activity HIV-RT. This scenario demonstrates that further studies are needed on the antiretroviral potential of *C. isthmocladum* to clarify the substances responsible for its anti-HIV action.

Other polar metabolites with high antiviral HIV-RT are proteins, mainly lectins. Lectins of red, brown and green algae (Singh and Walia, 2018) are considered as potential candidates for preventing sexual transmission of HIV as a microbicide (Alexandre et al., 2012). They do not only inhibit the infection of cells with HIV but can effectively prevent the transmission of the pathogen from infected cells to uninfected CD4+ T lymphocytes (Huskens and Schols, 2012). In a study carried out by Sato et al. (2011), a binding lectin was isolated from green macroalgae. The lectin showed antiviral activity against HIV-1 infections and influenza viruses (Queiroz et al., 2008). The mechanism of action anti-HIV-1 occurs when lectin or glycoproteins, bind to the carbohydrate moiety of the virus to inhibit its attachment to the target cells and also to hinder the replication of viral RNA (Sangtani et al., 2020).

Especially in methanolic extracts, red and green seaweeds can contain different amounts of phenolic compounds such as flavonoids, glycosylated flavonoids, phenolics acids, catechins and bromophenols responsible for antiviral activity in polar extracts, due to the reaction of these compounds with proteins, *e.g.*, enzymes or cellular receptors, while phlorotannins are the major polyphenolic secondary compounds synthesized mainly by marine brown seaweed (Yoshie-Stark et al., 2003; Deyab et al., 2016; Gómez-Guzmán et al., 2018).

Brown algae showed the highest antiviral activity among the studied beach-cast seaweeds, even in a very low extract concentration. Despite the high percentage of antiviral inhibition, the IC_{50} of the species was superior than the standard IC_{50} of Foscarnet (0.061 µg.mL^{-1}). However, it is still evident the antiviral potential of the beach-cast seaweeds, especially for *D. jolyana* (ES – methanolic extract) and *Z. tournefortii* (aqueous extract), which the IC_{50} could not be calculated due to the high activity reached in all concentrations tested. In the literature, these species showed promising antiviral activity for other viruses such as HSV (Bianco et al., 2013) and hepatitis B for the *Zonaria* genus (Premnathan et al., 1992).

Moreover, phlorotannins are polar metabolites rich in the chemical composition of brown macroalgae, commonly found in *Z. tournefortii and* species from *Dictyopteris* genus (Murray et al., 2018; Nunes et al., 2019). According to the mechanism of action, these substances can act at different stages of viral infection and can inhibit adsorption, reverse transcriptase and transcription (Ahn et al., 2004). Therefore, it is suggested that phlorotannins contributed to the high HIV-RT response of brown beach-cast algae.

In the literature, most studies with macroalgae evaluate the antiviral activity an isolated substance, such as Queiroz et al. (2008), Trinchero et al. (2009) and Cavalcanti et al. (2011), this explains the low IC_{50} values reported by the authors. Santos (2016) analyzed de antiviral activity in the hot water extract from *Sargassum vulgare* C. Agardh and observed seasonal differences in the antiviral activity of the species. Indicating that the environmental conditions in which the macroalgae are exposed may exert influence on the production of bioactive substances. According to Cos et al. (2006), extracts with promising biological activities should have an IC_{50} value below 100 $\mu g.mL^{-1}$. From this perspective, the most promising species were the aqueous extracts of the brown alga *Z. tournefortii* and the red algae *A. seaforthii*, *S. clavata* and *O. obtusiloba*, with IC_{50} values that ranged from <5 to 30.16 $\mu g.mL^{-1}$ and the methanolic extracts of *D.jolyana* from ES (100.61 $\mu g.mL^{-1}$) and PB (<50 $\mu g.mL^{-1}$).

Comparing the IC_{50} results of beach-cast algae from ES (present study) with the attached ones from the same place in the study of Amorim (2018), it is possible to note that the aqueous extracts of beach-cast algae, especially the red ones (*A. seaforthii* – 18.93 $\mu g.mL^{-1}$, *S. clavata* – 23.84 $\mu g.mL^{-1}$ and *O. obtusiloba* – 30.16 $\mu g.mL^{-1}$), showed higher antiviral activity than non-beach-cast macroalgae (*S. cymosum* – 50 $\mu g.mL^{-1}$, *P. gymnospora* – 70 $\mu g.mL^{-1}$, *C. minima* – 100 $\mu g.mL^{-1}$ and *D. plagiogramma* – 100 $\mu g.mL^{-1}$). These results can infer that beach-cast algae have a huge potential, similar to attached macroalgae. However, it is important to take into account the type of drying, extraction, solvents, biotic and abiotic factors that can influence these algal responses. Thus, further studies are needed to assess the fluctuation of the antiviral response of beach-cast seaweeds.

The present study is the first report that evaluated the biotechnological potential of beach-cast algae from the Brazilian coast as an antiviral agent. The biotechnological potential of the tested beach-cast seaweeds was highly promising, reaching percentages of inhibition above 90%. Methanolic and aqueous extracts showed an efficient inhibitory activity for brown and red algae; therefore, we can suggest that beach-cast seaweeds could be a potential renewable source of natural antiviral activity, including economically viable and abundant resource for prospective approaches. The *in vitro* results of the antiviral potential of beach-cast seaweeds suggest a greater investment of research to elucidate which substances are present in the methanolic and aqueous extracts that are responsible for the activity, in addition to understanding the mechanisms of action for inhibiting HIV-RT.

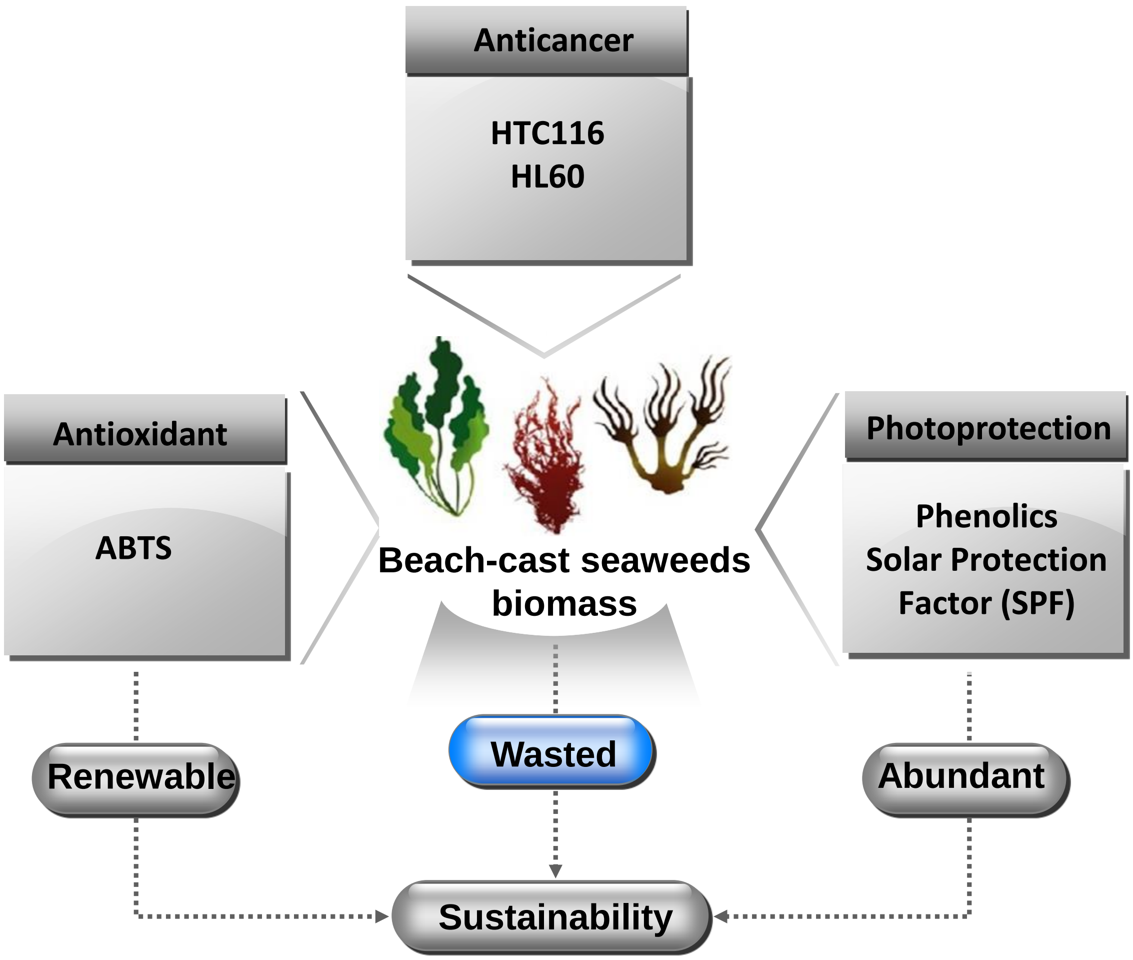
5. Conclusions

The results from the beach-cast seaweeds suggest the biotechnological potential application of this raw material, specifically as an antiviral agent. The aqueous extracts showed better responses than the methanolic extracts. Beach-cast algae have a great availability of biomass in Brazil; therefore they could be exploited as a functional ingredient with specific application for food, medical, pharmaceutical and cosmetic industries.

Chapter IV

Brazilian beach-cast seaweeds: antioxidant, photoprotection and cytotoxicity properties

Graphical abstract



Abstract

Seaweed-based products for cosmetic healthcare can be improved from natural secondary metabolites with antioxidant and photoprotection properties. Studies regading potential of algae extracts in cosmetic applications have increased with novel development and market. Benefical biological properties include the ability to fight aging (oxidative stress) anticancer agents, improve the immune system, protect against UV radiation and contribute to the treatment of diseases. A prospective study was carried out based on extracts of beach-cast algae from the Brazilian coast to select the species with the greatest potential to be used in cosmetic applications. Analysis of antioxidant activity by the ABTS assay, UV/Vis absorption and quantification of UV photoprotectors as phenolic compounds, mycosporine-like amino acids and total content of C, N and S were performed. The development of cosmetic creams to analyze the sun protection factor and evaluation of the cytotoxicity activity were also performed. The highest antioxidant activity was found in extracts of brown macroalgae, followed by extracts of red algae, with the lowest activity detected in the green macroalga *C. isthmocladum*. The same pattern was observed for the amount of phenolic compounds. No MAAs were detected in the red algae analyzed. The extracts of *Z. tournefortii*, *A. seaforthii* and *C. isthmocladum* did not show any cytotoxicity activity against human cells (HGF1). Cosmetic formulation was designed with extracts from beach-cast algae, but low SPF values were obtained. Other forms of extraction and incorporation of the extract into the base creams should be evaluated to try to increase the SPF values or isolation and purification of substances could increase the efficiency of the photoprotective capacity. This is the first report for beach-cast species evaluating the antioxidant activity, cytotoxicity potential against tumoral cells and photoprotection. The species analyzed 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 due to they are biodegradable.

1. Introduction

In recent years, marine macroalgae 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 (Gullón et al., 2020). As fundamental components of the marine ecosystem, macroalgae constitute one of the most diverse groups among photosynthetic organisms (Bianco et al., 2015), representing a strategic natural resource for biotechnological development. In Brazil, special attention has increased considerably due to its large coastline and wide biodiversity.

Marine macroalgae 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 algae, 28 from brown algae and 45 from red algae (Carrol et al., 2020), evidencing the increasing interest in the study of these organisms. As for strategical investment in research, development and innovation (RD&I), both cosmeceutical, evaluating antioxidant and photoprotection (Lourenço-Lopes et al., 2020; Rangel et al., 2020) and pharmaceutical areas, investigating cytotoxic and antitumor activities (Saeed et al., 2020), with 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 (Kim et al., 2018). Studies have delineated the potential of algae extracts in cosmetic applications, highlighting photoprotective, moisturizing, whitening and anti-cellulite properties and activities, which have been attributed to a variety of algae components (Bedoux et al., 2014). Besides, skincare and cosmetic company trials have been reported several seaweed compounds as having promising properties, such as anti-inflammatory and skin hydration, as well protection and enhance skin repair processes (Lange et al., 2020). These properties have been used in a broad spectrum of market segments, such as food and feed ingredients, skincare, cosmetics, fertilizer and biofuels (Stiger et al., 2016). Biological properties with positive benefits, such as the ability to fight aging (oxidative stress) and cancer, improve the immune system, protect against UV radiation and contribute to the treatment of diseases, have been also verified in several species of macroalgae (Cotas et al., 2020).

One of the major limitations in the search for new extracts, fractions or natural products with biological action is the reduced available biomass for studies and bioprospection. Nowadays, there is a limitation of sustainable biomass that includes population management practices and few macroalgae species are commercially cultivated (FAO, 2020). Besides that, 80% of the algae mass correspond to water, reducing significantly the amount of material for the studies (Behera et al., 2015). In this context, the availability of beach-cast seaweed biomass can be a sustainable and highly productive alternative.

The beach-cast seaweeds are those organisms that are detached from the natural substrate or drifted macroalgae that accumulate on the beaches, which are detached by sea turbulence caused by currents, winds and tides (do Nascimento Santos et al., 2013).

The literature of beach-cast algae in Brazil is still scarce; most studies have evaluated only the taxonomy and abundance of this biomass on different beaches for the country (Nascimento, 2010; Rios, 2010; do Nascimento Santos et al., 2013). These studies show that Brazil has an incredible potential for this waste and underused biomass and many of them evaluate their potential use as fertilizer (Britto et al., 2018; Coelho et al., 2018). On the other hand, the potential of beach-cast macroalgae as a raw material for several applications, combined with the large quantity available on the beaches of certain regions along the Brazilian coast, should be better understanding and suitable application must be proposed. However, scientific subsidies are still lacked to clearly establish its potential and application.

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

2. Material and Methods

2.1 Biological material

Seven materials of beach-cast macroalgae were collected in the northeast (Paraíba State) and southeast (Espírito Santo State) regions of Brazil as described in Table 11, four brown seaweeds, two red seaweeds and one green seaweed. These species were selected as they were abundant in the respective collection site. The biomass was collected by systematic sampling, in which only healthy appearance individuals were collected. The material was cleaned of macroepiphytes, washed three times in abundant tap water and then shadow air-drying. 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 São Paulo, Brazil (Table 11).

Table 11. List of selected species of beach-cast marine algae harvested from the Brazilian coast northeast (PB, Paraíba State) and southeast (ES, Espírito Santo State).

Taxonomical group **Species**	**Beach (State)**	**Localization**	**Nº Voucher (herbarium)**	**Collection date**
Ochrophyta/Phaeophyceae (brown algae)				
Dictyopteris jolyana	Pontal (ES)	20°58'22.5"S 40°48'38.6"W	SPF58249	06/09/2017
Dictyopteris jolyana	Coqueirinho (PB)	07°17'58"S 34°47'54"W	SPF58249	02/25/2012
Dictyopteris polypodioides	Ponta do Cabo Branco (PB)	07°08'43.6"S 34°48'20.7"W	SPF58249	07/18/2016
Zonaria tournefortii	Pontal (ES)	20°58'22.5"S 40°48'38.6"W	SPF58252	06/09/2017
Rhodophyta (red algae)				
Alsidium seaforthii	Piúma (ES)	20°50'31.5"S 40°43'46.0"W	SPF58253	06/11/2017
Osmundaria obtusiloba	Ponta do Cabo Branco (PB)	7°08'43.6"S 4°48'20.7"W	SPF58082	07/18/2016
Chlorophyta (green algae)				
Codium isthmocladum	Itaoca (ES)	20°54'18.0"S 40°46'42.3"W	SP470207	04/30/2018

2.2 Carbon, nitrogen, sulfur and total protein analysis

Elemental analysis of carbon (C), nitrogen (N) and sulfur (S) was performed from 5 mg of pulverized samples (n = 3; technical replicates) 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 CO_2, N_2 and SO_2 products were selectively separated into specific columns and thermal conductivity detected. EDTA (ethylenediaminetetraacetic acid) and sulfamethazine were used as standards 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 Lourenço et al. (2002). All measured parameters were expressed in percentage of dry matter (DM).

2.3 Mycosporine-like amino acids (MAAs)

Mycosporine-like amino acids were analyzed for the red beach-cast *A. seaforthii* and *O. obtusiloba* according to Karsten et al. (1998) and Peinado et al. (2004). Extraction of MAAs was performed using 20 mg DM and 1 mL of 20% methanol, in a thermostated bath at 45 °C for 2 h (*n* = 3; technical replicates). The supernatant was recovery after centrifugation at 4,000 rpm at room temperature for 10 min. Aliquots of 600-800 µL of the supernatant were vacuum-dried by centrifugation (Jouan RC 10-09, France) and the pellet resuspended in the same initial aliquot volume (600-800 µL) with methanol HPLC grade to eliminate salts and proteins. The samples were then filtered using a 0.2 µm filter for previous HPLC analysis (Waters Chromatography S.A. 600, Spain).

The HPLC system used a C8 column (Luna, Phenomenex, Germany), 5 µm, 250 x 4.6 mm with a C8 pre-column (Octyl, MOS, Phenomenex, Germany). The mobile phase was 2.5% aqueous methanol (v/v) plus 0.1% acetic acid (v/v) in water. Aliquots of 10 µL samples were injected into an isocratic flow of 0.5 $mL.min^{-1}$, with elution time of 15-20 min and read in a UV-vis photodiode-array detector (Waters, 996, Spain) at 290 and 400 nm. Data were analyzed using Empower 2 Chromatography Data Software.

After the analysis, MAAs were not detected in the samples, therefore, identification and quantification were not performed.

2.4 Extraction

Extracts from the seven materials collected were prepared in the proportion of 100 $mg.mL^{-1}$ with two different solvents, distilled water and hydroethanol (1:1 v/v; 50% EtOH) and an ultra-turrax homogenizer (T10, Ika Works Inc., USA) for 5 min, coupled to an ice bath to avoid thermal denaturation of the components. 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 algae) or sodium carbonate (for green and red algae) 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 algae biomass for better extract the substances (Kim and Chojnacka, 2015). For hydroethanolic extraction, the samples 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.

Supernatants from alkaline aqueous extracts were evaluated for phenolic compounds, antioxidant activity by ABTS assay, sun protection factor (SPF) and cytotoxicity activity. Supernatants from hydroethanolic extracts were assessed for phenolic compounds, ABTS assay, effective solar absorption radiation (ESAR), extract photoprotection index (EPI), SPF and cytotoxicity activity.

After evaluating the results obtained from the previous above extraction at a proportion of 100 $mg.mL^{-1}$, a second extraction was carried out only for *D. jolyana* (ES), *Z. tournefortii*, *A. seaforthii* and *C. isthmocladum* at the same conditions described previously for alkaline and hydroethanolic extracts, but at extraction proportion of 500 $mg.mL^{-1}$ as proposed by Álvarez-Gómez et al. (2016). For these extracts, UV-vis absorbance spectra, phenolic compounds, ABTS assay, ESAR, EPI and SPF were analyzed.

2.5 Analysis of the UV-visible absorption spectrum

The UV-vis absorption spectrum 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. For the UV wavelength (200-400 nm), all extracts were diluted seven times. For the visible wavelength (400-720 nm), all extracts were diluted five times. Dilution exception was performed for extracts of *C. isthmocladum* that no dilution was necessary for any of the evaluated wavelength intervals. 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 400nm and visible at 750 nm). Three measurement technical replicates were performed.

2.6 Phenolic compounds

The determination of phenolic compounds was carried out by the photocolorimetric method of Folin-Ciocalteu (Singleton et al., 1999), in which 250 µL of the extract was added to 1250 µL of distilled water and 125 µL of the Folin-Ciocalteu reagent, left to react for 2 h in the dark at 4 °C. Then, aliquots of 1000 µL of the reaction mixture were used for spectrophotometric absorbance 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 to 25 $\mu g.mL^{-1}$ and the equation y = 0.0757x – 0.021 (R^2 = 0.99) was obtained. Results were expressed as mg of PGE (phloroglucinol equivalent) per g of algal DM.

2.7 Antioxidant activity by ABTS assay

The antioxidant activity was determined by the ABTS radical cation assay (Re et al., 1999) and the assay was performed with three technical replicates. Aliquot of 50 µL of crude extract was added to 940 µL of sodium phosphate buffer (50 mM, pH 7) and 10 µL 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 (Oehlke et al., 2011) at concentrations of standard curve from 1 to 5 $\mu g.mL^{-1}$, with the equation y = 13.593x + 0.8717 (R^2 = 0. 99). Antioxidant activity was expressed as µmol of TE (Trolox equivalent) per g of algal DM.

2.8 Preparation of cosmetic products

An Unguator 2100 mixing machine (GAKO International GmbH, Munich, Germany) was used for allowing a perfect homogenization of the formulation to manufacture the cosmetic. All components (Table 12) were mixed until homogeneity was achieved (oil phase, water and algal extracts). All formulations were mixed at 750 rpm for 2 min. Figure 14 shows the absorption spectra of the oils from the base cream formulation. 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, ESAR and SPF.

Table 12. Components of the cream base formulation.

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	
Conservant	10
Step 4	
Algae liquid extract (pH 5.5)	250

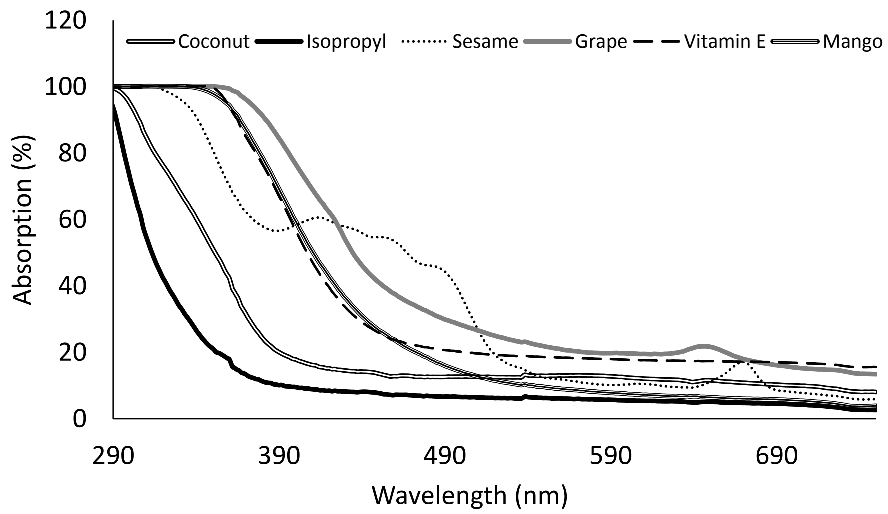


Figure 14. Absorption spectra of some components of step 1 from the base cream formulation.

2.9 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 EPI for the extracts according to Schneider et al. (2020). Both %ESAR and EPI are analogous to the sun protection factor (SPF) and it is possible to apply any action spectra in addition to the erythematic spectrum 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 (see Table 13 and Fig. 15) by the following formula:

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

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:

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

where: ΣtESAR(λ) stands for the sum of transmittance for ESAR in the respective wavelengths; ΣteSS(λ) 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 (Schneider et al., 2020) by applying 500 μL 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 Colipa (2011) 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, they were positioned in the trajectory of the radiation emitted by the solar simulator (Spectra-Physics Model 66902, 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.

Transmittance values were converted to absorptance values (A(λ)) as:

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

where: Tt(λ) is the transmittance by each sample at wavelength λ and To(λ) 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:

$$SA(\lambda) = A(\lambda) \text{ x } SS(\lambda)$$

where: A(λ) 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:

$$eSS(\lambda) = SS(\lambda) \text{ x } Act. Sp. (\lambda)$$

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

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

Table 13. Action spectra of biological responses driven by UV radiation utilized to calculate effective solar absorption radiation (%ESAR) and extract photoprotection index (EPI). Action spectra were divided according to their influence in different spectral regions, UVB (290-320 nm), UVA-I (320-340 nm), UVA-II (340-400 nm) and short-blue (400-420 nm).

Biological responses	**Action spectra**				**Reference**
	UVB	**UVA-I**	**UVA-II**	**Blue**	
Erythema	99.5	0.2	0.3	0.0	McKinley and Diffey (1987)
Persistent pigment darkening	2.9	54.9	42.3	0.0	Moyal et al. (2000)
Elastosis	63.4	24.1	12.4	0.0	Wulf et al. (1989)
Photoaging	3.6	61.6	34.8	0.0	Bisset et al. (1989)

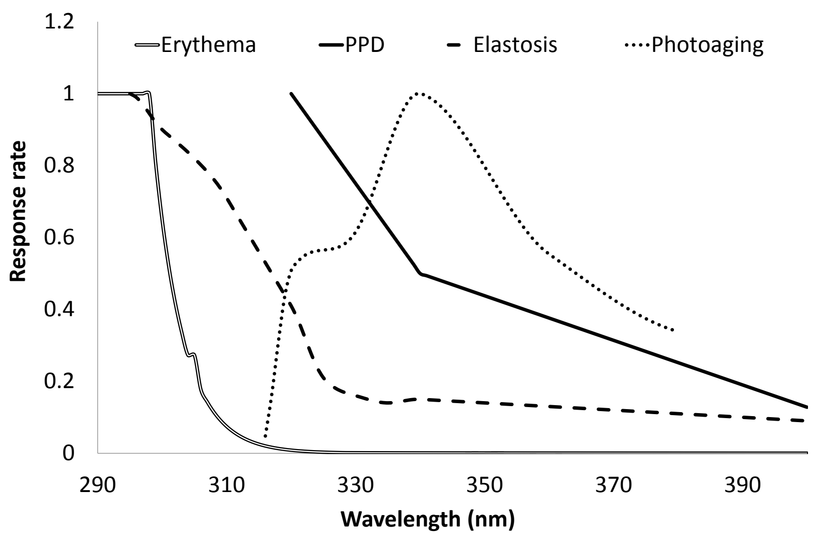


Figure 15. Biological response rate based on action spectra driven by UV radiation. Action spectra were applied to calculate the percentage of effective solar absorption radiation (%ESAR) from the creams formulations and EPI from algal extracts.

2.10 Sun Protection Factor (SPF)

The sun protection factor (SPF) indicates how much longer, a sunscreen, increases the skin's natural defense capacity before producing actinic erythema or reddening of the skin, using a photoprotection product against pre-burn erythema. For example, a person with fair skin (phototype I) who normally begins to suffer from erythema after 10 min in the sun, would take 15 times for a response, then a respective SPF 15 (150 min or 2.5 h) (Gilaberte et al., 2003). For the calculation of SPF, the 1.3

$mg.cm^{-2}$ of cream formulation was quickly applied to each PMMA plate according to the *in vitro* method Colipa (2011). The cream was distributed as evenly on the surface of the plate as described for the analysis %ESAR and EPI. After this period, the plates containing the cream formulation were placed between the path of the light beam of a solar simulator and the spectrometer. The solar simulator used was a Spectra-physics equipped (Model 66902, USA) with a mercury-xenon lamp (Lamp Power 50-500 W). This instrument generates the light source according to the reference standard, which must be between 51.4-63.7 $W.m^{-2}$ for total irradiance (290-400 nm) and the UVA/UVB ratio must be between 16.9-17.5 (Colipa 2011).

The percentage of transmission of UV radiation through the sample was measured at a spectral range of 280 to 400 nm, at 1 nm intervals and at four different sites on the plate. The blank was prepared using commercial PMMA plates according to the physicochemical properties described by Pissavini et al. (2009). This method describes the use of glycerol as a blank for the test (1.3 $mg.cm^{-2}$) in the same amount as the cosmetic product, since the Colipa (2011) method assumes that the photoprotectors manufactured today have in their composition high percentages of glycerol. The SPF for the UV radiation (290-400 nm) was calculated as:

$$\mathrm{SPF} = \frac{[\Sigma \mathrm{abs}(290 - 420\ \mathrm{nm}) \mathrm{E}(\lambda)\ \mathrm{x}\ \mathrm{S}(\lambda)]}{[\Sigma \mathrm{abs}(290 - 420\ \mathrm{nm}) \mathrm{E}(\lambda)\ \mathrm{x}\ \mathrm{S}(\lambda)\ \mathrm{x}\ \mathrm{T}(\lambda)]}$$

where: Σ_{abs} is the absorbances sum, E(λ) is the spectrum of erythematic action (ICD-1987), S(λ) is the spectral irradiance of the light source (solar simulator) and T(λ) is the monochromatic transmittance of the plate with the cosmetic formulation.

The UVA SPF was calculated as described above for SPF, using the spectral range for UVA radiation (320-400 nm). Instead of using the spectrum of erythematic action as in the SPF, the spectrum of action for persistent pigmentation darkening (PPD) is used for UVA SPF.

2.11 Cells cultures

Cytotoxicity activity 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 a tumourigenic colon cancer commonly used in therapeutic research and drug screening. HL60 is a leukemia cell line used for research on blood cell formation and physiology. HGF1 is a human gingival fibroblast, non-tumor connective tissue cells and used as negative cytotoxic cell viability. HaCat is a human keratinocyte, a non-tumor cell line and widely used in scientific research for its high capacity to differentiate and proliferate *in vitro* (Micallef et al., 2009).

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 100X (Biowset, Spain) and 0.5% of 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 100X (Biowset, Spain) and 0.5% of amphotericin B (Biowset, Spain). Cells were sub-confluent maintained at 37 °C in humidified air containing 5% CO_2. Cultured cells were collected by gentle scraping when confluence reached 75% for HTC-116, HGF-1 and adherent cells were obtained for HaCaT. HL-60 cells were collected by centrifugation at 1,500 rpm for 5 min due to the suspension trait.

2.12 Cytotoxicity assay

The proliferation of the cell lines was estimated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay according to Mosmann (1983). This method is simple and 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 insoluble form formazan (purple color) that is quantified spectrophotometrically. The ability of cells to reduce MTT constitutes a bioindicator of mitochondrial integrity and its functional activity being interpreted as a measure of cell viability (Jimenez et al., 2008).

A first previous screening of cytotoxicity activity 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 were tested (0.019, 0.039, 0.078, 0.156, 0.312, 0.625, 1.25, 2.5, 5 and 10 mg.mL^{-1}) with the four cell lines against HCT116, HL60, HGF1 and HaCat cell lines.

A second previous screening of cytotoxicity activity was performed with hydroethanolic and alkaline aqueous extracts from the beach-cast species *Z. tournefortii*, *A. seaforthii* and *C. isthmocladum* from the extraction at 100 mg.mL^{-1} and tested against the cell lines 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 x 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 type of 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 °C in a humid atmosphere with 5% CO_2 for 72 h. After this time, the microplate was centrifuged and the medium was removed from the wells. Then, 100 µL of medium (DMEM or RPMI) and 10 µL 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% CO_2 for 4 h. After this last incubation time, the crystals formed were dissolved by adding 150 µL 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:

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

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 IC_{50} values were calculated by linear regression and expressed as mg.mL^{-1}.

2.13 Data analysis

Statistical analyses were performed using Statistica 10 software. Data were tested for normality (Kolmogorov-Smirnov test) and homoscedasticity (Bartlett's test). One-way or bifactorial analysis of variance (ANOVA) was used to compare the results ($p < 0.05$). When differences were detected, Newman-Keuls *post-hoc* multiple comparison test was applied. To evaluate the ESAR values, a one-way analysis was performed for each species for the extracts.

3. Results

Elemental cell contents of carbon, nitrogen and sulfur, elemental stoichiometry and crude proteins of the beach-cast species are in Table 14. 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 algae *C. isthmocladum* had the lowest contents of carbon and nitrogen but showed the highest degree of sulfate among all 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 in *A. seaforthii* (14.95%), *O. obtusiloba* (11.78%) and *D. jolyana* (ES) (9.12%).

Table 14. Percentage of C, N and S, ratios of C:N, C:S and C:N:S and crude protein of selected species of beach-cast marine algae harvested from the Brazilian northeast (PB, Paraíba State) and southeast (ES, Espírito Santo State) coast.

Species	Carbon	Nitrogen	Sulfur	C:N	C:S	C:N:S	Crude Protein
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
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
C. isthmocladum	14.77	0.82	3.59	17.98	4.11	5.01	4.04

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. 16), regardless of, some patterns are relevant to highlight. The relative absorption spectra were more intense between 280 and 300 nm (UV spectrum) (Fig. 16A) and between 400 to 440 nm (visible spectrum) and small absorption bands at 640 to 700 nm (Fig. 16B).

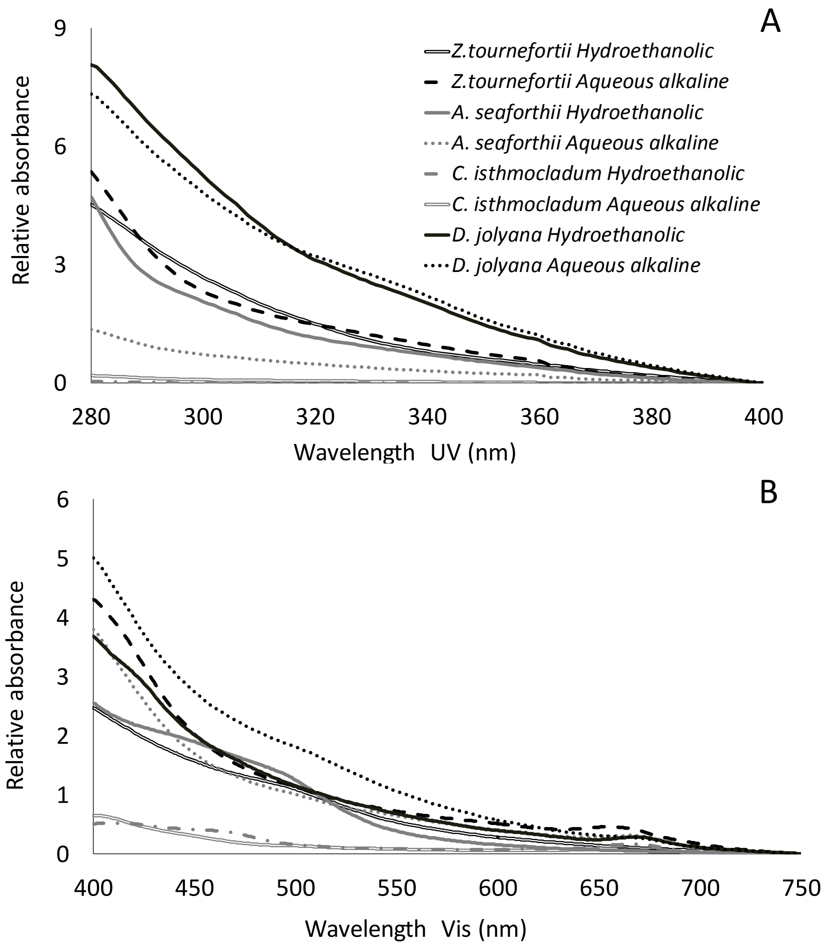


Figure 16. Relative absorption spectra (normalized data with the absorbance value of the longest wavelength for UV at 400nm and visible at 750 nm) for hydroethanolic and aqueous alkaline extracts from four selected beach-cast species for further concentration procedures (see details in section 2). (A) UV relative absorption spectra and (B) visible relative absorption spectra.

The hydroethanolic and aqueous extracts (at 100 $mg.mL^{-1}$ extraction proportion) from brown species (*D. jolyana*, *D. polypodioides* and *Z. tournefortii*) showed higher phenolic contents than red (*O. obtusiloba* and *A. seaforthii*) and green (*C. isthmocladum*) species (Fig. 17A). Additionally, phenolic compounds in the aqueous alkaline extracts were higher than hydroethanolic extracts (Figs. 17A), except for the extracts from the red macroalgae. Higher amounts of phenolic compounds were detected for both extracts at 500 mg.mL^{-1} extraction proportion from *D. jolyana* (ES), *Z. tournefortii*, *A. seaforthii* and *C. isthmocladum* (Fig. 17B) when compared to the 100 mg.mL^{-1} extraction proportion.

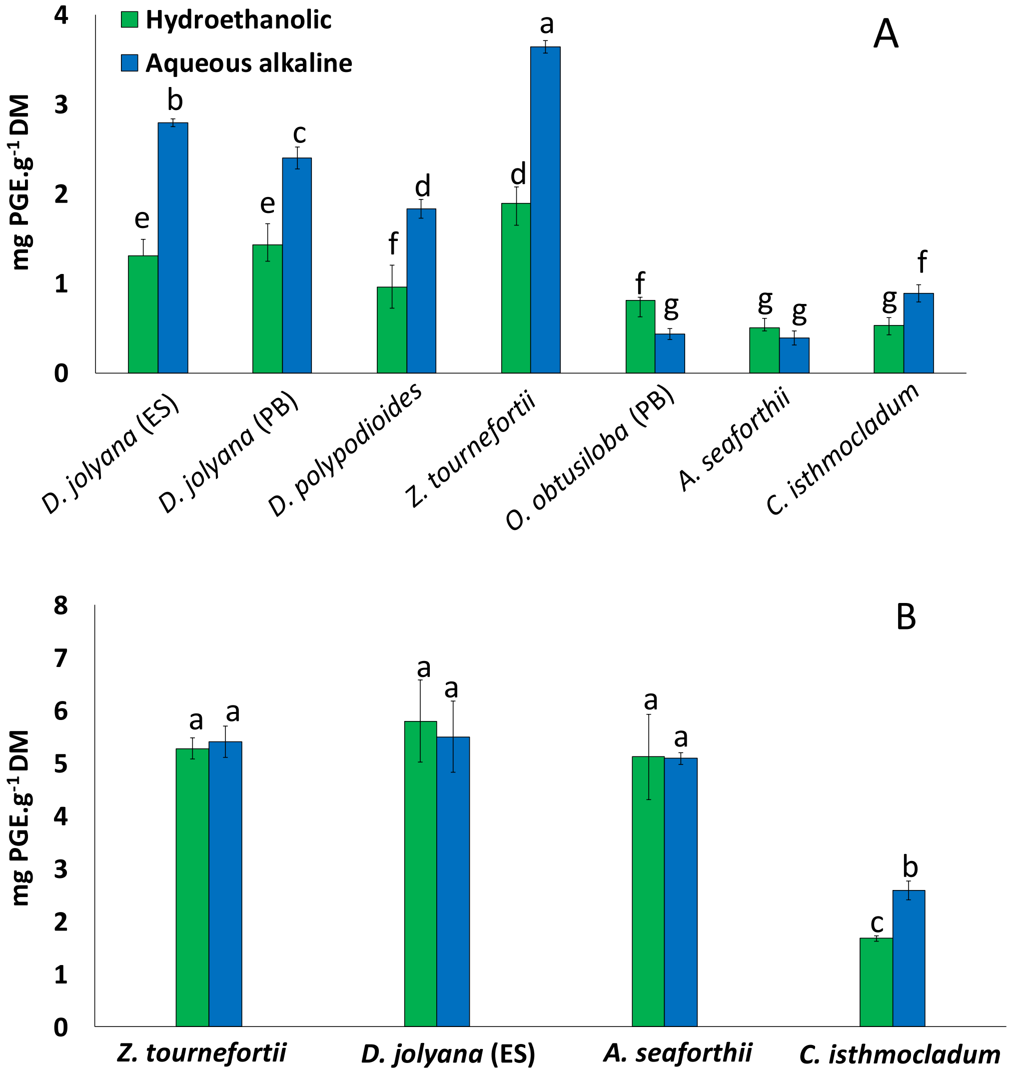


Figure 17. Level of phenolic compounds (mg PGE.g^{-1}) in hydroethanolic and aqueous alkaline extracts at extraction proportion of (A) 100 mg.mL^{-1} and (B) 500 mg.mL^{-1} from beach-cast algae species collected in the northeast Paraíba (PB) and southeast, Espírito Santo (ES) from Brazilian coast (Mean ± SD; n = 3). Letters indicate significant differences ($p < 0.05$) according to bifactorial ANOVA and Newman-Keuls *post-hoc* test.

Antioxidant activity obtained by the ABTS method was higher in both extracts from brown and red beach-cast algae at an extraction proportion of 100 mg.mL^{-1} compared to green alga extracts (Figs. 18A). Comparing the antioxidant activity between the extracts at 100 mg.mL^{-1} versus 500 mg.mL^{-1} extraction proportion similar levels were recorded (Fig. 18B), except for the extracts from *C. isthmocladum* and other species, in which 500 mg.mL^{-1} showed lower antioxidant power.

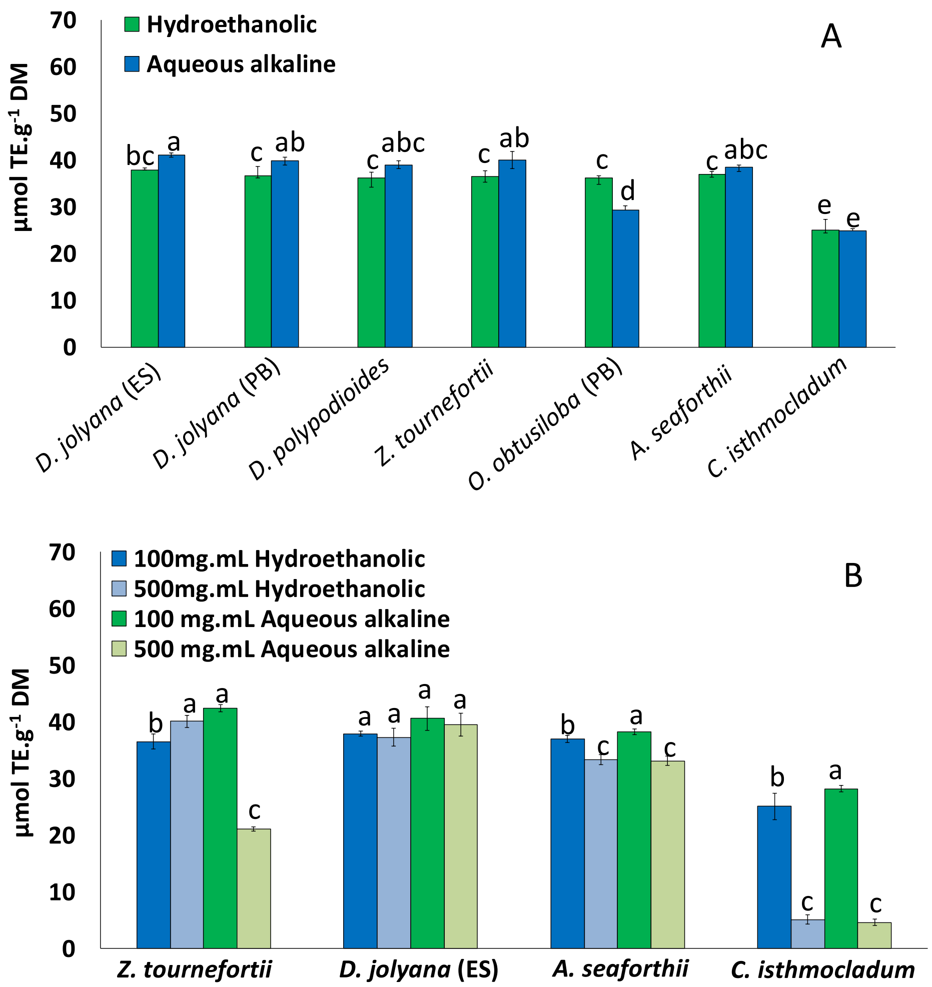


Figure 18. Antioxidant activity (Mean ± SD; *n* = 3) for the ABTS assay of hydroethanolic and aqueous alkaline extracts at (A) 100 mg.mL^{-1} and (B) the comparison of 100 mg.mL^{-1} and 500mg.mL^{-1} from beach-cast algae species collected in the northeast Paraíba (PB) and southeast, Espírito Santo (ES) from the Brazilian coast. Letters indicate significant differences ($p < 0.05$) according to bifactorial ANOVA and Newman-Keuls *post-hoc* test. For figure B, the bifactorial ANOVA was performed independently for each species.

The percentage of ESAR (effective solar absorption radiation) for hydroethanolic extracts at 100 $mg.mL^{-1}$ extraction proportion (Fig. 19A) and for both extracts at 500 $mg.mL^{-1}$ (Fig. 19B) were analyzed. From the extracts at 100 $mg.mL^{-1}$ the ESAR percentages were lower than 25% (Fig. 19A). The extracts at 500 $mg.mL^{-1}$ enhanced ESAR values were registered (Fig. 19B), with hydroethanolic extracts from *Z. tournefortii* and *A. seaforthii* and both extracts from *D. jolyana* exhibiting the highest values, 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 (Fig. 20) was higher at 10 mg/cm^2 from extract proportion of 500 $mg.mL^{-1}$ of hydroethanolic extracts from *D. jolyana* and *A. seaforthii*, reaching values of 47 and 17, respectively.

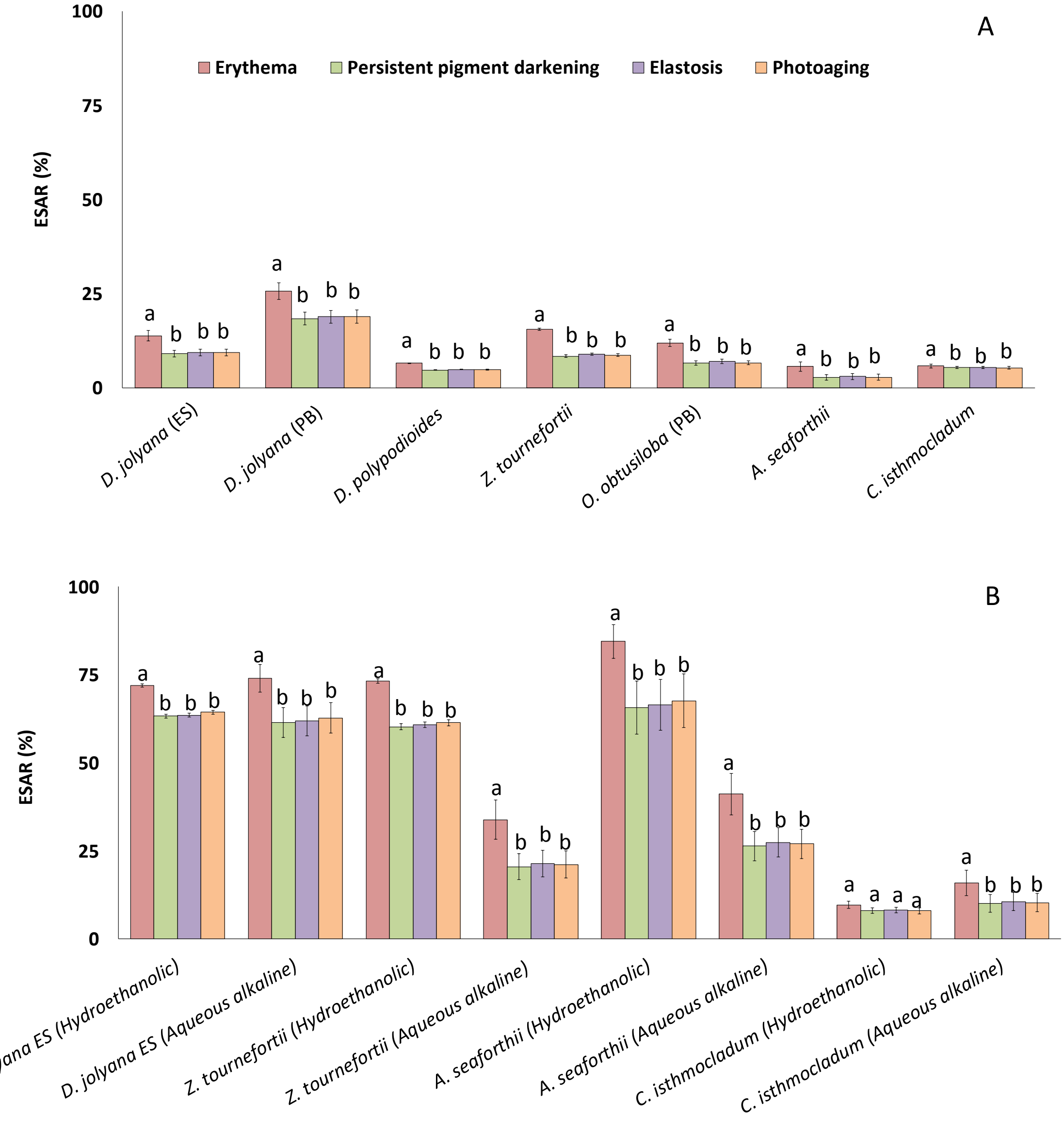


Figure 19. Percentage of effective solar absorption radiation (ESAR) (Mean ± SD; *n* = 3) evaluated for erythema, persistent pigment darkening, elastosis and photoaging action spectra of (A) hydroethanolic extracts at 100 mg.mL^{-1} extraction proportion and (B) hydroethanolic and aqueous alkaline extracts at 500mg.mL^{-1} extraction proportion from beach-cast algae 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.

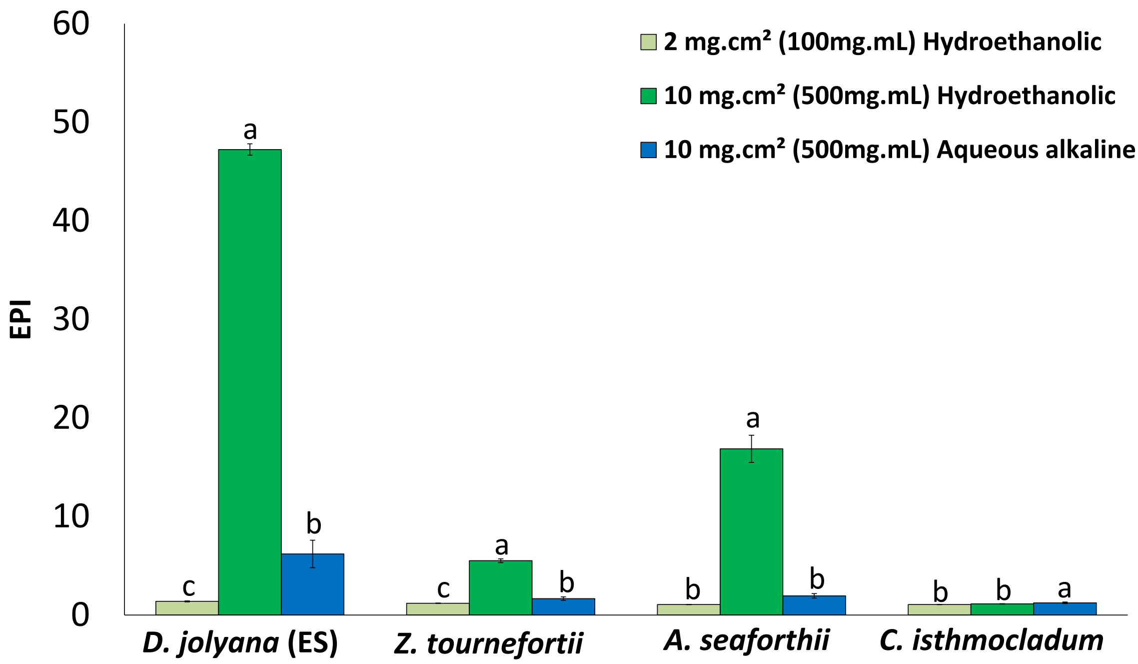


Figure 20. Extract photoprotection index (EPI) of hydroethanolic and aqueous alkaline extracts from four beach-cast algae species collected on the Brazilian coast (ES, Espírito Santo State). Letters indicate significant differences ($p < 0.05$) according to bifactorial ANOVA (extracts and concentrations were the variables) and Newman-Keuls *post-hoc* test.

Figure 21 evidenced a pattern of relationship between EPI versus %ESAR responses with the erythema action spectra. For hydroethanolic extracts at 2 mg.DM.cm^{-2} and from extract proportion of 100 mg.mL^{-1} a linear correlation among the species with the tendency of low ESAR and EPI and high ESAR and EPI results (Fig. 21A). The extract with the highest %ESAR and EPI was the hydroethanolic from *D. jolyana* (ES) with the concentration of extract 500 mg.mL^{-1}, 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. 21B). 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. 21C).

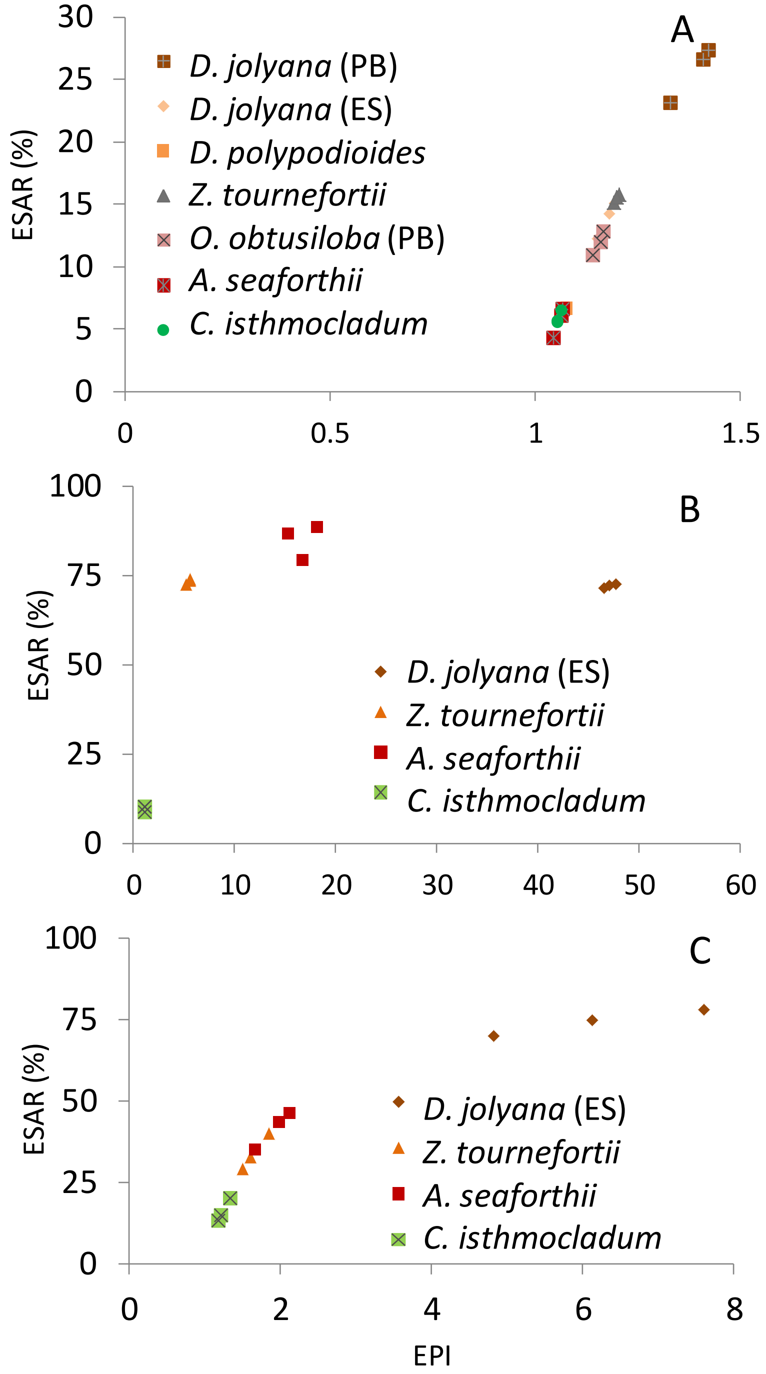


Figure 21. Extract photoprotection index (EPI) *versus* effective solar absorption radiation (%ESAR) for (A) hydroethanolic extracts at 2 mg.DM.cm^{-2} from extract proportion of 100 mg.mL^{-1}, (B) hydroethanolic at 10 mg.DM.cm^{-2} from extract proportion of 500 mg.mL^{-1} and (C) aqueous alkaline at 10 mg.DM.cm^{-2} from extract proportion of 500 mg.mL^{-1} of beach-cast algae species collected in the northeast (PB, Paraíba State) and southeast (ES, Espírito Santo State) from the Brazilian coast. The action spectra of biological responses applied were erythema. Data were plotted considering three replicates per each extract concentration of 2 or 10 mg.DM.cm^{-2}.

Cream formulations were made with hydroethanolic and aqueous alkaline extracts from the beach-cast algae and the sun protection factor (SPF) was evaluated. The SPF of the creams at the extraction proportion of 100 $mg.mL^{-1}$ were low for all species in both extracts, reaching a maximum SPF value of 1.6 in a concentration of 0.036 $mg.DM.cm^2$ on the PMMA plate (Fig. 22A), consider that the maximal SPF range is 130.

After the above test, increasing concentrations of the cream were evaluated for the hydroethanolic extracts at an extraction concentration of 500 $mg.mL^{-1}$ from *D. jolyana* (ES), *Z. tournefortii*, *A. seaforthii* and *C. isthmocladum*, ranging from 0.036 $mg.DM.cm^2$ to 0.6 mg $DM.cm^2$ (Fig. 22B). Increased SPF was observed for extracts of *D. jolyana*, *Z. tournefortii* and *A. seaforthii* at 0.18 $mg.DM.cm^2$ in the plate with hydroethanolic extracts at 500 $mg.mL^{-1}$, an increase in the SPF reaching up to 2.4 was observed for *D. jolyana* (ES) (Fig. 22B).

Cytotoxicity activity was evaluated for two tumoral cell lines, leukemia (HL60) and colon (HTC116) and non-tumoral cell lines, keratinocytes (HaCat) and human fibroblasts (HGF1), with crude extracts in DMEM (20 $mg.mL^{-1}$) for all the seven species of beach-cast seaweeds. It is important to point that a lower IC_{50} represents better activity. The extracts from *C isthmocladum*, *Z. tournefortii* and *D. jolyana* (ES) showed the best results against HL60 with IC_{50} values lower than 1 $mg.mL^{-1}$ (Fig. 23A). Regarding the cytotoxicity activity against HTC116, all the extract showed IC_{50} values lower than 1 $mg.mL^{-1}$, except for *D. polypodioides* that had 4.15 $mg.mL^{-1}$ (Fig. 23B). 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 tested, fibroblasts (HGF1) and keratinocytes (HaCat) (*data not show*).

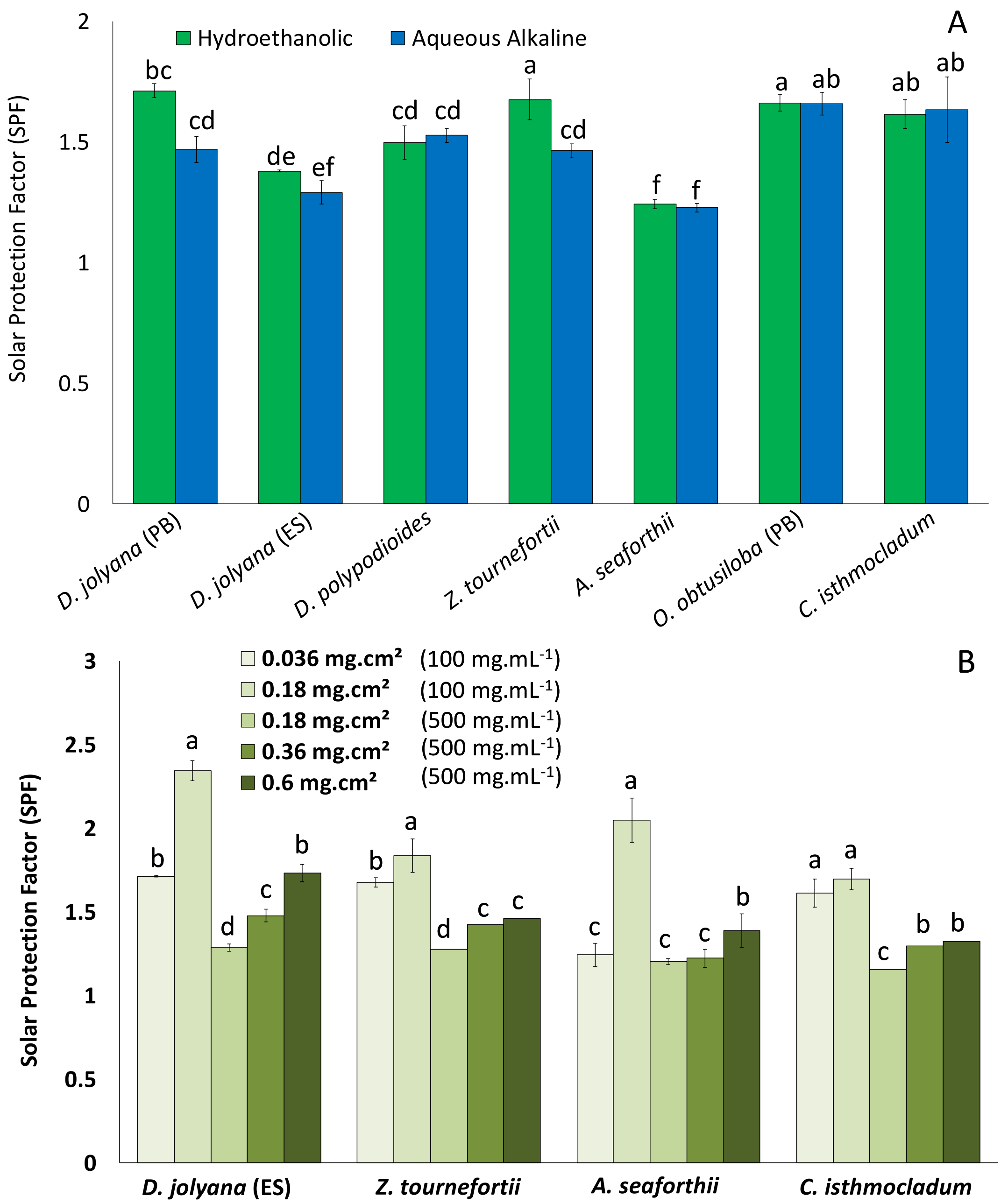


Figure 22. SPF *in vitro* (Solar protection factor) of (A) hydroethanolic and aqueous alkaline extracts at 100 mg.mL^{-1} and (B) hydroethanolic at 100 and 500 mg.mL^{-1} from beach-cast algae species collected in the northeast (PB, Paraíba State) and southeast (ES, Espírito Santo State) from the Brazilian coast. Data were plotted considering four replicates per each extract concentration of mg.DM.cm^{-2}. Letters indicate significant differences ($p < 0.05$) according to bifactorial and Newman-Keuls *post-hoc* test.

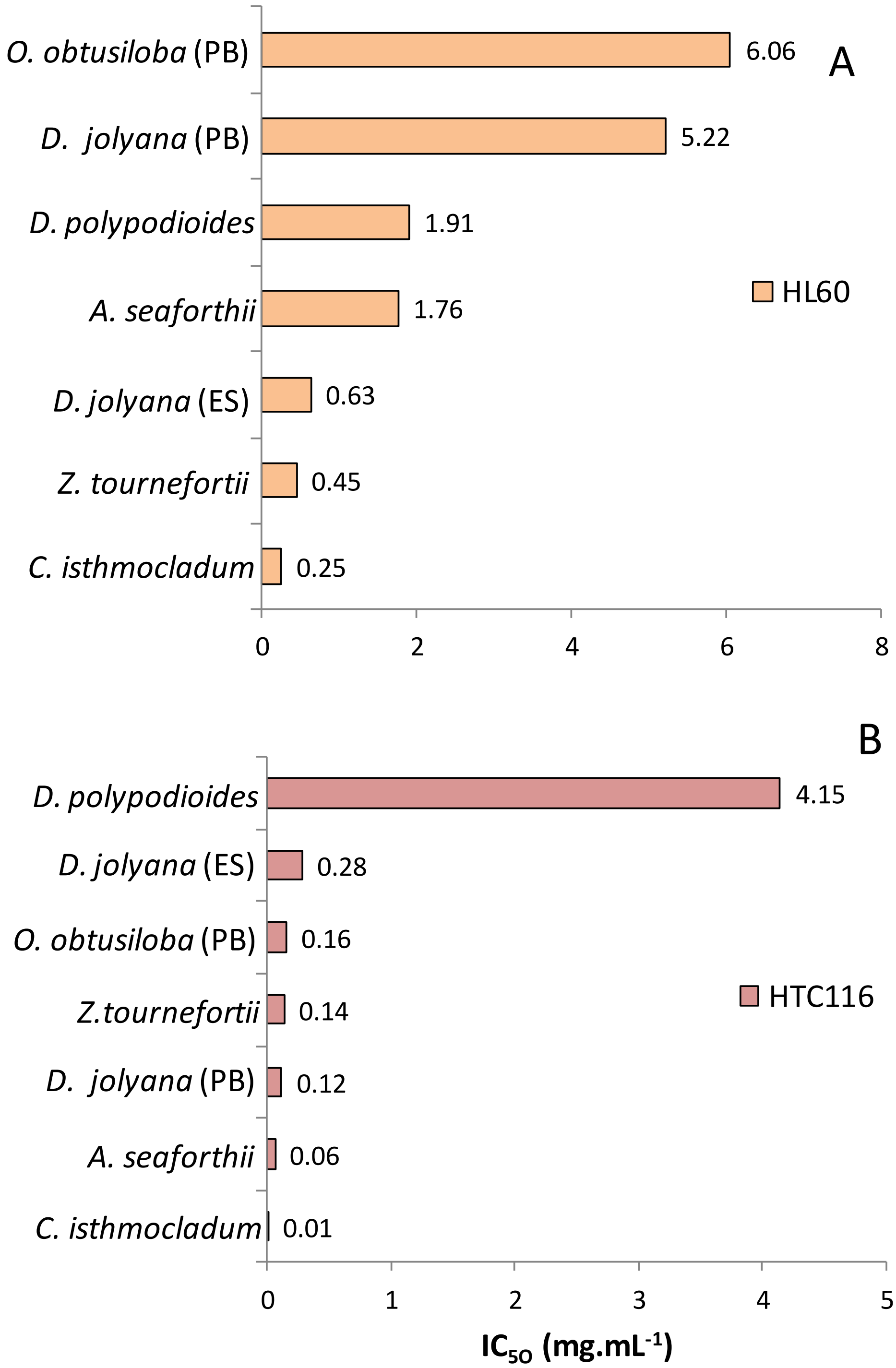


Figure 23. Cytotoxicity activity expressed as IC_{50} ($mg.mL^{-1}$) of the crude extract in DMEM for the tumoral lines (A) leukemia HL60 and (B) colon HTC116 from beach-cast algae species collected in the northeast (PB, Paraíba State) and southeast (ES, Espírito Santo State) from the Brazilian coast.

After the evaluation of the above results, only one specie was chosen from each macroalgae 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. 24).

Both extracts showed low IC_{50} values against leukemia cell lines (HL60) ranging from 0.06 $mg.mL^{-1}$ to 5.58 $mg.mL^{-1}$ (Fig. 24A). For the HL60 strain, *C. isthmocladum* showed better cytotoxicity activity for DMEM and aqueous alkaline extract, while for the brown beach-cast *Z. tournefortii* the hydroethanolic extract showed the lowest IC_{50} (0.06 $mg.mL^{-1}$). 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 achieved with the crude extract in DMEM (1.76 $mg.mL^{-1}$) (Fig. 24A).

For colon cell lines, all extracts showed low IC_{50} ranging from 0.01 $mg.mL^{-1}$ to 1.41 $mg.mL^{-1}$ (Fig. 24B), 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. 24B). In general, all the extracts of beach-cast seaweeds had a stronger cytotoxicity activity against HTC116 when compared to HL60 cell lines inhibition.

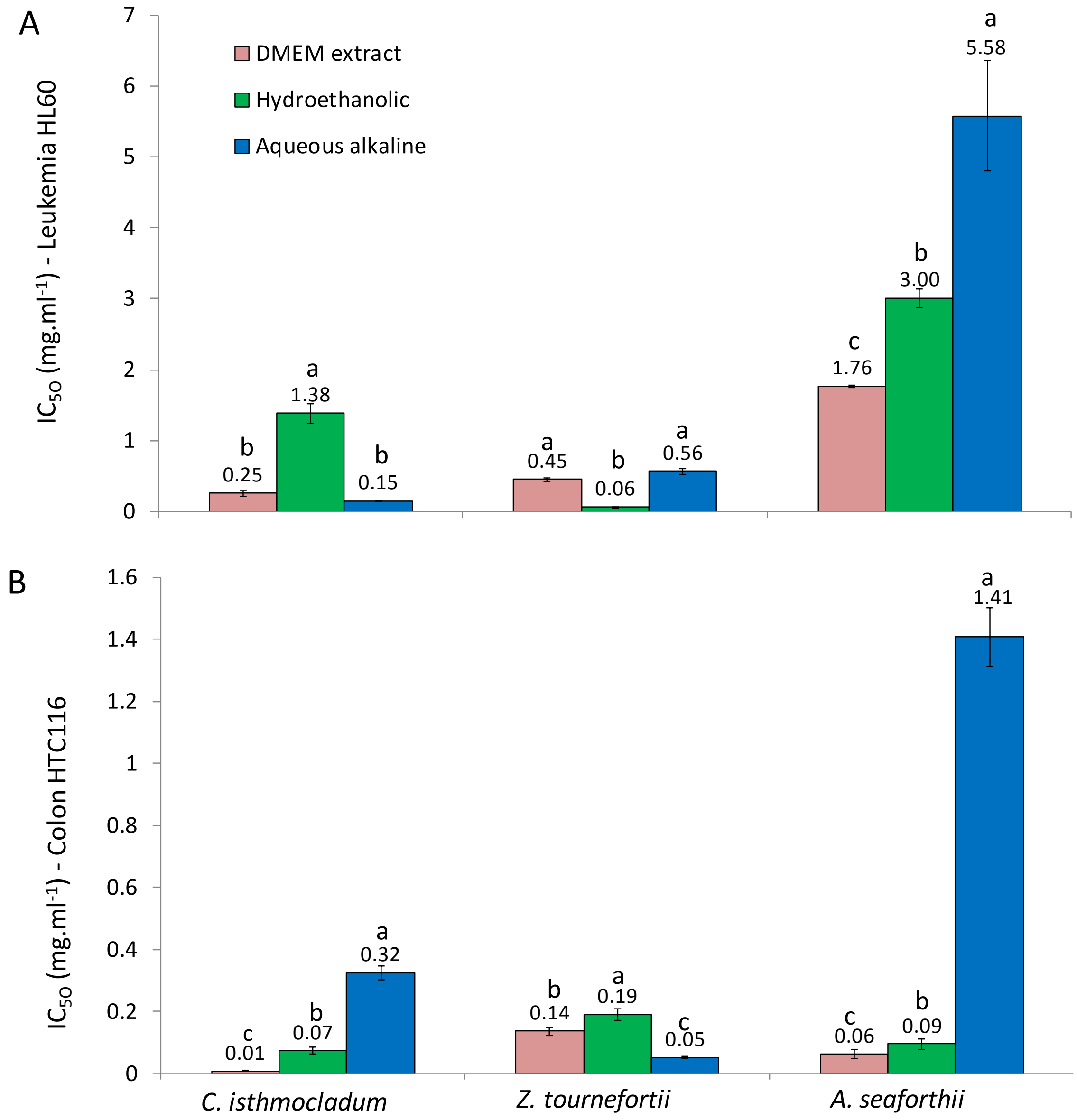


Figure 24. Cytotoxicity activity expressed as IC_{50} ($mg.mL^{-1}$) of crude extracts in DMEM and hydroethanolic and aqueous alkaline extracts at 100 $mg.mL^{-1}$ for the tumoral cell lines (A) leukemia HL60 and (B) colon HTC116 from beach-cast algae species collected in the southeast (ES, Espírito Santo State) 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.

4. Discussion

Macroalgae can be a sustainable source of natural metabolites due to their huge diversity, 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 and vitamins, and secondary metabolites such as phenolic compounds, terpenoids or pigments, which them great candidates for industrial applications (Maschek and Baker, 2008; Williamson et al., 2019).

There is a growing interest in the search for new ingredients for cosmetic industry with biological actions and multifunctional properties from marine macroalgae, where one of the limitations is the availability of biomass (Wells et al., 2017), currently, most of the biomass is destined for food and phycocolloids. In Brazil, one of the main challenges in the exploitation of algae 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 7,000 km, offers great potential for the use of different beach-cast seaweeds species with tonnes of biomass available all over the year.

Chemical composition

Most of the species evaluated in the present study showed a high content of total internal carbon (>30%), similar to results for C content describe in literature for macroalgae (Vega et al., 2020a). The N content obtained in this study varied between 0.82 to 3.04% in beach-cast algae, lower than results obtained by other authors, which ranged from 2.50 to 4.50% with macroalgae (Vega et al., 2020a,b). This study found that brown beach-cast algae had high C:N ratios values, and optimal C:N values are considered close to 10 (Lapoint and Ryther,1989). In this study, most of the species showed a C:N ratio higher than 10, indicating that these beach-cast species produce high contents of secondary metabolites, especially carbohydrates. Lindroth (2010) 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 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 was correlated with high C:N ratios and low C:N ratios were correlated with high amino acids and proteins in macroalgae (Handå et al., 2013; Schiener et al., 2015; Ometto et al., 2018).

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 (Kim et al., 2013). A patent filed in 2009 by founder of the Bioderma-Esthederm group, proposes the use of an aqueous extract of *Blidingia minima* (Nägeli ex Kützing) Kylin rich in polysaccharides with moisturizing properties in order to improve the state of the skin (Couteau and Coiffard, 2016). Sulfated polysaccharide composition in cosmetic and skincare products has often been associated to antioxidant, tonifying, cleaning, hydrating and revitalizing bioactivities (Pangestuti and Kim, 2014). An aqueous extract of the brown alga *M. pyrifera* is available on the market and seems to stimulate the synthesis of hyaluronic acid (Couteau and Coiffard, 2016), which is a principal component of the extracellular matrix of the skin (Price et al., 2007). In addition, macroalgae have been marketed as functional foods and nutraceuticals, which include foods that contain bioactive compounds or phytochemicals that could benefit human health (Hafting et al., 2012). In this context, our studied extracts of beach-cast seaweeds could be a valuable source as active ingredients for moisturizing and antiaging products, functional foods, nutraceuticals and improving nutritional diet.

Antioxidant activity and phenolic compounds

The antioxidant activity is one of the most studied bifunctional properties, driven by the search for natural antioxidants that have minimal side effects, in order to the replacement of synthetic antioxidants that can be harmful to health (Vijayabaskar et al., 2012). 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 (Biris-Dorhoi et al., 2020). 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 antitumor properties.

In the last decades, phenolic compounds present in different marine algae species have been used in cosmetic and nutraceutical products (oral or topically). Brown algae are the main producers of these compounds, which phlorotannins are the most relevant substances in these algae; however, green and red algae can also produce phenolic compounds in lower concentrations, and bromophenols are the main molecules for the last group (Jimenez-Lopez et al., 2020). Purified phlorotannins are included in cosmetic formulations, these compounds can prevent and decrease the skin aging process, which is mainly associated with free radical damage and reduction of hyaluronic acid concentration (Ferreres et al., 2012). The levels of phenolic compound registered for the extracts of the red beach-cast algae could be attributed to the presence of bromophenols, commonly found in Rhodophyta species. Cian et al. (2014), studying the red macroalga *Porphyra columbina*, pointed out phenolic compounds as the main responsible for the antioxidant capacity in Rhodophyta species.

A large number of macroalgae extracts were 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 activity (Couteau and Coiffard, 2016). In this study, beach-cast seaweeds with high amounts of phenolic compounds also showed higher antioxidant activity. Similar to our results, a positive correlation between ABTS assay and phenolic compounds was described for aqueous extracts from macroalgae (Vega et al., 2020b).

Our findings are very promising because indicate the potential of beach-cast macroalgae 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 (HGF 1), 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

Macroalgae have developed chemical protection mechanisms, including MAAs throughout evolution (Williamson et al., 2019). MAAs are secondary N-metabolites that can act in the protection against UV radiation (Lalegerie et al., 2020). 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 (Fernandes et al., 2015). These compounds absorb within the UV range and are characterized by having a single peak with maximum absorption between 310 and 360 nm (Wada et al., 2015).

Our results of UV absorption spectra, no prominent peaks of MAAs were observed. The analyzed red beach-cast seaweed species did not have large amounts of MAAs naturally, findings in accordance with Briani et al. (2018) which evaluated the content of MAAs in red algae 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 other species could still be more concentrated to confer a higher response of %ESAR and EPI. Schneider et al. (2020), observed a clear pattern of hyperbolic responses between %ESAR and EPI with two species from macroalgae, suggesting that other species could still be studied and higher concentrations can be tested to reach the same pattern of hyperbolic responses.

Another parameter desired in a cosmetic formulation is SPF. Our study formulated cosmetic products with antioxidant activity and photoprotective capacity using aqueous and hydroethanolic extracts from beach-cast algae and other ingredients used in natural cosmetics, but low SPF values were obtained in the present study. Zárate et al. (2020) showed that methanolic and hydroethanolic extracts from 15 materials of beach-cast seaweeds from Portugal presented UV protection, with SPF values above 30. Consider our results, we suggest that other forms of extraction and incorporation of the extract into the base creams should be evaluated in order to improve the SPF values. The isolation and purification of substances could also increase the efficiency of the photoprotective capacity.

The search for photoprotection properties is not limited to applications in formulations of sunscreens or creams. 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 (Turnbull and Parisi, 2005). However, most research with macroalgae 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 activity, cytotoxicity activity and photoprotection are the sulfated polysaccharides, specific metabolites from macroalgae. These molecules are not present in terrestrial vegetables and are commonly found in the three groups of seaweeds, brown algae (Ochrophyta), red algae (Rhodophyta) and green algae (Chlorophyta) (Pangestuti et al., 2018). Sulfate is a typical substitution of seaweed's polysaccharides (Jiao et al., 2011). This anion is linked to the polysaccharide through an ester bond ($O\text{-}SO3^{-}$) and plays a very important role from the point of view of the biological properties of algae. Several studies showed a positive correlation between sulfate content and biological properties such as antioxidants, anticoagulants, anticancer, antiviral, antiallergic and anti-inflammatory (Croci et al., 2011; Holdt and Kraan, 2011; Wijesekara et al., 2011).

The extracts of *C. isthmocladum* showed low antioxidant activity and phenolic compounds; however, they showed the highest cytotoxicity activity against the cell lines of leukemia (HL60) and colon (HTC116), possibly due 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 (Pinteus et al., 2017). The bioactivity of aqueous extracts from non-beach-cast *C. ishtmocladum* was also observed by Amorim (2018), 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.

Recently, Bellan et al. (2020) 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 show promising antitumor activities without the commonly collateral effects of the disease. Despite the unclear action mechanism of metabolites from macroalgae, it has been reported tumor cells inhibition by suppressing their expression and consequently showing anticancer property (Guedes et al., 2013).

The extracts of the brown and red beach-cast seaweeds analyzed have also antitumoral activity against leukemia and colon cancer. Popplewell and Northcote (2009) showed that bromophenols from the red alga *Osmundaria colensoi* (J.D. Hooker & Harvey) R.E. Norris 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 (Zárate et al., 2020). These findings reinforce the importance of this underused waste biomass.

Extracts with promising biological activities should have an IC_{50} value below to 0.1 $mg.mL^{-1}$ (Cos et al., 2006). In this context, the most promising species with citotoxic activity for HL60 was the hydroethanolic extract of *Z. tournefortii* (IC_{50} = 0.06 $mg.mL^{-1}$). Regarding HTC116, the extracts with promising activity were crude extracts in DMEM and hydroethanolic extracts from *C.isthmoclaum* (IC_{50} = 0.01 $mg.mL^{-1}$ and 0.07 $mg.mL^{-1}$, respectively) and *A. seaforthii* (IC_{50} = 0.06 $mg.mL^{-1}$ and 0.09 $mg.mL^{-1}$, respectively). The aqueous alkaline extract from *Z. tournefortii* also showed promising activity with IC_{50} 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 algae 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 also promoting 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.

5. Conclusions

Extracts from brown and red beach-cast algae 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 explore 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 non toxicity, therefore extracts of beach-cast algae may become valuable for the development of cosmetic products.

Challenges and final considerations

The biomass of beach-cast seaweeds is an easily accessible resource and can be used for innovative products with broad functional properties, as evidenced in this study.

The species of brown beach-cast algae proved to be good sources of bioactive compounds (antioxidant, anti-HIV-RT). The extracts of *C. isthmocladum* were very promising as anti-HIV-RT. The Mix samples showed medium antioxidant and anti-HIV-RT activity, however it is recommended its use for medium economic value products, with the advantage of not requiring biomass separation.

Complementary studies are recommended to evaluate the biofunctionality of beach-cast seaweeds for cosmetic purposes. The beach-cast species *Z. tournefortii* (brown), *A. seaforthii* (red) and *C. isthmocladum* (green) showed high bioactivity against tumor cells and further studies are suggested to identify bioactive compounds with metabolomic approaches and testing other bioactivities.

Discontinuous and unreliable supply of beach-cast seaweeds is one of the major challenges. Low-cost methods of preservation such as solar drying may address the problem of biomass storage. The biomass of beach-cast seaweeds, distribution and removal of detritus should be monitored in regions of occurrence to better understand the potential long-term effects of harvesting this material and could establish whether the population is expanding, remaining constant, or shrinking.

There are also significant challenges associated with the development of beach-cast seaweed-based ingredients due to varying levels of bioactive compounds in species and seasonal factors; therefore, the characteristic of the habitat can force these organisms to produce different contents or classes of metabolites. Then, studies are recommended to monitor the occurrence, total volume and specific abundance as well as seasonal and local bioactive assessment. Depending on the intended use, harvest times could be selected.

The beach-cast algae are a resource that can provide employment and economic benefit for local communities and commercial industry; however, the harvesting methods must be sustainable. If the activity becomes commercially viable, a management plan for harvest quotas is recommended in order to reduce ecological impacts. It is also recommended to incorporate a political plan that includes the local population and generates additional income for the most vulnerable communities.

Taking these challenges into account, the harvest and use of beach-cast seaweeds create a circular flow of energy and materials with dynamic scenarios in which all gains can reduce the consumption of unnatural resources, improving the social, environmental and economic value of coastal areas.

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