Exploring the photoprotective potential of seaweeds through alternative methods to animal testing

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Abstract

Seeking for new sources of substances with cosmetic/biotechnological potential sparks interest in seaweeds, marine organisms known for their adaptability. Despite its extensive coastline, marine resources in Bahia remain unexplored. This study aimed to evaluate the photoprotective potential, antioxidant activity, and chemical composition of seaweed collected in Bahia. Nine species were collected and identified through taxonomy, and the samples were processed and subjected to sequential extraction. The extracts were analyzed for absorption in the ultraviolet region, showing good absorption in UVA, UVB, and visible light. Three species were selected: *Caulerpa sertularioides*, *Solieria filiformis* and *Padina* sp. (showing the best UV absorption). All

three species underwent photo-stability tests, with *S. filiformis* showing the least photodegradation. In the assay for protection against reactive oxygen species (ROS) induced by UVA in human keratinocytes (HaCat) cells, *C. sertularioides* showed the greatest reduction in ROS. In short-term exposure eye irritation assays (STE), all three extracts were classified as non-irritating. Additionally, a molecular network was constructed using GC-MS, which indicates the compounds of interest: fatty acids, fatty alcohols, diterpenes, steroids, and alkaloids. The results highlight the potential of Brazilian seaweed extracts for innovative formulations of multifunctional skincare and/or haircare products.

Keywords: marine seaweed; photoprotection; antioxidant; alternative methods; metabolomics.

Introduction.

Seaweeds, photosynthetic organisms vital to marine ecosystems, provide oxygen, habitat, and food for various species. They also hold significant commercial value, with global seaweed aquaculture producing over 30 million tons of fresh biomass, valued at over $13.3 billion. This biotechnological potential attracts attention from the pharmaceutical, cosmetic, and chemical industries [1,2].

Climate change increases UV radiation exposure, affecting human health and organisms. Although essential for vitamin D synthesis, excessive exposure can cause acute and chronic damage, including premature aging, pigmentation, and skin cancer. This raises concerns about biochemical and biological changes [3].

Seaweeds are a rich source of extracts and compounds used in cosmetics, including UVA radiation absorbers as demonstrated by *Porphyra umbilicalis* extract, anti-aging agents such as *Coenochloris signiensis* extract, and skin elasticity and hydration restorers, showed by *Padina pavonica thallus* extract. These examples highlight the importance of exploring marine products such as sunscreens and promoting bioprospection for sustainable ingredients.

Seaweeds have developed defense mechanisms against solar radiation damage and, therefore, might be a source of innovative compounds for solar protection [4].

Seaweed extracts offer a sustainable alternative to traditional photoprotective compounds, which can harm the environment and cause coral bleaching. They enhance the protection spectrum and promote skin and hair hydration through carrageenans, fucoidans, carotenoids, and mycosporines. Incorporating seaweed extracts into cosmetics can meet consumer demand for natural and eco-friendly products [5].

Brazil's 8000 km coastline is home to diverse marine species, including endemic seaweeds. Bahia, the state with the longest coastline, is still in the early stages of exploring the biotechnological applications of its marine resources, particularly seaweeds. The unique biodiversity and tropical macroalgae species present great research opportunities [6]. Therefore, this study aimed to evaluate the chemodiversity, photoprotective potential, and antioxidant capacity of seaweed extracts collected in Villas do Atlântico, on the northern coast of Bahia, Brazil.

Materials and Methods.

Collection, Identification, and Extraction of Species

Authorization for material collection for research purposes was obtained from the Ministry of the Environment (MMA) (authentication code: 0881250120230420). The species were collected from the beachfront area of Vilas do Atlântico, Lauro de Freitas, BA, in 2023. Samples were identified through a taxonomic analysis conducted by Professor Dr. José Marcos de Castro Nunes at the Marine Algae Laboratory (LAMAR) at the Federal University of Bahia. Subsequently, the samples were dried and extracts were obtained using methanol-ethyl acetate (M-EA) [7].

Photoprotective Potential

The study evaluated the photoprotective potential of seaweeds through the determination of the UV/VIS absorption spectrum (UVA and UVB region - 280 to 400 nm) of the crude extracts using a spectrophotometer - Cary 60 UV-Vis – Agilent.

Photostability assay was conducted by comparing extracts exposed to UVA radiation of 9 J/cm² with a control group kept in the dark. Subsequently, the absorption in the UV (280 to 400 nm) was obtained using a spectrophotometer. The photodegradation percentage was calculated by comparing the area of the absorbance spectrum curve of irradiated samples to the area of non-irradiated samples, considered 100% [8].

Antioxidant Potential

The quantification of intracellular reactive oxygen species (ROS) in HaCat cells was conducted following UVA radiation exposure, using the probe $DCFH_2$-DA (2',7'-dichlorodihydrofluorescein diacetate). Cells were seeded in 96-well plates, incubated for 24 hours, treated with the extracts and controls, and maintained at 37ºC for 1 hour. Quercetin and Norfloxacin were used as ROS inhibitor and ROS generator controls, at the concentrations of 10 and 100 µg/mL, respectively. $DCFH_2$-DA solution was added, and the plates were re-incubated for 30 minutes and then exposed to UVA radiation with a total dose of 4 J/cm². Fluorescence was measured in a microplate reader (BioT já ek Synergy HT) at an excitation wavelength of 485 nm and an emission wavelength of 528 nm. The fluorescence of untreated irradiated cells was considered 100% to calculate the relative percentage of ROS in the samples [9].

Irritation Potential

The Short-Time Exposure (STE) assay was used for cytotoxicity evaluation of the extracts. The assay was conducted on rabbit corneal cells (SIRC), according to the OECD guideline nº 491, an alternative method to animal use. SIRC cells were seeded in 96-well plates. Following the incubation, the extracts, prepared in solutions of 0.05% and 5% of the

concentration 250 µg/mL, were applied to the wells in triplicate for 5 minutes at room temperature. The cells were incubated with MTT solution for 2 hours at 37ºC. After removing the MTT, a hydrochloric acid solution was added to extract the MTT, and the absorbance was measured in a microplate reader (BioTek Synergy HT) at 570 nm. Cell viability was calculated by subtracting blank absorbance values, and the samples were evaluated according to remaining cell concentration, categorizing the extracts as Category 1 (causing serious and irreversible damage); Category 2 (causing reversible effects); No category (non-irritant) [10].

Chemical Profile

The samples were analyzed using a gas chromatography system (GC 5977C/MSD – GC8890 - Agilent) coupled with mass spectrometry. The compounds were ionized, fragmented, and their mass spectra were recorded and compared with spectrum libraries (NIST11) for compound identification [11].

Results.

Identification and performance of the extractive procedure

Nine species of marine algae were collected and classified into three phyla: Chlorophyta (*Ulva lactuca, Caulerpa cupressoides var. lycopodium, Caulerpa sertularioides, Caulerpa mexicana*), Rhodophyta (*Centroceras gasparrini, Gracilaria caudata, Solieria filiformis*), and Ochrophyta (*Sargassum vulgare* and *Padina* sp.), as illustrated in Figure 1.

After the extraction procedure, with ethyl acetate and methanol, the yields were calculated, ranging from 2% to 19% of crude extract. The crude extracts with the highest yields were from the species: *C. sertularioides* (19.1%), *C. gasparrini* (4.0%), and *Padina* sp. (3.9%). Considering the phylum classification, absorption spectrum (in Figure 2A), and available literature regarding biotechnological potential, three species were selected for further

investigation of their antioxidant, photoprotective potential, safety profile, and chemical composition, namely: *Padina* sp.*, Caulerpa sertularioides*, and *Solieria filiformis*.

Figure 1. Taxonomic identification. A - *Ulva lactuca*; B - *Caulerpa cupressoides var. lycopodium*; C - *Caulerpa sertularioides*; D - *Caulerpa Mexicana*; E - *Centroceras gasparrini*; F - *Gracilaria caudata*; G - *Solieria filiformis*; H- *Sargassum vulgare*; I - *Padina* sp.

Photoprotective potential

The extract absorption spectrums in the UVB and UVA for each seaweed are demonstrated in Figure 2A. The photostability test was performed for *Padina* sp.*, C. sertularioides,* and *S. filiformis* and the extracts were considered photo-unstable, but without great variation in the absorption spectra (Figure 2B). The photostability predictions are presented in Table 1. *C. sertularioides* and *Padina* sp. showed similar photodegradation in the UV region, around 47% for UVA and 72% for UVB. On the other hand, *S. filiformis* exhibited photodegradation rates of 35% for UVA and 60% for UVB.

Figure 2. (A) Scanning spectrum of crude extracts from Bahia collected seaweed. (B) Photostability test of crude extracts of the chosen species *Caulerpa sertularioides*, *Padina* sp., and *Solieria filiformis*. NIR: non-irradiated. IR: irradiated

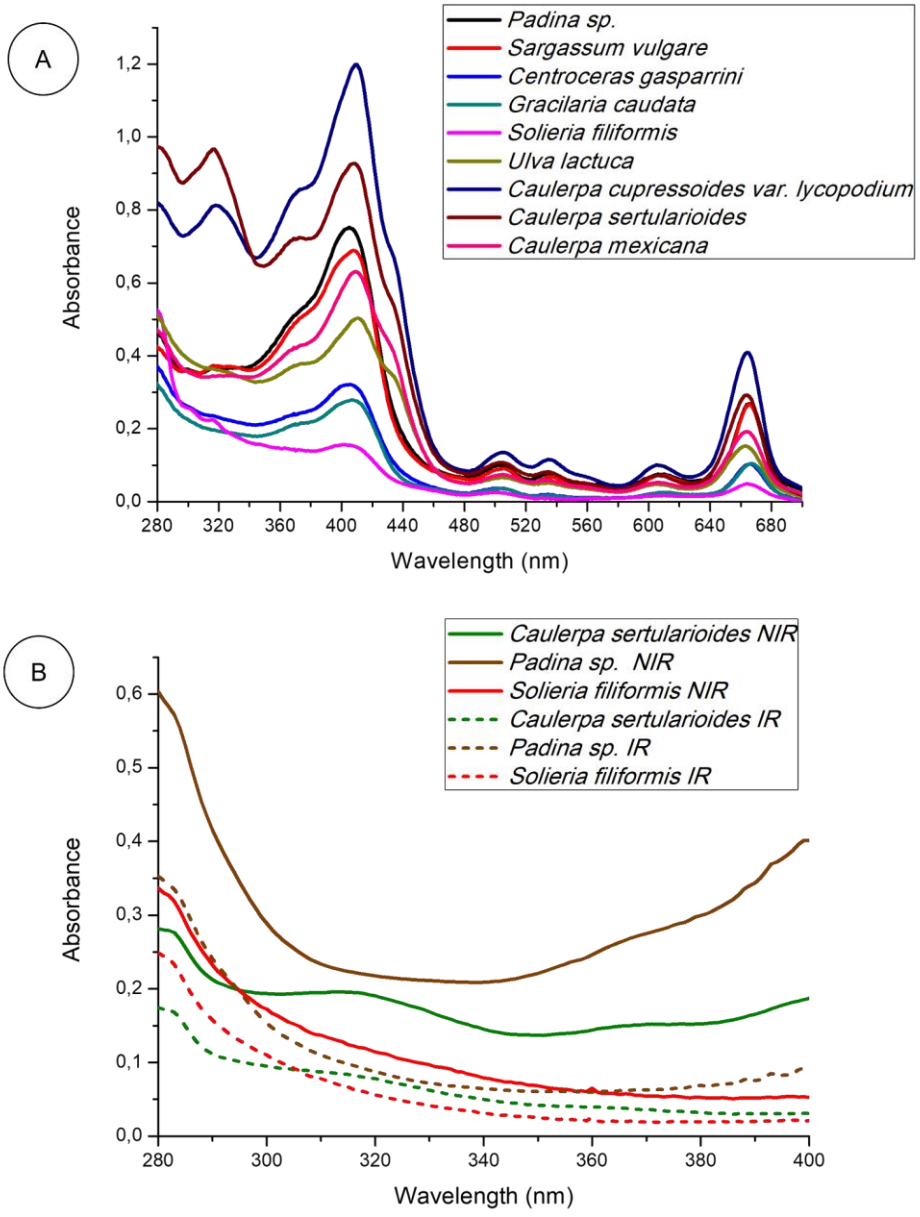


Table 1. UV radiation degradation of the crude extracts of *Padina* sp., *C. sertularioides, and S. filiformis*.

Crude Extracts	Photodegradation	
	UVA	UVB
Caulerpa sertularioides	72.45	49.75
Solieria filiformis	60.47	35.21
Padina sp.	73.90	46.08

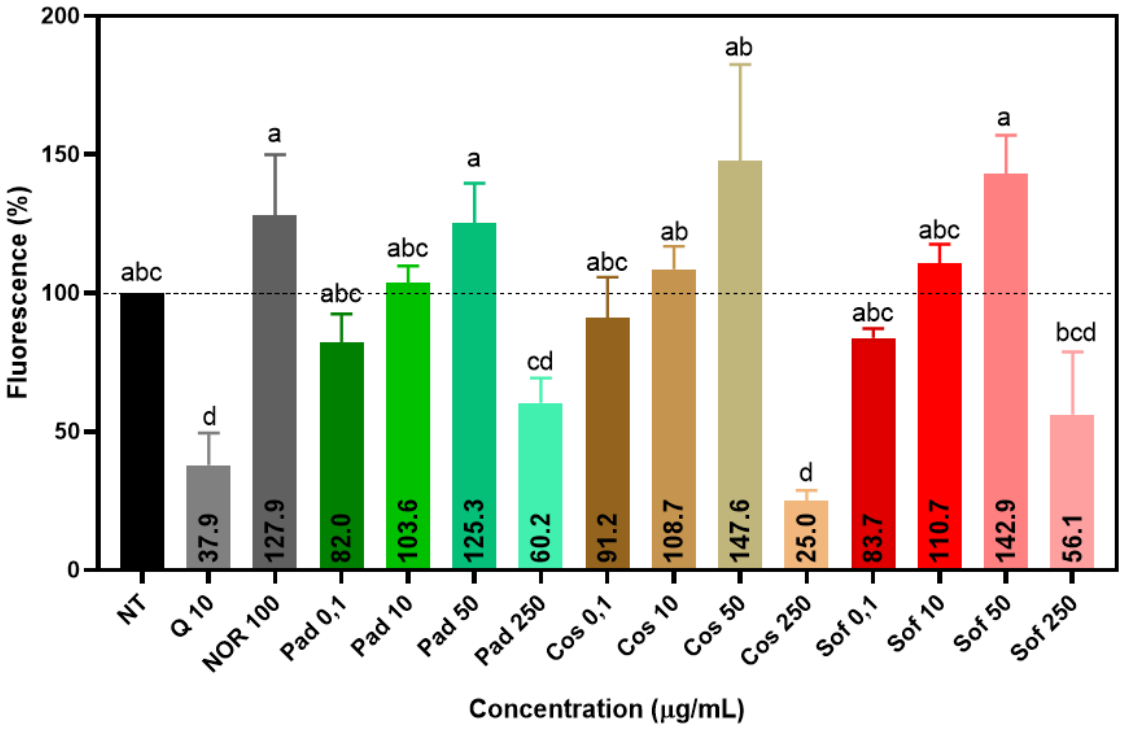
Antioxidant potential

The protection against reactive oxygen species (ROS) production induced by UVA in human keratinocytes (HaCat) was evaluated using the $DCFH_2$-DA probe.

Firstly, the cell viability was not significantly impacted when in contact with the extracts for none of the concentrations analyzed (0.1, 10, 50, and 250 µg/mL), remaining above 80%, indicating that all tested concentrations were not cytotoxic and would not present false-positive results.

Additionally, the crude extracts of *Padina* sp. and *S. filiformis* reduced ROS by approximately 42%, being statistically similar to quercetin (Q) and the non-treated irradiated (NT) ($p > 0.05$). Meanwhile, at a concentration of 250 µg/mL (Figure 3) *C. sertularioides* extract reduced by 75% the ROS production, statistically equivalent to the inhibitory control quercetin (Q) ($p > 0.05$), however different from the NT ($p < 0.05$).

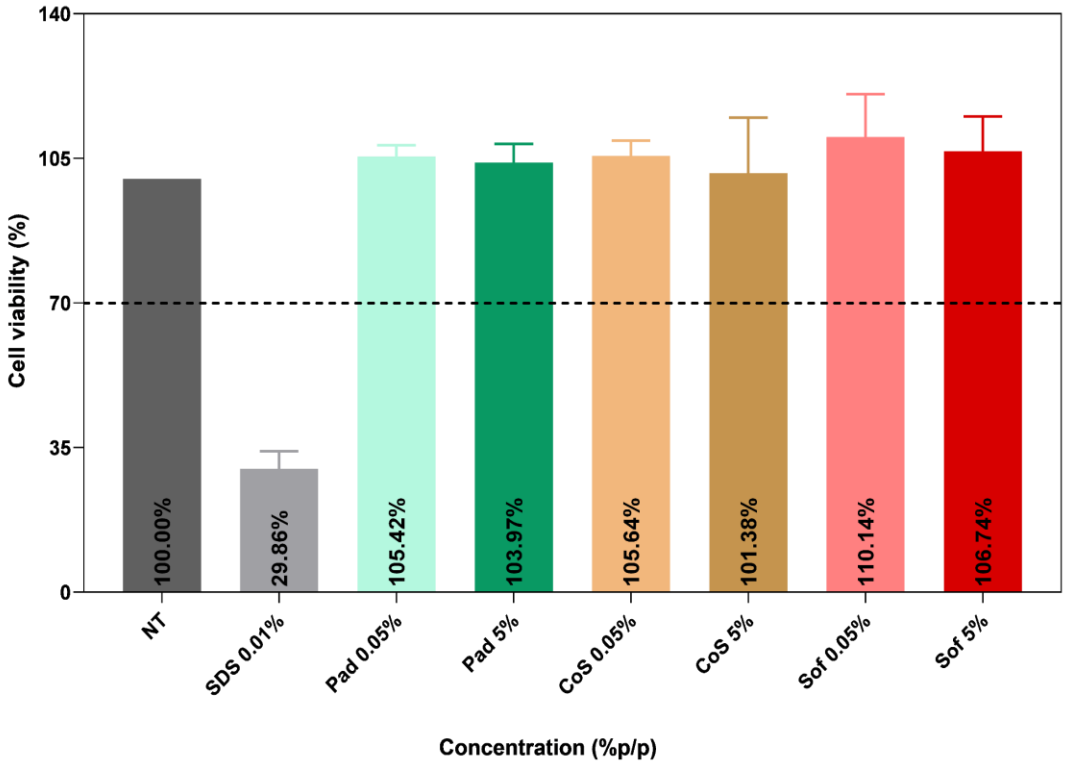
Figure 3. Production of ROS in HaCat cells for different concentrations (µg/mL) of *Padina* sp. (Pad), *C. sertularioides* (CoS) and *S. filiformis* (Sof) extracts (n=3, independent experiments)



Irritant potential

The extracts did not show any irritant potential in the assessment of ocular irritation in rabbit corneal cells, since the samples exhibited cell viability above 70% (Figure 4 and Table 4). Thus, all three samples were predicted as non-categorized, meaning they did not demonstrate any irritant potential.

Figure 4. Cell viability for STE performed with *Padina* sp. *(Pad), C. sertularioides* (CoS) and *S. filiformis* (Sof) extracts in 5% and 0.05% /(n=3, independent experiments).



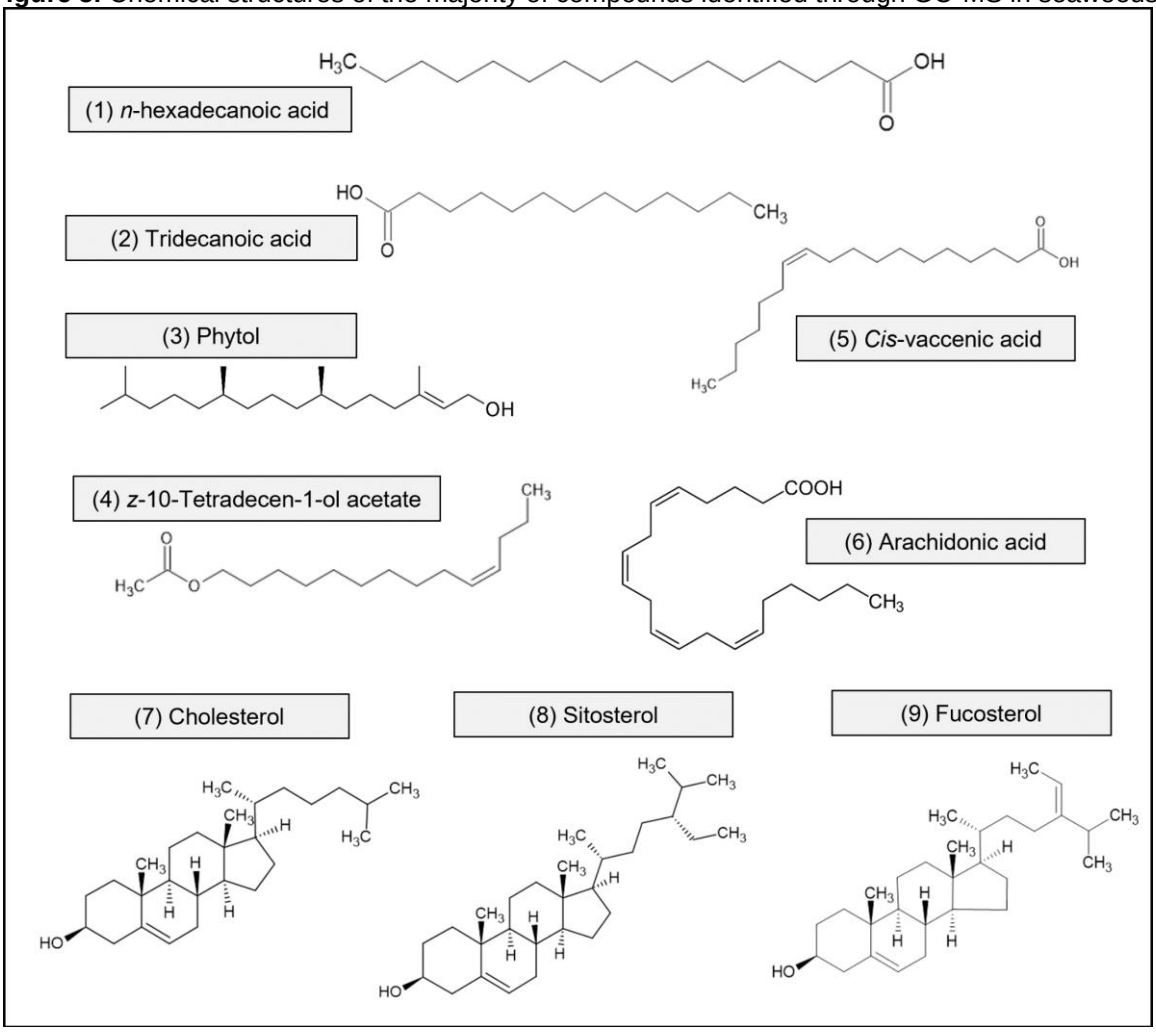
Chemical profile

The extracts are chemically characterized by GC-MS analysis. Using the fragmentation spectrum and chromatograms, a molecular network was constructed to visualize the chemical space and diversity. Compounds were annotated based on their fragmentation patterns and are described in Table 5 and Figure 5.

Table 5. Compounds annotated in crude seaweed extracts. MF: molecular formula. RT lit: retention time in literature (min). RT exp: experimental retention time (min).

	Compound	MF	RT lit.	RT exp.	Agilent Similarity %	Cosine GNPS Index	Classification
1	*n*-hexadecanoic acid	$C_{16}H_{32}O_2$	15.05[12]	32,20	98.8	0.95	Fatty acid
2	Tridecanoic acid	$C_{13}H_{26}O_2$	26.10[13]	32,30	88.9	0.91	Fatty acid
3	Phytol	$C_{20}H_{40}O$	17.83[14]	34.89	94.0	0.93	Alcohol
4	*z*-10-Tetradecen-1-ol acetate	$C_{16}H_{30}O_2$	66.26[15]	35,30	83.5	0.91	Ester
5	*Cis*-vaccenic acid	$C_{18}H_{34}O_2$	15.95[12]	35.38	92.1	0.94	Fatty acid
6	Arachidonic acid	$C_{20}H_{32}O_2$	29.70[12]	38,20	83.6	0.95	Fatty acid
7	Cholesterol	$C_{27}H_{46}O$	48.53[16]	50,00	89.7	0.96	Steroid
8	Sitosterol	$C_{29}H_{50}O$	21,64[13]	52.48	90.8	0.95	Steroid
9	Fucosterol	$C_{29}H_{48}O$	29.86[17]	52.50	96.6	0.96	Steroid

Figure 5. Chemical structures of the majority of compounds identified through GC-MS in seaweeds.



Discussion.

To ensure the safety and efficacy of new ingredients used in cosmetic formulations is essential to evaluate their stability against ultraviolet radiation. The assessment of photostability involves monitoring changes in UV absorption after exposure to ultraviolet rays. Compounds that offer sun protection contain chromophore groups capable of absorbing energy in the ultraviolet or visible region. These chromophores, when exposed to radiation, absorb the energy and enter an excited state. The absorbed energy is then rapidly dissipated, often within seconds, through processes such as fluorescence, phosphorescence, fragmentation, isomerization, reactions with other molecules, and the generation of free radicals. However, during this process, the degradation of these molecules can occur, thereby affecting their protective capabilities [9,21].

Therefore, for the photostability test, it is possible to observe the crude extracts of the seaweed *Padina* sp., *C. sertularioides*, and *S. filiformis,* exhibited photodegradation in the UVA region above 40%, being considered photo-unstable. For the UVB region, only *S. filiformis* was photostable, leading to 35% of photodegradation. However, most of the sunscreens currently available on the market contain UV filters, such as avobenzone, which absorbs in the UVA region but is photo-unstable. Avobenzone stability is improved by its association with UV photostabilizers molecules, such as bis-ethylhexyloxyphenol methoxyphenyl triazine. To improve the photostability profile of crude extracts, it is possible to perform fractionation using more sustainable organic solvents. Additionally, a substance or a set of substances may be photo-unstable without producing phototoxic products [9, 21-22].

The crude extracts of *Padina sp.* and *S. filiformis* reduced UVA-induced ROS generation by approximately 40%, at a concentration of 250 µg/mL. Meanwhile, the *C. sertularioides* extract reduced 75% of UVA-induced ROS production, also in 250 µg/mL, being statistically equal to the positive control quercetin ($p > 0.05$) (Figure 3B). The findings suggest that the extract possesses significant antioxidant properties, which act to neutralize the free radicals generated by UVA rays. This implies that its application may help preserve the integrity of cellular DNA and protect

cellular structures from oxidative stress-induced damage. Thus, it could be suggested that the extract not only prevents premature skin aging induced by UVA rays but also contributes to maintaining skin health and vitality by providing an effective defense against the harmful effects of ultraviolet radiation [8-9,21-23].

The evaluation of ocular irritation, using rabbit corneal cells, revealed that the extracts from the three investigated species showed no irritant potential, maintaining cell viability above 70%. Therefore, it is predicted that the crude extracts of *Padina* sp.*, C. sertularioides,* and *S. filiformis* are not irritants, indicating their suitability for use in multifunctional cosmetics. Emphasizing the importance of exploring new compounds' safety for cosmetic applications [10, 23, 24].

The GC-MS analysis allowed the identification of compounds present in the crude extract of the seaweed. The predominant compounds in the sample belong to the classes of fatty acids, alcohols, and steroids, all of which play a significant role in antioxidant and photoprotective activities. Fatty acids such as n-hexadecanoic acid, tridecanoic acid, z-10-tetradecen-1-ol acetate, cis-vaccenic acid, arachidonic acid, cholesterol, sitosterol, and fucosterol can neutralize free radicals, increase the fluidity of cell membranes, and protect the hair shaft, such as 18-methyl eicosanoic acid (18-MEA) present in hair does. The ester acetate of z-10-tetradecen-1-ol acetate can act as a vehicle for lipophilic antioxidants, enhancing product penetration and stability. The alcohol phytol can neutralize free radicals, inhibit lipid peroxidation, activate antioxidant enzymes, and contribute to UV radiation absorption [11-16, 25-26].

The identified steroids, which are characteristic of the respective phyla of each species: cholesterol (Rhodophyta), sitosterol (Ochrophyta), and fucosterol (Chlorophyta) likely regulate antioxidant genes, reduce oxidative inflammation, protect against UV damage, and/or inhibit enzymes that degrade the extracellular skin matrix. Consequently, this compound combination enhances skin and/or hair protection against oxidative stress and UV radiation damage [16-18, 26-28].

Conclusion.

The bioprospection of seaweed from Bahia has highlighted the biotechnological potential of their natural products, emphasizing their photoprotective and antioxidant properties. The seaweed extracts demonstrated significant absorption in the UVA and UVB regions, indicating the presence of compounds with photoprotective potential and the ability to reduce UVA-induced ROS generation in human keratinocytes, without any irritation potential. The identified compounds, including fatty acids, esters, alcohols, and sterols, highlight the chemical diversity and potential application of *Padina* sp., *C. sertularioides, and S. filiformis* in multifunctional cosmetic products. These results underscore the importance of exploring marine resources from the coast of Bahia, fostering innovation in the sector. Moreover, the crude extracts have potential applications for skin protection against radiation in sunscreens and/or in hair protection when incorporated into hair protection sprays.

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Conflict of Interest Statement.

The authors of this study declare no financial, commercial, or other conflicts of interest that could have influenced the results or interpretation of the data presented in this research.

The study phases, from conception and data collection to analysis and manuscript writing, were conducted independently and objectively.

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