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ORIGINAL PAPER

Processing *Sargassum tenerrimum*-Derived Polysaccharides for Antioxidant Face Cream Formulation and Characterization

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Abstract

The rising demand for sustainable and natural cosmetics has spurred interest in marine-derived polysaccharides for their bioactive properties in skincare applications. This study focuses on the development of antioxidant face creams formulated using polysaccharides extracted from marine macroalgae *Sargassum tenerrimum*, collected along the eastern coastline. These polysaccharides exhibited exceptional antioxidant, hydrating, and skin-rejuvenating properties, making them ideal candidates for cosmeceutical formulations. A systematic approach was undertaken to extract, characterize, and incorporate sulfated polysaccharides into a stable cream formulation. Key evaluations included assessment of their ability to neutralize free radicals, promote skin health, and maintain biocompatibility. The bioactivity of the formulations was assessed through photo-haemolysis inhibition assays, antioxidant activity measurements, and in vitro cell viability tests to validate their safety and functional efficacy. The formulated cream exhibited a sun protection factor (SPF) of 18.413, and an average polysaccharide yield of 14.68%. The findings underscore the potential of marine macroalgae polysaccharides as sustainable and effective ingredients for skincare, highlighting their potential in advancing environmentally friendly cosmeceutical innovations. This research contributes to the growing field of marine-derived bioproducts, emphasizing their applicability in meeting consumer demand for natural, high-performance skincare solutions.

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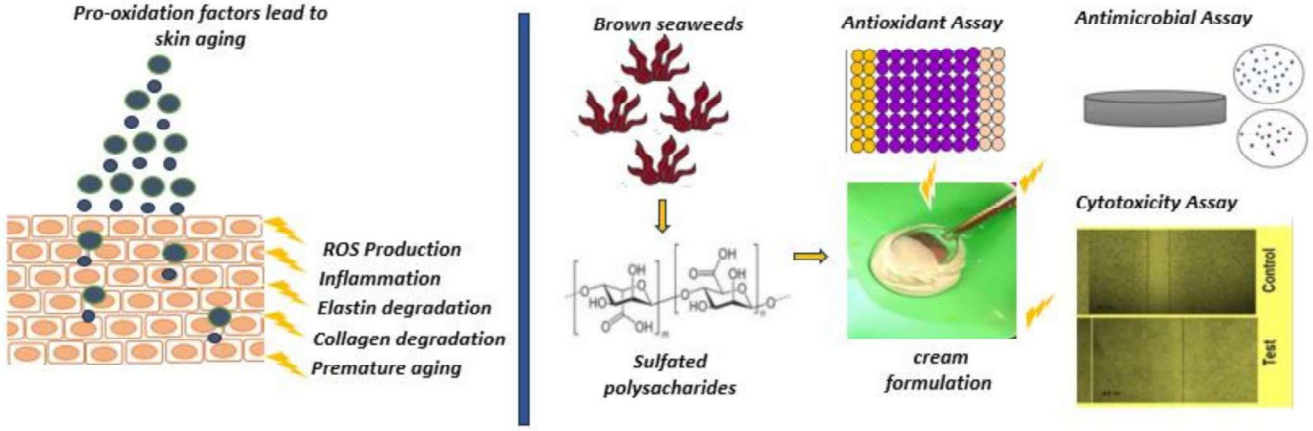
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Graphical Abstract



Highlights

- Cosmeceuticals incorporate bioactive ingredients to specifically target and address skincare.
- Higher sulfated polysaccharides (SPs) in seaweeds exert significant antioxidant effects on skin.
- SPs help to protect skin from oxidative stress, preventing premature aging and damage by free radicals.
- Cell viability assays revealed cosmeceutical cream with SPs effectively contribute to the prevention of aging.

Keywords Marine macroalgae · Sulfated polysaccharides · Sustainable cosmeceuticals · Photo-haemolysis

Introduction

Skin aging is a complex biological phenomenon influenced by a combination of physiological, environmental, and psychosocial factors. Among these, psychological stress has been identified as a critical accelerator of premature aging. Chronic stress triggers sustained cortisol release, disrupting the skin's barrier function, impairing collagen synthesis, and activating inflammatory pathways. These disruptions manifest visibly as wrinkles, reduced elasticity, and hyperpigmentation, which are further exacerbated by urban lifestyles characterized by pollution, suboptimal dietary habits, and limited access to preventative skincare measures.

A key mechanism underlying stress-induced aging is oxidative stress, driven by excessive production of reactive oxygen species (ROS) [1]. These free radicals degrade essential structural proteins such as collagen and elastin, leading to compromised skin integrity and resilience. Over time, the cumulative burden of oxidative damage impairs the skin's natural reparative and regenerative capacities, leaving it more vulnerable to external stressors, including ultraviolet radiation and environmental pollutants. Consequently, populations exposed to chronic psychosocial and occupational stressors experience accelerated aging, underscoring the need for effective, science-backed skincare interventions.

The global anti-aging cosmetics market, projected to reach approximately USD 60.26 billion by 2026, reflects increasing consumer demand for solutions addressing the root causes of skin aging [2]. While aesthetic surgeries offer structural modifications, cosmeceuticals have emerged as non-invasive alternatives [3]. These topical formulations, containing bioactive compounds such as peptides, antioxidants, polysaccharides, and phytochemicals, target the molecular pathways associated with aging [4]. Cosmeceuticals provide benefits including photoprotection, hydration, anti-inflammatory activity, and antimicrobial effects, making them a cornerstone of modern skincare strategies [5, 6].

Marine-derived compounds, particularly from seaweeds, have garnered significant attention as sustainable and effective cosmeceutical resources [7]. Seaweeds, a type of macroalgae, are abundant in bioactive constituents, including sulfated polysaccharides, essential minerals, and vitamins [8]. Among these, sulfated polysaccharides stand out for their remarkable antioxidant properties, neutralizing free radicals, reducing oxidative stress, and enhance skin rejuvenation [9]. Their gel-forming capabilities also make them ideal for integration into creams and lotions, providing stability and preventing phase separation [10].

The application of marine polysaccharides extends to sunscreen formulations, where compounds like alginates, carrageenan, fucoidan, and chitosan offer protection against UV-induced oxidative damage and support the skin's defence against environmental aggressors [11].

Additionally, these polysaccharides improve skin hydration, enhance barrier function, and maintain elasticity, making them valuable in advanced skincare formulations [7]. Although the production of serums from seaweed-derived lipids, such as those from *Padina australis* and *Euchema-cottonii*, have been explored [12], lipid extraction involves complex chromatographic techniques, rendering it more labor-intensive compared to polysaccharide extraction [13].

The increasing consumer preference for natural, sustainable skincare solutions has positioned marine polysaccharides as a promising alternative to conventional synthetic ingredients [14]. Their biocompatibility, antioxidant activity, and gel-forming properties make them highly desirable for developing innovative cosmeceuticals [15, 16]. This study aims to investigate the cosmeceutical potential of *S. tenerrimum*, with a particular focus on its antioxidant and anti-aging properties, to harness marine polysaccharides for the developing sustainable and effective antioxidant face cream formulations.

Materials and Methods

Collection of Seaweeds and Preparation of Algal Powder

The seaweeds were harvested from the eastern seashores of Dhanushkodi Latitude 9.1589° N and Longitude 79.4390° E, Rameshwaram in December 2022. The collected seaweeds were placed in clean containers to minimize damage. The species was tested for its authenticity and quality by a phycologist from Madras University, Chennai, South India. The species was identified as *Sargassum tenerrimum (Sargassaceae)* by Botanical Survey of India (Southern Circle, Coimbatore (Ref. no.: BSI/SRC/5/23/2023-24/Tech-477). Information regarding the collection site, species, and date was documented to effectively track and manage the seaweed collection.

Extraction of the Crude Sulfated Polysaccharide

Sulfated polysaccharides were extracted from seaweed following a method adapted from Jiao et al. [17], with minor modifications. Fresh seaweed was collected during low tide, rinsed thoroughly with cold distilled water to remove surface debris, and optionally blanched to inactivate endogenous enzymes. The material was air-dried, ground into a fine powder, sieved for uniformity, and stored in airtight containers under cool, dry conditions until use. The powdered seaweed was examined for quality and contamination before processing.

For extraction, 500 g of seaweed powder was soaked in 0.3 N KOH overnight to remove alkali-soluble impurities. The pretreated biomass was macerated in absolute ethanol at a 1:10 (w/v) ratio and heated to 60 °C, followed by thorough rinsing with tap water. Residual alkali was neutralized with 0.1 N HCl. The neutralized material was subjected to hot-water extraction by suspending it in distilled water and heating at 80 °C for 1 h with continuous stirring. The extract was clarified sequentially by filtration through muslin cloth and Whatman No. 1 filter paper.

Crude polysaccharides were precipitated by the addition of 95% ethanol (final concentration ≥ 70% v/v) and recovered by centrifugation at 8,000 × g for 20 min. The resulting pellet was dissolved in equilibration buffer (20 mM Tris-HCl, pH 7.4, or sodium acetate buffer, pH 5.5–6.0), filtered through a 0.45 µm membrane, and subjected to ion-exchange chromatography.

Purification was carried out using a DEAE-Sephadex A-25 column (2.5 × 30–40 cm; ~150 mL bed volume) pre-equilibrated with equilibration buffer at a flow rate of 0.5–1.0 mL/min. The crude extract was applied at 0.3–0.5 mL/min, ensuring the sample volume did not exceed 10% of the column bed volume. After removal of unbound components with equilibration buffer, bound polysaccharides were eluted using a stepwise NaCl gradient (0.5–2.0 M). Fractions were collected systematically, pooled in sets of four, and dialyzed against distilled water.

Chromatographic separation yielded four distinct fractions. The third fraction, eluting at 1.5 M NaCl, corresponded to the most strongly bound sulfated polysaccharides. All purified fractions were lyophilized and stored for subsequent physicochemical and biological characterization. Polysaccharide yield was determined according to established protocols [18, 19].

Quantification of Carbohydrates Phenol–Sulphuric Acid Assay

The phenol-sulfuric acid method, renowned for its efficiency, stands out as a valuable colorimetric technique due to its versatility and precision making it a fundamental tool for accurately quantifying neutral sugars within various biomolecules. Among the many colorimetric methods for carbohydrate analysis, the phenol-sulfuric acid method is widely used to measure neutral sugars in oligosaccharides, proteoglycans, glycoproteins, and glycolipids. A stock solution of dextrose was prepared at a concentration of 1 mg/mL. Different concentrations of stock solution were mixed with 0.5 mL of extracted polysaccharide solution. Then 0.5mL of 80% phenol solution (w/w) was added. The mixture was vortexed followed by the addition of 2 mL of concentrated sulphuric acid was added. The solution was left

undisturbed at room temperature for 15–20 min. A mixture of 2 mL concentrated sulfuric acid and 0.5 mL of phenol servedas the comparative reference blank. The absorbance was read at 490 nm using a colorimeter [20]. The carbohydrate content of the resultant sample was determined using phenol-sulphuric method [21].

Quantification of Sulfate Content by Barium Chloride Assay

To ascertain the presence of sulfate ions SO_4^{2-} in the extracted solution, the test involves the utilization of barium chloride. The test solution was acidified using few drops of dilute hydrochloric acid, which removes the presence of any carbonate ions that could produce false-positive result. Subsequently, a few drops of barium chloride solution were added to the mixture. The formation of a white solid formation serves as an unequivocal indication of the sulfate ions' occurrence within the solution. Various volumes (0.2, 0.4, 0.6, 0.8 and 1.0 mL) of extracted polysaccharide were taken and made up to a final volume of 1 mL, an appropriate volume of barium chloride solution was introduced into individual test tubes. The test tubes were set aside at room temperature for a 10 min incubation period until the white precipitation occurred. Then the absorbance was read at 405 nm using a colorimeter [22].

Antioxidant Activity Analysis of the Extracted Polysaccharide

To evaluate the antioxidant potential of the extracted algal polysaccharides, DPPH radical scavenging activity was performed using a method previously investigated in my earlier studies. To the 2 mL of DPPH solution, various concentrations of 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, and 4.0 mg/mL purified polysaccharide extract were added which was then made to equal volume using Tris HCl solution. The absorbance was quantified at a wavelength of 517 nm, and the scavenging activity percentage was ascertained using the following mathematical expression:

$$\text{Scavenging activity } (\%) = \frac{(Control\ absorbance - Sample\ absorbance)}{Control\ absorbance} \text{ X } 100$$

Table 1 Ingredients used in the formulation of anti-aging face cream

Ingredient	Role of the ingredient	References
Almond oil and glycerol	Surfactant and emulsion producing component	[23]
Shea butter andbee wax	Thickening agents	[24]
Distilled water	Mixing component and dilution	
Pectin	Weak emulsifier for the emulsifying agent	[25]
Lecithin	Emulsifying and solubilizing agent	
Starch (polysaccharide)	Binder and component that produces an antioxidant effect	
Rosemary extract	Preservatives	[26]

Preparation of Antioxidant Face Cream

The usual lotions are often prepared by using synthetic compounds but for this preparation the ingredients mentioned in Table 1 such as almond oil, shea butter, beewax, distilled water, pectin and rosemary extract. The extracted polysaccharide is added instead of starch powder to provide a gelling effect & antioxidant activity along with other natural ingredients.

Characterization of the Prepared Cream

The colour, odour and pH of the cream are analysed along with zeta potential and ultrasonication tests to evaluate the cream's stability under stresss [27]. The cream formulation underwent an analysis to evaluate its sensory properties. To assess homogeneity, the formulations were visually and tactilely inspected. The analysis also encompassed the assessment of consistency and the identification of any coarse particles to ascertain the overall texture and evenness of the formulations. The texture, bacteriological shelf life, phototoxicity, and sun protection factor (SPF) in the cream formulations were assessed using sensory evaluation tests, microbiological analysis, phototoxicity assays, and SPF testing methods [28]. Cell viability assays of the cream were performed to assess the potential cytotoxicity of the cream formulations [29].

Assessment of Physical Attributes of the Cream

The sensory properties of the formulated cream, including appearance, consistency, transparency, smoothness, aroma, and flavor, were systematically evaluated. Prior to testing, the cream was equilibrated to room temperature, maintained within the range of 25–30 °C. A clean spatula was utilized to collect a 1 g sample of the cream, which was subsequently diluted with 9 mL of distilled water to facilitate further analysis. The pH of the sample was measured under controlled experimental conditions, specifically at 29 °C, using calibrated instrumentation. Following pH measurement, detailed visual and tactile evaluations were performed to assess cream consistency, with particular attention to irregularities in color, texture, or particulate presence. Spreadability was evaluated in accordance with standardized ICH guideline protocols to ensure reproducibility and reliability.

All measurements were performed in triplicate to account for variability and enhance the statistical robustness of the findings.

Assessment of Texture Characteristics of Formulated Cream

Texture Profile Analysis (TPA) is a widely recognized technique for evaluating the textural properties of creams, playing a crucial role in the cosmetics industry to ensure products meet desired texture and quality standards. For the analysis, cream samples were prepared and presented in a standardized format, typically as uniform discs or within cups, to maintain consistency in testing conditions. A mechanical probe, 35 mm in diameter, was employed to penetrate the cream at a controlled speed of 1 mm/s, measuring the force required to deform the sample. Key TPA parameters, including adhesiveness, viscosity, and springiness, were quantified using a texture analyzer, which captured force-deformation characteristics with high precision and reliability [30].

Assessment of Microbial Shelf Life of Formulated Cream

Cream samples were collected aseptically for microbiological analysis to establish the initial microbial load. This involved conducting tests for total viable microbial count, yeast, mold, and specific pathogens. A portion of the herbal cream was placed in a controlled environment where conditions, such as temperature and humidity, were set to accelerate aging. Typical conditions included 40 °C and 75% relative humidity. The cream's appearance, odour, texture, and colourwere systematically evaluated at regular intervals, with any observed changes recorded through detailed visual and sensory assessments.

A suitable quantity of the cream sample (e.g., 1 g) was weighed and placed it in a sterile container. A known volume of sterile diluent (e.g., saline solution) to the cream sample was added, forming a 1:10 dilution (10 g of diluent for every gram of cream). The contents were mixed thoroughly to create a homogeneous mixture. Performed serial dilutions by transferring a known volume (e.g., 1 mL) of the diluted cream solution to another container with sterile diluent. Continued this process to create dilutions with decreasing concentrations (e.g., 10^{-1}, 10^{-2}, etc.). By using a sterile pipette 0.1 mL of the appropriate dilutions onto sterile nutrient agar plates was done. The sample was spread evenly over the agar surface using a sterile spreader or glass rod. The agar plates were incubated upside down at the appropriate temperature (usually 30–37 °C) for 24 to 48 h. After incubation, the agar plates were observed for the growth of visible colonies. The colonies on plates with a suitable number of colonies (typically between 30 and 300) were counted for 180 days to ensure accuracy [31].

Assessment of Phototoxic Potential of Formulated Cream

Superoxide generation assay is a test used to assess the phototoxic potential of cream to determine their safety and potential risks associated with prepared cream when exposed to light, aiding in the development and regulation of products to ensure consumer safety. The test samples were prepared by adding 50 ppm of the cream to separate cuvettes. To each cuvette containing the cream sample, an equal volume of NBT solution prepared at a concentration of 1.67×10^{-4} M in 0.01 M carbonate buffer was added. They were mixed gently. One tube contained only the NBT solution and carbonate buffer (negative control), while the other contained the positive control. They were placed in direct sunlight for the incubation of the cuvettes. The cuvettes containing the cream with NBT, as well as the control cuvettes, were placed on the sunlight-exposed platform. They were allowed to incubate under sunlight for 10 min. During the incubation period, the cuvettes were visually monitored for the formation of nitro blue formazan, which was indicated by a change in color from yellow to blue-purple. After 10 min of incubation, a UV-visible spectrophotometer was used to measure the absorbance of each cuvette at the appropriate wavelength (usually around 560–600 nm). The absorbance values for each cuvette, including the cream samples, negative control, and positive control, were recorded. The difference in absorbance between the test samples (containing the cream) and the negative control was calculated. This difference represented the extent of superoxide generation caused by the cream [29].

Assessment of SPF of Formulated Cream

For this study, a portion of the cream was acquired and it was imperative to ensure that the sample exhibited homogeneity and lacked any discernible clumping. The UV spectrophotometer underwent calibration, a process executed with the utilization of standard sunscreens with known SPF values. Subsequently, the formulated cream, appropriately diluted to a concentration of 1% (w/v), was meticulously applied onto the surfaces of UV-protective quartz cuvettes. A thorough record was maintained of the absorbance readings obtained across various wavelengths, encompassing the region between 290 and 400 nm. In conjunction with this, the absorbance levels of a cuvette devoid of cream (serving as the control) were also measured. The measurement protocol was replicated for standard sunscreens featuring established SPF values. In accordance with established

procedures, the SPF value of the herbal cream was ascertained by discerning the ratio between the intensities of UV radiation necessary to elicit a Minimal Erythema Dose (MED) on skin shielded by the cream and that required to induce a MED on unprotected skin. The assessment of MED was conventionally conducted within the wavelength range of 290–400 nm [32].

Assessment of the Antimicrobial Potential of Formulated Cream

The antibacterial activity of seaweed concentrate was performed using an agar well diffusion assay [33]. According to Bauer et al. Mueller-Hinton (MH) agar plates were prepared as they are commonly used in antimicrobial studies due to their standardized formulation, which ensures consistent and reproducible results [34]. The medium's rich nutrient content supports the growth of a wide range of bacteria, both Gram-positive and Gram-negative, while its stable pH maintains accuracy in testing even under anaerobic conditions [35]. The organisms such as *E. coli* (MTCC 449) and *B. subtilis* (MTCC 441) were inoculated on the surface of the plates. Stock concentrations of seaweed concentrate were prepared at 50 µg/mL. Wells were made using a sterile steel cork borer and 25 µLof seaweed concentrate was added and incubated at 37 °C for 24 h and the wells were observed for the zone of inhibition to measure antibacterial activity.

Assessment of Cytotoxicity of Formulated Cream

Cultured cells were verified to be in their log-phase of growth. Subsequently, the cells were introduced into a 96-well cell culture plate at the appropriate density and incubated in a standard cell culture medium. Upon cell attachment, the culture medium was replaced with the formulated cream at concentrations ranging from 10 to 150 µg/mL. These cells were then subjected to a specified incubation period under standard culture conditions. An MTT solution was meticulously prepared by dissolving MTT in PBS to achieve a concentration of 5 mg/mL. Following the incubation, the medium containing the cream was carefully aspirated, and the MTT solution was added to each well, undergoing incubation for a designated time frame, typically ranging from 0 to 48 h, facilitating the formation of formazan crystals. The MTT solution was subsequently aspirated, and DMSO was introduced to dissolve the formazan crystals. Absorbance measurements of the resultant formazan solution in each well were taken at the typically recommended wavelength range of 570–600 nm employing a microplate reader. Cell viability or cytotoxicity was computed through a comparative analysis of the absorbance values derived from the treated cells with the control cells [36].

Results and Discussion

Analysis and Purification of Crude Polysaccharide from Brown Algae

The crude polysaccharide obtained from *S. tenerrimum* was dehydrated until a significant decrease in its water content was attained. Subsequently, it underwent further purification through anionic exchange chromatography, a process that segregates the polysaccharide based on its charge and its affinity for the resin. A suitable precipitant, namely ethanol, was introduced to each of the fractions, leading to the precipitation of the polysaccharide and its subsequent separation from the solution in solid form. The average yield of the precipitate was found to be 14.68% after centrifugation and washing. Previous reports indicate that the yield of sulfated polysaccharides (fucoidan) from brown seaweeds varies considerably depending on the extraction conditions and species used. Fucoidan yields of approximately 4.5% of dry seaweed weight have been reported in earlier studies [37], which fall within the lower range typically observed for *Sargassum* and related species. In contrast, significantly higher yields of around 12% (dry weight) were obtained from *Chorda filum* using acid-assisted extraction [38], underscoring the influence of both algal species and extraction methodology on recovery efficiency. Moreover, variability in yield across studies has been highlighted, with factors such as seasonal harvesting, pretreatment steps, and precipitation techniques contributing to fluctuations in sulfated polysaccharide recovery [39]. Collectively, these findings demonstrate that yields can range from modest (≈4–5%) to relatively high (≈12%), providing a useful benchmark for assessing the efficiency of new extraction protocols. The amount of sulfate content present was found to be 0.0153 g/mL using barium chloride method which was further confirmed using FTIR and GCMS analysis.

FTIR Analysis of Purified Polysaccharide

The infrared spectroscopic analysis of *S. tenerrimum* seaweed and its purified polysaccharides, conducted using a Spectrum One FTIR spectrometer within the 800–3800 cm^{-1} range (Figs. 1 and 2), revealed distinct functional groups indicative of bioactive polysaccharides (Fig. 2) revealed the absorption band observed at 711.73 cm^{-1} can be attributed to C–O–S bending vibrations of sulfate esters, a characteristic feature of fucoidan and related sulfated polysaccharides

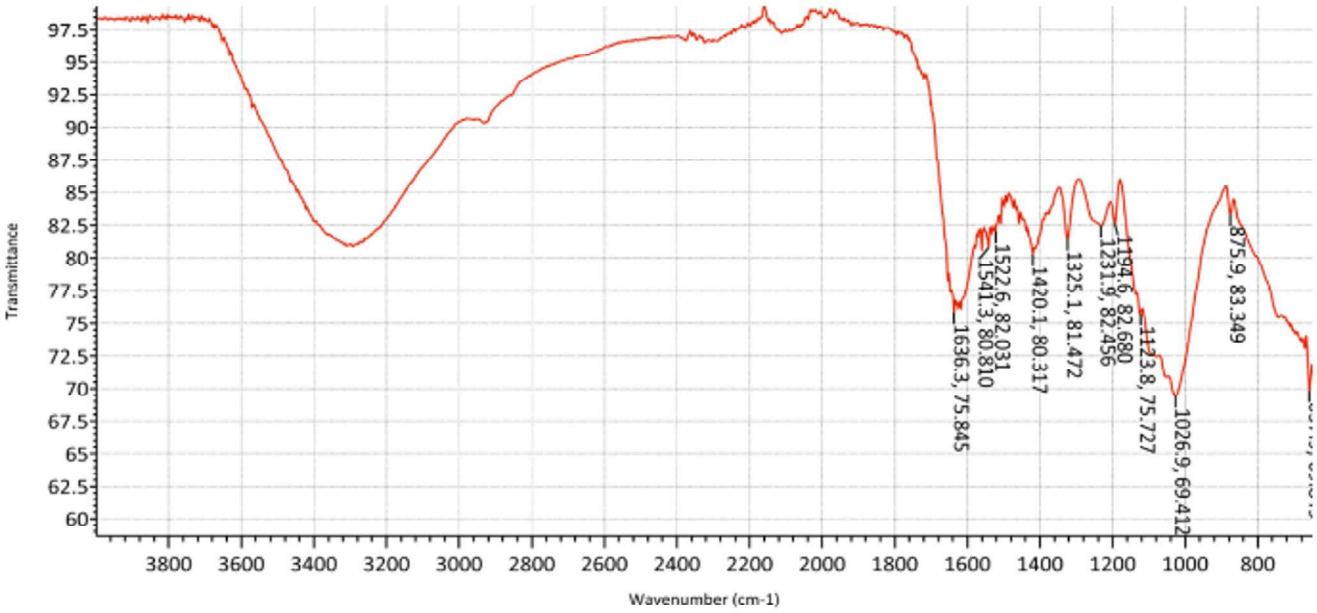


Fig. 1 FTIR spectroscopic analysis of the dried marine macroalage species

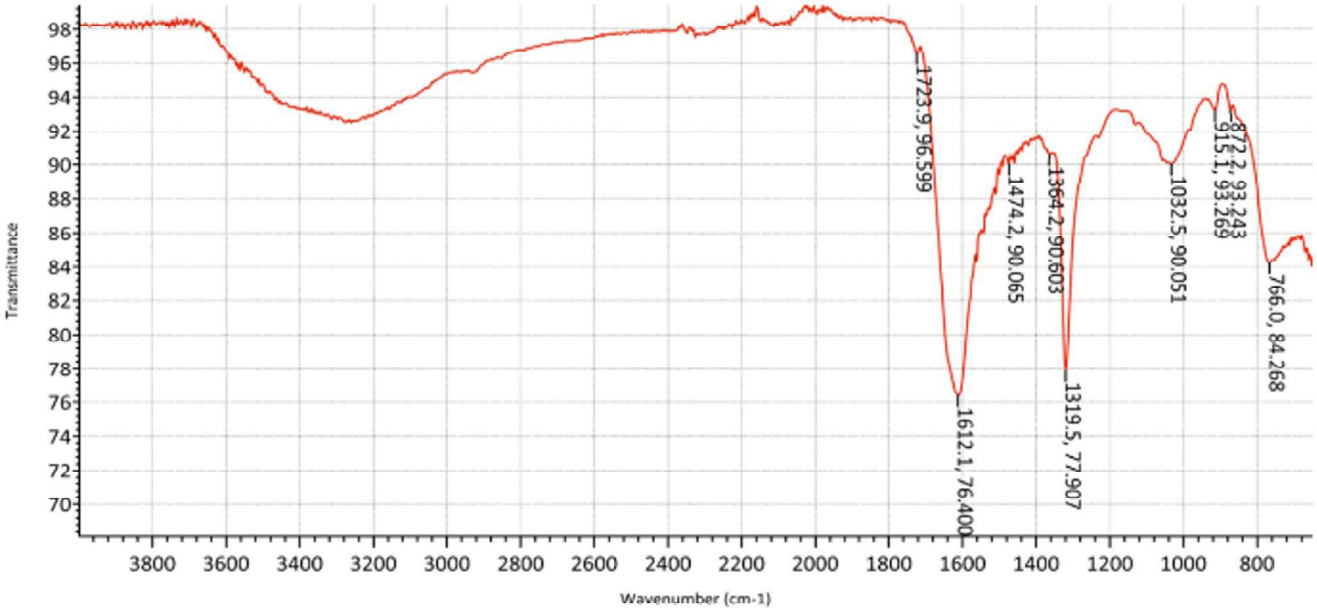


Fig. 2 FTIR spectroscopic analysis of the purified polysaccharide from marine macroalgae

commonly found in brown seaweeds. However, *Sargassum* species are also known to contain alginates composed of mannuronic (M) and guluronic (G) acid residues. Vibrations associated with the skeletal ring deformations of these uronic acid units may also appear in the 700–750 cm^{-1} regions, thereby overlapping with sulfate-related signals. Thus, the band at 711.73 cm^{-1} in the present extract likely represents a combined contribution from sulfate ester bending in fucoidan and ring vibrations of mannuronic/guluronic residues in alginate, reflecting the co-occurrence of these two classes of polysaccharides in brown algae, 859.39 cm^{-1} peak is characteristic of C–O–S stretching vibrations in sulfate esters (S = O bending), indicating the presence of sulfated groups in the polysaccharide backbone, which is typical for fucoidan or other sulfated polysaccharides in brown algae and 871.82 cm^{-1} corresponds to C–H deformation vibrations of α-glycosidic linkages in polysaccharides, or can also be associated with C–O–S stretching in sulfated sugar residues, confirming sulfation. In polysaccharides, it may also indicate skeletal vibrations or bending modes

associated with sugar rings., indicating the presence of phenolic units known for antioxidant properties critical in skincare applications. Peaks at 1031.92 cm^{-1}, 1228.66 cm^{-1}, and 1321.24 cm^{-1} are attributed to C-O stretching vibrations characteristic of polysaccharide backbones, which contribute to gel-forming and water-retention properties valued in moisturizing formulations. The presence of sulfate groups is confirmed by peaks at 859.39 cm^{-1} and 1421.82 cm^{-1}, particularly at C-4 axial positions of the alginate backbone, highlighting their antioxidant, anti-inflammatory, and anticoagulant properties [40, 41]. A peak at 1631.78 cm^{-1} corresponds to C=C stretching vibrations, suggesting conjugated aromatic structures often linked to antioxidant activity. These findings corroborate previous studies on marine algae, such as *Fucus vesiculosus*, and underscore the potential of *S. tenerrimum* as a source of sulfated polysaccharides with significant bioactivity [42]. The identified functional groups, particularly sulfated polysaccharides, support the application of *S. tenerrimum* in developing skincare products with therapeutic and protective benefits, leveraging its antioxidant and hydrating properties.

GCMS Analysis of Purified Polysaccharide

The GC-MS analysis(Fig. 3), revealed three prominent peaks with significant areas, indicating the presence of specific compounds in the *Sargassum* species. These peaks correspond to retention times of 17.183 (alginate compound), 19.458 (3,7,11,17 tetramethyl-2-hexadecen-1-ol), and 19.68 (di-n-decylsulfone) [e-Supplementary data]. The identified compounds in this study were found to be present in higher concentrations compared to those reported in previous research This suggests that the *Sargassum* speciesutilized in the current study may contain a more concentrated form of these bioactive compounds [42]. The first compound, alginate, identified at a retention time of 17.183 min, is a renowned polysaccharide celebrated for its remarkable hydrating, anti-inflammatory, and wound-healing properties. The elevated concentration of alginate in *Sargassum* indicates that thisalga may serve as a potent source of alginate, contributing significantly to enhanced moisturization and skin regeneration in cosmetic formulations, as evidenced by its wide use in the skincare and pharmaceutical industries [43].

The second compound, 3,7,11,17 tetramethyl-2-hexadecen-1-ol, detected at 19.458 min, belongs to the terpenoid class, a group of compounds recognized for their antioxidant and anti-inflammatory attributes. This compound's presence suggests that it could offer substantial protection against oxidative stress, mitigate inflammation, and provide therapeutic benefits for sensitive or acne-prone skin. Furthermore, its antibacterial properties enhance the cream's efficacy in preventing skin infections, positioning it as a potential agent for treating acne and other inflammatory dermatoses [44]. The third compound, di-n-decylsulfone, identified at 19.68 min, is a sulfur-containing compound with potent antioxidant and antibacterial properties. It is known for its use as an emollient, it contributes to improved texture and skin-conditioning properties in topical formulations. Additionally, its anti-aging potential arises from its ability to neutralize free radicals, safeguarding the skin from premature aging and microbial damage [45]. These findings underscore the multifaceted bioactivity of *Sargassum* species, underscoring their promise as a rich source of bioactive compounds with anti-aging, anti-inflammatory, and antibacterial properties, making them highly valuable for advanced skincare formulations.

Antimicrobial Activity

The antimicrobial efficacy of sulphated polysaccharides was systematically evaluated using the agar well-diffusion assay, a reliable and widely employed method for assessing antibacterial properties, as documented in earlier studies [46]. The investigation focused on their activity against two representative bacterial strains: *Escherichia coli* (MTCC 449), a Gram-negative bacterium, and *Bacillus subtilis* (MTCC 441), a Gram-positive bacterium. These organisms were selected due to their clinical and industrial relevance, as well as their differing cell wall compositions, which provide a basis for understanding the spectrum of activity of the polysaccharides.

As illustrated in Fig. 4, the sulphated polysaccharide extract demonstrated a pronounced antibacterial effect, evidenced by the zones of inhibition surrounding the wells. The inhibition zones measured approximately 5.35 cm for *E. coli* and 4.98 cm for *B. subtilis*, suggesting a slightly higher susceptibility of *E. coli* to the polysaccharide extract. This observation may be attributed to differences in the structural composition of the bacterial cell walls. The outer membrane of Gram-negative bacteria, while often providing resistance to certain compounds, may be more permeable to the sulphated polysaccharides due to specific interactions with the sulphate groups. Conversely, the thicker peptidoglycan layer in Gram-positive bacteria, such as *B. subtilis*, may impede the polysaccharide's penetration, resulting in a comparatively smaller zone of inhibition. The concentration-dependent activity of the polysaccharides was also examined, with the highest antibacterial efficacy observed at a concentration of 1 mg/mL, as depicted in Fig. 5. This finding highlights the potential of sulphated polysaccharides as effective antimicrobial agents at relatively low concentrations, making them promising candidates for applications

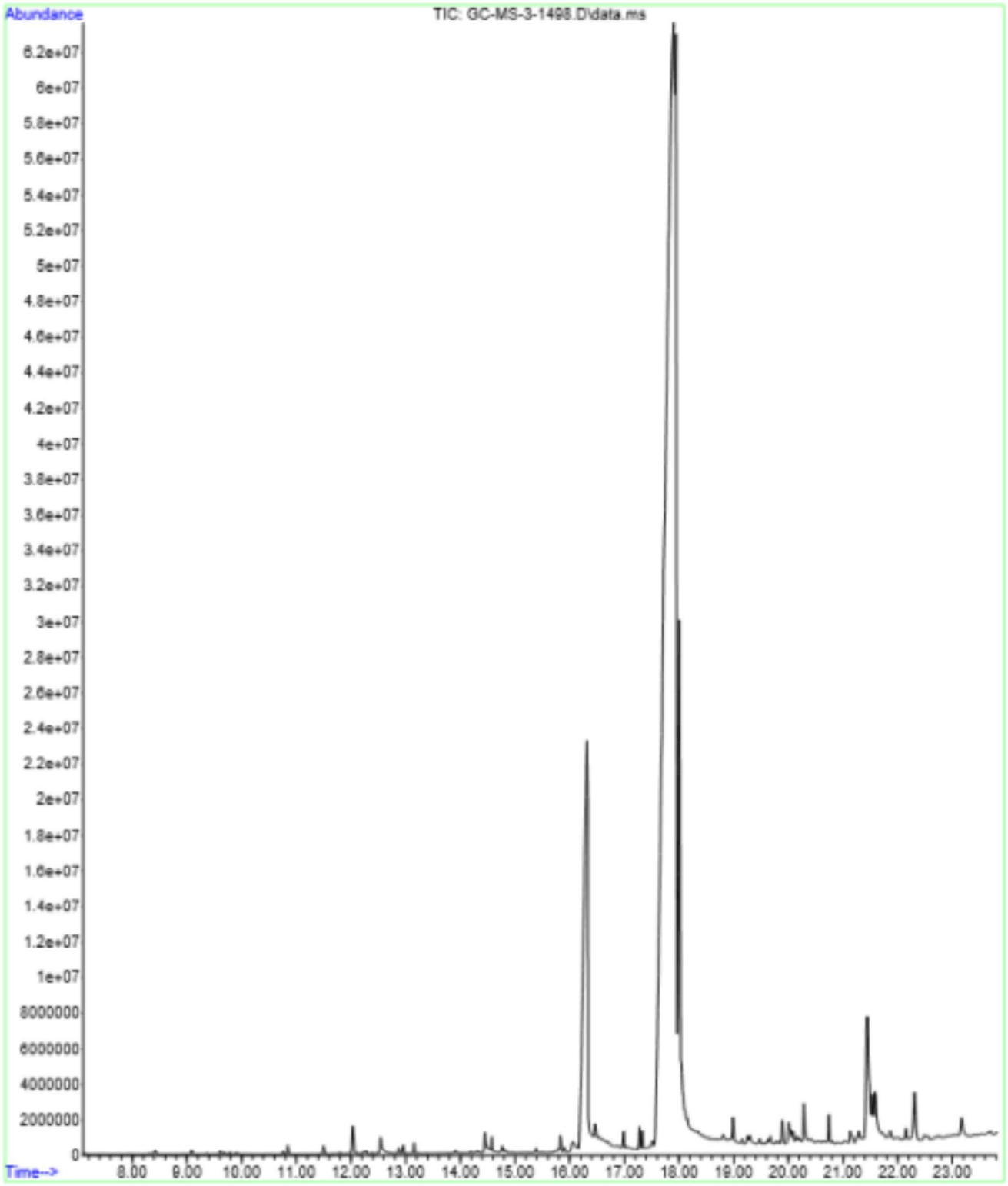


Fig. 3 GC MS analysis of the extracted sulphated polysaccharide from the marine macroalgae (µg/mg)

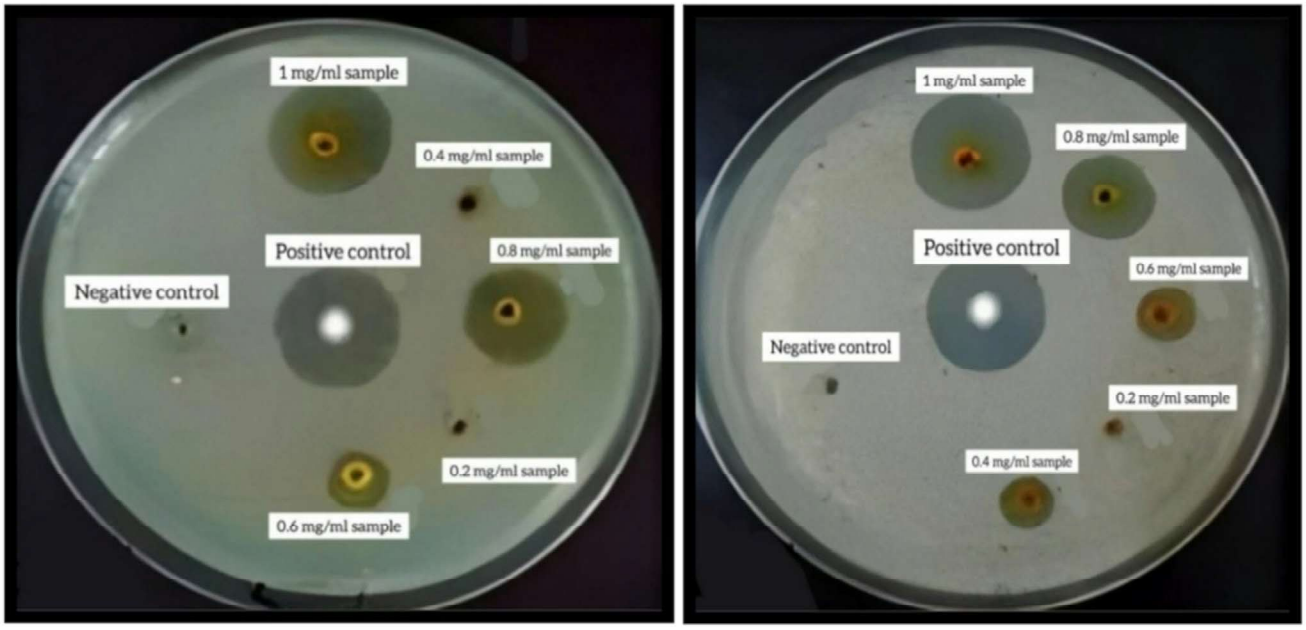


Fig. 4 Zone of inhibition formed by the sulphated polysaccharides at different concentrations(mg/mL) against *Escherichia coli* and *Bacillus subtilis*

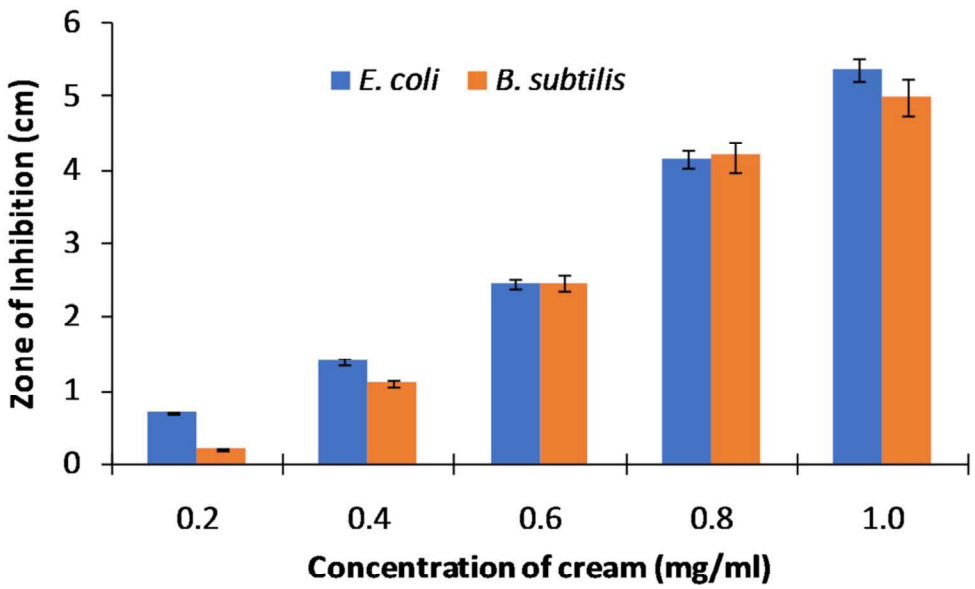


Fig. 5 Different concentrations of sulphated polysaccharides against *Escherichia coli* and *Bacillus subtilis* (mg/mL)

in pharmaceutical, food preservation, and biomedical industries.

The observed antibacterial activity can be contextualized within the broader framework of the bioactivity of sulphated polysaccharides. The sulphate groups within the polysaccharide structure are known to enhance antimicrobial activity by interacting with the bacterial cell surface, disrupting membrane integrity, and inhibiting essential cellular processes. Moreover, the high molecular weight and unique structural features of the algal polysaccharides likely contribute to their efficacy by facilitating stronger interactions with bacterial cells.

Free Radical Scavenging Activity

Natural polysaccharides are intrinsically associated with bioactive phytochemicals, such as polyphenolic compounds, which are hypothesized to contribute significantly to their pronounced antioxidant properties [47]. The free radical scavenging potential of the algal polysaccharides was quantitatively evaluated using the DPPH

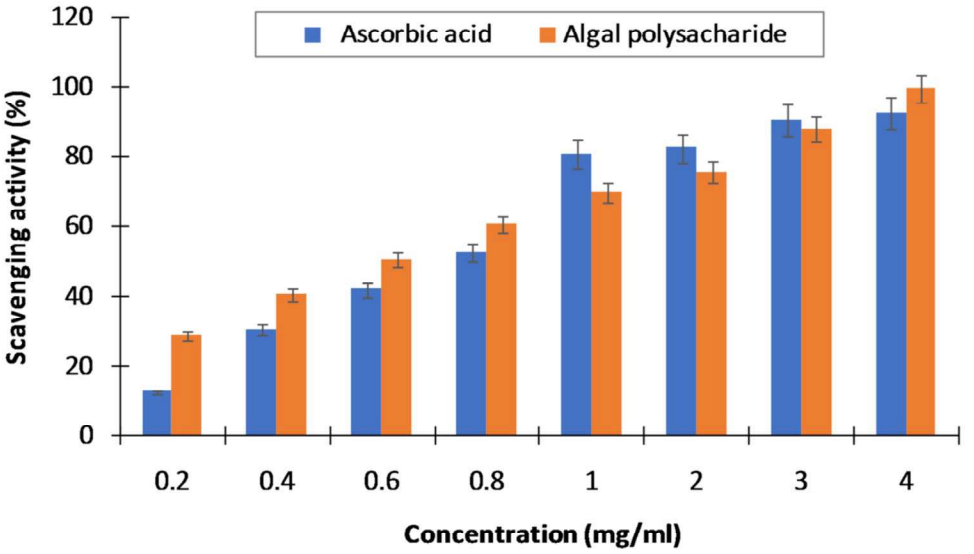


Fig. 6 DPPH free radical scavenging activity of the sulphated polysaccharides at various concentrations (mg/mL)

(2,2-diphenyl-1-picrylhydrazyl) assay. The results demonstrated a clear dose-dependent relationship, wherein the scavenging activity increasing proportionally with the concentration of the polysaccharides, ranging from 0.2 mg/mL to 4 mg/mL. As illustrated in Fig. 6, the maximum scavenging activity was recorded at 99.3% at a concentration of 4 mg/mL, while the minimum activity was observed at 28.75% at a concentration of 0.2 mg/mL. This dose-responsive behaviour underscores the efficacy of the algal polysaccharides in mitigating oxidative stress. Substantial variations in antioxidant efficacy observed across different seaweed species can be attributed to intrinsic factors such as species-specific biochemical composition and extrinsic factors such as the extraction methodologies employed [46]. Furthermore, critical parameters such as the structural configuration of the polysaccharides, their compositional heterogeneity, physicochemical characteristics, and molecular weight were identified as pivotal determinants ofantioxidant capacity [48]. These findings highlight the complex interplay of structural and functional attributes governing the antioxidative potential of marine-derived polysaccharides.

Cream Formulation

The cream formulation was meticulously developed by incorporating sulphated polysaccharides into a matrix of bio-derived cream ingredients to achieve a biphasic composition through precise homogenization, adhering to established methodologies detailed in prior research [49]. A comprehensive evaluation of the organoleptic parameters, including chromaticity, pH equilibrium, rheological viscosity, spreadability, and textural integrity, was conducted following validated protocols outlined in the literature [50]. The resultant physicochemical properties of the optimized formulation are systematically represented in Table 2. The preparation process involved the precise gravimetric measurement of components such as lecithin, pectin, rosemary extract, and polysaccharide derivatives. The procedure initiated with the thermal liquefaction of the lipophilic phase constituents in a controlled vessel, typically a borosilicate beaker, under continuous heating at a target temperature of approximately 75 °C. In parallel, glycerol, employed as a hygroscopic humectant, was subjected to isothermal conditions matching the oil phase to ensure compatibility.

Table 2 Comparison of physical parameters of the prepared anti-aging cream with the commercially available cream

Parameter	Prepared cream formulation	Commercial anti-aging cream
Colour	Light yellow	Pale pink
pH	5.25	5.05
Viscosity	Not highly viscous	Not highly viscous
Spread ability	Able to spread evenly on skin(2.3 cm)	Able to spread evenly on skin (2.2 cm)
Texture	Semi solid	Semi solid
Immediate effect on skin	Cooling effect, no film formation	Cooling and moisturizing effect no film formation
Sun protection factor(SPF)	18.413	18.256
Shelf life	No change in colour or odour for 20 days	No change in colour or odour for 20 days.

Upon achieving thermal equilibrium between the lipophilic and hydrophilic phases, the aqueous polysaccharide solution was incrementally incorporated into the molten oil phase under sustained high-shear mixing to facilitate a stable emulsification. This critical emulsification phase harmonized the biphasic system, promoting the formation of a kinetically stable and thermodynamically metastable emulsion. The emulsified system was subsequently subjected to

gradual ambient cooling, allowing the emulsifying agents to establish molecular interactions requisite for the stabilization of the final product. This process culminated in the generation of a structurally robust cream with optimal biphasic characteristics, as illustrated in Fig. 7. The finalized formulation demonstrates excellent stability and uniformity, aligning with the desired specifications for advanced dermocosmetic applications.

Determination of Toxicological Parameters by Cell Viability Assay

A cell viability assay was conducted to evaluate the potential cytotoxicity of the formulated cream [51]. Absorbance values were meticulously measured at varying concentrations (10, 25, 50, 100, and 150 µg/mL) over a time span extending from 0 to 48 h. This experimental framework facilitated a comprehensive assessment of the formulation's impact on L929 cell lines, specifically in terms of cytotoxicity and cellular integrity. The results unequivocally demonstrated that the cream formulation exhibited significantly elevated cell viability across all tested concentrations. In triplicate trials, an average cell viability of 92.46% was recorded at a concentration of 50 µg/mL, and 88.54% at 150 µg/mL. The average cell viability of 92.46% observed at 50 µg/mL suggests that the cream formulation does not significantly harm the cells at this concentration. This high level of cell viability is a positive indicator, as it shows that the cream is relatively non-toxic to L929 cells at this dose. A cell viability rate above 90% typically indicates that the formulation is safe and does not lead to substantial cytotoxicity under the tested conditions. Even at a higher concentration of 150 µg/mL, the cream demonstrated 88.54% cell viability, indicating that its cytotoxic effect remains minimal. While the cell viability decreases slightly with increasing concentration, the viability remains relatively high, further supporting the cream's favorable safety profile (Fig. 8).

A decrease in viability at higher concentrations can be expected; however, the fact that it remains above 80% suggests that the formulation is still safe and well-tolerated at this dose. These findings substantiate the formulation's minimal cytotoxic effects, thereby affirming its promising safety profile and potential suitability for dermatological applications. The formulated cream showed better cell viability compared to other formulations tested on HaCaT cells (human keratinocytes) using Spirulina platensis Powder, Polysorbate 60, and Cremophor surfactants. The latter formulations showed lower cell viability, indicating higher toxicity to the cells. This comparison highlights that the cream formulation developed in this study exhibits a significantly safer profile in terms of cell survival, making it a preferable choice over other surfactant-based formulations for potential therapeutic or cosmetic applications [52].

Conclusions

The crude polysaccharide extracts from *S. tenerrimum* initially demonstrated a yield of 4.5%, which significantly increased to 14.68% following purification using DEAE-Sephadex resin. The yield of sulfated polysaccharides was about 14.68% significantly higher than the crude polysaccharide around 5% following purification using DEAE-Sephadex resin. This significant enhancement can be attributed

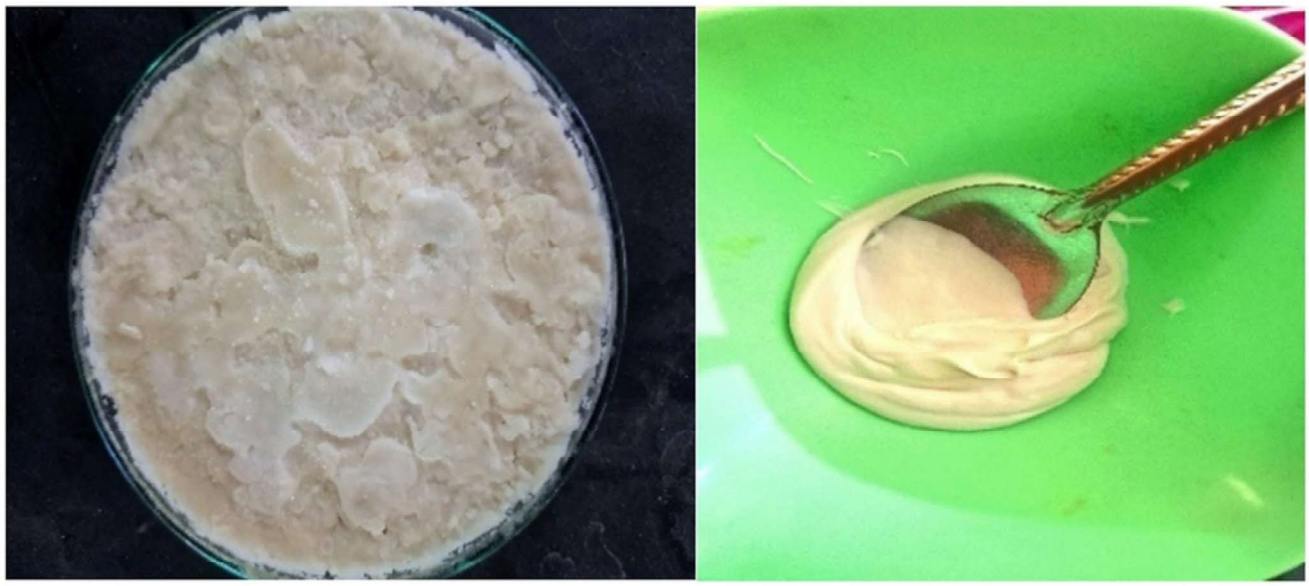


Fig. 7 (**A**) sulphated polysaccharides; (**B**) Cream formulation

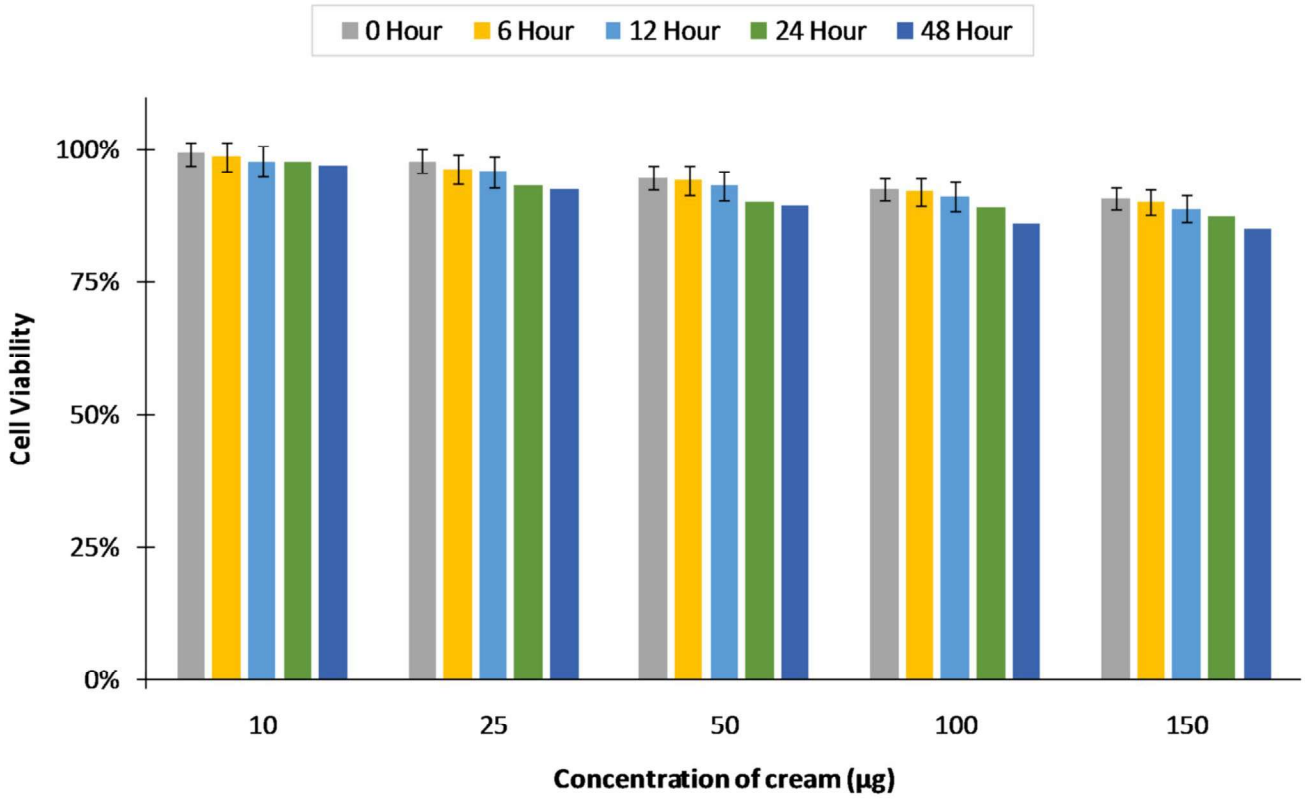


Fig. 8 Cell viability rate over the period from 0 h to 48 h

to the removal of non-polysaccharide impurities during ion-exchange chromatography, which enriched the target sulfated polysaccharides. Since yields were calculated per 100 g of extract, the increase reflected the improved purity and selectivity of the DEAE-based purification process rather than an absolute increase in polysaccharide mass. This highlights the efficiency of purification techniques in enhancing the polysaccharide content, which is critical for achieving a high-quality product suitable for commercial applications. The formulated cream demonstrated remarkable radical scavenging activity, with a peak efficiency of 87.82% at a concentration of 4 mg/mL and optimal performance at 2 mg/mL (79.82%). This high antioxidant activity confirmed the role of sulfated polysaccharides in neutralizing free radicals, a key factor in mitigating oxidative stress-induced skin aging. The cream's Sun Protection Factor (SPF) of 18.413 further substantiates its potential as a photoprotective agent, offering effective protection against UV-induced damage. These findings are particularly relevant in the context of growing consumer demand for natural, multifunctional skincare products that provide both anti-aging and UV protection benefits.

The cell viability assessment revealed high viability rates of 92.46% at 50 µg/mL and 88.54% at 150 µg/mL, demonstrating the cream's biocompatibility and safety for dermal application. These results were consistent with the non-toxic nature of marine-derived polysaccharides, reinforcing their suitability for prolonged use without adverse effects. The verified shelf life of 20 days indicated the cream's stability, which is a critical parameter for commercial viability. However, rosemary oil was added and to ensure complete preservation. It also contributed to the cream's aromatic profile and skin-soothing properties, making it a multifunctional ingredient in natural and herbal skincare formulations. The current study adds to this growing body of evidence by showcasing the potential of *S. tenerrimum* as a sustainable source of sulfated polysaccharides, thereby expanding the range of algae species explored for cosmeceutical applications. Moreover, the study's emphasis on radical scavenging and photoprotective efficacy addressed key consumer concerns related to premature skin aging and environmental damage. This positioned the formulated cream as a competitive alternative to synthetic cosmetics, which often raise concerns regarding safety, sustainability, and long-term health implications.

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Data Availability The data used to support the findings of this study are included in the article.

Declarations

Conflict of Interest The authors declare that there is no conflict of interest regarding the publication of this article.

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