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Therapeutic potential of *Stypocaulon scoparium* extract in antioxidant, antimicrobial, anti-inflammatory, and anticancer applications

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Abstract: Seaweeds are widely recognized as valuable sources of bioactive secondary metabolites with significant medicinal and industrial potential. The biological properties of the brown seaweed *Stypocaulon scoparium* crude extract were investigated. An algal sample was collected from the coastal region of Tipaza, Algeria, and the extract was evaluated for total polyphenol and flavonoid contents, and antioxidant, antibacterial, anti-inflammatory, and anticancer activities. The extract is rich in polyphenols and flavonoids, exhibiting strong antioxidant capacity. It showed notable antimicrobial activity was observed against various pathogens, with pronounced strong antifungal efficacy, and significantly reduced MCF7 breast cancer cell viability, indicating anticancer potential. Anti-inflammatory activity was demonstrated by inhibition of protein denaturation, with the extract exhibiting a distinct concentration-dependent effect. In summary, *Stypocaulon scoparium* was identified as a promising source of bioactive compounds. However, further investigations are required to elucidate their mechanisms of action and to validate their pharmaceutical applications.

Keywords: brown seaweed, *Stypocaulon scoparium*, polyphenol, flavonoid, bioactive compounds, MCF7 breast cancer cell

INTRODUCTION

As some of the oldest organisms on Earth, algae have long been utilized for their beneficial properties [1]. Often referred to as seaweeds, these photosynthetic creatures are important for marine ecosystems since they are primary producers that generate oxygen and help to sequester carbon [2]. In addition to their environmental relevance, algae are abundant in vitamins, minerals, proteins, polysaccharides, and functional polyphenols. They are highly diverse, with chemical composition varying by species, geographic area, seasonal changes, and environmental conditions [3]. This versatility reflects their ecological resilience and underlines their importance in a variety of aquatic settings.

Algae synthesize a range of bioactive compounds to counter biotic stresses such as predation and competition, as well as abiotic stresses from changing environmental conditions. These include pigments such as fucoxanthin, astaxanthin, and carotenoids, as well as alkaloids, terpenoids, and polyphenols such as phenolic acids, tannins, and flavonoids, all of which hold economic and environmental significance [4]. Marine algae extracts are considered a rich source of bioactive compounds with a wide range of biological activities [5]. Their antioxidant, antimicrobial, antidiabetic, anti-inflammatory, and anticancer potential [6] highlights algae as a valuable resource for innovation in pharmaceuticals, nutraceuticals, and biotechnology.

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The global interest in algae extends beyond their ecological roles to their industrial applications. Historically, seaweeds have been harvested for use in food, fertilizers, and traditional medicine [7]. Today, their bioactive compounds are increasingly incorporated into cosmetics, biofuels, and functional foods, driven by a growing demand for sustainable and natural products [8]. Brown algae, such as *Stypocaulon scoparium* from the Stypocaulaceae family (Phaeophyceae class), demonstrate this potential [9]. Although their full therapeutic and commercial potential is underexplored, especially in certain regions, these species are notable for their phytochemical profiles and biological activities.

Algeria, with its long coastline stretching along the Mediterranean Sea, represents one of the largest natural reservoirs of marine algae in the Mediterranean Basin [10]. Although thousands of algal species inhabit these marine environments, many remain unidentified despite existing research. Most studies have concentrated on cataloging algal flora and its practical applications, leaving the in-depth exploration of their phytochemical and bioactive properties largely unexplored[11].

This study focused on the brown macroalgae *Stypocaulon scoparium* collected from the coastal region of Tipaza. This brown macroalga was selected both for its abundance in this region of the Mediterranean and for preliminary findings indicating promising bioactivity, suggesting potential pharmaceutical and biotechnological applications [12]. We aim to address a gap in current knowledge by examining the phytochemical composition and biological properties of this locally sourced alga. It represents a step toward recognizing and evaluating Algeria's rich but underexplored marine biodiversity. By shedding light on the potential of these natural resources, the research seeks to promote a broader appreciation of algal diversity and encourage further research into untapped marine treasures.

MATERIALS AND METHODS

Ethics statement

The research study was conducted *in vitro* with no human subjects or animals involved.

Collection of samples

Specimens of the seaweed *Stypocaulon scoparium* were collected in May 2023 from the coast of Hadjeret Ennous (36°34′24″N, 2°03′07″E), in Tipaza Province, Algeria. Collection was carried out manually by uprooting or through underwater diving. Samples were stored in seawater at 0°C until washing to remove epiphytes and necroses by tap water, followed by distilled water [13], and air-dried in the dark at room temperature for 3 to 4 weeks until full dehydration. The dried samples were ground into a fine powder and stored in light-protected and dry conditions until use.

Extract preparation

Bioactive compounds were extracted by maceration. Initially, 20 g of algal powder were mixed with 400 mL of 80% ethanol at 25°C, and the mixture was gently agitated for 24 h at ambient temperature. The extract was then subjected to ultrasound-assisted extraction (UAE) for 2 h using an ultrasonic bath (Transsonic 100H, Elma Schmidbauer GmbH, Singen, Germany) operating at 37 kHz and 100 W. The mixture was filtered through Whatman No. 2 filter paper to remove residual plant material. The resulting filtrate was concentrated using a rotary evaporator (Witeg, Model 2600000, Germany), and the concentrated extract was subsequently freeze-dried to yield a fine powder. The dry extract was weighed to determine the extraction yield and stored in opaque, airtight containers under refrigeration to maintain its bioactive properties.

Phytochemical analysis

Quantification of polyphenols and flavonoids

Total phenolic content was measured using the Folin-Ciocalteu method, modified according to Wang et al. [14]. A 96-well microplate with 20 µL of extract treated in 80% methanol received 100 µL of diluted (1:10) Folin-Ciocalteu reagent. After a 5-min incubation at room temperature in the dark, 80 µL of sodium carbonate solution (75 g/L) was added to each well, and the microplate was incubated for 2 h at room temperature in the dark with moderate shaking. Using a Thermo MULTISCAN EX355 microplate reader, absorbance was obtained at 765 nm. Total phenolic content was

expressed as milligrams of gallic acid equivalents per gram of extract, calculated using a gallic acid standard curve.

The $AlCl_3$ colorimetric technique [15] adjusted for a 96-well plate format was used to measure the total flavonoid concentration in the extract. To 80 µL of distilled water and 20 µL of algal extract, 10 µL of 5% $NaNO_2$ was added. After mixing for 1 min, 10 mL of 10% $AlCl_3$, 80 µL of NaO, and 50 µL of water were added. The mixture was then incubated in the dark at room temperature for 40 min. Using a microplate reader, absorbance was recorded at 415 nm. Total flavonoid content was expressed as mg of quercetin equivalents (QE) per g of extract.

Fourier transform infrared (FT-IR) spectra analysis

The extracts were analyzed by attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy [16] using a PerkinElmer FTIR spectrometer (MA, USA) in absorbance mode over the mid-infrared range of 4000-400 cm^{-1}. Origin Pro 8 software was used to analyze spectral data, and IR correlation maps and notes helped identify their functional categories.

Antioxidant capacity determination assays

Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical-scavenging activity was measured using the method described in [17]. The stock solution was prepared by dissolving 24 mg of DPPH in 100 mL of methanol and stored at 20°C until use. A 250-µL aliquot of this solution was mixed with 50 µL of the sample at different doses. Ascorbic acid was used as a positive control, and ethanol and DPPH as a negative control. The reaction mixture was agitated and incubated in the dark for 15 min at ambient temperature. Absorbance was measured at 517 nm using a Thermo MULTISCAN EX355 microplate reader. Scavenging activity was calculated as the percentage of DPPH radicals scavenged using the following equation:

$$I(\%) = \frac{(\text{Control abs} - \text{Sample abs})}{(\text{Control abs})} \times 100$$

β-carotene bleaching (BCB) assay

The β-carotene bleaching inhibitory activity of each sample was measured using the standard method [18]. Briefly, 0.5 mg of β-carotene dissolved in 1 mL of chloroform was mixed with 25 µL of linoleic acid and 200 µL of Tween-80. The chloroform was evaporated under vacuum at 45°C, then 10 mL of 30% hydrogen peroxide was added to the residue to dilute it. In the wells of the microtitration plate, 40 µL of the extract or standards at varying concentrations were combined with 160 µL of the *β*-carotene solution. The plate was incubated at 45 °C, and absorbance at 470 nm was recorded every 30 min over a total period of 2 h. Butylated hydroxytoluene (BHT) was used as the positive control. *β*-Carotene bleaching inhibition was calculated using the following equation:

$$\text{Bleaching inhibition}(\%) = \left[\frac{\text{A120E} - \text{A120c}}{\text{A0c} - \text{A120c}}\right] \text{x100}$$

Ferric reducing antioxidant power (FRAP) assay

The reducing power of the extracts and positive controls was assessed using the potassium ferricyanide reduction method, with minor modifications to the procedure of Oyaizu [19]. Ten µL of extract at varying concentrations were combined with 40 µL of phosphate buffer (0.2 mol/L, pH 6.6) and 50 µL of 1% $K_3Fe(CN)_6$. The mixture was incubated for 20 min at 50°C, after which 50 µL of 10% trichloroacetic acid (TCA) was added to stop the reaction. Subsequently, 40 µL of distilled water and 10 µL of 0.1% ferric chloride were added. Absorbance was measured at 700 nm, using the extraction buffer as the blank reference. Ascorbic acid served as the positive control at concentrations ranging from 0.01 to 1 mg/mL. Data were expressed as the effective concentration (IC_{50}, in µg/mL). This value was determined using interpolation on a linear regression curve.

Hydroxyl radical scavenging activity assay

The scavenging activity for hydroxyl radicals was evaluated according to [20]. We prepared a reaction mixture containing 24 µL of 8 mM $FeSO_4 \cdot 7H_2O$, 80 µL of 3 mM salicylic acid, 40 µL of the extract solution, and 20 µL of 6 mM hydrogen peroxide. After incubation

for 30 min at 37°C in a water bath, the absorbance of the reaction mixture was determined at 510 nm using a Thermo MULTISCAN EX355 microplate reader. Ascorbic acid served as the standard control. The scavenging activity of the extract was calculated using the following equation:

$$I(\%) = \frac{(\text{Control abs} - \text{Sample abs})}{(\text{Control abs})} \times 100$$

ABTS+ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay

The antioxidant activity was assessed using the method established by Re et al. [21] and modified for a microplate format. The $ABTS^+$ radical cation was produced by combining 5 mL of a 7 mM aqueous ABTS solution with 2.45 mM $K_2S_2O_8$ and incubating the mixture in the dark at 4°C for 16 h. The solution was diluted with methanol to attain an absorbance of 0.7±0.02 at 734 nm before use. Subsequently, 160 µL of the $ABTS^+$ solution was mixed with 40 µL of the sample at various concentrations or with the standard antioxidant butylated hydroxytoluene (BHT). The blank was prepared in the same way, utilizing methanol instead of the sample. Following a 10-min incubation, absorbance was assessed at 734 nm using a Thermo MULTISCAN EX355 microplate reader.

Antimicrobial activity

Test microorganisms

The microbial strains used in this investigation included both reference strains (ATCC) and multidrug-resistant (MDR) clinical isolates. Bacterial isolates were obtained from the Bacteriology Laboratory of Kolea Hospital (Tipaza, Algeria), while fungal strains, including ATCC reference strains and pathogenic clinical isolates, were sourced from the Mycology Laboratory of the same institution. The sources of microbial strains are detailed in Supplementary Table S1. All microorganisms were cultured on selective and differential media suitable for each species. Bacterial cultures were incubated at 37°C for 18-24 h, while fungal isolates were incubated at 28 °C for 7 days to obtain fresh cultures with isolated colonies.

Agar disk-diffusion method

In vitro antibacterial activities were assessed using the agar diffusion method [22]. Briefly, Petri dishes were prepared with 20 mL of Mueller-Hinton agar. Sterile 6 mm Whatman paper disks were impregnated with 10 µL of extract at 10 mg/mL and 5 mg/mL, dissolved in 2 mL of 10% dimethyl sulfoxide (DMSO), and placed on agar surfaces previously inoculated with 200 µL of a cell suspension adjusted to a 0.5 McFarland standard. The plates were incubated at 37°C for 24 h. A disk impregnated with DMSO served as the negative control, while a disk containing an antibiotic was used as the positive control. The inhibitory zones were then identified by measurements taken with Vernier calipers, and all experiments were performed in triplicate.

Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays

The minimum inhibitory concentration (MIC) of the antimicrobials was determined using the broth microdilution method, following Clinical and Laboratory Standards Institute (CLSI, USA) guidelines [22]. Briefly, a sequence of two-fold serial dilutions of the extract, starting at 10 mg/mL, was prepared in Muller-Hinton broth. Ten µL of a standardized bacterial suspension with an optical density of 0.4 at 600 nm was introduced to each well, resulting in a total volume of 100 µL per well. Two vertical rows of wells served as controls: the positive control comprised 10 µL of the standardized microbial suspension (10^8 CFU/mL) and 90 µL of Muller-Hinton broth, and the negative control comprised only 100 µL of Muller-Hinton broth. The plates were prepared in triplicate and placed in an incubator at 37°C for 18-24 h. To assess cell viability, we used resazurin as an indicator dye [23]. Any observable color change from purple to pink or colorless was recorded as a positive result. The lowest concentration at which this color change occurred was considered the MIC value [24-27].

Antifungal activity (*in vitro* assay)

Agar well diffusion method

The agar well diffusion method was used to evaluate the antimicrobial activity of the natural extracts [28]. Similar to the procedure used in the disk diffusion

method, the agar plate surface was inoculated with 200 µL cell suspension adjusted to a McFarland 0.5 standard. An 8 mm well was aseptically created using a sterile cork borer, and 100 µL of the extract solution at 10 mg/mL and 5 mg/mL was added. A separate well was filled with 100 µL of DMSO, serving as a negative control. A positive control was incorporated using two synthetic antifungals (Econazole). The plates were incubated at 30°C for 24 h for *Candida albicans* and for 6 days for other fungal species [29].

Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) assays

MIC and MFC values were determined [30] with minor modifications. Serial dilutions of crude extracts were prepared (ranging from 5 to 0.039 mg/mL). Each well of a 96-well microplate was filled with 100 µL of the extract dilution and 100 µL of a fungal spore suspension (10^6 spores/mL) prepared in fresh potato dextrose broth (PDB). The plates were incubated at 27°C for 2 to 3 days with daily monitoring for visible changes. All tests were conducted in triplicate. To determine MIC values, resazurin was used as a growth indicator. For MFC determination, 20 µL from wells showing no visible growth were transferred onto potato dextrose agar (PDA) plates using a sterile rod. These plates were incubated at 37°C for 24 h. The MFC was defined as the minimal concentration of the extract that inhibited fungal growth on solid media [31].

Anti-inflammatory activity

The protein denaturation assay was employed to evaluate the anti-inflammatory potential of the samples, using egg albumin and bovine serum albumin (BSA) to assess the inhibition of protein denaturation [32]. Denaturation was induced by heating the reaction mixtures at 70°C for 10 min. The reaction mixtures contained varying concentrations of algal extract (100-500 µg/mL), 200 µL of egg albumin or 450 µL of a 5% solution of BSA, and 1400 µL of phosphate-buffered saline (PBS). Distilled water served as a negative control. After a 15-min incubation at 37 °C, the mixtures were heated at 70 °C for 5 min and then cooled under running water. Absorbance was quantified at 660 nm. Sodium diclofenac served as a positive control. The experiment was performed in triplicate, and the percentage of protein denaturation inhibition indicating anti-inflammatory activity of the extract was calculated using the following equation [33]:

$$\% \text{ Inhibition} = \left(1 - \frac{C}{D}\right) \times 100$$

where D represents the absorbance of the sample, and C is the absorbance of the negative control.

Potential anti-cancer activity

To assess the potential anticancer properties of the extract, we performed the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay using the MCF7 breast cancer cell line obtained from Tokat Gaziosmanpasa University, Cancer Research Laboratory. MCF7 cells were grown in RPMI 1640 medium (Roswell Park Memorial Institute 1640 Medium) supplemented with 10 % fetal bovine serum (FBS), 5 mM L-glutamine, and 5 mM penicillin/streptomycin. The cells were then incubated at 37°C, 5% CO_2 until they reached 80% confluence. Once the desired confluence was achieved, the cells were harvested using trypsin, followed by centrifugation at 698.75×g for 3 min. The supernatant was carefully removed, and the resulting pellet was retained for further processing. The cells were stained with trypan blue and counted by an Invitrogen Countess 3 Automated cell counter (Thermo Fisher). The cells were seeded into two separate 96-well cell culture plates at a density of 1×10^4 cells per well. The plates were then incubated at 37°C for 24 h at 5% CO_2 to allow cell attachment. After the incubation period, cells were treated with compound concentrations of 0.04 mg/mL, 0.4 mg/mL, and 4 mg/mL, with untreated cells serving as the negative control. Cells were further incubated for 48 h at 37°C [34]. The media from the incubated cells was aspirated, and the cells were gently washed with PBS. Then, 100 µL of the MTT solution was added to each well, and the plate was incubated for 4 h at a temperature of 37°C. After incubation, 100 µL DMSO was added to each well to dissolve the formazan crystals formed by the viable cells. Absorbance was read using a microplate spectrophotometer at a wavelength of 540-570 nm [34]. The percentage cell viability was calculated using the formula:

Table 1. Extraction yield, phenolic content and flavonoid content of *Stypocaulon scoparium* marine algae.

Sample	Extraction yield (%)	Phenolic content (GAE mg/g)	Flavonoid content (QE mg/g)
Stypocaulon scoparium	8.505	22.06	45.25

$$\text{Cell viability \%} = \frac{\text{Absorbance of treated cells} - \text{background absorbance (b)}}{\text{bsorbance of untreated (c)} - \text{background absorbance (b)}} \times 100$$

where (b) is the blank, and (c) is the negative control.

Statistical analysis

Mean comparisons were performed using Duncan's multiple range test, and one-way analysis of variance (ANOVA) was applied. Statistical analyses were conducted with GraphPad Prism 10 version (GraphPad Software, Inc., San Diego, CA 92121 USA). The statistical variations between the two groups were evaluated using Student's t-test. Significance was set as $P<0.05$, $P<0.005$, $P<0.0005$, $P<0.00005$.

RESULTS

Extraction yields phenolic content and flavonoid content

Table 1 presents the extraction yield and the phenolic and flavonoid contents of the marine algae extract *Stypocaulon scoparium*. Maceration yielded 8.50%, with the polyphenol and flavonoid contents of 22.06 mg EAG/g and 45.25 mg QE/g, respectively.

Fourier transform infrared analysis (ATR/FTIR)

The IR spectroscopic analysis results for the two extracts, illustrated in Fig. 1, disclose multiple shared characteristic bands. A broad band observed between 3600 and 3200 cm^{-1} indicates the presence of alcohol and phenol (OH) groups in both extracts. A band near 1052 cm^{-1} corresponds to aliphatic C-O bonds, while bands between 1378 and 1415 cm^{-1} confirm the presence of C-O-C groups, characteristic of aromatic or α,β-unsaturated structures. Out-of-plane deformation vibrations detected between 500-600 cm^{-1} indicate aromaticity and unsaturation in both extracts. These findings indicate a similar functional composition for the two extracts, despite possible variations in band intensities.

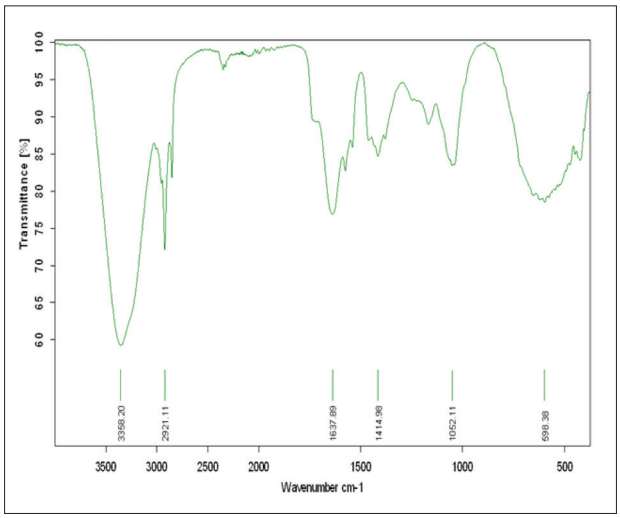

Fig. 1. ATR-FTIR spectra of the *S. scoparium* extract recorded over the full spectral range (4000-400 cm^{-1}). The spectrum exhibits characteristic absorption bands corresponding to hydroxyl groups (3600-3200 cm^{-1}), aliphatic C-O (≈1052 cm^{-1}), C-O-C linkages (≈1415 cm^{-1}), and aromatic out-of-plane deformations (500–600 cm^{-1}).

The IR spectroscopic analysis results for the extract (Fig. 1) reveal several characteristic bands. A broad and intense band between 3600 and 3200 cm^{-1} indicates the presence of alcohol and phenol (OH) groups. A band near 1052 cm^{-1} corresponds to aliphatic C-O bonds, while a band at 1415 cm^{-1} confirms the presence of C-O-C groups, characteristic of aromatic or α,β-unsaturated structures. Out-of-plane deformation vibrations observed between 500 and 600 cm^{-1} highlight the aromaticity and unsaturation of the extract.

Antioxidant activity

The antioxidant capacity of *Stypocaulon scoparium* extract was assessed using several tests, including DPPH, β-carotene bleaching, ABTS, FRAP, and hydroxyl radical scavenging. The results are expressed in IC_{50} values (µg/mL), as summarized in Table 2. In the DPPH assay, *S. scoparium* demonstrated significant antioxidant activity with an IC_{50} of 352.46 ± 28.93 µg/mL, while standard ascorbic acid showed much stronger inhibition (37 ± 0.06 µg/mL), reflecting the superior radical

Table 2. IC_{50} values for *Stypocaulon scoparium* extract in DPPH, β-carotene bleaching, ABTS, FRAP, and hydroxyl radical scavenging assays

IC_{50} values(μg/mL) of radical scavenging					
Marine extract/control	**DPPH radical**	**Beta-Carotene Bleaching Assay**	**FRAP**	**Hydroxyl Radical**	**ABTS**
S. scoparium	352.46±28.93	234.19±37.12	139.94±21.44	338.74±62.05	615.37±42.19
Controls					
- Ascorbic acid	37± 0.06	ND	ND	32 ± 1.7	ND
- BHT	ND	8.99± 1.79	4.4 ± 0.17	ND	0.8001± 0.001

Each value in the table is represented as the mean±SD (n = 3). ND – not determined. P<0.05, P<0.005, P<0.0005, P<0.00005.

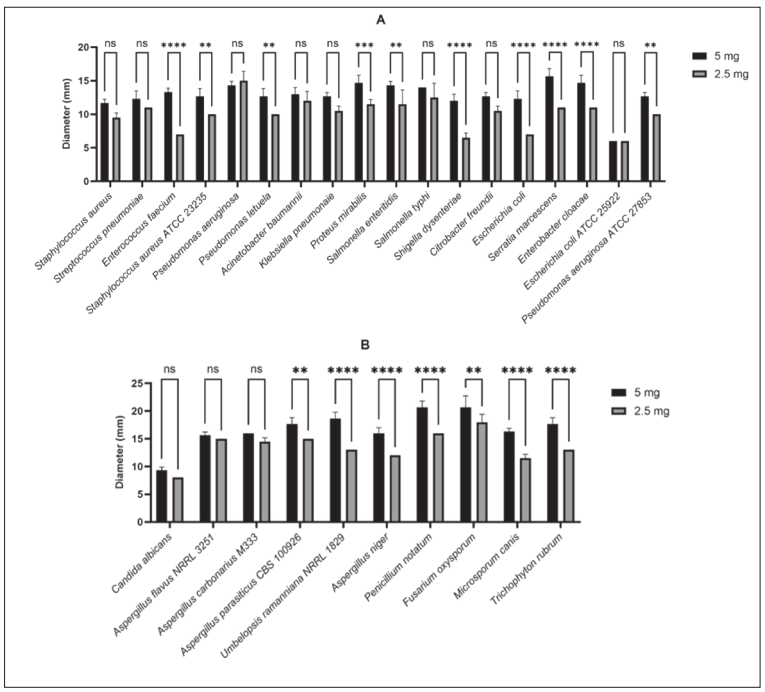

Fig. 2. Disk and well diffusion assay against microbial strains. **A** – Antibacterial activity of *S. scoparium* extract at two concentrations (5 mg/mL and 2.5 mg/mL) against the same bacterial panel (disk diffusion method). **B** – Antifungal activity of the *S. scoparium* extract at two concentrations (5 mg/mL and 2.5 mg/mL) against fungal strains (well diffusion method).

scavenging capacity of the standard. The β-carotene bleaching assay showed that S. scoparium had antioxidant activity with an IC_{50} of 234.19 ± 37 μg/mL, while BHT was significantly more potent (8.99 ± 1.79 μg/mL). In the $ABTS^{+}$ assay, *S. scoparium* exhibited an IC_{50} of 615.37±42.19 μg/mL, while BHT showed significantly higher activity (0.8001±0.001 μg/mL). The FRAP assay revealed strong reducing activity for *S. scoparium* (139.94±21.44 μg/mL), though lower than that of BHT (4.4±0.17 μg/mL). In the hydroxyl radical scavenging test, *S. scoparium* showed an average value of 338.74±62.05 μg/m, while ascorbic acid exhibited markedly higher activity (32±1.7 μg/mL).

Antimicrobial activity

Agar diffusion method

The antibacterial activity of the *S. scoparium* extract was evaluated using the agar disk diffusion method against common human pathogenic bacteria and ATCC strains (Fig. 2A). The results showed that the extract significantly inhibited the proliferation of most Gram-negative bacilli and Gram-positive cocci. *S. marcescens* was highly susceptible, with inhibition zones of 15.66±1.15 mm at 5 mg/mL and 11±0 mm at 2.5 mg/mL. *E. cloacae* exhibited inhibition zones of 14.66±1.15 mm at 5 mg/mL and 11±0 mm at 2.5 mg/mL, while those of *P. mirabilis* were 14.66±1.15 mm at 5 mg/mL and 11.5±0.7 mm at 2.5 mg/mL. Well diffusion assay results showed that the tested fungi were sensitive to the extract, with stronger activity observed at 5 mg/mL (Fig. 2B). The *S. scoparium* extract demonstrated strong antifungal activity, producing inhibition zones of 20.66 ± 1.15 mm against *P. notatum* and 20.66 ± 2.08 mm against *F. oxysporum.*

Minimum inhibitory concentrations (MIC) of algae extract

The MIC of *S. scoparium* extract was evaluated against all bacterial and fungal strains (Table 3), demonstrating notable antimicrobial activity. The extract demonstrated significant activity against *Pseudomonas letuola*, *Salmonella enteritidis*, and *Serratia marcescens*, with respective MICs of 0.26±0.09 mg/mL, 0.3125±0 mg/mL, and 0.3125±0 mg/mL. Against fungi, the extract showed notable efficacy, with MICs of 0.104 ± 0.045

Table 3. Bactericidal and fungicidal activities of the *S. scoparium* crude extract

		Strains	S. *scoparium* extract		
			MIC	MBC	BA
Bacterial strains	Gram positive cocci	*Staphylococcus aureus*	1.25±0	3.33±1.44	Bactericidal
		Streptococcus pneumoniae	1.25±0	2.5±0	Bactericidal
		Enterococcus faecium	1.25±0	5±0	Bacteriostatic
		Staphylococcus aureus ATCC 23235	1.25±0	2.5±0	Bactericidal
	Gram negative bacilli	*Pseudomonas aeruginosa*	0.625±0	0.625±0	Bactericidal
		Pseudomonas letuola,	0.26±0.09	0.625±0	Bactericidal
		Acinetobacter baumannii	0.625±0	1.25±0	Bactericidal
		Klebsiella pneumonaie	0.625±0	1.04±0.36	Bactericidal
		Proteus mirabilis	1.25±0	1.25±0	Bactericidal
		Salmonella enteritidis	0.312±0	0.52±0.18	Bactericidal
		Salmonella typhi	0.625±0	0.625±0	Bactericidal
		Shigella dysenteriae	1.04±0.36	2.08±0.72	Bactericidal
		Citrobacter freundii	0.625±0	0.625±0	Bactericidal
		Escherichia coli	1.66±0.72	1.66±0.72	Bactericidal
		Serratia marcescens	0.312±0	0.312±0	Bactericidal
		Enterobacter cloacae	0.625±0	0.83±0.36	Bactericidal
		Escherichia coli ATCC 25922	0.625±0	0.625±0	Bactericidal
		Pseudomonas aeruginosa ATCC 27853	1.25±0	4.16±1.44	Bactericidal
			MIC	**MFC**	**FA**
Fungal strains		*Candida albicans*	0.10±0.04	0.156±0	Fungicidal
		Aspergillus flavus NRRL 3251	0.312±0	1.25±0	Fungistatic
		Aspergillus carbonarius M333	0.312±0	2.08±0.72	Fungistatic
		Aspergillus parasiticus CBS 100926	0.625±0	1.66±0.72	Fungicidal
		Umbelopsis ramanniana NRRL 1829	1.25±0	5±0	Fungistatic
		Aspergillus niger	1.25±0	3.33±1.44	Fungicidal
		Penicillium notatum	0.625±0	2.5±0	Fungistatic
		Fusarium oxysporum	0.625±0	2.5±0	Fungistatic
		Microsporum canis	0.312±0	1.25±0	Fungistatic
		Trichophyton rubrum	0.41±0.18	1.25±0	Fungicidal

MIC – minimal inhibitory concentration; MBC – minimal bactericidal concentration; MFC – minimal fungicidal concentration; BA – bactericidal activity; FA – fungicidal activity.

mg/mL for *Candida albicans*, 0.3125 ± 0 mg/mL for *Aspergillus flavus* NRRL 3251, 0.3125 ± 0 mg/mL for *Aspergillus carbonarius* M333, and 0.3125 ± 0 mg/mL for *Microsporum canis*. Bactericidal activity was observed for most bacterial strains, based on MBC/MIC ratios, whereas *Enterococcus faecium* showed a bacteriostatic effect. Among fungi, some species displayed fungicidal sensitivity, while others exhibited a fungistatic response (Table 3).

Anti-inflammatory activity

The *in vitro* anti-inflammatory efficacy of the *S. scoparium* extract was assessed by its capacity to impede protein denaturation, using BSA and egg albumin as models. The findings, illustrated in Table 4, showed significant concentration-dependent inhibition at doses ranging from 156.25 to 2500 µg/mL. At the maximum concentration of 2,500 µg/mL, the *S. scoparium* extract inhibited BSA denaturation by 62.24%, reflecting significant efficacy. In contrast, diclofenac sodium, used as a reference standard at 100 µg/mL, demonstrated 84.28% inhibition. Assessment of egg albumin denaturation inhibition revealed a concentration-dependent increase in inhibition. The *S. scoparium* extract exhibited a maximum inhibition of 75.60% at 2,500 µg/mL, comparable to 76.13% inhibition by diclofenac sodium at 100 µg/mL, suggesting substantial anti-inflammatory potential, particularly at high concentrations.

Anticancer activity

The cytotoxicity of the *S. scoparium* extract on the MCF7 breast cancer cell line was evaluated using the MTT assay (Fig. 3). At the highest tested concentration (4 mg/mL), the extract reduced cell viability to 32.47%, whereas lower concentrations (0.04 and 0.4 mg/mL) caused markedly smaller reductions.

DISCUSSION

Marine macroalgae are increasingly recognized as a valuable resource because of their rich nutritional profile and high content of bioactive compounds, offering potential health and industrial benefits [35]. Bioactive compounds extracted from the Algerian marine brown alga *Stypocaulon scoparium* were investigated for their phytochemical composition and diverse biological activities. This study aimed to assess chemical richness by measuring the total polyphenol and flavonoid contents. Additionally, *in vitro* assays evaluated the extract's antioxidant, antibacterial, anti-inflammatory, and anticancer activities. The main goal was to clarify

Table 4. Anti-inflammatory activity assessed using egg albumin and bovine serum albumin denaturation assays

		Egg albumin denaturation		Bovine serum albumin denaturation	
Treatment (s)	Concentration (µg/mL)	Absorbance at 660 nm	% inhibition of protein denaturation	Absorbance at 660 nm	% inhibition of protein denaturation
Control	--	1.902± 0.017		0.791± 0.07	
***S. scoparium* extract**	2500	0.464± 0.1071	75.60	0.298± 0.02	62.24
	625	1.311±0.3	31.05	0.5513±0.11	30.29
	156.25	1.578±0.3	17.01	0.6533±0.12	17.40
Diclofenac sodium	100	0.39± 0.08	76.13	0.124± 0.04	84.28

P<0.05, P<0.005, P<0.0005, P<0.00005.

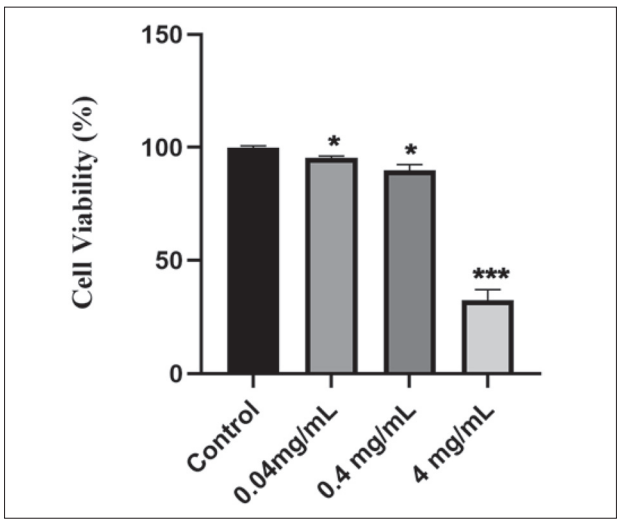

Fig. 3. Percentage of MCF7 breast cancer cell viability after treatment with *S. scoparium* extract at different concentrations, measured by the MTT assay. The extract exhibited dose-dependent cytotoxic effects (P<0.05, P<0.005, P<0.0005, P<0.00005).

the therapeutic potential of these macroalgal bioactive compounds and explore their application as natural bioactive agents in the pharmaceutical and biotechnological industries.

The *S. scoparium* algae had an extraction yield of 8.50%. These findings align with earlier research on marine macroalgae extracted with hydroalcoholic solvents, which suggests an efficient recovery of phenolic components [36]. In this study, the total phenolic content of *S. scoparium* (22.06 mg GAE/g extract) was substantially higher than previously reported values (1.232-3.287 mg GAE/g dry alga powder) [37]. Higher TPC values have been reported [38] in a study of six brown algae species from the order Dictyotales, with TPC ranging from 250 to 540 mg GAE/g extract. Lower TPC values have been found in *Sargassum* species [39], with values between 6 and 13 mg GAE/g. This comparison indicates that *S. scoparium* falls within a moderate phenolic range. The total flavonoid content of the *S. scoparium* extract was 45.25 mg QE/g extract, a value that is comparatively high among brown algae. TFC values ranging from 9.89 to 29.39 mg CAE/g extract have been reported across six brown algal species [40]. Seasonal variation, algal growth circumstances, or methodological discrepancies in the extraction and quantification of phenolic chemicals may account for this variability. Geographical location and environmental conditions may alter the chemical composition of *S. scoparium*, affecting the levels of secondary metabolites. The efficiency of polyphenol and flavonoid extraction is greatly influenced by the choice of solvent and experimental parameters, including temperature and extraction duration. Previous studies show that hydroalcoholic combinations improve the extraction of flavonoids, while polar solvents such as methanol and ethanol are effective for phenolic chemicals [37]. These methodological factors may explain the differences observed between this study and previous reports.

The antioxidant capability of an extract is reflected in its reducing power. Extracts with strong reducing activity can donate electrons more efficiently, facilitating interactions with free radicals to generate stable molecules and reduce oxidative stress [41]. The *S. scoparium* extract exhibits significant reducing properties, indicating an enhanced capacity to donate electrons and neutralize free radicals, thereby contributing to oxidative stress prevention. However, its reducing power is compared with ascorbic acid and BHT, which are established, highly efficient reference antioxidants.

The antioxidant potential of *S. scoparium* extract was evaluated using multiple *in vitro* assays. Among these, the FRAP assay produced the lowest IC_{50} values (139.94±21.44 µg/mL), indicating a relatively stronger

ferric-reducing capacity compared to other radical scavenging activities. Previous findings [37] show lower antioxidant activity, likely due to differences in the extraction methods and the solvent systems. Although the antioxidant efficacy of *S. scoparium* extract is lower than that of standard compounds, the results suggest its potential for development into products with antioxidant properties.

Marine algae are an important source of bioactive compounds with antibacterial properties against both Gram-negative and Gram-positive pathogenic bacteria [42]. In this study, the hydroethanolic extract of *S. scoparium* demonstrated promising antibacterial activity against both types of bacteria. The strongest activity, assessed by the disk diffusion method, was recorded against Gram-negative bacilli (*Serratia marcescens*, *Enterobacter cloacae*, and *Proteus mirabilis*), with inhibition zones of 14-16 mm, followed by Gram-positive cocci (*Enterococcus faecium*), averaging 13 mm. These observations are consistent with previous work [43], which reported that ethanolic extracts of eight algal species belonging to Chlorophyta, Phaeophyta, and Rhodophyta exhibited significant antibacterial and antifungal properties. The results also corroborate those in [44], which reported antibacterial activity of *S. scoparium* (brown algae) and *Halopitys incurvus* (red algae) against at least one of the tested bacteria. The ethanolic extract of *S. scoparium* produced inhibition zones greater than 10 mm for all bacterial strains analyzed. Supercritical fluid extracts of *Halopteris scoparia* (brown algae) progressively inhibited the growth of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Propionibacterium acnes* in a concentration-dependent manner [45]. The MIC tests confirmed the antimicrobial efficacy of the hydroethanolic extract of *S. scoparium*, with values ≤1.66 mg/mL. The most notable activity was observed against *Pseudomonas letuola* (Gram-negative). The brown seaweed *Padina pavonica* extract exhibited moderate activity against *Pseudomonas aeruginosa*, with an MIC≤10 mg/mL and an MBC>10 mg/mL [46], suggesting that the *S. scoparium* extract may be more active and potentially richer in antimicrobial compounds. Many secondary metabolites from brown seaweed are known for their broad-spectrum antibacterial properties. In this study, the MIC of the hydroethanolic extract of *S. scoparium* also demonstrated effective antifungal activity at concentrations ≤1.25 mg/mL, with the most pronounced effect against *Candida albicans* (MIC=0.1042 mg/mL). The MFC was less than 5 mg/mL. These results are consistent with those of Khelil-Radji et al.[44], who showed that the aqueous extract of *S. scoparium* reduced the growth of *Fusarium oxysporum* f. sp. *albedinis* by 60%, surpassing the effectiveness of ethanolic extracts. These data confirm the strong antimicrobial and antifungal potential of brown algae, particularly *S. scoparium*.

The *S. scoparium* extract showed significant inhibition (62.24%), highlighting its effectiveness in preventing bovine serum albumin (BSA) denaturation. Extracts from the microalga *N. incerta* showed moderate inhibitory effects on BSA denaturation, with less than 50% inhibition at the tested concentrations 47]. These results support our findings on albumin denaturation inhibition, showing only minor variations in the observed effect. Albumin denaturation was inhibited in a concentration-dependent manner by fucans from *Cystoseira crinita* extract, as previously reported [48]. This variation may be attributed to differences in algal species, extraction methods, and the type of albumin used across the three studies.

In the egg albumin denaturation assay, *S. scoparium* also displayed substantial inhibitory activity, with 75.60% inhibition. This level is comparable to or exceeds that reported for certain brown seaweeds, where purified fucoidan from five species inhibited protein denaturation with IC_{50} values ranging from 0.05 to 0.75 mg/mL [49].

Limitations of current cancer treatments, such as drug resistance, non-specific delivery, and severe side effects, have increased interest in natural bioactive compounds as potential alternatives. The present study demonstrated that *S. scoparium* extract exerted dose-dependent cytotoxic effects against MCF-7 breast cancer cells, reducing cell viability to 32.47%. These findings are in agreement with previous reports on the antiproliferative potential of seaweed-derived polyphenols against a variety of cancer cell lines. For instance, the methanolic extract of *H. scoparia*, a brown alga from the same family as *S. scoparium*, exhibited an IC_{50} of 26.8 µg/mL on MCF-7 cells and induced apoptosis both through extrinsic and intrinsic pathways. [47,50,51]. Several studies have attributed these effects to bioactive compounds such as phlorotannins,

flavonoids, and bromophenols, which can induce apoptosis, inhibit cell cycle progression, or disrupt cancer-related signaling pathways [52]. While our findings highlight *S. scoparium* as a promising source of anticancer agents, further research is needed to isolate and characterize the active compounds and to confirm their mechanisms of action in more extensive *in vitro* and *in vivo* cancer models.

The findings of this study underscore the potential of marine algae extracts, with phytochemical analysis revealing a wealth of polyphenols and flavonoids. Antioxidant assays demonstrated substantial ability to reduce oxidative stress and scavenge free radicals. *S. scoparium* extract also showed notable antibacterial action against multiple pathogens, which is vital for preventing chronic infections. The anti-inflammatory activity is further evidenced by inhibition of protein denaturation, highlighting the therapeutic potential of marine algae, consistent with previous studies. The use of *S. scoparium* extract presents new opportunities for applications in food, cosmetics, and pharmaceuticals, supporting the development of natural alternatives to synthetic agents.

CONCLUSIONS

This study highlights the bioactive compounds of *S. scoparium* marine algae, which exhibit antioxidant, anti-inflammatory, antibacterial, antifungal, and anticancer activities. These effects are linked to high levels of polyphenols and flavonoids. The pronounced antioxidant activity, particularly in reducing oxidative stress, supports their potential use in natural health and cosmetic products. The anti-inflammatory effects suggest potential biomedical applications. The extract exhibits antibacterial and antifungal activity, highlighting its potential as a natural alternative to conventional antimicrobial drugs. Further studies are needed to identify the active compounds, evaluate their safety, and explore large-scale applications, thereby promoting the sustainable use of marine resources in pharmaceutical, cosmetic, and food industries.

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Conflict of interest disclosure: The authors declare no conflict of interest.

Data availability: The data supporting this article are available in the online dataset: https://www.serbiosoc.org.rs/NewUploads/Uploads/Abidi%20et%20al_Dataset.xlsx

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SUPPLEMENTARY MATERIAL

Supplementary Table S1. Origin and resistance profile of microbials strains

Bacterial strains	Origin	Resistance profile
Staphylococcus aureus	Blood culture	*Multiresistant*
Streptococcus pneumoniae	Cracha	/
Enterococcus faecium	Stool	Resistant to vancomycin
Pseudomonas aeruginosa	Urine	*Multiresistant*
Pseudomonas letuela	Diabetic pus	/
Acinetobacter baumannii	Pus	*Multiresistant*
Klebsiella pneumonaie	Urine	Resistant to carbapenems
Proteus mirabilis	Pus	Resistant to cephalosporins
Salmonella enteritidis	Cerebrospinal fluid (CSF)	/
Salmonella typhi	Stool	Resistant to cephalosporins
Shigella dysenteriae	Stool	/
Citrobacter freundii	Pus	/
Escherichia coli	Urine	Resistant to cephalosporins
Serratia marcescens	Pus	Resistant to cephalosporins
Staphylococcus aureus ATCC 23235	Pasteur Institute of Algeria	ATCC strains
Escherichia coli ATCC 25922	Pasteur Institute of Algeria	ATCC strains
Pseudomonas aeruginosa ATCC 27853	Pasteur Institute of Algeria	ATCC strains
Candida albicans	Urine	/
Aspergillus flavus NRRL 3251	Pasteur Institute of Algeria	/
Aspergillus carbonarius M333	Pasteur Institute of Algeria	/
Aspergillus parasiticus CBS 100926	Pasteur Institute of Algeria	/
Umbelopsis ramanniana NRRL 1829	Pasteur Institute of Algeria	/
Aspergillus niger	Pasteur Institute of Algeria	/
Penicillium notatum	Soil	/
Fusarium oxysporum	Soil	/
Microsporum canis	Human skin	/
Trichophyton rubrum	Human skin	/