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Phytochemical Constituents, Antioxidant and Antimicrobial Activities of Red Seaweed *Corallina elongata* (Syn. *Ellisolandia elongata*) from the Mediterranean Sea (Algeria)

Abdellatif Alioui[1]*, Asmaa Belmimoun[1], Zouaoui Benattouche[1], Samira Karoune[2], Somia Saad[2], Abdelhamid Foughalia[2], Halima Okaben[3], Keltoum Rais[3], Hamdi Bendif[4]

[1]*Department of Biology, Laboratory of bioconversion, Microbiology Engineering and health safety (LBGMSS), University of Mascara, Mascara29000, Algeria*
[2]*Center for Scientific and Technical Research on Arid Regions CRSTRA, Biskra 07000, Algeria*
[3]*Departement of biology, Faculty of Nature and Life Sciences, University of Mascara, Mascara29000, Algeria*
[4]*Department of Nature and Life Sciences, Faculty of Sciences, University of M'sila; University pole, Bordj Bou Arreridj road, M'sila 28000 Algeria*

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ABSTRACT

In recent years, seaweeds have garnered significant attention due to their chemical and bioactive properties, presenting an avenue for the discovery of novel molecules with valuable applications. Among the major metabolites of seaweeds are phenolic compounds, which exhibit the greatest structural diversity. This study aimed to assess the phytochemical profile, antioxidant and antimicrobial activities of various solvent (hexane, chloroform, methanol and water) extracts of the red seaweed *Corallina elongata*, sourced from the Algerian West Coast. The phytoconstituents of the methanol extract were identified by high-pressure liquid chromatography-electrospray ionization-tandem mass spectrometry (HPLC-ESI-MS-MS), followed by quantitative evaluation of the total phenolic, flavonoid, and condensed tannin contents. Antioxidant activity was assessed using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) scavenging, ferric reducing antioxidant power (FRAP), and phenanthroline assays. The antimicrobial potential was assessed using the disc diffusion method. HPLC-ESI-MS-MS analysis identified 21 compounds, with *p*-hydroxy coumaric acid as the major compound. The methanol extract displayed the highest phenolic content (142.44 µg GAE/mg extract), while the aqueous extract had the highest flavonoid and condensed tannin contents (23.32 µg QE/mg extract and 20.00 mg CE/g extract, respectively). The aqueous extract exhibited superior antioxidant activity, with IC_{50} values of 148.15, 27.01, and 45.85 µg/mL for DPPH, ABTS, and phenanthroline assays, respectively. Both the aqueous and methanol extracts demonstrated promising antibacterial activity against all tested bacterial strains, with inhibition zones ranging from 8.83 to 20.66 mm. Overall, the aqueous and methanol seaweed extracts proved to be the most effective, highlighting their potential as sources of bioactive compounds.

Keywords: Red seaweeds, *Corallina elongata*, Polyphenols, Flavonoids, Antioxidant, Antimicrobial

Introduction

Seaweeds, often referred to as marine vegetables, form the foundation of life in aquatic ecosystems and have served various purposes, including use as fertilizer, human food, and animal feed, spanning ancient to modern times.[1] These marine organisms are categorized into three primary groups: Chlorophyta (green seaweed), Rhodophyta (red seaweeds), and Phaeophyceae (brown seaweeds). The classification is based on their pigment composition, and these groups exhibit significant variations in their metabolite compositions.[2] Red seaweeds, belonging to the phylum Rhodophyta, constitute a diverse and ecologically significant group of marine macroalgae. Characterized by their vibrant red to purple pigmentation, these seaweeds play a crucial role in marine ecosystems worldwide.[3]

*Corresponding author. Email: alioui.latifbio@gmail.com
Tel: 00213671565675

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With a staggering diversity of over 7,000 identified species, red seaweeds inhabit a range of marine environments, from intertidal zones to the deep sea.[4] Phylogenetically distinct, red seaweeds owe their distinctive colour to the presence of phycoerythrins, a class of photosynthetic pigments that allows them to efficiently capture light, enabling their survival in environments with lower light availability. Outside their ecological roles, red seaweeds have garnered increasing attention due to their potential applications in various industries. From the food and pharmaceutical sectors to cosmetics and agriculture, red seaweed exhibits a rich reservoir of bioactive compounds with diverse functionalities.[5] This has spurred research into the extraction and utilization of these compounds for human benefit. This exploration of red seaweed aims to provide an overview of its biological characteristics and the manifold ways in which it intersects with human activities. In recent times, there has been a notable surge in interest concerning marine macroalgae due to their status as natural reservoirs of various bioactive compounds, which present valuable applications across various industrial sectors, including textiles, materials, cosmetics, biomedicine, pharmaceuticals, and notably, the food industry.[6] From a nutritional perspective, seaweeds serve as significant sources of minerals (ranging from 7% to 36%), encompassing essential elements such as calcium, iron, copper, and iodine. They also contain polysaccharides (15% to 76%), including agar-agar, alginate, and carrageenan; proteins (5% to 47%), featuring all essential amino acids; and lipids (1% to 5%), notably rich in polyunsaturated fatty acids.

Additionally, seaweed is generally abundant in numerous micronutrients, including vitamins A, B1, B12, C, D, and E. Furthermore, they exhibit a diverse array of phenolic compounds, such as phlorotannins, bromophenols, flavonoids, phenolic terpenoids, and mycosporine-like amino acids.[7]

Phenolic compounds are present in both terrestrial plants and seaweeds.[8] Particularly noteworthy are the polyphenols synthesized by seaweeds, constituting one of the most extensive and widely distributed groups of phytochemicals in these organisms. Their pharmacological activity and diverse health-promoting benefits have attracted considerable attention, given the significant role played by polyphenols in the varied biological activities exhibited by seaweeds.[9] Seaweeds appear as valuable sources of polyphenolic compounds, including phlorotannins, bromophenols, flavonoids, phenolic terpenoids, and mycosporine-like amino acids. Natural antioxidants possessing versatile functionalities have garnered significant attention as substitutes for synthetic antioxidants, particularly in safeguarding complex biological systems like muscle tissues from oxidation. Extensive research has been dedicated to exploring natural antioxidants derived from terrestrial plants and their applications in preventing oxidation in biological systems. In this regard, aquatic plants are emerging as promising reservoirs of antioxidants.[8] Studies have demonstrated that marine macroalgae represent a rich source of diverse natural antioxidants, notably polyphenols, which play a crucial role in averting lipid peroxidation. Methanol extracts from red algae have been shown to contain various polyphenolic compounds, including catechins such as gallocatechin, epicatechin, and catechin gallate, as well as flavonols and flavonol glycosides.[8] This underscores the potential of marine macroalgae as valuable contributors to the pool of natural antioxidants.[10] In this particular context, the objective of this research is to assess the chemical composition of red seaweed (*Corallina elongata*) and examine its phenolic and flavonoid content, antimicrobial and antioxidant activities of extracts obtained from red seaweed gathered from the northwest of Algeria, specifically in the province of Mostaganem on the Mediterranean coast.

Materials and Methods

Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade. The following were purchased from Sigma-Aldrich (St. Louis, MO, USA; via Tlemcen branch, Algeria): gallic acid, quercetin, catechin, Folin–Ciocalteu reagent, sodium carbonate (Na_2CO_3), aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$), sodium acetate, vanillin, methanol, hydrochloric acid (HCl), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium ferricyanide, phosphate buffer, trichloroacetic acid, ferric chloride ($FeCl_3$), 1,10-phenanthroline, formic acid, acetonitrile, and butylated hydroxytoluene (BHT). Mueller Hinton Agar and nutrient broth were obtained from Biokar Diagnostics (Beauvais, France). Iodonitrotetrazolium chloride was purchased from Merck (Darmstadt, Germany). Dimethyl sulfoxide (DMSO) was obtained from Fisher Scientific (Loughborough, UK). Sterile 6 mm Whatman filter paper discs were obtained from GE Healthcare Life Sciences (UK).

Plant sampling and preparation

Samples of red seaweed (Figure 1) were collected at the Kharouba beach (Sid Mejdoub), situated 4 km north of the province of Mostaganem (35°56'N, 0°05'E), in January 2022. The plant material was identified and authenticated by Prof. Bachir Bouiadjra Benabdellah. at the University of Mostaganem, Algeria, and an herbarium specimen with voucher number BMMOS101 was deposited in the same university. The plant material was washed thoroughly with tap water to remove sand and other earthy particles. Subsequently, the samples were dried away from direct sun light and humid condition for several weeks. The dried plant material was broken into small fragments and then ground into a fine powder.

Extraction

In a Soxhlet apparatus, 10 g of the plant material was subjected to an initial extraction with 100 mL of hexane. Subsequently, an exhaustive extraction of the remaining plant materials was carried out using chloroform, methanol, and water successively, aiming to facilitate the release of the maximum number of bioactive compounds. Each solvent extraction cycle lasted for 6 hours, maintaining a constant temperature of 60°C for the solvents, except for distilled water, which was set at 100°C. For another set of experiments, ten grams (10 g) of plant material was placed in an Erlenmeyer flask, and 100 mL of methanol was added. This mixture was allowed to macerate with stirring in the dark at room temperature for 24 hours.

Figure 1: Sample of red seaweed (*Corallina elongata*)

Characterization by HPLC-ESI-MS-MS

The high-pressure liquid chromatography electrospray ionization tandem mass spectrometry (HPLC-ESI-MS-MS) method (Shimadzu Kyoto, Japan) was conducted using an orbitrap Thermo q-Exactive mass spectrometer coupled to a Vanquish HPLC. A Kinetex XB-C18 column (Phenomenex) with a particle size of 2.6 µm, a length of 100 mm, and a diameter of 2.1 mm served as the column. The mobile phases consisted of a 0.1% formic acid aqueous solution (A) and acetonitrile (B). The gradient program (time (min), % B) was set as follows: (0.00, 50); (20.00, 100); (25.00, 100); (26.00, 50). The flow rate was maintained at 0.200 mL min^{-1}, and the injection volume was 10 µL. Electrospray ionization in negative mode was employed, utilizing the following analysis parameters: electrospray voltage of −3.8 kV, sheath gas flow rate of 30, auxiliary gas unit flow rate of 10, drying gas temperature of 310°C, capillary temperature of 320°C, and S-lens and RF level set at 55. The acquisition spanned a mass range from 100 to 1000 a.m.u. An auto MS2 program was implemented with a fragmentation voltage of 30.

Determination of polyphenols content

Total phenols content (TPC)

The total phenolic content of the extracted samples was determined using the Folin-Ciocalteu reagent following the method outlined by Müller *et al*. (2010).[11] Specifically, 100 µL of Folin-Ciocalteu reagent (10-fold dilution) and 75 µL of sodium carbonate (Na_2CO_3, 7.5%) were added to 20 µL of each extract (1 mg/mL, triplicate per sample). Subsequently, this mixture was incubated at room temperature for 2 hours in the dark. The absorbance was then measured at 765 nm using a 96-well microplate reader (Thermo ScientificTM Multiskan Sky). The results were expressed in micrograms of gallic acid equivalents per milligram of dry extract (µg GAE/mg DE). Gallic acid at various concentrations (0.0 to 250 µg/mL) was used to generate the calibration curve ($y = 0.0043x + 0.0363$; $R^2 = 0.9914$).

Total flavonoids content (TFC)

The total flavonoid content was assessed using the aluminum chloride colorimetric method as described by Topçu *et al*. (2007).[12] In this method, 50 µL of each extract solution (1 mg/mL) was combined with 50 µL of aluminum chloride ($AlCl_3 \cdot 6H_2O$, 2%) and 150 µL of 5% sodium acetate in the wells of a 96-well microplate. The mixture was incubated in the dark at room temperature for 2.5 hours. Absorbance was measured at 440 nm using a UV–Vis spectrophotometer (Aqualabo, France). The results were expressed as µg quercetin

equivalents (µg QE/mg) of the extract (µg EQ/mg), with referenced to the calibration curve of quercetin (y = 0.0049x + 0.015 R^2 = 0.9963).obtained at various concentrations (0.0 to 250 µg/mL).

Determination of condensed tannins

The method used for determining condensed tannins was the vanillin assay in an acidic medium.[13] Vanillin reacts with condensed tannins in the presence of sulfuric acid, causing depolymerization and producing a measurable yellow complex at 500 nm. To perform the assay, 500 µL of extract solution (1 mg/mL) was added to 1500 µL of a 4% vanillin/methanol solution, followed by 750 µL of concentrated hydrochloric acid (HCl), and the mixture was homogenized using a vortexer. The resulting mixture was left at room temperature for 20 minutes, and the absorbance was measured at 550 nm using a UV–Vis spectrophotometer (Aqualabo, France). A calibration curve (y = 1.1748x + 0.0665; R^2 = 0.9972) was created using catechin (0.0 to 300 µg/mL) as the standard, and the total condensed tannin content was calculated in mg of catechin equivalents per gram of extract (mg CE/g).

Determination of Antioxidant activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH radical scavenging activity of the plant extracts was assessed following the method described by Bouchoukh *et al.* (2019).[14] In summary, 40 µL of each extract at various concentrations (12.5, 25, 50, 100, 200, 400, and 800 µg/mL) were mixed with 160 µL of 0.1mM DPPH radical solution in methanol. After a 30-minute incubation in the dark, the absorbance was measured at 517 nm using a microplate reader. Butylated hydroxytoluene (BHT) served as positive control. DPPH radical scavenging effect was calculated by the following equation 1:

$$DPPH\ scavenging\ effect\ (\%) = \frac{\text{Control absorbance} - \text{Extract absorbance at 30min}}{\text{Control absorbance}} X100 \quad \text{(Eq. 1)}$$

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

The ABTS radical scavenging effect was conducted according to the procedure outlined by Re *et al*. (1999).[15] Specifically, 160 µL of 2 mM ABTS solution was combined with 40 µL of each extract at different concentrations (12.5 - 800 µg/mL), with BHT serving as the positive control. Following a 10-minute incubation, the absorbance was measured at 734 nm. The ABTS scavenging effect was determined using the following equation 2;

$$\% \text{ inhibition} = [(AC - AS) / AS] \times 100 \quad \text{(Eq. 2)}$$

Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power was evaluated following the method described by Okafor *et al.* (2024),[16] with slight modifications. Briefly, 10 µL of each extract at various concentrations (3.125, 6.25, 12.5, 25, 50, 100, and 200 µg/mL), 40 µL of phosphate buffer (pH 6.6), and 50 µL of potassium ferricyanide (1%) were combined. After a 20-minute incubation at 50°C, 50 µL of trichloroacetic acid (10%), 40 µL of distilled water, and 10 µL of ferric chloride ($FeCl_3$, 0.1%) were added. Butylated hydroxytoluene (BHT) was used as the positive control. The absorbance was measured at 700 nm.

Phenanthroline (Phen) assay

The phenanthroline assay was conducted in accordance with the method outlined by Szydłowska-Czerniak *et al*. (2008).[17] In summary, 10 µL of each extract at various concentrations (ranging from 3.125 to 200 µg/mL), 50 µL of $FeCl_3$ (0.2%), 30 µL of 1,10-phenanthroline (0.5%), and 110 µL of methanol were combined and placed into a 96-well microplate. Absorbance was measured at 510 nm after a 20-minute incubation at 30°C. BHT was employed as the positive control.

Determination of antimicrobial activity

Bacterial growth inhibition assay

The antibacterial effect of the extracts was conducted in according to the method outlined by Ijoma and Ajiwe (2023),[18] using six bacterial strains: *Escherichia coli* (ATCC25922), *Salmonella typhimurium* (ATCC14028), *Vibrio cholerae* (ATCC14035), *Listleria monocytogenes* (ATCC35152), *Bacillus cereus* (ATCC14579), and *Staphylococcus aureus* (ATCC25923). Bacterial suspensions were prepared using sterile saline solution and adjusted to 0.5 McFarland standard (≈1.5 × 10^8 CFU/mL). The disc diffusion method was employed to evaluate the antibacterial activity of the extracts. The surface of the Mueller Hinton Agar was inoculated with one of the bacterial suspensions using sterile swabs. Subsequently, 6 mm sterile discs (Whatman filter paper) were impregnated with the extracts (10 mg/mL) (dissolved in 2.5% DMSO and sterilized through 0.45 µm filters) and placed on the surface of the inoculated agar. Negative and positive controls were represented by DMSO and gentamicin (10 µg/disc), respectively. The plates were then incubated at 37°C for 24 hours, and the diameter of the zones of inhibition was measured using Vernier calipers. The experiment was conducted in triplicate.

Minimum Inhibitory Concentration (MIC)

The microdilution method in liquid medium was used to determine the Minimum Inhibitory Concentration (MIC), with slight modifications.[19] Broth inoculated with the test organism was incubated at 37°C for 18 hours, then diluted to a concentration of 1 × 10^6 CFU/mL. A range of extract concentrations from 10 mg/mL to 0.078 mg/mL was tested, with acetone-water as the negative control. All tests were performed in triplicate, and the microdilution plates were incubated at 37°C for 24 hours. Antibacterial activity was assessed by adding 25 µL of 0.01% aqueous Iodonitrotetrazolium to each well after incubation, followed by a 30-minute incubation.

Statistical analysis

Data were represented as mean ± standard deviation (SD), n = 3. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's HSD test for multiple comparisons between studied groups. Statistical significant differences between mean values were established at P-value < 0.05.

Results and Discussion

Polyphenols identified by HPLC-ESI-MS/MS analysis

The HPLC–ESI-MS/MS analysis of the methanol extract of the red seaweed *Corallina elongata* identified 21 compounds (Table 1, Figure 2). *p*-Coumaric acid was identified as the predominant metabolite, constituting 92.17% of the extract. Phenolic acids were identified as crucial compounds in *Corallina elongata* seaweed. The phytochemical profile of the methanol extract showcased the presence of bioactive phenolic and flavonoid compounds and other compounds. The findings indicated a high phenolic content in the extract, aligning with prior research.[20,21] Similar studies on the ethanol extract of the green seaweed *Caulerpa racemosa* and the methanol extract of *Corallina elongata* demonstrated substantial presence of phenolic and flavonoid compounds, such as rutin and quercetin.[22] Elmosallamy *et al.*[23] confirmed the presence of flavonoid compounds in *U. lactuca* extract. Previous studies have identified polymeric structures in red, green, and brown seaweed species, with flavonoids and phenolic acids being major constituents in red and green seaweeds.[24,25]

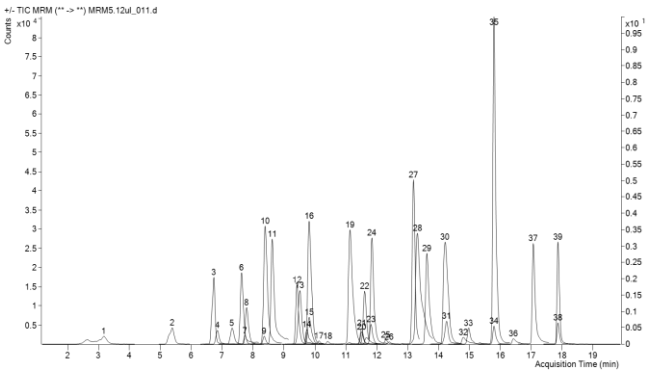

Figure 2: HPLC-ESI-MS/MS Chromatogram of methanol extract of red seaweed *Corallina elongata* (Syn. *Ellisolandia elongata*)

Polyphenolic content of Corallina elongata

The assessment of total phenolic and flavonoid contents in *Corallina elongata* involved the use of various solvent extracts (hexane, chloroform, methanol, and water).

Table 1: Phytochemicals identified from the HPLC-ESI-MS/MS analysis of *Corallina elongata* methanol extract

Compound Name	Compound Class	Rt	Area%
Keampferol	Flavonoid	0.9	0.018
Vanillin	Phenolic aldehyde	1.0	2.976
p-Coumaric acid	Phenolic acids	1.1	92.172
2-tert-Butyl-4-hydroxyanisole	Phenolic	1.4	0.013
Butylated Hydroxytoluene	Phenolic	1.2	1.941
Lawsone / 1,4-Naphthoquinone	Organic compounds	1.1	0.018
Chrysin/5,7-Dihydroxyflavone	Flavonoid	5.3	0.709
Caffeic acid	Phenolic acids	9.3	0.215
Cinnamic acid	Organic compounds	12.5	0.007
Picric Acid	Organic compound	13.6	0.094
Esculin/ 6-*O*-beta-D-glucoside of esculetin	Glycosyl Compounds	17.7	0.033
Rutin	Flavonoid	17.7	0.149
Hesperidin	Flavanoid	38.8	0.154
Folic Acid	Vitamin	39.6	0.038
Beta-Carotene	Terpenoids	43.6	0.078
Ascorbic Acid	Vitamins	47.5	0.711
Benzoic Acid	Organic compound	47.6	0.530
Quercetin	Flavonoid	47.5	0.020
Maleic Acid	Organic compound	46.8	0.098
Naringenin	Flavonoid	47.4	0.014
4-Hydroxy coumaric acid	Vitamin	48	0.010

Rt: Retention time in minutes; Area%: Relative abundance of each compound expressed as a percentage of the total peak area.

The total phenolic content (TPC) ranged from 31.33 to 142.44 µg GAE/mg dry extract, while the total flavonoid content (TFC) ranged from 1.94 to 23.32 µg QE/mg dry extract (Table 2). Notably, TPC values were higher in polar extracts, particularly in the methanol and aqueous extracts. Similarly, the TFC contents of the methanol and aqueous extracts surpassed those of hexane and chloroform extracts. There is no study on the total phenolic content of *Corallina elongata* in existing literature. Zouaoui and Ghalem[26] compared the total phenolic contents in different *Corallina elongata* extracts and reported the highest phenolic content in the diethyl ether extract, which, however, was lower than the values obtained for the methanol and aqueous extracts in the present study. Pinteus *et al*.[27] studied the methanol extract of *Corallina elongata* and reported a considerably lower total phenolic content compared to that found in the present study.

Table 2: Total polyphenolic contents of extracts of *Corallina elongata* collected from Mostaganem (western Algeria).

Extraction Method	Extract	TPC (µg GAE/mg DE)	TFC (µg EQ/mg DE)	Condensed Tannins mg CE/g DE)
Soxhlet	Hexane extract	31.33 ± 0.30^{a}	1.94 ± 0.20^{a}	1.10 ± 0.15^{a}
	Chloroform extract	44.76 ± 3.60^{b}	3.41 ± 1.20^{ab}	3.20 ± 0.05^{a}
	Methanol extract	142.44 ± 0.60^{c}	4.43 ± 0.10^{b}	5.60 ± 0.07^{a}
	Aqueous extract	56.23 ± 2.50^{d}	23.16 ± 0.50^{c}	20.00 ± 1.05^{b}
Maceration	Methanol extract	104.63 ± 2.20^{e}	23.32 ± 1.40^{c}	21.20 ± 1.02

Data are expressed as Mean ± SD, (n = 3). Values in the same column followed by a different letter are significantly different ($P < 0.05$). TPC: Total phenolic content; TFC: Total flavonoid content; DE: dry extract.

Similarly, Oucif *et al*.[28] reported a considerably lower total phenolic content in the methanol extract of *C. elongata*. The observed variations in phenolic content within the same species can be attributed to external factors such as the choice of solvent, environmental conditions, etc.[29] Phenolic compounds typically exhibit higher solubility in polar organic solvents than in water, and aqueous mixtures of methanol, ethanol, and acetone are recommended as effective extractants.[30] Red algae, in general, are known to contain higher amounts of polyphenols.[8] In the case of TFC, differences between TFC values among the different extracts were found to be statistically insignificant ($P>0.05$). It was found out that *C. elongata* methanol and aqueous extracts had the highest flavonoid content. This value was found to be significantly higher than the TFC values of the other extracts ($P<0.05$). There are no comparative studies in the literature examining flavonoid content in *Corallina elongata* extracts. When compared to other species, the acetone extract of *C. amentacea* was found to contain a higher amount of total flavonoids than the methanol extract in our study.[31]

The results from the present study showed that *Corallina elongata* had a higher tannin content in its methanol extract obtained by Soxhlet extraction (5.6 ± 0.07 mg CE/g DM), followed by the chloroform extract (3.2 ± 0.05 mg CE/g DM), which were comparable to the findings of Sahnouni *et al*.[32] They reported that the methanol extract of *C. elongata* had a higher tannin content compared to *A. taxiformis* and *H. musciformis*, with values ranging from 1.48±0.01 to 6.56±0.015 mg CE/g extract. The results from the present study also fall within the range of values found in other studies.[33] The increased condensed tannin content in the methanol extract obtained by Soxhlet extraction may be due to the heat-induced destruction of polyphenol oxidases (PPO), which reduce polyphenol content, as well as the breaking of bonds between polyphenols and other substances (proteins, polysaccharides), making the active compounds more accessible. Generally, the brown algae contain higher polyphenol levels than green and red algae. However, it is noted that phenolic compounds are more soluble in polar solvents like methanol, ethanol, and acetone, which are recommended for algae extraction. Nonetheless, comparison with literature is challenging, as several factors can influence the qualitative and quantitative distribution of phenolic compounds in extracts.

Antioxidant activity

The absence of a standardized test for evaluating antioxidant capacities is a well-recognized challenge. Therefore, the assessment of the antioxidant potential of *Corallina elongata* extracts was conducted by assessing their capacity to neutralize free radicals, specifically 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid7) (ABTS) radicals. This neutralization can occur through mechanisms involving electron or hydrogen donation. Additionally, the reducing power was evaluated using the Ferric Reducing Antioxidant Power (FRAP) and phenanthroline assays, which examine the samples' ability to reduce metallic ions via the electron transfer mechanism (Cu^{2+} to Cu^{1+} and Fe^{3+} to Fe^{2+}, respectively). The summarized results are presented in Table 3. The results showed that all extracts had moderate antioxidant activity compared to BHT which was used as a positive control.

Table 3: Antioxidant activity (IC_{50} and $A_{0.5}$) of *Corallina elongata* extracts

Extraction method	Extract and standard	IC_{50} (µg/mL)		$A_{0.5}$ (µg/mL)	
		DPPH	ABTS	Phenanthroline	FRAP
Standard	BHT	<12.5[c]	<12.5[a]	9.71 ± 0.90[b]	6.52 ± 0.07[a]
Soxhlet	HES	>800[b]	282.39 ± 8.50[d]	>800[a]	>800[b]
	CES	653.09 ± 5.70[a]	<12.5[a]	127.14 ± 1.60[d]	>800[b]
	MES	661.32 ± 23.80[a]	128.95 ± 2.90[b]	>800[a]	>800[b]
	AES	148.15 ± 2.70[d]	27.01 ± 2.10[a]	45.85 ± 3.03[c]	>800[b]
Maceration	MEM	>800[b]	234.23 ± 30.30[c]	>800[a]	>800[b]

Data are expressed as Mean ± SD, (n = 3). Values in the same column followed by a different letter are significantly different ($P < 0.05$). BHT: Butylated hydroxytoluene, HES: hexane extract (Soxhlet), CES: chloroform extract (Soxhlet), MES: methanol extract (Soxhlet), AES: aqueous extract (Soxhlet), MEM: methanol extract (maceration).

In general, the Soxhlet extract demonstrated significant antioxidant potential. Specifically, the aqueous extract (Soxhlet) AES showed the highest antioxidant activity among the extracts, with IC_{50} values of 148.15 ± 2.7 µg/mL for DPPH and 45.85 ± 3.03 µg/mL for the phenanthroline assay, while the chloroform extract (Soxhlet) CES displayed remarkable ABTS scavenging activity with an IC_{50} value of <12.5 µg/mL, which was comparable to the positive control BHT. These values indicated higher activity and greater promise of the aqueous and chloroform extracts compared to the other extracts including hexane extract (HES), methanol extract (MES), and methanol extract (maceration) (MEM). The only exception to this trend was observed in the FRAP assay, where all extracts showed no significant activity, registering IC_{50} values greater than 800 µg/mL. Based on the IC_{50} values, the order of antioxidant activity for the five seaweed extracts was observed as follows: AES > CES > MEM > MES > HES. It is important to state that here are no prior studies investigating the antioxidant activities of Hexane, Chloroform, Methanol, and Aqueous extracts of *Corallina elongata* in existing literature. This study marks the first report on the antioxidant activity of this species, and it is remarkable that CES exhibited the highest scavenging effect among the examined extracts. Previous research by Pinteus *et al*.[27] and Oucif *et al*.[28] reported IC_{50} values for the methanol extract of *Corallina elongata* as >1000 µg/mL and 1780 µg/mL, respectively for DPPH scavenging activity. In contrast, findings from the present study showed a higher free radical scavenging activity for the *Corallina elongata* extract, with IC_{50} values of 148.15, 653.09, 661.32, >800, and >800 µg/mL for AES, CES, MES, HES, and MEM, respectively. Regarding other species, Stanojković *et al*.[34] and Kosanić *et al*.[31] determined the DPPH radical scavenging activity of acetone extract of *Cystoseira amentacea* and reported IC_{50} values of 150.2 and 409.81 µg/mL, respectively. Notably, the methanol extract of *C. amentacea* used in this study showed a very high IC_{50} value, suggesting lower antioxidant activity compared to findings in other studies. The difference in solvents (acetone vs. methanol) may account for variations in activity, indicating that different components in the algae respond differently to solvents. Studies on the DPPH radical scavenging activity of methanol extracts of *Jania rubens* from different locations reported varied IC_{50} values ranging from 8.43 mg/mL to 0.13 mg/mL.[27,35-37] Additionally, it has been noted that antioxidant activity and phenolic compound levels may vary with different seasons.[35]

Antimicrobial activity

Bacterial growth inhibitory activity

Extensive documentation has highlighted the capacity of marine algae to produce secondary metabolites with potential significance.[38] Previous studies have indicated that the antibacterial efficacy of algae is contingent upon factors such as the algal species, the efficiency of the extraction method, and the resistance exhibited by the tested bacteria.[39] In the current study, the most potent activity against the tested bacteria was observed in the aqueous and methanol extracts of *Corallina elongata*, followed by other extracts like chloroform and hexane, which displayed comparatively lower activity against nearly all tested strains (Table 4).

Table 4: Antimicrobial activity of extracts of *Corallina elongata*

Extract	Inhibition Zone Diameter (mm)					
	S. aureus	*Salmonella*	*E. coli*	*L. monocytogenes*	*B. cereus*	*V. cholerae*
MES	6.33 ± 0.51[f]	8.50 ± 1.64[c]	6.16 ± 0.40[e]	6.16 ± 0.40[e]	6.16 ± 0.40[d]	9.66 ± 0.51[b]
MEM	16.66 ± 0.51[c]	14.33 ± 1.03[c]	16.33 ± 0.49[c]	14.5 ± 1.22[c]	10.33 ± 0.81[c]	8.83 ± 0.40[bc]
CES	12.66 ± 1.96[d]	12.66 ± 3.61[c]	16.66 ± 0.51[c]	9.50 ± 1.22[d]	6.16 ± 0.40[d]	8.83 ± 0.40 [bc]

HES	9.66 ± 2.58[e]	13.66 ± 4.17[c]	11.33 ± 3.20[d]	9.50 ± 1.22[d]	9.33 ± 0.81[c]	7.83 ± 0.40[c]
AES	20.33 ± 0.51[b]	22.66 ± 5.71[b]	23.00 ± 1.54[b]	21.00 ± 3.52[b]	21.66 ± 2.58[b]	8.83 ± 0.40[bc]
Gentamicin	31.33 ± 1.63[a]	32.66 ± 1.63[a]	29.50 ± 1.22[a]	31.50 ± 1.87[a]	24.83 ± 3.97[a]	21.50 ± 1.76[a]

Data are expressed as Mean ± SD, (n = 3). Values in the same column followed by a different letter are significantly different (P < 0.05). BHT: Butylated hydroxytoluene, HES: hexane extract (Soxhlet), CES: chloroform extract (Soxhlet), MES: methanol extract (Soxhlet), AES: aqueous extract (Soxhlet), MEM: methanol extract (maceration).

The findings from a previous study indicated that chloroform extract derived from *Corallina elongata* displayed the most significant activity against *E. coli* and *Salmonella* spp, whereas, the methanol extract exhibited antifungal activity against *Candida albicans*.[26] The results of this study suggest that seaweeds from Algeria possess substantial potential as a reservoir of antibacterial and antifungal compounds, indicating their potential use in treating diseases caused by these organisms. The findings from the present study align with earlier reports.[40] Soliman *et al*.[41] investigated the impact of red macroalgae extracts on the mycelial growth of *Rhizoctonia solani*, *Macrophomina phaseolina*, and *Fusarium solani*. Their findings indicated a 25.9% reduction in *R. solani* growth with the methanol extract, while the chloroform extract achieved a 100% inhibition. In the case of *F. solani*, both the organic solvent and aqueous extracts moderately inhibited mycelial growth. The variability in inhibition outcomes could be attributed to the possibility that some or all algal extracts introduced additional minerals and nutrients to the medium, potentially masking the inhibitory effects.

According to Shan *et al*.[42], the membrane of Gram-negative bacteria is associated with enzymes in the periplasmic space that are capable of destroying invading molecules. The hydrophobic regions of lipoteichoic acid in Gram-positive bacterial cell walls facilitate the penetration of hydrophobic compounds,[43] such as tannins, which can reach the cytoplasmic membrane and disrupt the proton motive force, inhibit active transport, and cause coagulation of cellular contents.[44] The type and nature of bioactive substances produced influence the antibacterial potential.[45] Several factors may explain the difference between the present results and those obtained in previous studies. Among these factors is the intraspecific variability in the production of secondary metabolites

Minimum inhibitory concentration (MIC)

The previously obtained results showed that the extracts of red seaweed possess interesting antibacterial activity. For this reason, we aimed to determine their minimum inhibitory concentration (MIC) using liquid microplate assays. The MIC values of the algal extracts are presented in Table 5.

Table 5: Minimum Inhibitory Concentrations (MIC) of *Corallina elongata* extracts against gram-positive and gram-negative bacteria

Organism	**MIC (mg/mL)**			
	MEM	**MES**	**CES**	**HES**
E. coli	1.95 ± 0.30	3.90 ± 0.29	3.90 ± 0.33	7.81 ± 0.76
P. aeruginosa	3.90 ± 0.21	1.95 ± 0.24	3.90 ± 0.32	7.81 ± 0.88
B. cereus	1.95 ± 0.22	3.90 ± 0.22	15.62 ± 2.34	62.50 ± 2.45
S. aureus	15.62 ± 2.33	3.90 ± 0.3	62.50 ± 3.55	31.25 ± 1.34

Data are expressed as Mean ± SD, (n = 3). HES: hexane extract (Soxhlet), CES: chloroform extract (Soxhlet), MES: methanol extract (Soxhlet), MEM: methanol extract (maceration).

The inhibitory activity of the extracts was observed across a wide range of concentrations, ranging from 1.95 to 62.50 mg/mL for the polyphenolic extracts. Extracts obtained by Soxhlet extraction and maceration showed significant activity against *E. coli* and *P. aeruginosa*, with MIC values of 1.95 mg/mL for each strain. This effect could be attributed to the high polarity of methanol.[46] In contrast, a study by Sebaaly *et al*.[47] recorded much higher MICs, ranging from greater than 83.3 mg/mL to over 100 mg/mL, for the methanol extract of *Corallina* sp. against these same Gram-negative strains.

Conclusion

The red seaweed *Corallina elongata* from the Mediterranean Sea (Algeria) shows strong potential as a natural source of bioactive compounds. HPLC–MS/MS analysis identified 21 metabolites in its methanol extract, with *p*-Coumaric acid as the major component. High levels of phenolics and flavonoids were linked to notable antioxidant and antimicrobial activities, particularly in polar extracts like methanol and chloroform, which exhibited strong free radical scavenging activity and inhibitory effects against *E. coli* and *P. aeruginosa*. These findings highlight the value of *C. elongata* as a sustainable source of natural antioxidants and antibacterial agents. Future research should focus on isolating active compounds, exploring *in vivo* efficacy, and developing applications in food, pharmaceutical, and agricultural sectors.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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