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ORIGINAL ARTICLE

Bioactive potential of mediterranean macroalgae: comparative insights from the extracts of native (*Ericaria brachycarpa* and *Ericaria crinita)* and invasive (*Asparagopsis taxiformis)* species

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Abstract
Marine species are a rich source of bioactive molecules. Among them, marine algae are known to produce various secondary metabolites with a wide range of biological activities, such as immunomodulatory, antioxidant, and antimicrobial. This study aimed to characterize the extracts of three Mediterranean macroalgae species: two native, *Ericaria brachycarpa* and *Ericaria crinita,* and one invasive, *Asparagopsis taxiformis*, to subsequently evaluate their haemolytic and antimicrobial activities. The characterization of secondary metabolites was performed using untargeted HPLC/ESI/MS and confirmed the presence of meroterpenoids for *E. crinita*, oxidized fatty acids (oxylipins) in *E. brachycarpa,* and brominated compounds as constituents of *A. taxiformis*. The extracts were tested for their antagonistic activities against some representative strains of the human bacterial pathogens *Listeria monocytogenes* and *Staphylococcus aureus*, isolated from food matrices and human stools. The extracts from *E. brachycarpa* and *A. taxiformis* inhibited all tested strains, while *E. crinita* barely inhibited two *L. monocytogenes* strains. These extracts also showed low haemolytic activity towards mammalian erythrocytes. These preliminary results encourage future investigation on the biological efficacy of the metabolites from *E. brachycarpa* and *A. taxiformis*.

Keywords Meroterpenes · Oxylipins · Brominated compounds · Macroalgae · Foodborne pathogens

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Introduction

In recent decades, extensive research has been conducted to discover new bioactive molecules produced by living organisms for the development of innovative drugs (Pan et al. 2010; Librizzi et al. 2024). To this end, numerous compounds have been isolated from intertidal macroalgae that thrive in complex environments with extreme conditions like high salinity, temperature fluctuations, and intense solar radiation. These harsh conditions promote the production of diverse secondary metabolites (Schnitzler et al. 2001; Cardozo et al. 2007). Marine algae are particularly rich in structurally novel and biologically active compounds, including flavonoids, terpenoids, alkaloids, quinones, sterols, tannins, oxylipins, and polysaccharides (Barbosa et al. 2016; Fraly Erbabley and Junianto 2020). Macroalgae of the Sargassaceae family are distributed in the Atlantic Ocean and the Mediterranean Sea and show important antioxidant (Čagalj et al. 2022), anticancer (Abu-Khudir et al. 2021; Martino et al. 2024), and anti-inflammatory activities (Saraswati et al. 2019). Within the Sargassaceae family, Ericaria sp. pl. are canopy-forming brown algae living on the Mediterranean rocky coastline. Our previous research identified seven oxylipins in the extract of E. brachycarpa (J. Agardh) Molinari & Guiry, 2020 and highlighted that this oxylipin-containing extract caused a severe dose-dependent decrease in normal Arbacia lixula sea urchin embryo development and of neuroblastoma cells viability (Martino et al. 2024), suggesting E. brachycarpa's extract as a potential source for the development of innovative, environmentally friendly products with larvicide and antineoplastic activity and paving the road for further investigation. The species E. crinita (J. Agardh) Molinari & Guiry, 2020 is known for the numerous antioxidant properties of the meroterpenoid compounds it produces and for their potential applications.

On the other hand, various Asparagopsis sp. pl. (belonging to the red macroalgae) are known to produce natural antimicrobial compounds (Genovese et al., 2012; Pinteus et al. 2015) important for potential medical use, considering that the resistance to antibiotics represents a hot global health issue, due to the indiscriminate use of antimicrobial (Frieri et al. 2017). Asparagopsis taxiformis (Delile) Trevisan, 1845 is an invasive alien species, listed among the 100 worst invasive species in this Mediterranean basin (Streftaris et al., 2005), with potential dramatic effects on species composition and structure of the associated epifaunal assemblages (Mancuso et al. 2022).

The species Listeria monocytogenes and Staphylococcus aureus are among the most relevant foodborne pathogens transferred to humans via food ingestion (Miceli and Settanni 2019). L. monocytogenes is a Gram-positive bacterium facultative intracellular pathogen with a high adaptive behaviour; it grows and survives in very diverse environments such as soil, silage, marine and freshwater wastewater, vegetation, food processing plants, foods, domestic and wild animals, and humans (Sauders and Wiedmann 2007). This pathogen is responsible for listeriosis, a rare but severe human infection with a mortality rate of 20–30% (Allerberger and Wagner 2010). S. aureus is a Gram-positive anaerobic facultative bacterium that causes illness worldwide. Only in the United States, it annually causes about 2.41 million illnesses (Scallan et al. 2011).

The haemolysis test is an effective tool for evaluating the cytotoxicity of compounds, offering a first indication of their safety and potential application (Greco et al. 2020; Sæbø et al. 2023). In this regard, extracts and compounds produced by macroalgae could present some bioactive compounds with potential haemolytic properties (Paarvanova et al. 2023).

This study investigates three macroalgal species occurring along the Mediterranean coasts, particularly in the Gulf of Palermo. The choice of these species was motivated by

the presence of A. taxiformis, an invasive alien species that has colonized the infralittoral zone, a habitat typically occupied by the two native species E. brachycarpa and E. crinita. The aim of this work is to characterize the chemical composition of extracts from both the alien and native species, and to compare their biological activities in order to evaluate their bioactive potential. In particular, we evaluated their antioxidant, haemolytic and antimicrobial activities using S. aureus and, for the first time, L. monocytogenes as indicator strains. This approach was intended to highlight the potential applications of the invasive alga in sustainable biotechnological sectors, such as bio-preservation.

Materials and methods

Sampling and preparation of algal extracts

The sporophytes of *E. crinita* and *E. brachycarpa* and the gametophytes of *A. taxiformis* were sampled along the coast of Palermo, on the northern Sicilian coast, from March to June 2023, which corresponds to the main reproductive peak of the *Ericaria* genus and the period when the *A. taxiformis* gametophyte reaches its maximum size, and were processed as previously described (Martino et al. 2024). Briefly, they were washed and rinsed to remove debris and epiphytes and dried at a temperature of 40 °C in an oven for 48 h. The subsequently dried thalli were finely ground, obtaining a powder, and combined to obtain an average extract to reduce variability from seasonal changes.

Extractions were carried out on 1 g of dried mass with 10 mL of $CHCl_3$-MeOH (2:1 v/v) solvent, and the solvent was removed by Rotavapor (BUCHI), obtaining the crude extracts of *E. crinita* (ECE), *E. brachycarpa* (EBE), and *A. taxiformis* (ATE), which were then stored at—20 °C before use. All values are expressed as weight percentage (% w/w) of extract obtained from dry biomass.

Characterization of the metabolites present in the algal extracts by HPLC/ESI/MS

Qualitative analysis of the compounds from ECE, EBE and ATE was achieved by means of untargeted HPLC/ESI/MS equipped with QToF, in negative mode, allowing the annotation of 18 compounds from ECE, 19 from EBE and 10 from ATE (see Tables 1, 2, 3). HPLC/MS analysis of the extracts was performed as previously described (Faddetta et al. 2023). Compounds annotated by comparison with analytical standards or with literature, were indicated in Tables 1–3. MS/MS data were acquired in negative mode applying 10–40 V collision energies.

Mass spectrum data were analyzed for metabolites annotation using MassHunter Qualitative Analysis B.06.00 and the Metabolomic Workbench database [https://www.metabolomicsworkbench.org/search/ms.php].

Determination of the phenolic content of the extracts

The Folin-Ciocalteu reagent was used to determine the phenolic content of the algal extracts accordingly to previous methods (Emanuele et al. 2018). The extracts were solubilized in methanol at a concentration of 1 mg/mL and the calibration curve was prepared using solutions at different concentrations of gallic acid. The UV–vis spectra of the

Table 1 Metabolites present in the $CHCl_3$-MeOH extracts of the *Ericaria crinita*

Metabolites	Rt (min)	Exp. Mass [m/z]	Molecular Formula	Chemical class	Name (major MS/MS fragments)
1	1.04	181.0737	$C_6H_{14}O_6$	Sugar	Mannitol[a]
2	1.12	387.1186	$C_{12}H_{22}O_{11}$	Sugar	Cellobiose[a]
3	1.77	134.0488	$C_5H_5N_5$	Purine base	Adenine[a]
4	3.01	151.0277	$C_5H_4N_4O_2$	Purine base	Xanthine[a]
5	5.15	241.0851	$C_{10}H_{14}N_2O_5$	Pyrimidine nucleoside	Thymidine[a]
6	12.97	225.1522	$C_{13}H_{22}O_3$	Jasmonate	Methyl dihydrojasmonate[a]
7	16.78	286.2413	$C_{16}H_{33}NO_3$	Lipid	Lauroyl diethanolamide[a]
8	17.55	445.3008	$C_{27}H_{42}O_5$	Meroterpenoid	2-[(2,6,10-trimethyltrideca-2E-ene-6,7,10-triol)−13-yl]−6-hydroxy-2,8-dimethyl-2*H*-chromene
9	21.00	479.2612 $[M+Cl]^-$	$C_{27}H_{40}O_5$	Meroterpenoid	2,6,10-hexadecatrien-5-one,16-(2,5-dihydroxy-3-methylphenyl)−4,14-dihydroxy-2,6,10,14-tetramethyl
10	22.36	429.3046	$C_{27}H_{42}O_4$	Meroterpenoid	2-[(2'*E*,6'*E*)−10',11'-dihydroxygeranylgeranyl]−6-methyl-1,4-hydroquinone
11	23.82	427.2904	$C_{27}H_{40}O_4$	Meroterpenoid	2-[(2,6,10-trimethyltrideca-2,10*E*-diene-6S,7S-diol)−13-yl]−6-hydroxy-2,8-dimethyl-2*H*-chromene
12	24.28	455.2821 $[M+Cl]^-$	$C_{22}H_{44}O_7$	Lipid	D-Mannitol, 1-hexadecanoate
13	25.11	443.3215	$C_{28}H_{44}O_4$	Meroterpenoid	2-[(2'*E*,6'*E*)−10',11'-dihydroxygeranylgeranyl]−1-hydroxy-4-methoxy-6-methyl-benzene (427, 149)
14	25.55	427.2900	$C_{27}H_{40}O_4$	Meroterpenoid	(10'R*,11'R*)−2-[(2'*E*,6'*E*)−10',11'-dihydroxygeranylgeranyl]−6-methyl-1,4-benzoquinone
15	26.28	297.2463	$C_{18}H_{34}O_3$	Lipid	Hydroxyoleic acid[a]
17	28.15	483.2830	$C_{29}H_{40}O_6$	Meroterpenoid	1,4-Benzendiol, 2-[5-(acetyloxy)−3,7-dimethyl-11-(tetrahydro-4-hydroxy-5,5-dimethyl-2-furanyl)−2,6,10-dodecatrienyl]−6-methyl-
18	29.28	609.1809	$C_{28}H_{34}O_{15}$	Glycosylated flavanone	Hesperidin[a]

[a]Annotated by comparison with analytical standards

Table 2 Metabolites present in the $CHCl_3$-MeOH extracts of *Ericaria brachycarpa*

Metabolites	Rt (min)	Exp. Mass [m/z]	Molecular Formula	Chemical class	Name
1	1.09	193.815 $[M+Cl]^-$	$FeCl_3$	Inorganic salt	Iron chloride[a]
2	2.9	243.0645	$C_9H_{12}N_2O_6$	Pyrimidine nucleoside	Uridine[a]
3	5.14	241.0857	$C_{10}H_{14}N_2O_5$	Pyrimidine nucleoside	Thymidine[a]
4	16.74	322.2194 $[M+Cl]^-$	$C_7H_{13}NO_9S$	Monosaccharide sulfate	*N*-acetyl-6-O-sulfo-D-glucosamine[a]
5	18.63	224.0953	$C_{11}H_{15}NO_4$	Amino acid derivative	3-Hydroxytyrosine ethyl ester[a]
6	18.95	194.0845	$C_{10}H_{13}NO_3$	Amino acid derivative	Tyrosine methyl ester[a]
7	20.09	291.1999	$C_{18}H_{28}O_3$	Lipid	Oxo-phytodienoic acid
8	21.29	293.2160	$C_{18}H_{30}O_3$	Lipid	Hydroxy-octadecatrienoic acid
9	21.80	317.2158	$C_{20}H_{30}O_3$	Lipid	Hydroxy-eicosapentaenoic acid
10	23.04	319.2314	$C_{20}H_{32}O_3$	Lipid	Hydroxy-eicosatetraenoic acid
11	23.51	317.2158	$C_{20}H_{30}O_3$	Lipid	Hydroxy-eicosapentaenoic acid isomer
12	23.81	555.2903	$C_{32}H_{44}O_8$	Triterpenoid	Cucurbitacin
13	24.09	555.2903	$C_{32}H_{44}O_8$	Triterpenoid	Cucurbitacin isomer
14	24.26	455.2829 $[M+Cl]^-$	$C_{22}H_{44}O_7$	Lipid	Sorbitol monopalmitate
15	24.60	535.2521 $[M+Cl]^-$	$C_{29}H_{40}O_7$	-	Unknow
16	24.73	481.2989 $[M+Cl]^-$	$C_{24}H_{46}O_2$	Lipid	Sorbitol monooleate[a]
17	25.82	271.2311	$C_{16}H_{32}O_3$	Lipid	Hydroxypalmitic acid[a]
18	26.00	356.1909	$C_{15}H_{25}N_5O_5$	Tripeptide	Asn-Lys-Pro
19	26.33	297.2472	$C_{18}H_{34}O_3$	Lipid	Hydroxyoleic acid[a]

[a]Annotated by comparison with analytical standards

Table 3 Metabolites present in the $CHCl_3$-MeOH extracts of *Asparagopsis taxiformis*

Metabolites	Rt (min)	Exp. Mass [m/z]	Molecular Formula	Chemical class	Name (major MS/MS fragments)
1	1.09	371.6188 $[M+Br]^-$	$FeBr_3$	Inorganic salt	Iron bromide[a]
2	2.64	214.8373	$C_2H_2BrO_2$	Halogenated compound	Dibromoacetic acid[a]
3	7.32	121.0308	$C_7H_6O_2$	Carboxylic acid	Benzoic Acid[a]
4	8.50	226.8367	$C_3H_2Br_2O_2$	Halogenated compound	3,3-dibromoacrylic acid[a]
5	10.03	368.6792	$C_3H_2Br_4O$	Halogenated compound	tetrabromo acetone[a]
6	12.83	388.8223	$C_7H_4I_2O_3$	Halogenated compound	2-hydroxy-3,5-diiodobenzoic acid
7	18.92	194.0845	$C_{10}H_{13}NO_3$	Amino acid derivative	Tyrosine methyl ester[a]
8	21.26	488.6034	$C_5H_3Br_5O_2$	Halogenated compound	pentabromoacetylacetone
9	23.81	555.2903	$C_{32}H_{44}O_8$	Triterpenoid	Cucurbitacin
10	25.16	566.5148	$C_5H_2Br_6O_2$	Halogenated compound	hexabromoacetylacetone

[a]Annotated by comparison with analytical standards

different samples were recorded through the spectrophotometer SPECORD S 600 using a quartz cuvette with 1 cm of optical path. Total phenolic content (TPC) is expressed as mg gallic acid equivalents (GAE)/g.

Haemolytic effects on mammalian erythrocytes

The haemolytic activity assays of EBE, ECE, ATE were performed by incubating the samples with mammalian erythrocytes (sheep). Before using, the erythrocytes were resuspended in PBS (KH_2PO_4 6 mM, Na_2HPO_4 30 mM, NaCl 0.11 M) and centrifuged at 400 g for 10 min, the pellet was recovered and resuspended in ISO-Ca^{2+} (0.5 M NaCl, 20 mM Tris–HCl, 10 mM $CaCl_2$; pH 7.4) at 1% concentration (8×10^6 fresh erythrocytes). The erythrocytes were provided by the Istituto Zooprofilattico Sperimentale della Sicilia 'A. Mirri'. To perform the cytotoxic activity assay, 100 µl of sample were incubated with 100 µl of erythrocytes (1%) at 37 °C for 60 min. Afterwards, the samples were centrifuged for 10 min at 400 g at 4 °C. The amount of hemoglobin released in the supernatant was assessed at a wavelength of 541 nm using the spectrophotometer (GloMax®-Multi Detection System; Promega Corporation, Madison, Wisconsin, USA). The assays were performed in triplicate. The following formula was used to calculate the degree of haemolysis:

$$\frac{|sample| - |erythrocytes|}{|hemolysis|} * 100$$

Abs sample is the absorbance value measured for each sample.

Abs erythrocytes is the absorbance value measured by incubating the erythrocytes only with ISO-Ca^{2+}

Abs haemolysis is the absorbance value relative to total haemolysis obtained by incubating only the erythrocytes subsequently resuspended in distilled water.

Results were expressed as mean ± SD and experiments were conducted in triplicate.

Determination of antimicrobial activity of the extracts

ECE, EBE and ATE were tested for their antimicrobial activity against six strains of *Staphylococcus aureus* (ATCC 33862, 1313-MRSA, 4 ADI MRSA, 14 LUMRSA, E36GI MRSA, and C38/249,1-MSSA) and six strains of *Listeria monocytogenes* (ATCC 19114, 129, 182, 1 BO, 188, and 140). Except the strains from American Type Culture Collection (ATCC), all other strains belong to the culture collection of Agricultural Microbiology laboratory of University of Palermo and were isolated from food matrices or human stools. All bacteria were sub-cultured in Brain Heart Infusion (BHI) broth (Condalab) incubated at 37 °C for 24 h. The antimicrobial assay was conducted by the disc diffusion method as previously described (Vandepitte et al. 2003; Pinteus et al. 2015). Each bacterial strain was inoculated in BHI soft agar (0.7% w v^{-1}) (top layer), at a cell density of approximately 10^7 CFU/ml (Kelmanson et al. 2000), which was poured onto a water-agar medium (2%, w/v) used as support medium (bottom layer). Sterile paper discs (Wathman No. 1) with a diameter of 6 mm, previously soaked with 10 µL of algal extracts at different concentrations in DMSO to obtain 100 and 1000 µg/disc, were placed on the top of the double agar layer. Undiluted algal extracts were prepared putting 5000 µg of extract on paper discs. The sensitivity of staphylococci and listerias to algal extract was evaluated after 24 h at 37 °C by measuring the diameter of the halo around the discs with a caliper. Streptomycin

(5% w/v) and DMSO were used as positive and negative control, respectively. Experiments were performed in triplicate.

Data analysis

Quantitative data were analyzed by a one-way analysis of variance (ANOVA). Tukey's HSD test was used as a post hoc test for mean comparison. The homogeneity of variance was checked and confirmed using Levene's test. All statistical analyses were performed using the Statistica 13.2 software (StatSoft, Tulsa, OK, USA), and a p-value < 0.05 was considered significant.

Results

Characterization of the metabolites present in the extracts

A qualitative analysis of the compounds from ECE, EBE and ATE was achieved by means of untargeted HPLC/MS, allowing the identification of a total of 47 compounds. The $CHCl_3$-MeOH ECE shows the presence of 7 meroterpenoids (see Table 1 and SI, compounds **8–11**, **13**, **14**, **17**) as the most representative chemical class, while 2 sugars (see Table 1, compounds **1**, **2**), 2 purine bases (see Table 1, compounds **3**, **4**), the pyrimidine nucleoside thymidine (see Table 1, compound **5**), 3 lipid derivatives (see Table 1, compounds **7**, **12**, **15**), one jasmonate (see Table 1, compound **6**) and the glycosylated flavanone Hesperidin (see Table 1, compound **18**) were also identified.

EBE showed the presence of 19 annotated compounds (Table 2). Lipids represented the predominant class of compounds in the extract, with seven oxylipins being identified, among these we found cyclic derivative oxo-phytodienoic acid and linear hydroxylated fatty acids (see Table 2 and SI, compounds **7**–**11**, **17**, **19**), confirming our previous results (Martino et al. 2024), and two esters of sorbitol with fatty acids (see Table 2, compounds **14**, **16**). Among other chemical classes, we observed the presence of an iron salt (see Table 2, compound **1**), two pyrimidine nucleoside (see Table 2, compounds **2**, **3**), one monosaccharide derivative (see Table 2, compound **4**), two amino acid derivatives (see Table 2, compounds **5**, **6**), two triterpenoids (see Table 2, compounds **12**, **13**) and one tripeptide (see Table 2, compound **18**). An unknown compound with molecular formula $C_{29}H_{40}O_7$ was also present (see Table 2, compound **18**).

ATE shows the presence of 10 annotated compounds (Table 3). Halogenated compounds are the most represented class with 6 compounds, mainly (5 out of 6) brominated (see Table 3 and SI, compounds **2**, **4–6**, **8**, **10**). Among other chemical classes, we observed the presence of an iron salt (see Table 3, compound **1**), one carboxylic acid (see Table 3, compound **3**), one amino acid derivative (see Table 3, compound **7**), and one triterpenoid (see Table 3, compound **9**).

Determination of the phenolic content

The phenolic content of the algal extracts differed significantly among the three extracts ($F_{2,6} = 20.75$, $p = 0.002$). The highest concentration was detected in ECE (75.8 mg gallic acid equivalents (GAE)/g), followed by ATE (48.3 mg GAE/g) and EBE (41.5 mg

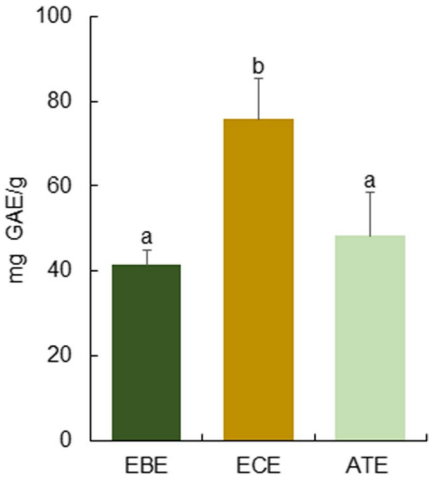

Fig. 1 Phenolic content of the extracts in $CHCl_3$-MeOH of macroalgae *Ericaria brachycarpa (EBE), Ericaria crinite (ECE), Asparagopsis taxiformis (ATE).* Values are reported as mean ± SD and significant differences are indicated by different letters (Tukey HSD)

GAE/g) (Fig. 1). The highest phenolic content found in ECE could be related to the presence of meroterpenoid, due the presence of phenolic groups.

Haemolytic effects on mammalian erythrocytes

EBE, ECE and ATE produced a significant dose-dependent haemolytic activity ($F_{11, 36} = 1728.1$, $p < 0.0001$) in the presence of mammalian erythrocytes (Table 4).

EBE 400 and 4000 showed the highest haemolytic activity of 35.1±1.7% and 44.4±1.40%, respectively. At lower concentrations (EBE 4 and 40), EBE had a very low haemolytic activity (< 5 %). ECE 400 and 4000 exhibited haemolytic activity of 12.5±0.9% and 24.7±0.4%, respectively (Table 4), remarkably lower than EBE at the same concentration. Notably, ECE 40 showed a similar activity than EBE. Interestingly, ATE presented low haemolytic activity (< 5 %) at all tested concentrations (Table 4).

Antimicrobial assay of extracts

Table 5 shows the results of the antimicrobial activity of the three extracts against six *L. monocytogenes* and six *S. aureus* strains. EBE had a significant effect on *L. monocytogenes* strains 19114 ($F_{2, 6} = 6.9$, $p = 0.02$), 129 ($F_{2, 6} = 89.6$, $p < 0.0001$), 182 ($F_{2, 6} = 9.2$, $p = 0.01$), 1 BO ($F_{2, 6} = 1365.3$, $p < 0.0001$), 188 ($F_{2, 6} = 3072$, $p < 0.0001$) and 140 ($F_{2, 6} = 3072$, $p < 0.0001$). While EBE 1000 was found effective against all six *L. monocytogenes* strains, with the highest antimicrobial activity against *L. monocytogenes* ATCC 19114 strain (12 mm), at lower concentrations (EBE 100) EBE showed a significantly lower activity. Similar results were obtained with the undiluted EBE, that manifested inhibitory activity only against the ATCC 19114, 129 and 182 strains.

EBE also exhibited good inhibitory activities against *S. aureus* strains 33862 ($F_{2, 6} = 20.2$, $p = 0.02$), 1313-MRSA ($F_{2, 6} = 39.1$, $p < 0.0005$), 4 ADI MRSA ($F_{2, 6} = 133.7$, $p < 0.001$), E36GI MRSA ($F_{2, 6} = 193.5$, $p < 0.001$), C38/249,1-MSSA ($F_{2, 6} = 312.9$, $p < 0.0001$) and 14 LUMRSA ($F_{2, 6} = 454.4$, $p < 0.001$). EBE100 showed activity against all tested strains of *S. aureus*, with a diameter of the clear area in the range 10–12 mm. EBE1000 displayed a

Table 4 Percentage haemolytic activity of the extracts of macroalgae *Ericaria brachycarpa, Ericaria crinita* and *Asparagopsios* taxiformis

Dose	EBE	ECE	ATE
4 µg/mL	0^{a}	0^{a}	0^{a}
40 µg/mL	0.3 ± 0.5^{a}	2.8 ± 0.7^{b}	0.9 ± 0.1^{a}
400 µg/mL	35.1 ± 1.7	12.5 ± 0.9	$1.5 \pm 0.1^{a, b}$
4000 µg/mL	44.4 ± 1.4	24.7 ± 0.4	3.1 ± 0.2^{b}

Values are reported as mean ± SD and significant differences are indicated by different letters (Tukey HSD)

more consistent inhibitory activity, with halos in the range 12–16 mm. Undiluted EBE had similar activity to EBE100, with an inhibition zone of 8–10 mm.

On the contrary, different doses of ECE (ECE 100, 1000 and undiluted ECE) were found inactive against all tested strains.

ATE showed a significant effect against *L. monocytogenes strains* 19114 ($F_{2, 6} = 10.5$, $p = 0.01$), 129 ($F_{2, 6} = 50.8$, $p < 0.001$), 182 ($F_{2, 6} = 177$, $p < 0.0001$), 1 BO ($F_{2, 6} = 154.5$, $p < 0.0001$), 188 ($F_{2, 6} = 678.6$, $p < 0.0001$) and 140 ($F_{2, 6} = 922.7$, $p < 0.0001$). ATE 1000 was found effective against all *L. monocytogenes* strains with a diameter of the inhibition area between 8 and 10 mm. Undiluted ATE showed comparable activity than ATE1000. At lower doses, ATE100 slightly inhibited only the strain ATCC 19114.

ATE also showed interesting results against *S. aureus* strains 33862 ($F_{2, 6} = 171.3$, $p < 0.001$), 1313-MRSA ($F_{2, 6} = 99.3$, $p < 0.0001$), 4 ADI MRSA ($F_{2, 6} = 27.3$, $p < 0.0001$), E36GI MRSA ($F_{2, 6} = 371.5$, $p = 0.003$), C38/249,1-MSSA ($F_{2, 6} = 651.2$, $p < 0.0001$) and 14 LUMRSA ($F_{2, 6} = 87.1$, $p < 0.001$). In particular, ATE1000 inhibited *S. aureus* growth consistently, with an inhibition area up to 20 mm against strains ATCC 33862, 4 ADI MRSA, and E36GI MRSA. Undiluted ATE generally showed a lower activity, as well as ATE100.

Discussion

To survive environmental stress conditions, marine algae produce numerous biomolecules with important biological activities. A qualitative analysis of the compounds from ECE, EBE and ATE, achieved by means of untargeted HPLC/MS, allowed the annotation of a total of 47 compounds belonging to different classes. The $CHCl_3$-MeOH ECE showed the presence of seven meroterpenoids compounds. According to literature, these compounds are widespread in brown algae of the family Sargassaceae (Sunassee and Davies-Coleman 2012) and present important antioxidant and antiproliferative properties (Fisch et al. 2003; Mhadhebi et al. 2011; Reyes Jiménez, et al., 2013; Zbakh et al. 2020).

EBE showed the presence of numerous lipid derivatives, confirming our previous findings (Martino et al. 2024), which identified seven oxylipins, including oxo-phytodienoic acid and linear hydroxylated fatty acids primarily derived from palmitic acid. Our results agree with previous results highlighting the presence of these derivatives in red-brown algae (Illijas et al. 2020).

ATE showed the relevant presence of low molecular weight halogenated compounds, particularly those containing bromine, in agreement with other authors (Burreson et al. 1975; McConnell and Fenical 1977; Parchemin et al. 2023). Extracts obtained from this genus are recognised as having important biological properties (Genovese et al., 2012;

Table 5 Antibacterial activity of the extracts of macroalgae *Ericaria brachycarpa, Ericaria crinita* and *Asparagopsios taxiformis* at different concentrations in DMSO (100, 1000 µg/disc or undiluted, *i.e.* 5000 µg/disc) expressed as inhibition zone diameter (mm)

	EBE 100	EBE 1000	U EBE	ECE 100	ECE 1000	U ECE	ATE 100	ATE 1000	U ATE	CP	CN
L. monocytogenes ATCC 19114	10 ± 1.0^{a}	12 ± 1.0^{a}	$9 \pm 1.0^{a,b}$	-	-	-	$6^{*} \pm 0.1^{c}$	$9 \pm 1.0^{a,b}$	$9 \pm 1.0^{a,b}$	35 ± 1.0^{g}	-
L. monocytogenes 129	-	8 ± 0.4^{b}	8 ± 1.4^{b}	-	-	-	-	8 ± 1.4^{b}	8 ± 1.4^{b}	$34 \pm 1.4^{g,h}$	-
L. monocytogenes 182	$9 \pm 1.0^{a,b}$	10 ± 1.4^{a}	$6^{*} \pm 0.3^{c}$	-	$6^{*} \pm 0.1^{c}$	-	-	10 ± 1.1^{a}	8 ± 0.4^{b}	36 ± 1.0^{g}	-
L. monocytogenes 1 BO	-	$6^{*} \pm 0.3^{c}$	-	-	-	-	-	10 ± 1.0^{a}	10 ± 1.0^{a}	35 ± 1.0^{g}	-
L. monocytogenes 188	-	$6^{*} \pm 0.2^{c}$	-	-	$6^{*} \pm 0.2^{c}$	-	-	8 ± 0.4^{b}	8 ± 0.4^{b}	$34 \pm 1.0^{g,h}$	-
L. monocytogenes 140	-	$6^{*} \pm 0.2^{c}$	-	-	-	-	-	8 ± 0.4^{b}	$6^{*} \pm 0.1^{c}$	36 ± 1.2^{g}	-
S. aureus ATCC 33862	10 ± 1.0^{a}	12 ± 0.6^{a}	8 ± 0.6^{b}	-	-	-	$6^{*} \pm 0.1^{c}$	20 ± 0.6^{f}	18 ± 0.8^{f}	33 ± 1.0^{h}	-
S. aureus 1313-MRSA	$12 \pm 1.0^{a,d}$	13 ± 1.0^{d}	10 ± 1.0^{a}	-	-	-	10 ± 0.4^{a}	11 ± 1.0^{a}	10 ± 1.0^{a}	24 ± 1.0^{i}	-
S. aureus 4 ADI MRSA	$12 \pm 1.0^{a,d}$	16 ± 0.4^{e}	10 ± 1.0^{a}	-	-	-	8 ± 0.4^{b}	20 ± 1.0^{f}	8 ± 0.6^{b}	31 ± 1.0^{j}	-
S. aureus E36GI MRSA	10 ± 1.0^{a}	13 ± 1.0^{d}	10 ± 1.0^{a}	-	-	-	8 ± 0.4^{b}	20 ± 0.4^{f}	$12 \pm 1.4^{a,d}$	31 ± 0.6^{j}	-
S. aureus C38/249,1-MSSA	10 ± 1.0^{a}	15 ± 0.4^{e}	10 ± 1.0^{a}	-	-	-	$9 \pm 0.4^{a,b}$	16 ± 1.4^{e}	15 ± 1.1^{e}	32 ± 1.4^{j}	-
S. aureus 14 LUMRSA	11 ± 1.0^{a}	12 ± 0.4^{a}	$9 \pm 0.6^{a,b}$	-	-	-	$9 \pm 0.6^{a,b}$	$9 \pm 0.4^{a,b}$	$6^{*} \pm 0.3^{c}$	32 ± 0.6^{j}	-

(-) no activity; (*) activity equal to inhibition halo of 6.4 mm; CN = Control negative; CP = Control positive

U EBE/ECE/ATE = Undiluted EBE/ECE/ATE. Values are reported as mean ± SD and significant differences are indicated by different letters (Tukey HSD)

Genovese et al., 2013; Pinteus et al. 2015), mainly due the presence of brominated derivatives (Parchemin et al. 2023).

Total phenolic content of macroalgae varies with seasonal and environmental changes (Generalić Mekinić et al. 2019), and for the *Ericaria* sp. pl. it was also demonstrated that the geographical distribution in the Mediterranean coasts influences it (Mancuso et al. 2019). The highest phenolic content found in ECE could be related to the presence of meroterpenoid as already reported by others (Fisch et al. 2003). On the other hand, the phenolic content determined into the extracts of these macroalgae agrees with previous work on *Cystoseira compressa* (Čagalj et al. 2022).

Considering the presence of bioactive compounds and the good antioxidant activity, we also tested haemolytic effect of obtained extract to further assess their potential toxicity.

EBE, ECE, and ATE produced a dose-dependent haemolytic activity in the presence of mammalian erythrocytes. Both EBE and ECE extracts exhibited a clear dose-dependent haemolytic activity, with EBE showing the strongest effect. This activity can reasonably be ascribed to their lipid derivatives, particularly oxylipins, which are known to interact with cell membranes, as already observed in other models (Martino et al. 2024). These results are consistent with previous evidence of haemolytic effects reported in other Sargassum species (Gerasimenko et al. 2010), supporting the view that such compounds may contribute to the overall toxicity of the extracts.

Notably, ATE showed very low haemolytic activity at all tested concentrations. This interesting result can be ascribed to the predominant presence of short-chain compounds, unable to interfere with the membranes of the erythrocytes. These results confirm the potential of the extracts for further testing on their antimicrobial activity.

Marine macroalgae extracts are known for their numerous antimicrobial properties (Pinteus et al. 2015; Avila-Romero et al. 2023); nevertheless, these algae were poorly tested against foodborne pathogens such as *S. aureus* and, to the best of our knowledge, were not previously tested against *L. monocytogenes*.

We tested the three extracts against a total of twelve strains, with also some resistant bacteria. The results of the antimicrobial activity of the three extracts against *L. monocytogenes* and *S. aureus* (Table 5) reveal that EBE and ATE present good activity in most of the cases, while ECE was ineffective against all tested bacteria, with the only exception of *L. monocytogenes* 182 and 188.

In general, tested extracts, in particular ATE, were more active against *S. aureus* strains than against *L. monocytogenes*. Interestingly, ATE1000 exhibited broad-spectrum activity against all tested strains, while EBE showed a comparable profile, albeit with generally lower potency.

The observed antibacterial activity for ATE could be ascribed to the presence of brominated compounds, in particular dibromoacetic acid, pentabromoacetylacetone and hexabromoacetylacetone (compound 2, 8 and 10, respectively, see Table 3), as already suggested by other authors (Parchemin et al. 2023).

These results clearly indicate that EBE and ATE can exert an important antimicrobial activity against the strains of *L. monocytogenes* and, especially, *S. aureus*. The limited activity observed with undiluted extracts, together with the lack of a clear dose-dependent effect in some cases, suggests that the bioactive compounds may diffuse only weakly into the medium, while the presence of the solvent likely facilitates, albeit marginally, the release and delivery of these metabolites. However, for perspective applications, different food matrices could improve the release, solubilization and absorption of lipophilic and polar bioactive compounds, thus improving transport and overall biological activity (Tan and McClements 2021; Martínez-Sánchez et al. 2024).

Therefore, further improvement on the delivery system could enhance the antimicrobial activity of the extracts. Considering these encouraging results, the application of solvent-free extracts in the food sector, as bio-preservative, is currently undergoing in our labs.

Conclusions

In this study, extracts of *E. brachycarpa*, *E. crinita*, and *A. taxiformis* were investigated as potential sources of natural antibacterial compounds for use in bio-conservation. The extracts were characterized by HPLC–MS, which identified meroterpenes as the main compounds in *E. crinita*, oxylipins in *E. brachycarpa*, and brominated derivatives in *A. taxiformis*.

Regarding antioxidant activity, *E. crinita* had the highest phenolic content. The extracts were also evaluated for hemolytic activity and tested against the foodborne pathogens *S. aureus* and *L. monocytogenes*. Notably, the extract from *A. taxiformis* showed low hemolytic activity and strong antimicrobial effects, especially against *S. aureus*. While less pronounced, activity against *L. monocytogenes* was also observed and is reported here for the first time. In conclusion, this study provides new insights into the potential use of extracts from the edible algae *E. brachycarpa* and *A. taxiformis* as natural alternatives to chemical preservatives for controlling microbiological risks in the food industry.

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Data availability Data will be available upon request.

Declarations

Conflicts of interest The authors declare no conflicts of interest.

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