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Extraction and characterization of volatile compounds and fatty acids from red and green macroalgae from the Romanian Black Sea in order to obtain valuable bioadditives and biopreservatives

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Abstract Three species of macroalgae, *Ceramium virgatum* (Rhodophyta), *Ulva intestinalis*, and *Cladophora vagabunda* (Chlorophyta), harvested from the Romanian Black Sea coast, were studied as sources of valuable compounds that could be used as additives and biopreservatives. Volatile compounds including hexanal (11.2 %), octane (9.8 %), nonanal (7.0 %), octanal (6.7 %), 2,5,5-trimethyl-2-hexene (4.7 %), 3-hexen-2-one (4 %), and o-cymene (3.6 %) were identified as the major components in the biomass extract of *C. vagabunda*. In *C. virgatum*, the major volatile components were 3-hexen-2-one (27.9 %), acetone (12.4 %), hexanal (3.4 %), and o-cymene (2.7 %). The major volatile compounds of *U. intestinalis* were hexanal (14.6 %), trichloromethane (7.3 %), nonanal (5.6 %), 3-hexen-2-one (5.3 %), and octanal (3.1 %). Some of these compounds have industrial applications as additives in the food, pharmaceutical, or cosmetics industries. The *U. intestinalis* extract had a greater content of mono- and polyunsaturated fatty acids around 46.0 % as compared with 42.0 % for *C. vagabunda* and 31.9 % for *C. virgatum*. The most abundant fatty acids were palmitic acid (C16:0), arachidonic acid (C20:4n-6), and oleic acid (C18:1ω-9cis). The antimicrobial effect of fatty acid extracts was tested against four pathogenic bacteria. The minimum inhibitory concentrations of *C. vagabunda*, *C. virgatum*, and *U. intestinalis* fatty acids extracts were 1.8, 3.8, and 3.8 mg mL^{-1}, respectively, for all bacterial strains. This study can help the efforts of finding new, value-added uses for natural marine resources.

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Introduction

Oceans are the natural habitat of many plants, animals, and microorganisms and cover more than 70 % of the Earth surface. Marine algae represent a significant part of the coastal biomass and are classified as red (Rhodophyta), brown (Phaeophyta), or green algae (Chlorophyta) depending on their chemical composition and color (Dawczynski et al. 2007). Many algae species have been used for the extraction of phycocolloids (alginate, carrageenan, and agar) and as a source of pharmaceutical substances. They have also been used as herbal medicine, fertilizers, fungicides, herbicides, and for direct use in human nutrition (Aguilera-Morales et al. 2005; Cardozo et al. 2007; Ortiz et al. 2006). Aside from these uses, seaweeds are potential sources of bioactive compounds, since they are able to produce a great variety of secondary metabolites with a broad spectrum of biological activities. Compounds with antioxidant, antiviral, antifungal, and antimicrobial activities have been detected in brown, red, and green algae (Yuan et al. 2005; Bansemir et al. 2006; Chew et al. 2008). The use of bioactive compounds extracted from algae to

foods could enhance their shelf life and nutraceutical potential (Chandini et al. 2008).

Algae also contain fatty acids, which are important for human and animal health. They act as precursors in the biosynthesis of eicosanoids, which are viewed as bioregulators of many cellular processes (Khotimchenko 2005). Moreover, polyunsaturated fatty acids (PUFAs) play key roles in cellular and tissue metabolism, including the regulation of membrane fluidity, electron and oxygen transport, and thermal adaptation (Funk 2001). Marine algae possess very complex and diverse lipid composition. The concentration of PUFAs in some species is relatively high, and this represents a practical interest for drugs and foods (Elenkov et al. 1996).

Ceramium virgatum, *Ulva intestinalis*, and *Cladophora vagabunda* are common red and green species of seaweeds found in abundance around the Romanian coastline, but little effort has been made to explore their biological potential. In the present study, one red macroalga (*C. virgatum*) and two green macroalgae (*U. intestinalis* and *C. vagabunda*) harvested from the Romanian Black Sea coast were evaluated as sources of volatile compounds and fatty acids with valuable potential as additives or biopreservatives. The antimicrobial activity of fatty acid extracts obtained from crude oils extracted from seaweed biomass was evaluated against four pathogenic bacterial strains with incidence in food spoilage and food safety (*Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella enteritidis*).

Materials and methods

The macroalgae species *Ceramium virgatum* Roth, *Ulva intestinalis* L., *Cladophora vagabunda* (L.) Hoek were identified using online algae databases (www.algaebase.org). The biomass was harvested from sea water and washed with tap water to remove extraneous materials. The cleaned biomass was frozen at −70 °C and then lyophilized for 24 h. The powder samples were kept in freezer at −20 °C for future application use.

Listeria monocytogenes 56 LY, *Bacillus cereus* DSM 10, *E. coli* 555, and *Salmonella enteritidis* 15S from the Central Microbial Cultures Collection, Department of Microbiology, Facolta di Scienze degli Alimenti, Cesena, Universita di Bologna, Italy have been used as indicator microorganisms for antimicrobial activity. Analytical grade chemical reagents (hexane, sodium sulfate, sulfuric acid, ethyl ether, heptane, diazomethane, and methanolic potassium hydroxide) and brain–heart infusion (BHI) medium for the cultivation of indicator bacteria were purchased from Sigma–Aldrich GmbH (Germany).

Oil extraction

For extraction of oil, 10 g of powdered seaweed biomass was mixed with 200 mL of hexane and sonicated for 30 min at 40 °C. The mixtures were stored for maceration at 4 °C for 24 h in the dark. Further, all the extracts were filtered using 0.45-μm filter paper and stored in the dark at −20 °C until the next steps.

Fatty acid extraction

For fatty acid extraction, 0.5 g of extracted seaweed oil was mixed with Na_2SO_4 (2 g), 2.5 M H_2SO_4 (0.2 mL), and 1 mL of ethyl ether and heptane (1:1), and agitated using a vortex mixer for 5 min in Falcon tubes (Ukeda et al. 1992). After centrifugation at 3,000 rpm for 5 min, the supernatant was filtered through a filter paper coated with 1 g Na_2SO_4. The filtrate was subjected to derivatization and then to gas chromatography–mass spectrometry (GC–MS) analysis.

The diazomethane method was used for the preparation of analytical quantities of methyl esters (Hartman and Lago 1973). For methyl esterification, four to five drops of diazomethane were added after drying the fatty acid samples with liquid nitrogen. The reaction of diazomethane with carboxylic acid is quantitative and essentially instantaneous in ether solutions. Methyl esters were prepared by titration of ether solution with diazomethane until the yellow color persisted. After, 1 mL of 2 N methanolic KOH and 1 mL hexane were added using vortex mixing and separated using separation funnels. After separation, the upper phase containing fatty acids was collected and stored at −20 °C until GC–MS analysis.

Fatty acid analysis by gas chromatography–mass spectrometry

Fatty acid methyl ester analysis was performed both in SCAN and SIM modes using an Agilent Technologies gas chromatograph 6890 N (USA) equipped with an Agilent Network Mass Selective detector HP 5973 (USA) and a capillary column SPB-5 (30 m×0.25 mm×0.25 mm (Supelco USA)). The injector and the detector were both held at 250 °C. The temperature was increased from 120 °C (held for 5 min) to 215 °C at a rate of 3 °C min^{-1}, then from 215 to 225 °C at a rate of 0.5 °C min^{-1}, and the final temperature was held for 5 min.

The carrier gas was helium with a flow rate of 1 mL min^{-1} and a split ratio of 1:10. Fatty acids were identified by comparing their retention time and mass fragmentation profiles with those of the standards mix FAME 37 (Sigma–Aldrich). The results were expressed as relative percentage of each fatty acid as a fraction of total fatty acids (TFA).

Analysis of volatile compounds

Volatile compounds were monitored using a GC–MS coupled with solid phase microextraction (SPME GC–MS) according to the protocol of Iucci et al. (2007). For each seaweed sample, 0.2 g of biomass was placed in 5-mL

sterile vials, sealed using PTFE/silicon septa. The samples were then equilibrated for 10 min at 60 °C, and the volatiles were adsorbed on a fused silica fiber covered by 65-μm polydimethylsiloxane-divinyl benzene (Supelco, Germany). Adsorbed molecules were desorbed in the gas chromatograph for 5 min. For peak detection, an Agilent Hewlett–Packard 6890 GC gas chromatograph equipped with a MS detector 5970 MSD (Hewlett–Packard, Switzerland) and a 50 m×0.32 i.d. fused silica capillary column coated with a 1.2-μm poly ethylene glycol film (Chrompack CP-Wax 52 CB, Netherlands) as stationary phase were used. The conditions were as follows: injection temperature 250 °C, detector temperature 220 °C, carrier gas (helium) flow rate 1 mL min^{-1}, and splitting ratio 1:20 (*v/v*). The oven temperature was programmed as follows: 50 °C for 2 min, from 50 to 65 °C (at 1 °C min^{-1}), from 65 to 220 °C (at 5 °C min^{-1}), followed by holding for 22 min. Volatile peak identification was carried out by computer matching of mass spectral data with those of the compounds in the Agilent Hewlett–Packard NIST 98 and Wiley version 6 mass spectral database.

Evaluation of antibacterial activity of fatty acids

The minimum inhibitory concentration (MIC) of fatty acid extracts was evaluated against four indicator bacterial strains using the microdilution method (Oke et al. 2009). Briefly, 100 μL of BHI, 100 μL of total fatty acid extract, and 100 μL of the test bacteria cell suspension were dispersed in each well. The inoculum of the test bacteria was prepared using 24-h grown cultures, and the suspensions were adjusted to 5 McFarland standard turbidity. Since turbidity is in part due to the inoculum itself, the inoculated tube kept in the refrigerator overnight was used as the standard for the determination of complete inhibition. A positive control (containing 100 μL of bacterial suspension and 100 μL of BHI) and negative control (containing 100 μL of fatty acid extract and 100 μL of BHI) were prepared to study the effect of bacterial culture age and hexane on MIC. The contents of the wells were mixed, and the microplates were incubated at 37 °C for 24 h.

Statistical analysis

All experiments were done in triplicate, and the data are reported as the mean of the three replicates. Data related to the zone of inhibition due to fatty acids were subjected to analysis of variance (one way ANOVA) using the SPSS (version 10) statistical software.

Results

The volatile composition analysis of *C. virgatum*, *C. vagabunda*, and *U. intestinalis* from the Romanian coast of the Black Sea highlighted a wide range of compounds with different structures (Fig. 1). A total number of 69 volatile compounds were identified in these extracts. Of these compounds, 13 were classified as aldehydes, 18 as ketones, 7 as alcohols, 4 as monoterpene hydrocarbons, 3 as oxygenated monoterpenes, 4 as phenolic monoterpenes, and 10 as non-terpene hydrocarbons (Table 1).

The composition and quantity of the particular compounds were different in different seaweeds. Hexanal (11.2 %), octane (9.8 %), nonanal (6.7 %), octanal (6.7 %), 2,5,5-trimethyl-2-hexene (4.7 %), 3-hexen-2-one (4 %), and o-cymene (3.6 %) were the major volatile compounds in *C. vagabunda*. The major compounds in *U. intestinalis* were hexanal (14.6 %), trichloromethane (7.3 %), nonanal (5.6 %), 3-hexen-2-one (5.3 %), and octanal (3.1 %). In *C. virgatum*, the major components were 3-hexen-2-one (27.9 %), acetone (12.4 %), hexanal (3.4 %), and o-cymene (2.7 %).

The fatty acid composition of lipids separated from the biomass of the three studied seaweed species is shown in Table 2. The studied macroalgae were rich in saturated and unsaturated fatty acids. In the three studied samples, the most abundant was palmitic acid (C16:0), which accounted for 24.6±2.0 % in *C. vagabunda*, 23.4±2.2 % in *C. virgatum*, and 20.1±1.8 % in *U. intestinalis*. Arachidonic acid (C20:4n-6) represented 23.0±3.0 % in *C. vagabunda*, 20.6±2.8 % in *U. intestinalis*, and 14.5±1.4 % in *C. virgatum*, respectively. Oleic acid (C18:1ω-9cis) represented more than 10 % of TFA, whereas elaidic acid (C18:1 ω-9-trans) accounted for less than 1 % of TFA in all samples. Stearic (C18:0) and myristic (C14:0) acids were found in all samples, with a concentration ranging between 4.7 and 5.4 % of TFA and between 6.3 and 10.2 % of TFA, respectively. The extract of *U. intestinalis* had a greater content of MUFAs and PUFAs (around 46.0 %) as compared with 42.0 % for *C. vagabunda* and 31.9 % for *C. virgatum*.

Seaweed oil extracts had substantial antimicrobial potential against both Gram-positive (*B. cereus* and *L. monocytogenes*) and Gram-negative (*E. coli* and *S. enteritidis*) bacteria (Fig. 2). The MIC of *C. vagabunda* and *C. virgatum* extracts varied from 1.8 to 3.8 mg mL^{-1}, while the MIC of *U. intestinalis* extract was 3.8 mg mL^{-1} for all bacterial strains.

Discussion

In the present study, a high content of volatile compounds was found in the biomass of *C. vagabunda*, *C. virgatum*, and *U. intestinalis* seaweeds. The quantitative analysis of the concentration of volatile compounds revealed that ketones, aldehydes, and alcohols are the major volatile compounds in the biomass of the three macroalgae. These

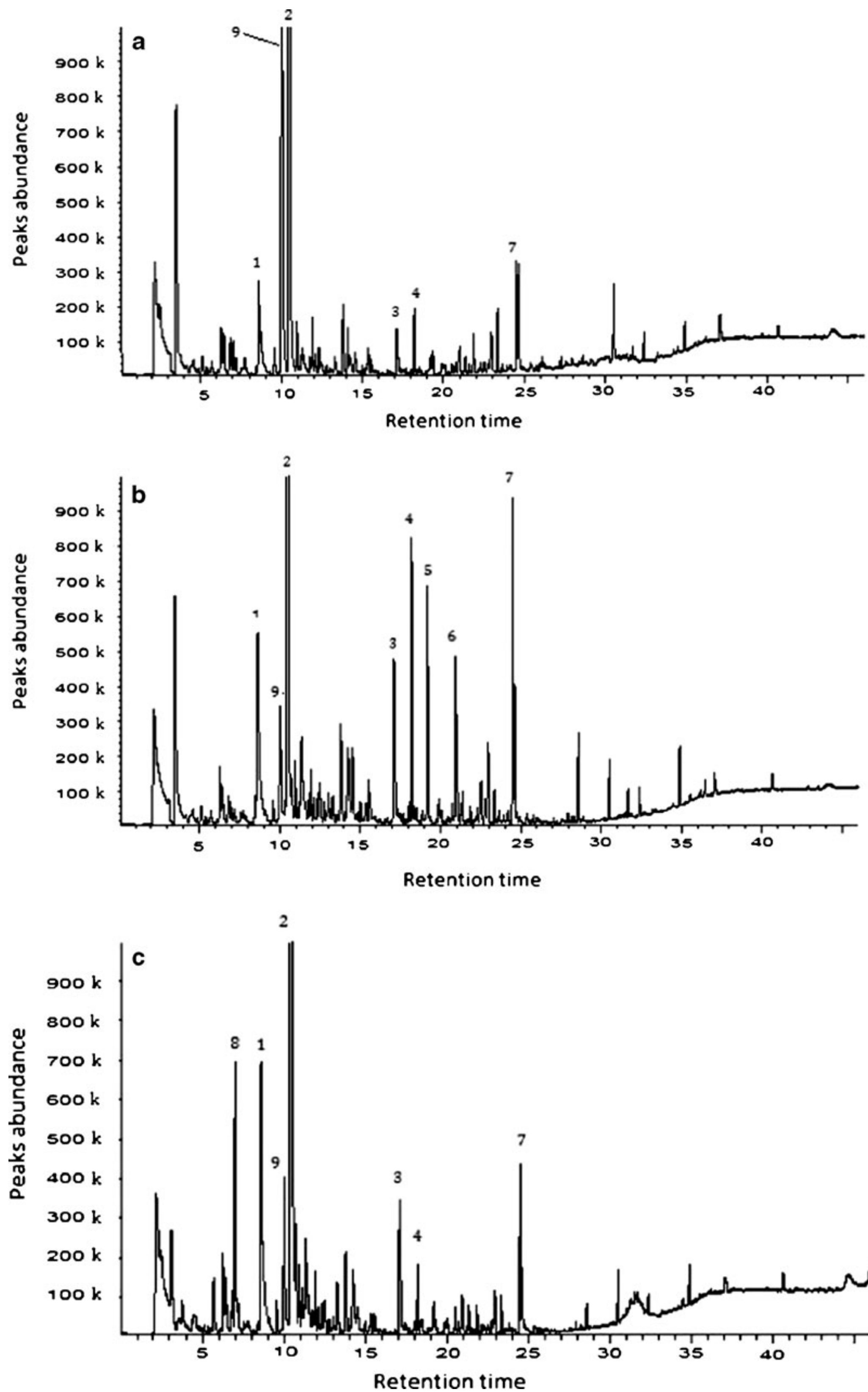

Fig. 1 SPME GC–MS chromatograms of the volatile compounds extracted from **a** *Ceramium virgatum*, **b** *Cladophora vagabunda*, and **c** *Ulva intestinalis*. *Peak 1* hexanal, *peak 2* internal standard, *peak 3* nonanal, *peak 4* 1-octen-3-ol, *peak 5* 3,4,4-trimethyl-2-hexene, *peak 6* benzaldehyde, *peak 7* furanone a, *peak 8* trichloromethane, *peak 9* 3-hexene-2-one; $k=10^3$

Table 1 Composition of volatile compounds of red and green macroalgae

Volatile compounds	*Ceramium virgatum*	*Cladophora vagabunda*	*Ulva intestinalis*
Aldehydes			
3-Methyl butanal	0.0	0.7	0.3
Pentanal	1.7	1.6	2.3
2-Butenal	0.0	0.2	0.6
2-Hexenal	0.0	0.2	0.0
Octanal	0.0	3.5	3.1
2-Heptenal	0.6	0.4	0.4
Nonanal	2.3	7.0	6.0
Decanal	0.0	0.6	0.0
Benzaldehyde	0.7	3.0	0.5
2,6-Nonadienal	0.7	0.2	0.0
β-Cyclocitral	0.0	0.3	0.0
Safranal	0.0	0.2	0.0
3,7-Dimethyl-2,6-octanedienal	0.0	0.2	0.0
Total	6.0	18.1	13.2
Ketones			
Acetone	12.4	0.0	1.0
Methyl isobutyl ketone	1.5	1.0	1.5
Trichloromethane	1.0	0.3	7.3
Hexanal	3.4	11.1	14.
4-Methyl-2-hexanone	0.9	0.7	1.2
3-Hexen-2-one	27.9	4.0	5.3
2,6-Dimethyl-4-heptanone	1.6	1.4	1.9
2-Heptanone	0.3	2.9	2.3
2-Octanone	1.3	0.0	0.0
2,5-Octanedione	0.0	0.6	0.4
6-Methyl-5-hepten-2-one	0.2	0.7	0.3
3,5,5-Trimethyl-2-cyclohexen-1-one	0.0	0.2	0.0
Acetophenone	0.0	0.1	0.0
Verbenone	0.0	0.1	0.0
Trans-geranylacetone	0.0	0.1	0.0
α-Ionone	0.0	2.0	0.0
β-Ionone	2.3	1.3	1.4
β-Ionon-5,6-epoxide	0.0	0.7	0.0
Total	52.8	27.2	37.2
Alcohols			
1-Penten-3-ol	0.0	0.1	0.0
2-Hexanol	1.3	1.1	1.3
5-Methyl-3-hexanol	0.5	0.4	0.6
2-Penten-1-ol	0.0	0.1	0.0
1-Octen-3-ol	1.8	6.7	1.9
Heptanol	0.0	0.3	0.2
1-Octanol	0.6	0.5	0.4
Total	4.2	9.2	4.4
Monoterpene hydrocarbons			
O-cymene	2.8	3.6	3.0
Carene-4,5-epoxy trans	0.0	0.3	0.0
Menthone	0.5	0.0	0.2
Limonene	0.5	0.4	0.0
Total	3.8	4.3	3.2
Oxygenated monoterpenes			
Eucalyptol	0.5	0.6	0.7
Menthol	1.7	0.0	0.9
α-Terpineol	0.0	0.2	0.0
Total	2.2	0.8	1.6
Phenolic monoterpenes			
Carvacrol	0.0	0.5	0.0
Thymol	0.7	0.6	0.9
Phenol	0.0	0.1	0.0
M-tert-butylphenol	0.4	0.5	0.7
Total	1.1	1.7	1.6
Non-terpene hydrocarbons			
2,4,4-Trimethyl-1-pentene	0.0	0.0	3.1
Octane	0.0	9.8	0.0
1-Chloro-octane	0.0	0.6	0.0
1H-pyrazole,4,5-dihydro-5,5-dimethyl-4-isopropylidene	0.0	0.0	0.1
(Z)-3-ethyl-2-methyl-1,3-hexadiene	0.0	0.5	0.0
2-Cyclohexen-1-one	0.0	0.2	0.0
3,4,4-Trimethyl-2-hexene, 2,5,5-trimethyl-2-hexene	0.5	4.7	0.9
Hexadecane	1.2	0.2	1.0
1,3-Cyclooctadiene	0.0	0.1	0.0
5-Hepten-3-yn-2-ol	0.2	0.0	0.7
Total	1.9	16.1	5.8
Others			
2-Pentyl furan	0.0	0.5	1.4
2-Ethyl furan	0.0	0.4	2.1
Furanone a	3.0	7.2	3.9
Furanone b	3.2	2.8	1.3
2-(2,2-dimethylvinyl) thiophene	0.0	0.1	0.0
Linalyl anthranilate	0.2	0.3	0.8
Trichloromethane	1.0	0.3	7.3
Butylacetamide	0.0	0.0	0.8
Hexanoic acid	0.0	0.2	0.0
Decanoic acid	0.0	0.3	0.0
Total	7.4	12.1	17.6
Total identified compounds (%)	79.4	89.5	84.6

volatile compounds are of special importance; for instance, hexanal can be used as a reagent in the flavor industry to produce fruity flavor similar to freshly cut grass.

Table 2 The fatty acid composition of the red and green seaweeds

Fatty acids	Composition[a]		
	Ceramium virgatum	*Cladophora vagabunda*	*Ulva intestinalis*
Saturated			
C14:0	6.3±0.80	9.0±0.5	10.2±2.00
C15:0	n.d.	0.5±0.08	0.1±0.02
C16:0	23.4±2.20	24.6±2.00	20.1±1.8
C17:0	n.d.	n.d.	0.3±0.02
C18:0	4.7±0.500	5.4±0.80	5.1±0.90
C 20:0	0.4±0.08	0.1±0.03	0.1±0.03
Total	34.8±2.01	39.6±2.09	35.9±1.89
Monounsaturated			
C16:1n-7	n.d.	0.3±0.05	0.2±0.06
C16:1n-9	n.d.	0.7±0.10	0.1±0.06
C18:1n-9 cis	10.8±1.50	11.3±1.30	13.5±1.30
C20:1n-11	n.d.	0.5±0.02	0.6±0.08
Total	10.8±1.53	12.8±1.16	14.4±1.62
Polyunsaturated			
C16:2n-7,10	n.d.	n.d.	0.2±0.04
C18:2n-9	2.1±0.60	2.4±0.30	3.2±0.70
C20:2n-6	n.d.	0.4±0.02	0.2±0.05
C20:3n-6	0.4±0.07	0.8±0.10	1.9±0.50
C20:4n-6	14.5±1.40	23.0±3.00	20.6±2.80
C20:5n-6	4.1±0.80	2.7±0.70	5.6±1.10
Total	21.1±2.82	29.3±3.83	31.7±4.27
Trans			
C18:1n-9 trans	0.7±0.04	0.1±0.02	0.4±0.03

n.d. not determined

[a] All data are expressed as a percentage of total fatty acid content

Algal carotenoids are usually represented by isoprenoids, polyene pigments that are mainly responsible for the cell protection against photodynamic damage and auxiliary light absorption for photosynthesis and phototaxis by these seaweeds. Flament and Ohloff (1984) reported that the thermal degradation of β-carotene from seaweeds produces

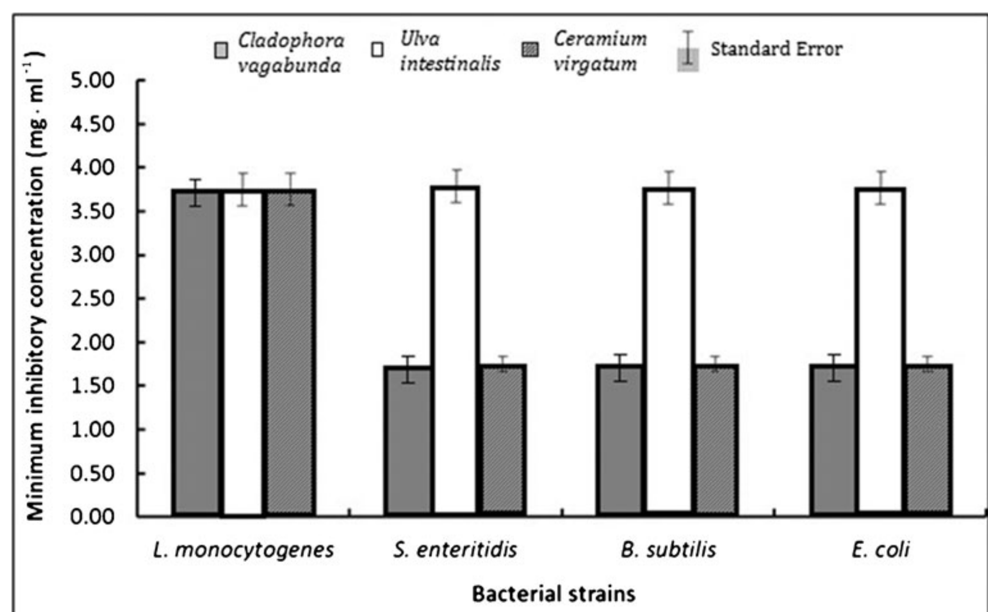

Fig. 2 Minimum inhibitory concentration of seaweed fatty acids against pathogenic bacteria

polyenes and aromatic hydrocarbons. Some of the hydrocarbons present in these algae are chemical messengers for male gametes; they are highly unsaturated aliphatic or cyclic hydrocarbons with saturated side chains (Pape et al. 2004). These compounds were identified in all three studied algae species.

It was suggested before that the aldehydes and ketones present in most seaweeds originate from the degradation of unsaturated fatty acid and carotenoids (Rzama et al. 1995). Tridecanone can be produced from fatty acid oxidation, but its exact origin remains unknown (Pape et al. 2004). This can explain the high content of aldehydes and ketones identified in this study. The alcohol fraction 1-octen-3-ol from green seaweed identified in this study has previously been detected in brown seaweeds by Takahashi et al. (2002). However, these authors did not refer to its origin. Its formation might be due to the decomposition of secondary hydroperoxides of fatty acids. In the present study, halogenated compounds such as trichloromethane (7.3 %) were found in *U. intestinalis*. Other studies (Rzama et al. 1995; Pape et al. 2004) also reported haloforms and other halogenated compounds in seaweed, especially in brown species. Limonene that is widely used in the pharmaceutical industry was identified at a lower concentration level in the red algae *C. virgatum*. This compound is found in highest quantities in lemons, giving them specific flavor.

Another compound identified in seaweed extracts was thymol, which was reported to have antibacterial activity against *Aeromoans hydrophila* and *Staphylococcus aureus* strains (Dorman and Deans 2000). Due to its antiseptic properties, this compound is widely used in cosmetics and in products for dental and oral cavity hygiene, such as mouthwashes and toothpaste (Filoche et al. 2005). Thymol, a compound used for its antifungal properties, was identified in all samples of algae at low levels.

Seaweed extracts are considered natural sources of long-chain polyunsaturated essential fatty acids from the omega-3 family (LC-PUFAs-ω-3), such as eicosapentaenoic acid, C20:5-ω-3 (Khotimchenko et al. 2002), which may reduce the risk of heart disease, thrombosis, and atherosclerosis (Mishra et al. 1993). Although seaweeds have lower lipid content, they contain a higher level of essential polyunsaturated fatty acids as compared with traditional vegetables (Ortiz et al. 2006).

The content of saturated fatty acids was almost similar to that of unsaturated fatty acids. The percentages of fatty acids from different marine algal species were relatively constant under similar cultivation conditions. However, there were differences in the proportions of specific fatty acids; for example, the percentage of C14:0 in *Chlorella* spp. reached about 10 % of TFA, while the content in freshwater algae usually did not exceed 1 %. These differences are considered characteristic of marine phenotypes (Ben-Amotz et al. 1985).

The obtained results are in good agreement with those reported by Johns et al. (1979) who reported 23.9 % palmitic acid in green algae, 27.9 % in brown algae, and 33.8 % in red algae. Ortiz et al. (2006) also suggested that C16:0 was the predominant saturated fatty acid (14.0–12.1 %) in *Ulva lactuca* and *Durvillaea antarctica*.

The occurrence of C18 PUFAs is important both in the nutrition of humans and fish, who are not able to synthesize them (Sànchez-Machado et al. 2004). The fatty-acid content of the lipids extracted from the seaweed biomass makes it very interesting for use as an antimicrobial agent (Parfene et al. 2013). The results of the current study are in good agreement with those by Taskin et al. (2007) who studied the antibacterial activity of marine Rhodophyceae (*Corallina officinalis*), Phaeophyceae (*Cystoseira barbata*, *Drosera dichotoma*, *Halopteris filicina*, *Cladostephus spongiosus* f. *verticillatus*), and Chlorophyceae (*Ulva rigida*) species against some pathogenic bacteria (*S. aureus*, *Micrococcus luteus*, *E. coli*, *Enterobacter aerogenes*, *Enterococcus faecalis*, and *E. coli* O157:H7). These authors reported no significant difference in antimicrobial efficacy of Gram-positive and Gram-negative bacteria. In the present study, the MIC for the Gram-positive bacteria was higher or equal to that of the Gram-negative bacteria. This may be due to the presence of higher content of arachidonic acid (C20:4, n-6). It was reported that long-chain fatty acids stimulated oxygen uptake by Gram-positive bacteria at bactericidal and protoplast lytic concentrations and produce inhibition at higher levels. The order of activity between individual acids and effects of reversal agents on the respiratory activity corresponds to those responsible for bactericidal activity. Protoplasts are more susceptible to inhibition than whole cells. Gram-negative bacteria are inhibited to a limited extent at high fatty acid concentrations, but spheroplasts are highly sensitive. Fatty acids inhibit amino acid uptake both aerobically and anaerobically at sub-bactericidal levels (Galbraith and Miller 1978; Branen et al. 1980).

Kandhasamy and Arunachalam (2008) observed that the methanol extract of *Hypnea musciformis* (red algae) had similar efficacy for Gram-negative (a MIC value of 13.0± 0.6 mg mL^{-1} for *K. pneumonia* and 12.0±0.8 mg mL^{-1} for *E. faecalis*) and Gram-positive bacteria (12.0±0.7 mg mL^{-1} for *S. aureus*). In previous studies, antibacterial and antifouling effects of marine macroalgae due to antibacterial compounds were detected in nonpolar (hexane) extracts of *C. virgatum* (named *C. rubrum*) with moderate to strong levels of growth inhibition (Hellio et al. 2001; Bansemir et al. 2006). Dubber and Harder (2008) showed that polar (methanol) extracts of *C. virgatum* (named *C. rubrum*) have higher antibacterial activity than the nonpolar (hexane) extracts. However, Ozdemir et al. (2004) indicated that the methanol extracts of *D. membranacea* and *C. barbata* did show lower inhibitor activity than those that are transformed

into powder hexane extracts, which contradicts the other studies mentioned above. They also found a variation in antimicrobial activity due to seasonal variation. It was also reported that antibacterial activity in the same algal species may vary on different geographic scales and local adaptations (Sandsdalen et al. 2003; Freile-Pelegrin and Morales 2004). Therefore, it can be concluded that macroalgae have significant antimicrobial activity, but this can vary from species to species, location, and probably depends on several other factors.

Studying seaweeds is very important for several reasons. First of all, they grow in abundance during summer in sea water and, thus, represent a cheap raw material for extraction of many biologically active compounds. In addition, their exploitation contributes to environmental protection by cleaning the seaside.

From the chemical characterization studies, it can be concluded that seaweeds represent natural reservoirs of bioactive compounds with high potential for food and pharmaceutical applications. The bioactive compounds of these seaweeds have a significant in vitro antimicrobial effect against pathogenic bacteria such as *S. enteritidis*, *E. coli*, *L. monocytogenes*, and *B. cereus*. Nonetheless, further studies are needed in order to evaluate the antimicrobial and food preservation activity of these seaweed extracts in real food systems.

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