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RESEARCH NOTE

Fatty acid profile, total phenolic content and antioxidant capacity of seaweeds in Salinas Bay, Ecuador

Perfil de ácidos grasos, contenido total de polifenoles y capacidad antioxidante de macroalgas en Bahía Salinas, Ecuador

Haydelba D'Armas[1*], Carmita Jaramillo-Jaramillo[2], Mayra D'Armas[3] and Gabriel José Ordaz-González[1]

[1]Departamento de Química, Escuela de Ciencia, Facultad de Ciencias Químicas y de la Salud, Universidad de Oriente, Núcleo Sucre, Cumaná 6101, Venezuela
[2]Facultad de Ciencias Químicas y de la Salud, Universidad Técnica de Machala, Machala 070102, Provincia del Oro, Ecuador
[3]Facultad de Ciencias de la Salud, Universidad Estatal de Milagro, Ciudadela Universitaria km 1 ½, Milagro 091050, Provincia de Guayas, Ecuador
*Corresponding author: hdarmasr@gmail.com

Abstract.- The coastal waters of Ecuador have emerged as a favorable environment for the growth and development of several species of edible macroalgae or seaweeds. In this study, fatty acid profiles, total soluble polyphenol content, and antioxidant capacity of seven species of macroalgae (four red algae, two brown algae and one green algae) collected from Salinas Bay, Santa Elena Province, Ecuador, were evaluated. Saturated fatty acids C14:0, C15:0, C16:0 and C18:0 were found in almost all organisms, from which palmitic acid showed the highest proportion (10.7-37.1%). Green and brown seaweeds exhibited a major content of unsaturated fatty acids. Monounsaturated fatty acid composition was represented by several isomers of C16:1 and C18:1, mainly palmitoleic (3.0-6.6%) and oleic (0.8-11.1%) acids. Polyunsaturated fatty acid composition ranged from 5.47 to 26.61%, with ratios of total *n*-6/*n*-3 acids lower than 3.3. Linoleic (C18:2, *n*-6, 1.16-7.73%) and eicosapentaenoic (C22:5, EPA, *n*-3, 1.44-5.92%) acids were found in the majority of the seaweeds. The total polyphenol content of the seaweeds was determined by the Folin-Ciocalteu method (765 nm), and ranged from 147.28 to 1,062.20 mg equivalents of gallic acid (GAE) per 100 g of dry seaweed. The antioxidant capacity was determined by the Trolox equivalent antioxidant capacity (517 nm). Only *Acanthophora spicifera*, *Spatoglossum schroederi*, and *Ulva lactuca* showed good radical-scavenging (14.18-3,379.47 mg Trolox equivalents (TE) per 100 g of dry seaweed). The results confirm the potential use of macroalgae in the study area as an important source of nutritional compounds, especially brown and green algae, which could be a recommended food supplement even in the treatment of some diseases.

Key words: Chlorophyta, Phaeophyceae, Rhodophyta, DPPH, nutraceuticals

INTRODUCTION

Macroalgae or seaweeds are marine organisms abundant in coastal lines around the world. They are classified as red (Rhodophyta), brown (Phaeophyceae) and green (Chlorophyta), due to their pigmentation and other features (Chapman & Chapman 1980, Mouritsen 2013, Hurd *et al*. 2014). These organisms have been harvested for centuries, especially for the Asian population; and nowadays represent a source of nutritive and healthy products in many countries (Ganesan *et al*. 2019, Shannon & Abu-Ghannam 2019, Oucif *et al*. 2020). Nearly 221 species of algae worldwide (125 red, 64 brown, and 32 green) are used as human food, cosmetics, fertilizers, and for extraction of industrial gums and chemicals (Buschmann *et al*. 2017, Rioux *et al*. 2017, Ditchburn & Carballeira 2019). However, they still require extensive study, because the benefits and risks of seaweed consumption are not completely established (Tanna & Mishra 2018, Cherry *et al*. 2019, Cotas *et al*. 2021, Kumar & Sharma 2021).

Several studies have demonstrated that seaweeds have low content of lipids, but they are an important source of polyunsaturated fatty acids, especially those belonging to long-chain omega-3 polyunsaturated fatty acids (*n*-3 PUFAs) that are essential in human diet (Schmid *et al*. 2018, Susanto *et al*. 2019, Berneira *et al*. 2020). PUFAs can change membrane phospholipid composition and participate in several physiological processes, including the biosynthesis of prostanoids and the transportation as well as oxidation of cholesterol (Uracz *et al*. 2014). Their applications comprise cardiovascular and inflammatory diseases; brain development, function and disease; cancer, anti-aging and mental health (Nabavi *et al*. 2015, Cornish *et al*. 2017, Tanna & Mishra 2018, Li *et al*. 2019).

All groups of macroalgae are currently attracting the attention of the scientific community due to the bioactive substances they produce that can sustain human health, preventing or reducing the convalescence period for various diseases, due to their antioxidant, anti-inflammatory, anti-aging, antiproliferative, antiviral, and antibacterial activities (Biris-Dorhoi *et al*. 2020).

Phenolic compounds are secondary metabolites widespread in various terrestrial and sea plant, which have shown several biomedical applications. Structurally, phenolic compounds present in macroalgae vary from simple molecules, such as phenolic and cinnamic acids or flavonoids, to the more complex phlorotannin polymeric structures (Pereira 2021). Phlorotannins are found only in brown seaweeds, which contain the highest level of phenols, mainly phlorotannins such as phloroglucinol (1,3,5-trihydroxybenzene) polymers that can reach up to 15% of the dry weight of these algae (Gomez-Zavaglia *et al*. 2019). As many of the human age-related degenerative diseases are associated with oxidative processes, these phytochemicals have been important for their potential health benefits, exhibiting defensive effects against hyperglycemia, hyperlipidemia, inflammation and oxidative stress, known risk factors for cardiovascular disease and diabetic complications, in cell culture, animal studies and some human studies (Cotas *et al*. 2020).

The aim of this study was to evaluate the fatty acid profile, total polyphenol content, and antioxidant capacity of macroalgae collected in Salinas Bay, Ecuador, as a contribution of the nutritional knowledge of these organisms in this region.

Materials and methods

Sampling

Macroalgae were collected in the coastal waters of Salinas Bay (2°15'0"S and 80°56'0"W; 78-81% of relative humidity; average temperature and altitude of 27 °C and 8 m above sea level, respectively), located in the west of Santa Elena Province, Ecuador.

All the species were collected at the same time of the year (March 2017) and were chosen on account of their abundance in Salinas Bay during that season. Each sample was cleaned with distillated water and allowed to drip off under sun-light, and then subjected to a drying process under shade at room temperature for 96 h. Subsequently, were dried in a stove (MEMMERT SNB 400 with air flow, Büchenbach, Germany) at 40 °C for 24 h. Afterwards, the dried seaweeds were pulverized with a grinder (Laboratory Mill serial No. 56969, Type AR 400 Erweka®, Germany).

Organisms were taxonomically identified as four red seaweeds, *Acanthophora spicifera* (M.Vahl) Børgesen (Rhodomelaceae), *Centroceras clavulatum* (C.Agardh) Montagne (Ceramiaceae), *Hypnea spinella* (C.Agardh) Kützing (Cystocloniaceae) and non-native *Kappaphycus alvarezii* (Doty) L.M.Liao (Solieriaceae); two brown seaweeds, *Padina pavonica* (Linnaeus) Thivy (Dictyotaceae) and *Spatoglossum schroederi* (C.Agardh) Kützing (Dictyotaceae); and one green seaweed, *Ulva lactuca* Linnaeus (Ulvaceae).

Fatty acid extracts

The crude lipid extracts from seaweeds were obtained following the methodology described by Chen *et al*. (2012). Dried seaweeds were extracted with a mix of chloroform/methanol (1:2, v/v) at 65 °C for 1 h under stirring. Mixtures were centrifuged at 950 rpm, and the respective supernatants were collected. The residuals were submitted to the same extraction process twice more. In each case 1% sodium chloride solution was added to the combined supernatants. The solutions were allowed to settle, and carefully transferred to a vial and dried to constant weight at 60 °C under nitrogen flow.

Fatty acid methyl esters were obtained following the Brockerhoff method (Litchfield 1972). In each case, 1 g of lipid extract dissolved in diethyl ether was mixed with 1 mol·L^{-1} KOH methanol solution. The mixture was agitated for five minutes and allowed to settle for 15 min. Then, 1 mol·L^{-1} HCl solution was added, and the mixture was extracted with diethyl ether. The methyl ester extracts were combined and the solvent evaporated via drying with gaseous nitrogen.

Analysis of fatty acid content

Solutions of 20-30 µL of esterified extract dissolved in 1 mL of chloroform were injected in an Agilent Technologies 7890A chromatograph, with source of electronic impact ionization (70 eV) and an injector type Split (10:1) at 250 °C. The apparatus was equipped with a SB5-MS column of 30 m × 0.25 mm DI × 0.25 mm of thickness at Centro de Investigaciones Biotecnológicas del Ecuador-CIBE, Escuela Superior Politécnica del Litoral, Guayaquil. Initial oven temperature was 150 °C (for 4 min), with a ramp of 2 °C min^{-1} until 250-300 °C. Helium was used as eluent with a flux of 1 mL min^{-1}. An Agilent Technologies 5975C inert XL MSD with triple axel detector was coupled to the chromatograph. Analyses were performed by triplicate. Quantification was established according to the relative area under each peak, and identification was based on the comparison of obtained mass spectra with spectra stored in the libraries NIST-2011 and WILEY®. (McLafferty 2011), selecting the compounds with a confidence match above 90%.

Total polyphenols contents

Quantification of total polyphenols was carried out using the Folin-Ciocalteu reagent, following a slightly modified method described by Ainsworth & Gillespie (2007). 200 µL of the extracts (~ 20 mg dry seaweed / 2 mL of methanol 95%) or standard (50 mM gallic acid in 95% (v/v) methanol) was added to the cuvettes (10 × 45 mm, 3 mL), followed by 400 µL 10% (v/v) Folin–Ciocalteu reagent in super distilled water. Further, 1600 µL 700 mM Na_2CO_3 in super distilled water was added to the cuvettes. Then, each assay was incubated at room temperature for 2 h. The absorbance of each well was measured at 765 nm. Methanol 95% (v/v) was used as blank. The polyphenols content was expressed as mg of gallic acid equivalents (GAE) per 100 g of sample (dry weight - DW).

Antioxidant capacity

The antioxidant capacity was evaluated by the ability as free radical scavenger of the samples (Ebada *et al*. 2008). 200 µL of the extracts (~ 20 mg dry seaweed / 2 mL of methanol 95%) were added to 3.2 mL of a 0.004% DPPH solution in methanol. Seven concentrations, ranging from 1 to 100 µM, were prepared for each sample and analyzed in triplicate. 3.2 mL of methanol plus 200 µL of each compound solution were used as blank solutions. 3.2 mL of 0.004% DPPH solution plus 200 µL of methanol were used as negative control. The absorbance at 517 nm was determined after 30 min of incubation, and the percentage of DPPH reduction was calculated taking into account the absorbance of the blank solutions and the negative control. The results were expressed as mg of Trolox equivalent (TE) per 100 g of dry sample.

Statistical analysis

All data was expressed in terms of mean ± standard deviation. Statistical Package for Social Science software (SPSS Version 23.0 for Windows) was used to calculate mean percentage and standard deviation. To determine whether there were any differences amongst mean, one-way Analysis of Variance (ANOVA) and Duncan's multiple range tests were applied to the results and P values < 0.05 were considered significant. Test of normality was previously applied to the data, which showed being approximately normal.

Results & discussion

Table 1 shows the fatty acid composition of seaweeds collected in Salinas Bay, Ecuador. The total fat content in all species were more than 0.33 g per 100 g which contains more than 20 fatty acids. The main saturated fatty acid (SFA) identified in these organisms was palmitic acid (C16:0, 10.70-37.14%), except in the brown macroalga *P. pavonica*, which did not contain this compound. Other SFAs found in all species were myristic (C14:0, 1.03-8.61%), pentadecanoic (C15:0, 0.17-1.38%), and stearic (C18:0, 0.82-2.11%) acids. Unusual saturated fatty acids were found in some seaweeds, for instance 13-methylmyristic (C15:0) acid was identified in the red seaweeds *A. spicifera* (0.25%) and *H. spinella* (0.13%), while dihydrohydnocarpic (C16:0, 5.02%) and dihydrochaulmoogric (C18:0, 2.72%) acids were detected only in the red seaweed *K. alvarezii*.

Although *P. pavonica* did not contain palmitic acid, its fatty acid composition was represented by a monounsaturated analogue, gaidic acid (C16:1, 22.92%), which was also found in the green seaweed *U. lactuca* (3.02%). All red seaweeds showed palmitoleic (C16:1) and oleic (C18:1, *n*-9) acids as common monounsaturated fatty acid (MUFA), which were detected in the brown seaweeds *S. schroederi* and *P. pavonica*, respectively. Palmitoleic acid was the main MUFA of *A. spicifera* and *K. alvarezii*, while oleic was the main MUFA of *H. spinella*. (*Z*)-7-octadecenoic (C18:1, *n*-11) was identified only in the brown seaweed *S. schroederi* (14.39%) as its main MUFA, while (*Z*)-13-octadecenoic (C18:1, *n*-5) was found as main MUFA in *C. clavulatum* (7.54%) and *U. lactuca* (7.25%), being also identified in *H. spinella* (1.61%).

Linoleic (C18:2, *n*-6) acid was the polyunsaturated fatty acid (PUFA) found in all species (1.16-7.72%), being the main PUFA of *A. spicifera*. Meanwhile, α-linolenic (C18:3, *n*-3) acid was found only in four species (*C. clavulatum*, *P. pavonica*, *S. schroederi*, *U. lactuca*) as their main PUFA, ranging from 6.00 to 12.97%. Arachidonic (C20:4, *n*-6) was found only in three red seaweeds (1.79-3.83%), being the main PUFA in *H. spinella* and *K. alvarezii*. Eicosapentaenoic (EPA, or timnodonic, *n*-3) ranged from 1.44 to 5.92%, and was found in six species, except in the red seaweed *C. clavulatum*, which did not exhibit any C20 PUFA. In general, brown and green seaweeds showed higher unsaturated fatty acid content (MUFA > PUFA > SFA) than red seaweeds (SFA > MUFA > PUFA). All organisms showed an important content of PUFA *n*-3 (1.44-18.89%) and *n*-6 (4.03-10.90%), with a ratio *n*-6/*n*-3 that ranged from 0.4 to 3.3%. An important percentage of fatty acids were detected but could not be identified, especially in *A. spicifera* samples (71.5%).

Table 1. Fatty acid composition (as methyl esters) of seven seaweeds collected in Ecuador / Composición de ácidos grasos (como ésteres metílicos) en siete macroalgas recolectadas en Ecuador

Fatty acid		Composition (%)						
		Acanthophora spicifera	*Centroceras clavulatum*	*Hypnea spinella*	*Kappaphycus alvarezii*	*Padina pavonica*	*Spatoglossum schroederi*	*Ulva lactuca*
Saturated								
C12:0	Lauric	0.15 ± 0.02^{a}	-	0.26 ± 0.04^{b}	-	-	-	-
C13:0	Tridecylic	0.11 ± 0.02^{a}	-	-	-	-	-	-
C14:0	Mirystic	1.66 ± 0.09^{a}	2.13 ± 0.08^{b}	2.91 ± 0.15^{c}	1.03 ± 0.07^{d}	8.61 ± 0.51 ^{e}	6.37 ± 0.19^{f}	6.04 ± 0.19^{f}
C15:0	Pentadecanoic	0.17 ± 0.03^{a}	1.16 ± 0.04^{b}	0.22 ± 0.03^{a}	1.11 ± 0.09^{b}	0.29 ± 0.05^{a}	1.31 ± 0.09^{c}	1.38 ± 0.17^{c}
C15:0	13-methylmyristic	0.25 ± 0.03^{a}	-	0.13 ± 0.02^{b}	-	-	-	-
C16:0	Palmitic	10.7 ± 0.4^{a}	37.1 ± 0.6^{b}	17.2 ± 0.4^{c}	12.1 ± 0.4^{d}	-	23.6 ± 0.5^{e}	11.1 ± 0.2^{a}
C16:0	Dihydrohydnocarpic	-	-	-	5.0 ± 0.2^{a}	-	-	-
C17:0	Margaric	-	-	-	-	0.44 ± 0.06^{a}	-	-
C18:0	Stearic	0.82 ± 0.06^{a}	1.3 ± 0.1^{b}	1.8 ± 0.1^{c}	2.1 ± 0.1^{d}	1.5 ± 0.1^{b}	1.4 ± 0.2^{b}	1.7 ± 0.2^{c}
C18:0	Dihydrochaulmoogric	-	-	-	2.7 ± 0.2^{a}	-	-	-
C22:0	Behenic	-	0.17 ± 0.02^{a}	-	-	-	-	-
C24:0	Lignoceric	-	-	-	-	-	0.31 ± 0.06^{a}	-
Monounsaturated								
C16:1	Gaidic (Δ^{2}, *n*-14)	-	-	-	-	22.9 ± 0.3^{a}	-	3.0 ± 0.1^{b}
C16:1	Palmitoleic (Δ^{9}, *n*-7)	4.9 ± 0.2^{a}	3.0 ± 0.2^{b}	2.4 ± 0.1^{c}	4.2 ± 0.2^{d}	-	6.6 ± 0.3^{e}	-
C16:1	Palmitvaccenic (Δ^{11}, *n*-5)	-	1.6 ± 0.1^{a}	-	-	-	-	-
C17:1	(*Z*)-10-heptadecenoic (*n*-7)	-	-	0.61 ± 0.08^{a}	-	-	9.7 ± 0.3^{b}	-
C18:1	Petroselinic (Δ^{6}, *n*-12)	-	-	-	1.2 ± 0.1^{a}	-	-	-
C18:1	(*Z*)-7-octadecenoic (*n*-11)	-	-	-	-	-	14.4 ± 0.4	-
C18:1	Oleic (Δ^{9}, *n*-9)	0.80 ± 0.05^{a}	3.9 ± 0.2^{b}	11.1 ± 0.2^{c}	1.1 ± 0.1^{d}	10.8 ± 0.1^{e}	-	-
C18:1	(*Z*)-10-octadecenoic (*n*-8)	-	-	-	-	-	-	8.0 ± 0.2^{a}
C18:1	Vaccenic (Δ^{11}, *n*-7)	2.1 ± 0.1^{a}	-	-	2.4 ± 0.2^{b}	14.7 ± 0.2^{c}	4.4 ± 0.3^{d}	-
C18:1	(*Z*)-13-octadecenoic (*n*-5)	-	7.5 ± 0.3^{a}	1.61 ± 0.04^{b}	-	-	-	13.2 ± 0.2^{c}
C18:1	(*E*)-13-octadecenoic (*n*-5)	-	-	-	2.8 ± 0.2^{a}	-	-	-
C20:1	Gondoic (Δ^{11}, *n*-9)	-	-	-	-	-	-	3.3 ± 0.1^{a}
Polyunsaturated								
C16:2	(*Z*,*Z*)-7,10-hexadecadienoic (*n*-6)	-	1.18 ± 0.08^{a}	-	-	-	-	-
C16:3	Roughanic ($\Delta^{7,10,13}$, *n*-3)	-	0.82 ± 0.04^{a}	-	-	-	-	-
C18:2	Linoleic ($\Delta^{9,12}$, *n*-6)	2.98 ± 0.20^{a}	4.97 ± 0.18^{b}	1.65 ± 0.10^{c}	1.16 ± 0.12^{d}	7.45 ± 0.20^{e}	7.72 ± 0.24^{e}	5.47 ± 0.21^{f}
C18:3	γ-Linolenic ($\Delta^{6,9,12}$, *n*-6)	-	-	-	-	-	-	5.43 ± 0.16^{a}
C18:3	α-Linolenic ($\Delta^{9,12,15}$, *n*-3)	-	6.00 ± 0.35^{a}	-	-	12.36 ± 0.24^{b}	12.97 ± 0.21^{c}	7.31 ± 0.22^{d}
C20:3	Dihomo-γ-linolenic ($\Delta^{8,11,14}$, *n*-6)	-	-	0.59 ± 0.04^{a}	-	-	-	-
C20:4	Arachidonic ($\Delta^{5,8,11,14}$, *n*-6)	1.79 ± 0.15^{a}	-	1.79 ± 0.08^{a}	3.83 ± 0.16^{b}	-	-	-
C20:5	Timnodonic (EPA, $\Delta^{5,8,11,14,17}$, *n*-3)	1.89 ± 0.20^{a}	-	1.44 ± 0.05^{b}	1.52 ± 0.12^{b}	3.85 ± 0.07^{c}	5.92 ± 0.27^{d}	4.52 ± 0.21^{e}
Fat		0.55 ± 0.01^{a}	0.75 ± 0.02^{b}	1.44 ± 0.01^{c}	0.57 ± 0.01^{a}	0.83 ± 0.01^{d}	3.07 ± 0.02^{e}	0.33 ± 0.01^{f}
∑SFA		13.9 ± 0.3^{a}	41.9 ± 0.4^{b}	22.5 ± 0.1^{c}	24.1 ± 0.9^{d}	10.8 ± 0.7^{e}	33.0 ± 0.6^{f}	20.3 ± 0.4^{g}
∑MUFA		7.9 ± 0.2^{a}	16.0 ± 0.2^{b}	15.8 ± 0.3^{b}	11.8 ± 0.8^{c}	48.5 ± 0.6^{d}	35.2 ± 0.4^{e}	27.6 ± 0.2^{f}
∑PUFA		6.7 ± 0.5^{a}	13.0 ± 0.6^{b}	5.5 ± 0.2^{c}	6.5 ± 0.4^{a}	23.7 ± 0.4^{d}	26.6 ± 0.3^{e}	22.7 ± 0.1^{f}
Others*		71.5 ± 1.0	29.1 ± 1.2	56.2 ± 0.6	57.6 ± 2.1	17.0 ± 1.7	5.2 ± 1.3	29.4 ± 0.7
∑PUFA *n*-3		1.9 ± 0.2^{a}	6.8 ± 0.4^{b}	1.4 ± 0.1^{c}	1.5 ± 0.1$^{a, c}$	16.2 ± 0.2^{d}	18.9 ± 0.1^{e}	11.8 ± 0.4^{f}
∑PUFA *n*-6		4.8 ± 0.3^{a}	6.2 ± 0.3^{b}	4.0 ± 0.2^{c}	5.0 ± 0.3^{a}	7.4 ± 0.2^{d}	7.7 ± 0.2^{d}	10.9 ± 0.4^{e}
n-6/*n*-3		2.5 ± 0.1^{a}	0.9 ± 0.0^{b}	2.8 ± 0.2^{c}	3.3 ± 0.1^{d}	0.5 ± 0.0^{e}	0.4 ± 0.0^{e}	0.9 ± 0.1^{b}

SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid
Values are expressed as means of three replicate measurements ± standard deviation (fat is expressed on seaweed dry weight basis, while fatty acid is expressed on lipid extract basis)
Values with different letters within a row are significantly different ($P \leq 0.05$) according to ANOVA
(-) not detected, (*) non-identified fatty acids

In the majority of cases ANOVA indicated that each fatty acid was significantly different among the species ($P \leq 0.05$), even among species of the same type (red or brown). Duncan's multiple range tests indicated a minimum of three and a maximum of six homogeneous sub-conjuncts in the cases where a fatty acid was common for six or seven species.

Fatty acid composition of red, brown and green seaweeds collected in Ecuador (Table 1) showed close similarities with values reported for seaweeds from other regions around the world, such as India (Ganesan *et al*. 2020, Manam & Subbaiah 2020), Australia (McCauley *et al*. 2015, Schmid *et al*. 2018), Japan (Susanto *et al*. 2019), Turkey (Caf *et al*. 2019), and Brazil (Santos *et al*. 2019), among others; especially in their total SFA, where palmitic acid has been the main constituent, with concentrations between 10% and 60%. Although palmitic acid has been reported as the main constituent of *P. pavonica* from other regions (El-Maghraby & Fakhry 2015, El-Sheekh *et al*. 2021), this SFA was not found in Ecuadorian *P. pavonica*, which indicates possible environmental effects on biosynthesis or metabolism of palmitic acid by this species. Furthermore, it has suggested that the chemical composition of seaweeds could been affected by the drying methodology besides ecological and seasonal factors (Uribe *et al*. 2019).

13-methylmyristic acid, that was identified in *A. spicifera* and *H. spinella*, is a methyl-branched fatty acid that has exhibited antitumor activity at low concentration (10-25 µg mL^{-1}) against seven carcinoma cell lines and one leukemia cell line (Yang *et al*. 2000), possibly inducing mitochondrial-mediated apoptosis through regulation of the AKT and MAPK pathways (Lin *et al*. 2012, Cai *et al*. 2013). On the other hand, cyclopropane fatty acids found in non-native *K. alvarezii* have been identified in other red macroalgae that belong to the Solieriaceae family (Miralles *et al*. 1990), representing a possible chemotaxonomic feature of this family. However, although this kind of fatty acid can occur naturally in eukaryote organisms, it is more associated with phospholipids of many species of bacteria (Grogan & Cronan 1997).

The MUFAs content of seaweeds collected in Ecuador ranged from 7.86 to 48.47%, and was represented by several isomers of C16:1 and C18:1 acid, being palmitoleic (16:1c9) and oleic (18:1c9) acids the MUFAs found in five of seven species. Some MUFAs, such as palmitoleic and oleic acids, protect human β-cells from the toxic effects of glucose and saturated palmitic acid, allowing apoptosis and promoting β-cell proliferation (Maedler *et al*. 2003). The palmitoleic acid has shown to play an important role on white adipose tissue metabolic and mitochondrial function, suggesting an increase in energy expenditure of adipocytes by triacylglycerol/fatty acids cycle acceleration, fatty acid oxidation and oxygen consumption (Cruz *et al*. 2018). Furthermore, dietary supplemented palmitoleate (C16:1 *n*-7) reduces atherosclerosis development in LDL receptor knock-out mice, and was associated with improvement of lipid and glucose metabolism and favorable changes in regulatory genes involved in lipogenesis and inflammation (Yang *et al*. 2019). While oleic acid has protective effects against palmitic acid-induced apoptotic cell death by restoring the disturbed anti-apoptotic/pro-apoptotic proteins balance toward cell survival in pancreatic AR42J cells (Ahn *et al*. 2013), and has shown great potential to combat the saturated fatty acids induced hepatic lipotoxicity (Chen *et al*. 2018).

Brown seaweeds showed the highest content of PUFAs (23.66-26.61%), followed by green (22.73%) and red (5.47-12.97%). Other studies have also indicated that brown and green seaweeds have a higher content of PUFAs than red algae (Ismail 2017, Schmid *et al*. 2018, Susanto *et al*. 2019). Linoleic (C18:2, *n*-6) acid was found in all seaweeds. This PUFA and its conjugated isomers have been recommended to reduce the risk of coronary heart disease, and have other biological functions, such as anticarcinogenic, antiobesity, antidiabetic and antihypertensive properties (den Hartigh 2019, Marangoni *et al*. 2020). Eicosapentaenoic (EPA or timmodonic, C20:5 *n*-3) acid was found in six seaweeds. It has been shown to be the most effective agent inhibiting glucose-induced cholesterol domain formation (Mason *et al*. 2020), and is recommended for cancer patients for its potential to modulate nutritional status/body composition (Inoue-Yamauchi *et al*. 2018, Aktaş *et al*. 2021), among other benefits. Furthermore, the ratios of total *n*-6/*n*-3 fatty acids were lower than 3.3, which is recommended by the World Health Organization (< 10) in order to prevent inflammatory, cardiovascular and nervous system disorders (Schmid *et al*. 2018, Susanto *et al*. 2019, Zhang *et al*. 2020).

Other authors have also reported and important percentage of non-identified fatty acids in seaweed composition (Ganesan *et al*. 2020), without suggesting any explanation. In this study, these kinds of component were detected at highest time retention (> 55 min), which could imply the presence of very long chain fatty acids or very long chain polyunsaturated fatty acids (with 23 or more carbon atoms in the molecule). These fatty acids seem to play an important role in the development of the organisms (Bach & Faure 2010).

As shown in Table 2, the total content of polyphenol ranged from 147.28 to 1,062.20 mg GAE 100 g^{-1} DW. The highest phenolic content was present in the green seaweed *U. lactuca*, followed by the brown *S. schroederi*, and the red *A. spicifera*. Meanwhile, the lowest content was found in the red seaweed *K. alvarezii*, followed by brown *P. pavonica*. According to ANOVA, there were significant differences between species ($P \leq 0.05$) in their polyphenol content, except for the polyphenol content of the red seaweeds *C. clavulatum* and *H. spinella*, which belong to the same subset according to Duncan's multiple range tests.

Table 2. Total phenolic content and antioxidant capacity of extracts of seaweeds collected in Ecuador / Contenido total de polifenoles y capacidad antioxidante de los extractos de las macroalgas recolectadas en Ecuador

	Seaweed	Total polyphenol content (mg GAE 100 g^{-1} DW)	Antioxidant capacity (mg TE 100 g^{-1} DW)
1	*Acanthophora spicifera*	246.7 ± 3.5[a]	14.2 ± 0.7[a]
2	*Centroceras clavulatum*	206.3 ± 5.2[b]	*
3	*Hypnea spinella*	201.9 ± 3.4[b]	*
4	*Kappaphycus alvarezii*	147.3 ± 2.7[c]	*
5	*Padina pavonica*	184.9 ± 2.0[d]	*
6	*Spatoglossum schroederi*	347.7 ± 2.4[e]	152.6 ± 2.7[b]
7	*Ulva lactuca*	1062.2 ± 19.9[f]	3379.5 ± 41.9[c]

DW: dry weight, GAE: gallic acid equivalents, TE: Trolox equivalent
*<50 mg L^{-1} TE
Values are expressed as mean ± standard deviations, n= 3
Values with different letters within a column are significantly different ($P \leq 0.05$) according to ANOVA

In this study, only the three seaweeds that had the highest content of total polyphenols (*U. lactuca*, *S. schroederi*, *A. spicifera*) showed antioxidant capacity with a similar behavior (Table 1). The green seaweed *U. lactuca* exhibited the highest antioxidant capacity (3379.47 mg TE 100 g^{-1} DW), followed by the brown *S. schroederi* (152.65 mg TE 100 g^{-1} DW), and the red *A. spicifera* (14.18 mg TE 100 g^{-1} DW), while the other organisms did not show sufficient antioxidant capacity.

The total soluble polyphenol content of Ecuadorian seaweeds (Table 2) appear to be slightly different to those reported in other studies, which have indicated that green and red seaweeds have lower content of phenolic compounds compared to brown seaweeds (Gomez-Zavaglia 2019, Peñalver *et al*. 2020, Pereira 2021). The green seaweed *U. lactuca* showed the highest content of phenolic compounds and antioxidant capacity. The potential of this species as a source of antioxidant and antimicrobial agent has already been reported (Alagan *et al*. 2017). The fact that seaweeds with the highest total phenolic content had shown the highest DPPH radical scavenging activity could be due to a positive correlation between them, which has been shown by other studies (Mohamed *et al*. 2018).

Seasonal studies seem to indicate that antioxidant activity may not change throughout seasons, although total phenolic content does (Marinho *et al*. 2019). It is well known that content and diversity of metabolites of seaweed are subject to many abiotic and biotic factors (Marinho *et al*. 2019, Cotas *et al*. 2020), so phenolic content and antioxidant activity could also be affected by several experimental factors, such as drying method, solvent and method of extraction, and analysis technique, among others (Sasadara & Wirawan 2012, Wekre *et al*. 2019, Čagalj *et al*. 2021).

Results suggest that seaweeds collected in Ecuador are a source of nutritional and possible bioactive compounds, and their consumption as food in the human diet might have chemotherapeutic and chemoprotective benefits, as have been suggested by other authors (Ganesan *et al*. 2019, Cotas *et al*. 2021). However, future studies must be carried out to support their pharmaceutical and nutraceuticals properties.

Among saturated acids, methyl-branched and cyclopropane fatty acids seem to be important chemotaxonomic marks of some red seaweeds. Brown and green seaweeds were found to be a better source of UFAs, especially C18:1, C18:2, C18:3 and C20:5 acids. *S. schroederi* and *U. lactuca* seem to be the most interesting species as nutraceutical source, because they showed the highest content of PUFA, especially EPA (*n*-3), a recommended equivalent content of *n*-6/*n*-3 ratio lower than 0.1, and highest content of polyphenols.

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