Artículo Original / Original Article

Health-promoting activities of edible seaweed extracts from Chilean coasts: assessment of antioxidant, anti-diabetic, anti-inflammatory and antimicrobial potential

Actividades promotoras de la salud de extractos de algas comestibles de las costas chilenas: evaluación del potencial antioxidante, antidiabético, antiinflamatorio y antimicrobiano

ABSTRACT

The aim of this study was to evaluate the antioxidant, anti-diabetic, anti-inflammatory and antimicrobial activities of three edible seaweed extracts from Chilean coasts: Pyropia orbicularis, Ulva spp, and Durvillaea antarctica. Seaweed extracts in methanol and 70% acetone were performed to evaluate antioxidant and antidiabetic activities, whereas 60% methanol was used to measure anti-inflammatory and antimicrobial activities. Acetone extracts from D. antarctica had the highest total phenolic content and consequently exhibited the strongest antioxidant activity, while methanol extract of this seaweed presented the highest α-glucosidase inhibition (IC50= 0.004 mg mL^{-1}). In the tests against E. coli and Penicillium sp., the extracts obtained from Ulva spp. were the most effective and exhibited the maximum anti-inflammatory effect against phorbol 12-myristate 13-acetate irritant agent (61.8% inhibition) in mice. Results indicated that all evaluated Chilean seaweed extracts are promising candidates for application in functional foods and in the pharmaceutical industry.
Keywords: Antimicrobial; α- glucosidase; Bioactive compounds; Inflammatory inhibitors; Macroalgae.

Vivian García[1], Elsa Uribe[1,2], Antonio Vega-Gálvez[1]*,
Carla Delporte[3], Gabriela Valenzuela-Barra[3],
Jéssica López[4], Alexis Pastén[1].

1. Departamento de Ingeniería en Alimentos.
Universidad de La Serena, La Serena, Chile.
2. Instituto de Investigación Multidisciplinario en Ciencia y Tecnología,
Universidad de La Serena, La Serena, Chile.
3. Laboratorio de Productos Naturales, Facultad de Ciencias Químicas y
Farmacéuticas, Universidad de Chile, Santiago, Chile.
4. Escuela de Alimentos, Pontificia Universidad Católica de Valparaíso,
Valparaíso, Chile.

*Corresponding autor: Antonio Vega-Gálvez,
Food Engineering Department, Universidad de La Serena,
Av. Raúl Bitrán 1305, La Serena, Chile.
E-mail: avegag@userena.cl

RESUMEN

El objetivo de este estudio fue evaluar las actividades antioxidantes, antidiabéticas, antiinflamatorias y antimicrobianas de los extractos de tres algas marinas comestibles de las costas Chilenas (Pyropia orbicularis, Ulva spp. y Durvillaea antarctica). Se realizaron extractos de algas marinas en metanol y acetona al 70% para evaluar las actividades antioxidantes y antidiabéticas, mientras que el metanol al 60% se usó para actividades antiinflamatorias y antimicrobianas. Los extractos de acetona de D. antarctica tuvieron el mayor contenido de fenoles totales (TPC) y, en consecuencia, exhibieron la mayor actividad antioxidante, mientras que el extracto metanólico de estas algas presentó la mayor inhibición de la α-glucosidasa (IC50= 0,004 mg mL^{-1}). En las pruebas contra E. coli y Penicillium sp., los extractos obtenidos de Ulva spp., actuaron como los más efectivos y exhibieron el máximo efecto antiinflamatorio contra el agente irritante de forbol 12-miristato 13-acetato

(TPA) (inhibición del 61,8%) en ratones. Por lo tanto, los resultados indican que todos los extractos de algas chilenas evaluados pueden ser candidatos prometedores para su aplicación en alimentos funcionales y en las industrias farmacéuticas.
Palabras clave: Antimicrobianos; α- glucosidasa; Compuestos bioactivos; Inhibidores inflamatorios; Macroalgas.

INTRODUCTION

Globally, demand for contaminant-free seaweed for use in developing functional foods and pharmaceuticals appears to be increasing. This could be attributed to the richness of compounds that seaweeds have with applications for both food and health[1]. These compounds of interest include pigments,

lipids, polysaccharides, fatty acids and sterols, proteins and peptides, as well as many secondary metabolites such as mycosporine-like amino acids, phenolic compounds and terpenes, all of which are highly specific to different algal groups and even to species within these[2]. There is substantial evidence for the health benefits of algal-derived compounds[3]. For example, phlorotannins from the brown seaweed, *Eisenia arborea*[4], or pheophytin-a from the green seaweed, *Enteromorpha prolifera*[5], exhibited a significant suppression against mouse ear edema induced by several inflammatory inducers. Consequently, these compounds could provide protective effects over the pathogenesis of inflammatory diseases. Sanz-Pintos et al[6] analyzed the macromolecular antioxidants (or non-extractable polyphenols) of six edible seaweeds from Chile. They reported that this fraction possesses on average 42% of total polyphenol content and a remarkable antioxidant capacity. The efficacy of brown seaweed extracts (*F. vesiculosus* and *P. canaliculata*) to inhibit enzymes involved in intestinal carbohydrate digestion and assimilation have also been shown. Moreover, these algal extracts may also have a functional potential for use in food aimed at lowering glycemic response[7]. On the other hand, a broad spectrum of *in vitro* antibacterial activity has been associated with phenolic compounds from algae, specifically against *Staphylococcus aureus* and *Bacillus* spp[8]. Considering the extensive data that is available on the bioactivities of different seaweed compounds, it would be interesting to research Chilean species, given that local conditions at the harvesting site such as light, nutrient, and temperature regimes considerably impact metabolite levels and thus bioactive composition[2]. Despite the fact that Chile has a great variety of seaweeds, especially edible kinds, Chilean seaweed has been little exploited. Most research about Chilean seaweed has focused on nutritional aspects[9,10,11] or selected chemical compounds of interest[6,12,13,14,15]. No previous study has investigated the bioactivities of Chilean seaweed extracts. Hence, this study aimed to obtain extracts from three edible Chilean seaweeds (*Pyropia orbicularis*, Ulva spp. and *Durvillaea antarctica*) and assess antioxidant, anti-diabetic, anti-inflammatory and antimicrobial activities.

Materials and methods
2.1 Seaweed harvest and sample preparation
Fresh red, green and brown seaweeds, *Pyropia orbicularis*, *Ulva* spp. (*Ulva lactuca* Linnaeus and *Ulva rigida* Agardh) and *Durvillaea antarctica*, respectively, were harvested between April and October 2017 in the Coquimbo Region of Chile. Pyropia orbicularis (Tongoy coast: 30°15´27´´S and 71°20´33´´W); Ulva spp. (Guayacan: 29°58´50´´S and 71°23´00´´W) and *Durvillea antartica* (Puerto Aldea: 30°24´39´´S and 71°63´03´´W). Seaweeds were selected by visual inspection according to color and absence of foreign matter and transported to the laboratory in a cooler. Then, seaweeds were washed with distilled water to remove epiphytes, sand and debris and immediately placed (in freeze drying trays) in a -80 °C freezer for 24 h, a batch

was then placed horizontally onto three drying trays with a charge density of 2.19 kg m^{-2} and dried in a freeze dryer (VirTis Wizard 2.0 Advantage Plus XL-70, Gardiner, NY, USA) with 0.027 KPa pressure for 68 h. The freeze-dried samples were ground to powder using a basic analytical mill (IKA A-11, USA) and passed through a 35-mesh sieve of 500 μm (U.S. Standard Sieve Series, Dual Manufacturing Co., USA). Powdered samples were sealed and stored in plastic bags at 5 °C until further analysis.

2.2 Seaweed extraction procedure
P. orbicularis, Ulva spp. and *D. antarctica* extracts were obtained using pure methanol and a mixture of acetone/ water (70:30, v/v) as reported previously[12,16] due to their effectiveness in generating antioxidant rich extracts. Briefly, powdered freeze-dried seaweeds were thoroughly mixed with solvents at a ratio of 1:10 w/v in an orbital shaker (Boeco, OS20, Germany) at room temperature and 200 rpm for 24 h. Extractions were filtered through filter paper #1 into 250 mL round bottom flasks. Solvents were removed using a rotary evaporator (Büchi R-210, Flawil, Switzerland) with the water-bath set at 40 ºC. Dried residues were resuspended in 10 mL of pure methanol or a mixture of acetone/water (70:30, v/v). Extracts were obtained in triplicate, stored at -80 ºC and then used to measure total phenolic content (TPC), antioxidant capacity and α-glucosidase inhibitory activity.

2.3 Determination of total phenolic content (TPC)
TPC in the seaweed extracts was determined using a spectrophotometric method using Folin-Ciocalteau reagent[12]. The absorbance of the produced blue color was measured at 750 nm, using a spectrophotometer (Spectronic 20 Genesys, NY, and USA). Results of TPC were expressed, based on a standard curve of gallic acid (Merck, Germany), as mg gallic acid equivalent (GAE) per 100 grams of dry matter (d.m.). All measurements were performed in triplicate.

2.4 DPPH (2,2-diphenyl-1-picryl-hydrazyl) assay
The DPPH assay was performed by preparing a solution of 50 μM DPPH in methanol or acetone/water (70:30). One hundred microliters of each seaweed extract were allowed to react with 3.9 mL of the DPPH solution for 30 min under dark conditions. The absorbance was read at 517 nm and results were expressed as μmol Trolox equivalents (TE) per 100 g d.m.

2.5 ORAC (oxygen radical absorbance capacity) assay
The ORAC assay was carried out according to Zhang et al[17] in a Victor[3] Multilabel Plate Reader (Perkin–Elmer, Turku, Finland). To each well of a polystyrene 96-well microplate (OptiPlateTM-96 F HB, Perkin–Elmer, Turku, Finland), 40 μL of each seaweed extract produced with methanol or acetone/water (70:30) and resuspended in phosphate buffer pH 7.4 (see Section 2.1) were mixed with 200 μL of fluorescein (100 μmol L^{-1}) prepared in phosphate buffer (75 mmol L^{-1}, pH 7.4) and incubated for 20 min at

37 °C. Then, 35 µL of AAPH (0.36 mol L^{-1}) (2,2'-azobis (2-amidinopropane) dihydrochloride) was added to start the reaction. Fluorescence was read every 60 s (λex= 485 nm and λem= 535 nm), until fluorescence reading had declined to less than 5% of the initial value. Final ORAC values were calculated as µmol TE per 100 g d.m., using the regression equation between Trolox concentration and net area under curve.

2.6 α-glucosidase activity assay

Seaweed extracts obtained as described in Section 2.1 and their effects on α-glucosidase activity were measured in a range of varying concentrations, according to Lordan et al[7], slightly modified. A volume of 50 µL of extract solution and 100 µL α-glucosidase from *Saccharomyces cerevisiae* (Sigma G5003, USA) solution (0.5 U mL^{-1}) in 0.1 M sodium phosphate buffer (pH 6.9) was mixed in a 96-well microplate and incubated at 20 °C for 10 min. Phosphate buffer containing 50 µl of 4-nitrophenyl α-d-glucopyranoside (Sigma N1377, Switzerland) was then added to each well. Absorbance at 405 nm was recorded every 30 s for 10 min using a Multilabel Plate Reader (Perkin–Elmer, Victor3, Turku, Finland) set to 20 °C. α-glucosidase activity was calculated as the percentage obtained through the slope of each exponential curve. Inhibitory activity (%) against α-glucosidase vs seaweed extracts was also plotted. An exponential model was used to fit data so that IC50 was calculated as the concentration of seaweed extract required to produce 50 % α-glucosidase inhibition.

2.7 Determination of anti-inflammatory and antimicrobial activity

2.7.1 Preparation of seaweed extracts

Powdered freeze-dried seaweed (10.0 g) was extracted with 60% methanol (100 mL) in an orbital shaker (Boeco, OS20, Germany) set at 200 rpm at room temperature for 24 h and then filtrated. After filtration, methanol extracts were evaporated in a rotary evaporator (Büchi R-210, Flawil, Switzerland) with a water-bath set at 40 ºC, after which, all extracts were freeze-dried. 60% methanol was used in accordance with a previous study where the antimicrobial activity of extracts from brown seaweeds obtained with water, methanol and some mixtures (20, 40, 60 and 80%) as solvents were compared. That study showed that a higher antimicrobial activity was obtained by 60% methanol compared to other solvents[18].

2.7.2 Anti-inflammatory activity

Anti-inflammatory activity measurement of seaweed extracts was carried out according to Miño et al[19] with some modifications. Adult male CF-1 mice (20–25 g) obtained from the stock at the Chilean Public Health Institute (ISP) were used. Dried seaweed methanol extracts (SME) (dissolved in acetone) were applied topically to the inner and outer surfaces of the right ear of each mouse at different doses (3.0, 1.5 and 0.75 mg/ear). Likewise, 10 µL

of a solution of phorbol 12-myristate 13-acetate (TPA; 5 µg in 20 µL acetone) or arachidonic acid (AA; 2 mg in 20 µL acetone) were applied immediately after SME application. Negative control mice received only TPA or AA at the same concentration (100% of inflammation). Left ear received only acetone. Indomethacin (0.5 mg/ear/20 µL) was used as reference drug for TPA and nimesulide (1.0 mg/ear/20 µL) for AA, respectively. After 6 h (TPA administration) or 1 h (AA administration), the animals were sacrificed by CO$_2$ asphyxiation. Disks of 6 mm diameter were removed from each ear and weight was determined. Edema was measured as the difference of the weights between the punches from right and left ears of the negative control and the treated animal groups, respectively. The experiments were performed in accordance with current "Guidelines on the care and use of animals for scientific purpose" and approved by the Animal Care and Use Committee of the Facultad de Ciencias Químicas y Farmacéuticas (University of Chile and ISP) (code of approval CICUAL-ISP-19062018).

2.7.3 Antimicrobial activity
2.7.3.1 Microorganisms and growth conditions

Seaweed methanol extracts were tested for antimicrobial activity against four microorganisms, *Staphylococcus aureus* (ATCC 25923) (Gram-positive), *Escherichia coli* (ATCC 25922) (Gram-negative), *Saccharomyces cereceviceae* (yeast) and *Penicillium* sp. (fungus). Microbial strains were maintained as 20% glycerol stocks at -80 °C in a nutrient broth (Difco), in the Microbiology Laboratory, Department of Food Engineering (University of La Serena, Chile). Prior to the experiments, cultures were transferred to solid or liquid media. The bacterial and fungi strains were aerobically grown in 5 mL of Müeller Hinton Broth (MHB, Merck) for 48 h, continuously shaken at 120 rpm at 37 °C. Cultures were then subcultured in tryptone soy broth (TSB, Difco) incubated for 12 to 24 h and used as the source of inoculum for each experiment. The bacterial and fungal suspensions for inoculation were adjusted to 0.5 McFarland standard turbidity[13].

2.7.3.2. Minimal inhibitory concentration (MIC) determination

Antimicrobial activity of the seaweed methanol extracts was determined using the tube broth dilution assay[20]. A serial two-fold dilution ranging from 0.25 to 1000 mg mL^{-1} of extracts were prepared. Two milliliters of each dilution were incubated with 2 mL of MHB and an inoculum of 10 µL (106CFU mL^{-1}) at 37 °C for 24 h and at 30 °C for 48 h, bacteria and fungus, respectively. MIC was determined by observing the lowest concentration of extract that inhibited visual bacterial growth. All assays were performed in triplicate and the results expressed in mg mL^{-1}.

2.7.3.3. Minimum bactericidal concentration (MBC) determination

MBC was determined by inoculation of one aliquot of the last tube showing visible turbidity and negative tubes (absence of turbidity in MIC determination) on Mueller Hinton agar,

incubated for 24 h at 37 ºC for bacteria and 48 h at 30 ºC for fungus, using the spread plate technique. Plates showing no growth on the Mueller Hinton agar indicated bactericidal effect of the extracts. The number of individuals in plates showing growth were counted manually. MBC was defined as the lowest concentration of each extract that completely prevented microbial growth on plates without the presence of other antimicrobial agents. All assays were performed in triplicate and the results expressed in mg mL^{-1}.

2.8 Statistical analysis

The experiments were conducted in triplicate and the data points are mean values ± standard deviation (SD). When the statistical distribution was normal, one- or two-way ANOVA followed by Tukey's multiple comparisons test were conducted. When the statistical distribution was not normal, the Kruskal-Wallis non-parametric test was applied. Mann Whitney test was used for the individual comparisons. Significant differences was defined at the 5% level (p < 0.05) for all tests. The software employed for statistical analysis was Statgraphics Centurion XVI (Statistical Graphics Corp., Herdon, USA).

RESULTS
3.1 Total phenolic content and antioxidant activity of seaweed extracts

Table 1 presents the total phenolic content (TPC) and antioxidant activity from *P. orbicularis*, *Ulva* spp. and *D. antarctica* extracts obtained by two solvents, methanol and a mixture of acetone/water (70:30, v/v). Significant differences in TPC were found between seaweed species and between

solvents used for extraction (p<0.05). TPC ranged from 74.9 to 254 mg GAE 100 g^{-1} d.m. in methanol seaweed extracts and from 165 to 554 mg GAE 100 g^{-1} d.m. in 70% acetone extracts.

Furthermore, the same table shows the antioxidant activity measured by the DPPH and ORAC assays, where *Durvillea Antarctica* showed greater activity with 566 ± 5 µmol TE 100 g^{-1} d.m. for methanol and 673 ± 2 µmol TE 100 g^{-1} d.m. for acetone 70%. This trend is similar for other results. ORAC values of 5501 ± 1 and 8808 ± 2 µmol TE 100 g^{-1} d.m. for methanol and acetone 70%, respectively.

3.2. α-glucosidase activity of the seaweed extracts

The comparison of α-glucosidase activity of methanol and 70 % acetone seaweed extracts are shown in Figure 1. All seaweed extracts obtained with both solvents affected α-glucosidase activity in a dose-dependent manner, except for P. orbicularis methanol extract that did not present activity (Figure 1A). Nevertheless, the methanol extracts from *Ulva* spp. and *D. antarctica* showed better α-glucosidase activities than aqueous acetone extracts. As a result, these methanol extracts completely suppress the activity of the enzyme at much lower concentrations than aqueous acetone extract (Figure 1B and C). As shown in table 2, *D. antarctica* methanol extract possessed the highest α-glucosidase inhibition with the lowest IC50 value detected at 0.004 g mL^{-1}, which could be the result of a higher content of phytochemical constituents of brown seaweed as compared to red and green ones.

3.3. Anti-inflammatory activity of seaweed extracts

The anti-inflammatory activity of the SME was evaluated by the method of ear edema in mice induced

Table 1. Total phenolic content (TPC) and antioxidant capacity of methanol and aqueous acetone extracts of three edible Chilean seaweeds.

Parameters	Solvent	Seaweeds		
		Pyropia orbicularis	*Ulva spp.*	*Durvillea antarctica*
TPC,	Methanol	174 ± 2.42[by]	74.9 ± 1.89[bz]	254 ± 1.58[bx]
(mg GAE 100 g^{-1} d.m)	Acetone:water (70:30)	276 ± 2.84[ay]	165 ± 4.10[az]	554 ± 2.84[ax]
DPPH,	Methanol	79.8 ± 5.77[bz]	134 ± 2.90[by]	566 ± 4.76[bx]
(µmol TE 100 g^{-1} d.m)	Acetone:water (70:30)	221 ± 4.71[az]	258 ± 2.23[ay]	673 ± 2.06[ax]
ORAC,	Methanol	3958 ± 4.54[by]	1937 ± 2.76[bz]	5501 ± 1.00[bx]
(µmol TE 100 g^{-1} d.m)	Acetone:water (70:30)	4630 ± 0.28[ay]	3286 ± 3.65[az]	8808 ± 2.43[ax]

Data are expressed as mean ± standard deviation. [a–b] Different superscript numbers in the same column indicate significant difference (p<0.05) and [x–z]different superscript letters in the same row indicate significant difference (P<0.05).
2,2-diphenyl-1-picrylhydrazyl (DPPH); Radical Absorbance Capacity (ORAC); Gallic acid equivalents (GAE); Trolox equivalents (TE).

by topical administration of two irritant agents, phorbol 12-myristate 13-acetate (TPA) and arachidonic acid (AA). The SME (3.0, 1.5 and 0.75 mg/ear), indomethacin (0.5 mg/ear; positive control to TPA) and nimesulide (1.0 mg/ear; positive control to AA) were applied along with the irritant substances. Results showed that extracts from *Ulva* spp. and *P. orbicularis* significantly reduced (p<0.05) mice ear edema induced by both irritant agents in a dose-dependent manner (Table 3). The highest dose (3 mg/ear) of the extracts from *Ulva* spp. and *P. orbicularis* exhibited the maximum anti-inflammatory effect against TPA (61.8% inhibition) and AA (57.2% inhibition), respectively, comparable even using a reference drug in AA inflammatory model (nimesulide: 50.0% inhibition). In contrast, *D. antarctica* extract (3 mg/ear) was able to produce only a slight anti-inflammatory activity (22.9% inhibition) against TPA-induced mice ear edema model and was ineffective in the AA-induced mice ear edema model (Table 3).

3.4. Antimicrobial activity of seaweed extracts

Table 4 shows the results obtained for the minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for *P. orbicularis*, *Ulva* spp. and *D. antarctica* extracts against *S. aureus*, *E. coli*, *S. cereceviceae* and Penicillium sp.

All tested extracts showed significant antimicrobial activity. No differences were observed among the MIC and MBC values obtained for the different seaweed extracts tested against *S. aureus* with values 250 mg mL^{-1} and values <1000 mg mL^{-1} for MIC and MBC, respectively. Similarly, differences were observed for *S. cereceviceae* with 125 mgmL^{-1} and <1000 mg mL^{-1} for MIC and MBC, respectively. In the tests against *E. coli* and Penicillium sp, the extracts obtained from *Ulva* spp. were more effective when compared to the other seaweed extracts since these presented lower MIC values. However, when assessing MBC, both *Ulva* spp. and *D. antarctica* extracts showed the best values against Penicillium sp. (250 mg mL^{-1}).
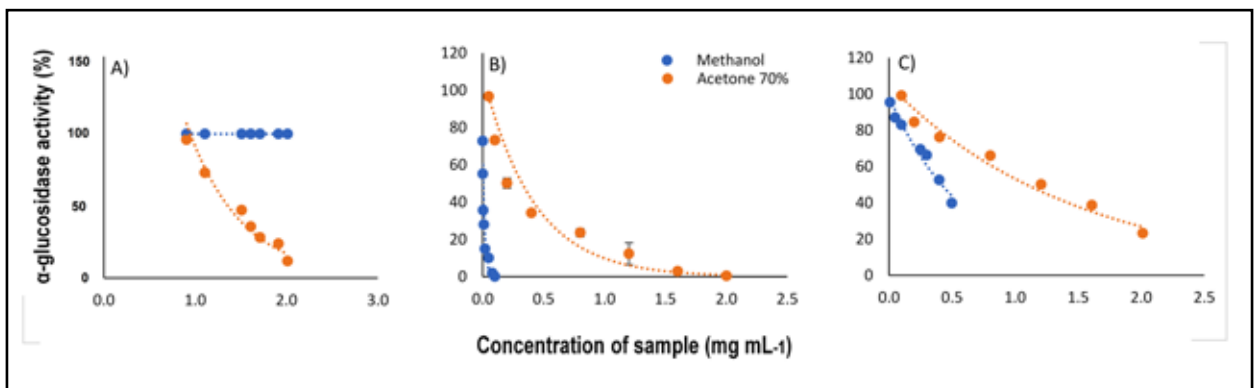

Figure 1: α-glucosidase activity of the methanol extracts and aqueous acetone of three edible Chilean seaweeds. A) Pyropia orbicularis; B) Ulva spp.; C) Durvillea antarctica. Values are averages (n= 3), error bars are standard deviations.

Table 2. IC50 values of α -glucosidase inhibitory activity of methanol and aqueous acetone extracts of three edible Chilean seaweeds.

Parameter	Solvent	Seaweeds		
		Pyropia orbicularis	*Ulva* **spp.**	*Durvillea antarctica*
IC 50 (g mL^{-1})	Methanol	ND	0.416 ± 0.005[by]	0.004 ± 0.000[bx]
	Acetone:water (70:30)	1.34 ± 0.010[ax]	1.09 ± 0.038[ay]	0.324 ± 0.003[az]

Data are expressed as mean ± standard deviation.
[a–b] Different superscript numbers in the same column indicate significant difference (p<0.05) and [x-z] different superscript letters in the same row indicate significant difference (P<0.05). ND: Not detected.

Table 3. Topical anti-inflammatory activity of the methanol extracts of three edible Chilean seaweeds against phorbol 12-myristate 13-acetate (TPA) and arachidonic acid (AA) - induced inflammation of mice ear edema.

Seaweeds	Topical anti-inflammatory effects		
	Dose (mg/ear)	%EA$_{TPA}$ ± SEM	%EA$_{AA}$ ± SEM
Ulva spp.	3.0	↑61.8 ± 6.1[*, α]	↑37.7 ± 5.0[*, α]
Ulva spp.	1.5	45.0 ± 7.1[*, b, α]	24.6 ± 3.8[*, b,α]
Ulva spp.	0.75	25.3 ± 7.5[*, a, α]	5.1 ± 8.6[a, α]
Pyropia oricularis	3.0	↑35.4 ± 3.9[*, b, α]	↑57.2 ± 6.1[*, c]
Pyropia oricularis	1.5	22.2 ± 6.7[*, a, α]	23.7 ± 8.9[*, b, α]
Pyropia oricularis	0.75	10.4 ± 4.7[*, α]	7.2 ± 7.0[*, a , α]
Durvillea antarctica	3.0	22.9 ± 3.9[*, a, α]	1.3 ± 8.3[a, α]
IND	0.5	↑92.9 ± 13.2[*]	n.d
NIM	1.0	n.d	50.0 ± 8.5[*, c]

↑maximum effect; EA$_{TPA}$: topical anti-inflammatory effect against phorbol 12-myristate 13-acetate; EA$_{AA}$: topical anti-inflammatory effect against arachidonic acid; IND: indomethacin; NIM: nimesulide; n.d: not determined. An asterisk (*) denotes significant differences (p<0.05) between samples respect to the negative control (100% of inflammation); [a-c]values in the same column with different superscripts indicate significant differences (p<0.05) for each sample. Alpha (α) represents significant differences between the samples and the reference drug (NIM or IND).

Table 4. Minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of methanol extracts of three edible Chilean seaweeds against gram-positive and gram-negative bacteria, yeast and mold.

Microorganisms	Parameter	Seaweeds		
	(mg mL^{-1})	*Pyropia orbicularis*	*Ulva* spp.	*Durvillea antarctica*
Staphylococcus aureus	MIC	250	250	250
	MBC	< 1000	< 1000	< 1000
Escherichia coli	MIC	500	250	500
	MBC	< 1000	< 1000	< 1000
Saccharomyces cereceviceae	MIC	125	125	125
	MBC	< 1000	< 1000	< 1000
Penicillium sp.	MIC	250	125	250
	MBC	< 1000	250	250

DISCUSSION

As shown in table 1, aqueous acetone was the most efficient solvent to extract TPC from the three kinds of edible seaweeds evaluated. This is due to the fact that polar solvents efficiently extract polar compounds, such as polyphenols bound to sugars or proteins, phlorotannins, glycosides and organic acids[18,21]. Farah Diyana et al[22] also reported higher TPC values in the extracts obtained with 70% acetone than with methanol of two red seaweed species, *K. alvarezii* and *K. striatum*. Cian et al[23] found that the TPC of the 80% acetone and methanol extracts of *P. columbina* extracts were approximately 50 and 30 mg

GAE 100 g⁻¹ dry algae, respectively. It has been suggested that acetone has the ability to inhibit protein–polyphenol complex formation during extraction and breaking down hydrogen bonds formed between phenolic group and protein carboxyl group, resulting in a higher amount of polyphenols extracted[18,21].

Among the evaluated seaweeds, *D. antarctica* showed the highest TPC level followed by *P. orbicularis* and *Ulva* spp. for both types of extracts (Table 1). This agrees with a previous study by Garcia-Casal et al[24], that reported lower TPC value in *Ulva* spp. extract compared to red and brown seaweeds. Green seaweed is known to produce chemicals similar to those of red seaweed but lack the same degree of halogenation[2]. It has been previously reported that brown seaweeds present higher levels of TPC than green and red ones[21,25]. This might be due to the higher content of polyphenol phlorotannin compounds that are exclusively found in brown algae[26], which ranges from 5 to 15% (dry weight)[1].

Indeed, phlorotannins have been reported to have a strong antioxidant activity by preventing oxidative stress–induced cell damage in lung fibroblast cells[3]. Our data indicate that the highest antioxidant activity was determined in the 70% acetone extract from *D. antarctica* measured by both assays (DPPH and ORAC) (Table 1), suggesting that phenols present in extracts of *D. antarctica* may be the main constituents responsible for antiradical properties of these extracts[26]. However, other classes of antioxidant compounds such as fucoxanthin and sterols could be partially and simultaneously extracted with this solvent and contribute to the overall antioxidant activity of brown seaweed[21].

Finally, our results were in agreement with others in which high antioxidant activity (by ORAC assay) was reported for 70% acetone extracts of brown seaweed species, including *F. vesiculosus* (2567 µmol TE g⁻¹ extract), *F. serratus* (2545 µmol TE g⁻¹ extract), *A. nodosum* (1417 µmol TE g⁻¹ extract), *L. hyperborea* (975 µmol TE g⁻¹ extract) and *D. antarctica* (8278 µmol TE 100 g⁻¹ d.m.)[12,21].

In this investigation, the activity of α-glucosidase was studied against different concentrations of the extracts with methanol and acetone 70% of three algae because the use of α-glucosidase activity suppressors could help to prevent diabetes as it suppresses carbohydrate digestion, thus delaying the process of glucose assimilation and resulting in a significant reduction of postprandial plasma glucose level[7,27]. The results support the findings of Lordan et al[7], who studied several brown, red and green edible seaweed species. They found that brown seaweed extracts, in particular *Fucus vesiculosus* Linnaeus, was amongst the most potent seaweed extract for α-glucosidase inhibition, exhibiting IC50 values of 0.32 (cold water extract) and 0.49 g mL⁻¹ (ethanol extract). Several *in vitro* studies have related this inhibitory activity with phlorotannins such as phloroglucinol, dioxinodehydroeckol, eckol, dieckol, fucodiphloroethol,

which are abundant in brown seaweeds[16,28] or bromophenols such as 2,4,6-tribromophenol, 2,4-dibromophenol, bis (2,3,6-tribromo-4,5-dihydroxyphenyl) methane, which are present in green and red seaweeds[29] or other polyphenols, such as chlorogenic acid[30]. The ability of polyphenols to inhibit certain enzymes is related to their interaction with proteins or polysaccharides (mainly in processed foods)[27].

Inflammation is a complex process usually involving the release of several mediators such as prostaglandins (PGs), histamine, thermo-attractants, cytokines and proteinases[31]. For the *in vivo* topical test, two inflammatory agents, TPA and AA, were used because TPA induces longer-lived skin edema associated to protein kinase C activation with the subsequent nuclear factor kappa B (NF-κB) activation[19,32]. This transcription factor promotes the over production of inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukins (IL-1b and IL-6) and cyclooxygenase 2 (COX-2)[33,34]. In contrast, inflammation induced by an AA irritant agent is related to increased activity of myeloperoxidase and elastase[32]. It has been established that this agent generates a rapid onset of short-lived edema associated with increases in prostaglandins (PGs), thromboxane TXB2 and leukotriene LTB4 with leukocyte extravasation[35].

In this study, the extract from *Ulva* spp. at different doses tested on AA-induced mouse ear edema tended to be weaker than that of TPA-induced mouse ear edema. On the other hand, the extract from *D. antarctica* was not able to inhibit the edema induced by AA, probably because the extract acts before AA metabolism[19]. Only extract obtained from *P. orbicularis* (3.0 mg/ear) produced an inhibition of AA edema in a much higher degree than the one obtained in the TPA (Table 3), suggesting an interference in AA metabolism[36].

In a previous study, in Chilean seaweeds extracts, the presence of terpenoids (pacifenol, stypotriol triacetate and epitaondiol)[37] was identified. They showed that these compounds possess anti-inflammatory actions, including mice ear edema attenuation induced by TPA. Terpenoid compounds have been established to possess an anti-inflammatory effect by inhibiting the phospholipase A2 and blocking arachidonic acid metabolism[31]. In other studies, the presence of other bioactive compounds were reported such as pheophytin from *Enteromorpha prolifera*[5], sterols (delta(5,7)-sterols, delta(5,7,9(11))-sterols among others) from Chlorella vulgaris[38] and phlorotannins (eckol, 8,8'-bieckol, phlorofucofuroeckol-A and -B) from Eisenia arborea[4] which also were effective against mouse ear inflammation induced by inflammatory inducers, especially TPA. Based on these results, the topical anti-inflammatory activity of our seaweed extracts could be attributed to the different bioactive compounds present in them, which should be investigated in subsequent studies.

This study demonstrated that all algae studied have an antimicrobial effect. However, MIC and MBC values

were higher than those reported in others studies. For example, Devi et al[39] evaluated the antimicrobial potential of *Haligra* sp. seaweed extracted with methanol and reported stronger bactericidal effect against *S. aureus* compared to a standard antimicrobial agent (sodium benzoate). The study reported MBC values of 7.5 mg mL^{-1} for seaweed extract and 30.0 mg mL^{-1} for sodium benzoate in Mueller Hinton agar, respectively. Wang et al[40] evaluated the antimicrobial activity of phlorotannins present in *Ascophyllum nodosum* and reported that the growth of *E. coli* O157:H7 was successfully inhibited, exhibiting a MIC of 0.025 mg mL^{-1}. El Shafay et al[41] assessed the antimicrobial potential of four red seaweeds against a wide range of gram positive and gram-negative bacteria, including *E. coli* and *S. aureus*. According to the study of El Shafay et al[41], the MICs of all extracts studied (diethyl ether, methanol, ethanol and chloroform extracts) ranged between 50 and 100 mg mL^{-1}.

The aforementioned differences are probably accounted for by the different strains used in the determination of antimicrobial activity, different extraction techniques, and different seaweed composition used in each assay[8]. One explanation may be that, in the literature, the antimicrobial power of algae is defined by a wide range of compounds which depend on the type of extraction and the solvent of choice. Among the compounds mentioned in the literature with antimicrobial activity are proteins, polysaccharides, amino acids and polyunsaturated fatty acids (PUFAs). Seaweeds are often rich sources of PUFAs, especially high in *P. orbicularis* (52.63 g 100 g^{-1} of total fatty acid)[13], *Ulva* spp. (41.94 g 100 g^{-1} of total fatty acid) 14 and *D. antarctica* (41.94 g 100 g^{-1} of total fatty acid)[15]. According to the review of Pina-Pérez et al, the mechanism of action of fatty acids as antimicrobials may be due to cell leakage derived from membrane damage.

CONCLUSIONS

In conclusion, all extracts from Chilean seaweed species (*P. orbicularis, Ulva* spp. and *D. antarctica*) presented different levels of antioxidant, anti-diabetic, anti-inflammatory and antimicrobial activities. The results revealed that *D. antarctica* aqueous acetone extract contained the highest TPC level and had the greatest antioxidant activity; *D. antarctica* extract in methanol was the most potent for α-glucosidase inhibition. In tests against *E. coli* and Penicillium sp., the extracts obtained from *Ulva* spp. were more effective when compared to the other seaweed extracts and showed the maximum anti-inflammatory effect against TPA. However, future studies should identify the bioactive compounds present in these seaweed extracts and determine the exact constituents responsible for such noteworthy activities.

REFERENCES

1. Agregán R, Munekata P.E.S, Franco D, Dominguez R, Carballo J, Lorenzo J.M. Phenolic compounds from three brown seaweed species using LC-DAD–ESI-MS/MS. *Food Res Int.* 2017; 99: 979-985.

2. Stengel DB, Connan S. Natural products from marine algae: methods and protocols. In Walker JM, Ed. Marine algae: a source of biomass for biotechnological applications. New York: Springer Science+Business Media. 2015; p. 1-30.

3. Bocanegra A, Bastida S, Benedí J, Ródenas S, Sánchez-Muniz FJ. Characteristics and nutritional and cardiovascular-health properties of seaweeds. *J Med Food.* 2009; 12: 236-258.

4. Sugiura Y, Tanaka R, Katsuzaki H, Imai K, Matsushita T. The anti-inflammatory effects of phlorotannins from Eisenia arborea on mouse ear edema by inflammatory inducers. *J Funct Foods.* 2013; 5: 2019-2023.

5. Okai Y, Higashi-Okai K. Potent anti-inflammatory activity of pheophytin aderived from edible green alga, Enteromorpha prolifera (Sujiao-nori). *Int J Immunopharmacol.* 1997; 19: 355-358.

6. Sanz-Pintos N, Pérez-Jiménez J, Buschmann AH, Vergara-Salinas JR, Pérez-Correa JR, Saura-Calixto F. Macromolecular antioxidants and dietary fiber in edible seaweeds. *J Food Sci.* 2017; 82: 289-295.

7. Lordan S, Smyth TJ, Soler-Vila A, Stanton C, Ross RP. The α-amylase and α-glucosidase inhibitory effects of Irish seaweed extracts. *Food Chem.* 2013; 141: 2170-2176.

8. Pina-Pérez MC, Rivas A, Martínez A, Rodrigo D. Antimicrobial potential of macro and microalgae against pathogenic and spoilage microorganisms in food. *Food Chem.* 2017; 235: 34-44.

9. Ortiz J, Romero N, Robert P, Araya J, López-Hernández J, Bozzo C, et al. Dietary fiber, amino acid, fatty acid and tocopherol contents of edible seaweeds Ulva lactuca and Durvillaea antarctica. *Food Chem.* 2006; 99: 98-104.

10. Astorga-España MS, Mansilla A. Sub-Antarctic macroalgae: Opportunities for gastronomic tourism and local fisheries in the Region of Magallanes and Chilean Antarctic Territory. *J Appl Phycol.* 2014; 26: 973-978.

11. Astorga-España MS, Rodríguez-Galdón B, Rodríguez-Rodríguez EM, Díaz-Romero C. Amino acid content in seaweeds from the Magellan Straits (Chile). *J Food Compos Anal.* 2016; 53: 77-84.

12. Uribe E, Vega-Gálvez A, Vargas N, Pásten A, Rodríguez K, Ah-Hen KS. Phytochemical components and amino acid profile of brown seaweed Durvillaea antarctica as affected by air drying temperature. *J Food Sci Technol.* 2018; 55: 4792-4801.

13. Uribe E, Vega-Gálvez A, García V, Pastén A, Rodríguez K, López J, et al. Evaluation of physicochemical composition and bioactivity of a red seaweed (Pyropia orbicularis) as affected by different drying technologies. *Dry Technol.* 2020; 38: 1218-1230.

14. Uribe E, Vega-Gálvez A, García V, Pastén A, López J, Goñi G. Effect of different drying methods on phytochemical content and amino acid and fatty acid profiles of the green

seaweed, Ulva spp. J Appl Phycol. 2019; 31: 1967-1979.

15. Uribe E, Pardo-Orellana C, Vega-Gálvez A, Ah-Hen KS, Pastén A, García V, et al. Effect of drying methods on bioactive compounds, nutritional, antioxidant, and antidiabetic potential of brown alga Durvillaea antarctica. Dry Technol. 2019.

16. Nwosu F, Morris J, Lund VA, Stewart D, Ross HA, McDougall GJ. Anti-proliferative and potential anti-diabetic effects of phenolic-rich extracts from edible marine algae. Food Chem. 2011; 126: 1006-1012.

17. Zhang L, Li J, Hogan S, Chung H, Welbaum GE, Zhou K. Inhibitory effect of raspberries on starch digestive enzyme and their antioxidant properties and phenolic composition. Food Chem. 2010; 119: 592-599.

18. Rajauria G, Jaiswal AK, Abu-Gannam N, Gupta S. Antimicrobial, antioxidant and free radical-scavenging capacity of brown seaweed Himanthalia elongata from western coast of Ireland. J Food Biochem. 2013; 37: 322-335.

19. Miño J, Moscatelli V, Hnatyszyn O, Gorzalczany S, Acevedo C, Ferraro G. Antinociceptive and antiinflammatory activities of Artemisia copa extracts. Pharmacol Res. 2004; 50: 59-63.

20. Beevi SS, Mangamoori LN, Hand V, Ramakrishna SD. Isothiocyanate profile and selective antibacterial activity of root, stem, and leaf extracts derived from Raphanus sativus L. Foodborne Pathog Dis. 2009; 6: 129-135.

21. Wang T, Jónsdóttir R, Ólafsdóttir G. Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. Food Chem. 2009; 116: 240-248.

22. Farah Diyana A, Abdullah A, Shahrul Hisham ZA, Chan KM. Antioxidant activity of red algae Kappaphycus alvarezii and Kappaphycus striatum. Int Food Res J. 2015; 22: 1977-1984.

23. Cian RE, Fajardo MA, Alaiz M, Vioque J, González RJ, Drago SR. Chemical composition, nutritional and antioxidant properties of the red edible seaweed Porphyra columbina. Int J Food Sci Nutr. 2014; 65: 299-305.

24. García-Casal MN, Ramírez J, Leets I, Pereira AC, Quiroga, MF. Antioxidant capacity, polyphenol content and iron bioavailability from algae (Ulva sp., Sargassum sp. and Porphyra sp.) in human subjects. Br J Nutr. 2009; 101: 79-85.

25. Gupta S, Abu-Ghannam N. Recent developments in the application of seaweeds or seaweed extracts as a means for enhancing the safety and quality attributes of foods. Innov Food Sci Emerg Technol. 2011; 12: 600-609.

26. Farvin KHS, Jacobsen C. Phenolic compounds and antioxidant activities of selected species of seaweeds from Danish coast. Food Chem. 2013; 138: 1670-1681.

27. Parada J, Pérez-Correa JR, Pérez-Jiménez J. Design of low glycemic response foods using polyphenols from seaweed. J Funct Foods. 2019; 56: 33-39.

28. Moon HE, Islam MN, Ahn BR, Chowdhury SS, Sohn HS, Jung HA, et al. Protein tyrosine phosphatase 1B and α-glucosidase inhibitory phlorotannins from edible brown algae, Ecklonia stolonifera and Eisenia bicyclis. Biosci Biotechnol Biochem. 2011; 75: 1472-1480.

29. Wang W, Okada Y, Shi H, Wang S, Okuyama T. Structures and aldose reductase inhibitory effects of bromophenols from the red alga Symphyocladia latiuscula. J Nat Prod. 2005; 68: 620-622.

30. Abirami RG, Kowsalya S. Quantification and correlation study on derived phenols and antioxidant activity of seaweeds from Gulf of Mannar. J Herbs Spices Med Plants. 2017; 23: 9-17.

31. Lajili S, Azouaou SA, Turki M, Muller CD, Bouraoui A. Anti-inflammatory, analgesic activities and gastro-protective effects of the phenolic contents of the red alga, Laurencia obtuse. Eur J Integr Med. 2016; 8: 298-306.

32. Ban J, Oh JH, Kim DJ, Jeong H-S, Han SB, Hong JT. Antiinflammatory and arthritic effects of thiacremonone, a novel sulfur compound isolated from garlic via inhibition of NF-κB. Arthritis Res Ther. 2009; 11: 1-13.

33. Gábor M. Models of acute inflammation in the ear. In Winyard, PG, Willoughby, DA Eds. Inflammation protocols. Humana Press, Totowa, NJ, 2003, pp 129-137.

34. Xian Y-F, Lin Z-X, Xu X-Y, Su Z-R, Chen J-N, Lai X-P, et al. Effect of Rhizoma Polygonati on 12-O-tetradecanoylphorbol-acetate-induced ear edema in mice. J Ethnopharmacol. 2012; 142: 851-856.

35. Griswold DE, Marshall PJ, Lee JC, Webb EF, Hillegass LM, Wartell J, et al. Hanna N. Pharmacology of the pyrroloimidazole, SK&F 105809—II: anti-inflammatory activity and inhibition of mediator production in vivo. Biochem Pharmacol. 1991; 42: 825-831.

36. Camponogara C, Casoti R, Brusco I, Piana M, Boligon AA, Cabrini DA, et al. Tabernaemontana catharinensis leaves exhibit topical anti-inflammatory activity without causing toxicity. J Ethnopharmacol. 2019; 231: 205-216.

37. Gil B, Ferrándiz M.L, Sanz MJ, Terencio MC, Ubeda A, Rovirosa J, et al. Inhibition of inflammatory responses byepitaondiol and other marine natural products. Life Sci. 1995; 57: PL25-PL30.

38. Yasukawa K, Akihisa T, Kanno H, Kaminaga T, Izumida M, Sakoh T, et al. Inhibitory effects of sterols isolated from Chlorella vulgaris on 12-O-tetradecanoylphorbol-13-acetate-induced inflammation and tumor promotion in mouse skin. Biol Pharm Bull. 1996; 19: 573-576.

39. Devi KP, Suganthy N, Kesika P, Pandian SK. Bioprotective properties of seaweeds: In vitro evaluation of antioxidant activity and antimicrobial activity against food borne bacteria in relation to polyphenolic content. BMC Complement. Altern Med. 2008; 8: 1-11.

40. Wang Y, Xu Z, Bach SJ, McAllister TA. Sensitivity of Escherichia coli to seaweed (Ascophyllum nodosum) phlorotannins and terrestrial tannins. Asian-Australas J Anim Sci. 2009; 22: 238-245.

41. El Shafay SM, Ali SS, El-Sheekh MM. Antimicrobial activity of some seaweeds species from Red sea, against multidrug resistant bacteria. Egypt J Aquat Res. 2016; 42: 65-74.