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Radical-scavenging capacity of phenol fractions in the brown seaweed *Ascophyllum nodosum*: An electrochemical approach

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ABSTRACT

In this article, the radical-scavenging capacity of phenol fractions extracted from the brown seaweed *Ascophyllum nodosum* was assessed using in parallel colorimetric methods (ABTS and DPPH) and electrochemistry (cyclic voltammetry). Results obtained by the three methods correlated in the case of global fractions, whereas only ABTS and DPPH correlated when activities were expressed on a phenol basis. The successive fractions separated by both their average molecular size and their polarity exhibited activities largely dependant on their phenol content, suggesting that phlorotannins are the main anti-oxidant molecules in hydro-alcoholic extracts of *A. nodosum*. In addition, phenol fractions of relative low molecular weight were clearly more active than others. This work opens new opportunities to better evaluate the radical-scavenging potential of phenol pools in algae using both bi-parametric fractionating and electrochemistry.

1. Introduction

Phenolic compounds are secondary metabolites present in various organisms, including higher plants [1], lichens [2] and algae [3]. There is considerable interest in the analysis of plant phenols due to their potential role as antioxidants [4,5], which can help neutralize free radicals linked to the development of degenerative diseases and conditions, including cancer, cardiovascular disease, cognitive impairment, immune dysfunction, cataracts and macular degeneration, asthma or diabetes [6–14]. In addition, many phenolic phytochemicals have antimicrobial, antiallergic and anti-inflammatory activities [15,16]. Significant amounts of phenolic compounds frequently occur in foods and beverages such as fruits, vegetables, fruit juices, tea, cider and wine and are routinely consumed in our diet [17,18].

Among algae, Phaeophyceae exhibit high levels of phlorotannins, i.e. both oligomers and polymers of phloroglucinol (1,3,5-trihydroxybenzene). These molecules have molecular weights (MW) in a large range (<200–600,000 Da) and may accumulate in Fucales and Dictyotales up to 30% dry weight (DW) [3,19]. Phlorotannins play important roles inthe protection of algae against marine grazers [20,21], pathogens [3] and epiphytes [22]. They are also involved in photoprotection mechanisms, particularly to counteract the cytotoxic effects of UV radiations [23]. Antibacterial activities against positive and negative Gram bacteria have been described for tannins extracted from the algae *Ecklonia kurome*, *E. cava* and *Fucus vesiculosus*, suggesting a potential use as natural preservatives in food industry or as antibacterial drugs [24,25]. Polymeric phlorotannins inhibit enzymes such as hyaluronidase [26], phospholipase A, lipoxygenase and cyclooxygenase-1 [27], or tyrosinase [28]. In addition, polyphenols from *E. cava* were reported to inhibit the metalloproteinase MMP-1 which is involved in skin aging processes [29].

Therefore, if phenolic compounds extracted from algae could be properly purified, fractionated and characterized, they could represent an important source of bioactive molecules in several fields such as medicine, cosmetology or alimentation, due to the occurrence of large, exploitable biomasses in temperate, maritime areas [30].

In this study, both fractionating and purification processes have been carried out on phlorotannins extracted from the brown seaweed *Ascophyllum nodosum*. The purity of the resulting fractions has been checked by dosing their phenol content and their radical-scavenging activity has been evaluated by both colorimetric, chemical methods and electrochemistry. The accuracy of these methods is discussed in the perspective of studies on both the functional role and the potential use of algal phenol fractions.

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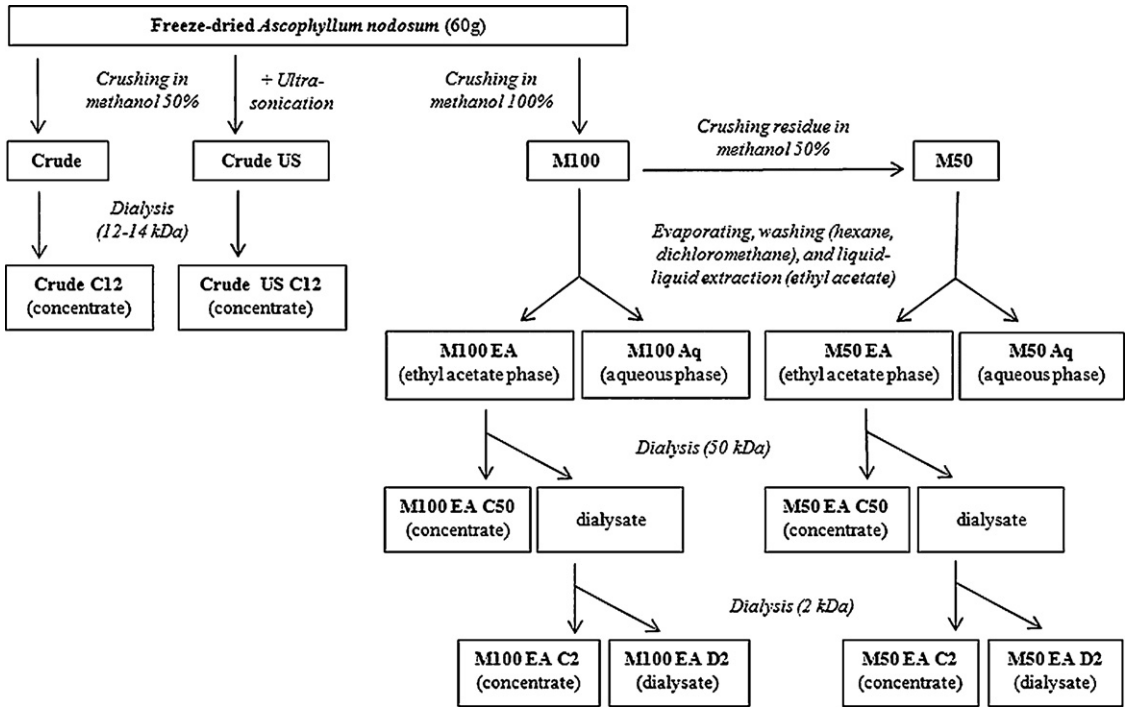

Fig. 1. Flowchart of purification steps in the production of phenol fractions in *A. nodosum*.

2. Material and methods

2.1. Seaweed material

Thalli of *A. nodosum* were collected in winter at Plouzané (Brittany, France). Typically, medium parts of 15 thalli about 1 m long were excised and pooled for a single extraction. In the laboratory, mature, lateral receptacles as well as epiphytes were removed and the samples were then quickly washed up with de-ionised water, cut off into small pieces and kept freeze-dried before extraction.

2.2. Extraction and fractionating procedures

Extraction of phlorotannins was carried out typically on 60 g freeze-dried tissue homogenized in a rotary shaker (60 rounds per minute) in 1 L solvent at 30 °C during 3 h. Both extraction and fractionating were realised using two alternative procedures. As developed by Connan et al. [31], a first procedure included a direct extraction step of polyphenols in methanol 50% (CH_3OH/H_2O, volume/volume) in order to quantify the global pool of phlorotannins in the tissues, resulting in total extracts (crude). Crude extracts were then fractionated on dialysis membranes at a cutting size of 12000–14000 Da. The concentrate was kept for further use (crude C12), whereas the dialysate was discarded. As a variation to this procedure, a soft ultrasound treatment could be added during the extraction step, giving corresponding crude extracts (crude US) and dialysis concentrates (crude US C12).

In order to study the effect of polarity on the distribution of phlorotannins, a second procedure was developed, involving a first extraction step in methanol 100%, giving a supernatant (M100) and a residue submitted to a second extraction step in methanol 50% (M50). Both M100 and M50 extracts were then freeze-dried after elimination of methanol in a Büchi rotary evaporator, dissolved in de-ionised water and washed several times by hexane and dichloromethane, successively. The organic fractions containing mainly lipids and pigments were discarded while the remaining aqueous solution was partitioned into ethyl acetate (M100 EA or M50 EA) and residual aqueous (M100 aq or M50 aq) phases by liquid–liquid extraction. Ethyl acetate fractions were dialyzed on 50,000 Da cutting size membranes giving concentrates (M100 EA C50 and M50 EA C50) and dialysates were submitted to a second dialysis step at a cutting size of 2000 Da. Final fractions of the process were therefore concentrates M100 EA C2 and M50 EA C2 and dialysates M100 EA D2 and M50 EA D2. All crude extracts and fractions were stored freeze-dried before analysis.

A fractionation chart is provided in Fig. 1 to show how the different fractions were generated.

2.3. Antioxidant standards

Phloroglucinol dihydrate (1,3,5-trihydroxybenzene), trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxilic acid), quercetin dihydrate, and rutin trihydrate were used in this study as antioxidant standards.

2.4. Determination of phlorotannins content

Total phlorotannins content in algal extracts were determined by the Folin–Ciocalteu's (FC) method which was adapted from Swain and Hillis [32]. Freeze-dried samples were previously dissolved in de-ionised water at room temperature. Then, 2 mL of each extract were mixed to 10 mL of de-ionised water and 12 mL of sodium carbonate decahydrate (29% m/v). One millilitre of the Folin–Ciocalteu reagent was added and coloration developed for 30 min in the dark at room temperature (20 °C). The absorbance was measured at 760 nm with a spectrophotometer (Novaspec II, Amersham Pharmacia Biotech, Orsay, France) and was taken into account between 0.3 and 0.8 A.U. (Absorbance Units).

Phloroglucinol (PG) was used as a standard at a concentration scale between 0 and 6.10^{-5} mol L^{-1}. Each solution belonging to this linear range was prepared daily in de-ionised water. Results were expressed in phloroglucinol equivalents (eq. PG g g^{-1} of dry extract) using the following standard regression equations: $y = 9590\,x - 0.025$ ($R^2 = 0.995$) and $y = 73.422\,x - 0.009$ ($R^2 = 0.997$),

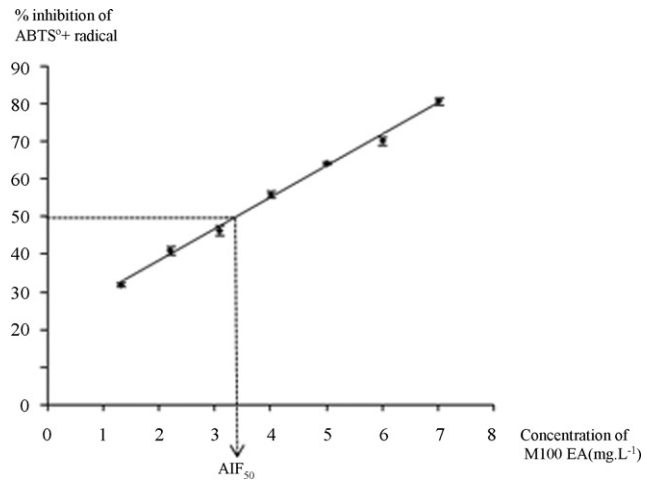

Fig. 2. Graph showing inhibition percentage obtained from $ABTS^{\bullet+}$ assay with standard deviation ($n = 3$) versus increasing concentrations of the fraction M100EA.

where y was the absorbance at 760 nm in A.U. and x the concentration in PG ($mol\,L^{-1}$ and $g\,L^{-1}$, respectively). These linear correlations have been calculated from the results of three different experiments and were performed using the AVA V3-1 qualilab software package.

2.5. Methods for the evaluation of the anti-oxidant activity

2.5.1. $ABTS^{\bullet+}$ radical assay

For the ABTS assay, the procedure followed the method developed by Re et al. [33] with minor modifications. The stock solution included 7 mM ABTS (2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid))–$(NH_4)_2$ dissolved in water, and 2.45 mM potassium persulfate (final concentration), producing cations of the ABTS radical ($ABTS^{\bullet+}$) and was let to develop for 12–16 h at room temperature in the dark. The radical cations were stable under that form for more than 2 days. Prior to assay, the stock solution was diluted by mixing about 1 mL with 60 mL ethanol to obtain an absorbance of 0.700 ± 0.02 A.U. at 734 nm. Fresh working $ABTS^{+}$ solution was prepared for each assay at room temperature. For each algal sample, 10 μL aliquots of each dilution in ethanol were mixed to 990 μL of diluted $ABTS^{+}$. Exactly 30 min after the initial mixing, addition of algal samples had to result in 20–80% inhibition of the blank absorbance. A minimum of five dilutions per algal fraction and corresponding solvent blanks were assayed in triplicate at 734 nm. The percentage inhibition of the blank absorbance was then both calculated and plotted as a function of the concentration (see for example regression curve in Fig. 2).

2.5.2. $DPPH^{\bullet}$ radical assay

The hydrogen donating and/or radical-scavenging capacity of the algal samples was evaluated as their ability to scavenge the free radical DPPH. The DPPH assay was done according to the method of Blois [34] with some modifications. A 1.10^{-3} M stock solution of DPPH was obtained by diluting 19.7 mg of DPPH (2,2-diphenyl-1-picryl-hydrazyl) in 50 mL ethanol and then stored in the dark at 20 °C until use. For each sample, at least 5 dilutions in ethanol were prepared and 2 mL aliquots of each dilution were then mixed to 150 μL of the DPPH stock solution. Exactly 1 h after the initial mixing, the absorbance at 517 nm was measured for all dilutions of each algal fraction, resulting in regression curves which were usually linear between 20% and 60% inhibition of the blank absorbance.

2.5.3. Antioxidant activity by the electrochemical method

The method used here has been developed by Le Bourvellec et al. [35] and it is based on the reaction kinetics of the antioxidant substrate, as either phloroglucinol or flavonoids, with the superoxide radical (O_2^{-}). A cyclic voltammetric technique was used to generate the radical O_2^{-} by reduction of molecular oxygen in an aprotic medium (N,N-dimethylformamide or DMF extra dry ($[H_2O] \leq 0.01\%$), stored over molecular sieve 3A). The consumption of radical O_2^{-} was directly measured by the anodic current decay resulting from its oxidation in the presence of increasing concentrations of antioxidant.

For all measurements, the following instrumentation was used: a dual potentio-galvanostat PGSTAT 30 (Autolab instrument, Eco Chemie B.V., Utrecht, The Netherlands), a GPES software (General Purpose Electrochemical System version 4.9, Eco Chemie B.V.), a three-electrode cell thermostated at 20 °C, with a glassy carbon disk working electrode (diameter 2 mm), a platinum wire counter electrode and a reference electrode Ag/AgCl in EtOH. The reference electrode was separated from the solution by a salt bridge containing 0.5 M of tetrabutylammonium hexafluorophosphate (Bu_4NPF_6) in DMF. The glassy carbon disk working electrode was polished using silicon carbide 2400 paper (Struers, Ballerup, Danemark).

A solution of 10 mL of an extra dry DMF containing the supporting electrolyte 0.1 M Bu_4NPF_6 was saturated by dry air during 10 min. The cyclic voltammogramm (CV) of the oxygen reduction was then recorded at a scan rate of $0.1\,V\,s^{-1}$, with the initial potential at 0 V and the reverse one at −1.5 V vs. Ag/AgCl. Aliquots of each stock solution of the algal extracts in $mg\,L^{-1}$ were successively added to the 10 mL oxygen solution in order to get an algal concentration in a linear range. After each aliquot addition, CV of the oxygen solution was recorded at a scan rate of $0.1\,V\,s^{-1}$ (see for example registred voltammograms in Fig. 3).

2.6. Expression of results

Results of the Folin–Ciocalteu's method were expressed in equivalent phloroglucinol units as g eq. PG g^{-1} of dry mass of the algal extracts and then reported either as percentages of the algal dry mass or as percentages of the fraction dry mass. The reactivity of the fractions was characterized by an antioxidant index AI_{50}, determined by analogy with the efficient/inhibiting concentration (EC_{50}/IC_{50}). AI_{50} is defined as the concentration in $mg\,L^{-1}$ of algal extract and in $\mu M\,L^{-1}$ of antioxidant standards which causes 50% loss of the radical activity. When estimated by both DPPH and ABTS methods, AI_{50} was related to absorbance variations and calculated from the linear portions of the regression curves (Fig. 2), whereas in the electrochemical method, AI_{50} corresponded to the concentration of algal extract or antioxidant standards needed to consume the radical $O_2^{\bullet-}$, as revealed by a current decrease of the initial anodic current Ip_a^0 and expressed by the equation $AI_{50} = (Ip_a^0 - Ip_a^S)/Ip_a^0 = 0.5$, where Ip_a^0 is the intensity of the anodic current peak of $O_2^{\bullet-}$ and Ip_a^S the intensity of the anodic current peak of O_2^{-} for the concentration S of the sample [35], (see Fig. 4 for an example).

The term AIF_{50} was used for the AI_{50} measured for the total fraction mass, whereas AIP_{50} corresponded to the AI_{50} of the phenols in the fractions, considered as the sole active molecules. AIF_{50} was expressed in $mg\,L^{-1}$ and AIP_{50} in equivalent phloroglucinol units as g eq. PG g^{-1} of dry extract or mM eq. PG L^{-1}.

2.7. Statistical analysis

Analysis of variance (ANOVA) was used to test any difference between both phenol contents and antioxidant activities, after testing the homoscedasticity by both Cochran C test and Bartlett's test. When useful, an LSD ranking test was applied to the values of

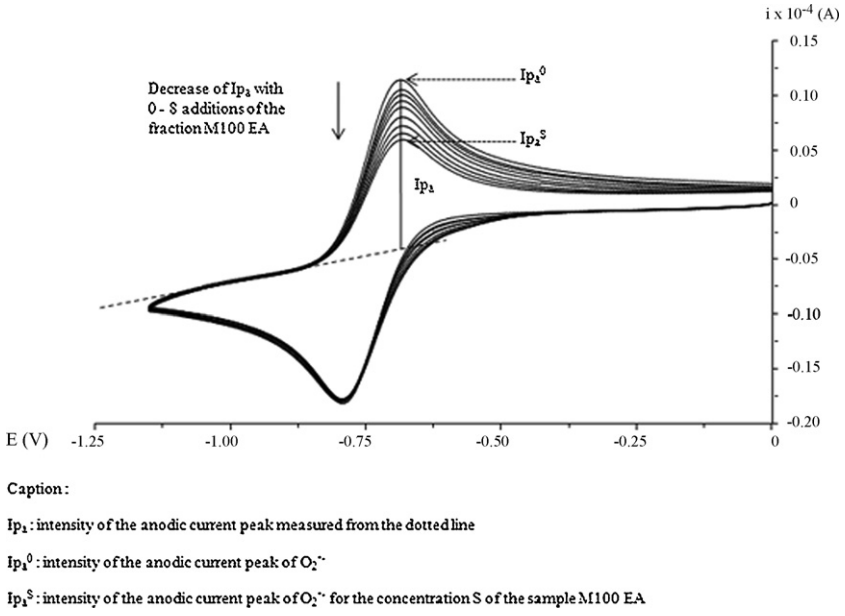

Fig. 3. Superposed representative cyclic voltamogramms obtained without and further eight addition of M100EA solution.

the same variable. Correlations among data were calculated using either Pearson's or Spearman's correlation coefficients (r). All tests were performed using either Statgraphics or Statistica softwares.

3. Results

3.1. Phlorotannin contents

The overall global content of phlorotannins extracted from *A. nodosum* was ca. 5% of dry tissues, in agreement with previous reports [31,36,37]. Total phlorotannin contents of the fractions (TPh) are shown in Table 1 and ranged between 2.8% in M100 Aq and ca. 75% in M50 EA C50. Crude extracts contained less than 20% phenols and the proportion of phlorotannins increased significantly (ANOVA, $F = 54.12$, $p = 0.0000$) with the average size of molecules separated by dialysis (Fig. 5), i.e. around 32% for phenols above 2000 Da (M100 EA C2 and M50 EA C2), ca. 43% above 12,000–14,000 Da (crude C12 and crude US C12), and around 70% above 50,000 Da (M100 EA C50 and M50 EA C50). The ranking test LSD showed that TPh did not differ significantly between crude extracts, aqueous and D2 fractions on the one hand, and between EA and C50 fractions on the other hand. Since TPh values are more than 20 times higher in EA fractions than in aqueous phases, 3–9% in Aq fractions versus about 65% in EA fractions, phenols of *A. nodosum* appear globally less polar than water and are therefore better extracted from crude extracts using ethyl acetate. However, the fact that fractions obtained from the M50 extract and those separated from the M100 extract vary in the same way (Spearman's test, $p = 0.9000$) suggests a simi-

Table 1
Phlorotannin content of the studied fractions, expressed as a percentage of phloroglucinol equivalents of the fraction dry matter (% of g eq. PG g^{-1} of dry extract).

Extracts	% Phlorotannins in fraction
Crude	22.4
Crude US	16.2
Crude C12	39.7
Crude US C12	47.4
M100 Aq	2.8
M50 Aq	8.8
M100 EA	62.3
M50 EA	69.1
M100 EA C50	65.5
M50 EA C50	74.8
M100 EA C2	33.5
M50 EA C2	29.8
M100 EA D2	14.5
M50 EA D2	6.8

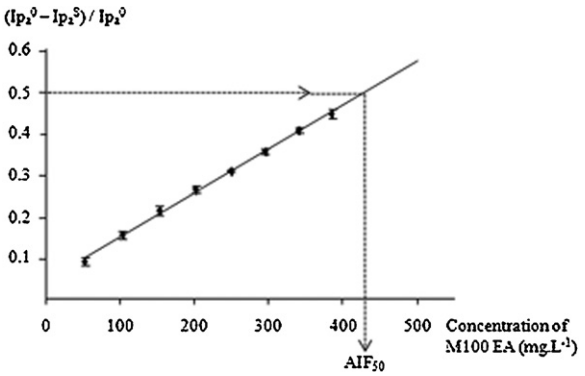

Fig. 4. Graph showing Ip ratios with standard deviation ($n = 3$) calculated from cyclic voltamogramms versus increasing concentrations of the fraction M100EA.

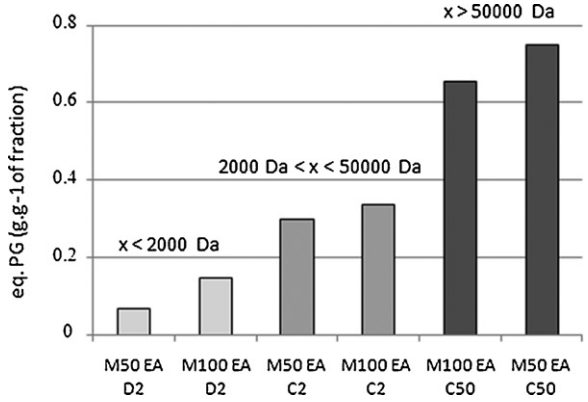

Fig. 5. Histograms showing phlorotannin contents of fractions, expressed as phloroglucinol equivalents, relative to the increase of the average size of phenol molecules.

Table 2
Total antioxidant activity of each fraction quantified by the three methods tested and expressed by the AIF_{50}.

Fractions	AIF_{50} (mg L^{-1})		
	Electrochemistry	$ABTS^{\bullet+}$	$DPPH^{\bullet}$
Crude	865.4	25.0	29.8
Crude US	1230.2	37.5	26.5
Crude C12	478.8	6.8	3.8
Crude US C12	449.5	3.2	3.9
M50 Aq	771.2	23.5	54.0
M100 Aq	372.2	37.8	385.7
M100 EA	431.5	3.5	5.9
M50 EA	nd	nd	7.5
M100 EA C50	315.4	2.6	2.3
M50 EA C50	391.0	4.1	5.0
M100 EA C2	318.1	1.9	7.1
M50 EA C2	446.6	18.7	39.1
M100 EA D2	677.5	11.9	24.7
M50 EA D2	1502.1	28.6	41.7

Table 3
Antioxidant activity of the phenols contained in each fraction and antioxidant activity of standards quantified by the three methods tested and expressed respectively by the AIP_{50} and the AI_{50}.

Compounds	Electrochemistry ($\times 10^3$)	$ABTS^{\bullet+}$	$DPPH^{\bullet}$
Fractions		AIP_{50} (μM eq. PG L^{-1})	
Crude	1.54	44.4	52.9
Crude US	1.58	47.9	33.8
Crude C12	1.51	21.4	12.0
Crude US C12	1.69	12.0	14.9
M50 Aq	0.08	8.4	85.6
M100 Aq	0.54	16.2	37.3
M100 EA	2.13	19.2	24.2
M50 EA	nd	nd	27.7
M100 EA C50	1.64	13.5	11.9
M50 EA C50	2.23	24.3	29.6
M100 EA C2	0.24	6.0	22.4
M50 EA C2	1.06	70.2	147
M100 EA D2	0.78	13.8	28.6
M50 EA D2	0.80	36.5	53.2
Antioxidant standards		AI_{50} (μM L^{-1})	
Phloroglucinol	1.77[a]	6.0	235.4
Trolox	1.97[a]	11.9	21.0
Quercetin	0.46[a]	4.0	10.7
Rutin	1.24[a]	12.0	8.5

[a] Electrochemical AI_{50} values published by Le Bourvellec et al., 2008 [35].

lar purification pattern, whatever the polarity of the extraction medium.

3.2. Antioxidant capacity

Fig. 3 shows a typical example of cyclic voltamogramms obtained in this study both in the absence and with increasing concentrations of a fraction. The values obtained from these voltamogramms permitted to generate a regression curve as shown in Fig. 4 to determine the AIF_{50} by electrochemistry. A determination example of AIF_{50} by chemical methods was also presented for $ABTS^{+}$ assay in Fig. 2. The global activity of both fractions and antioxidant standards were expressed respectively as AIF_{50} (i.e. the global activity of the fractions), AIP_{50} and AI_{50}, which are shown respectively in Table 2 and in Table 3. Values scale up between 315.4 mg L^{-1} (high activity) for M100 EA C50 and 1502.1 mg L^{-1} (low activity) for M50 EA D2, when tested by electrochemical method, between 1.9 mg L^{-1} for crude M100 EA C2 and 37.8 mg L^{-1} for M100 Aq when assayed by ABTS and between 2.3 mg L^{-1} for M100 EA C50 and 385.7 mg L^{-1} for M100 Aq when evaluated by DPPH. When based on the phenol content, activities expressed as AIP_{50} were in a 0.08–2.23.10^{-3} μM eq. PG L^{-1} range as tested by electrochemistry, between 6 and 47.9 μM eq. PG L^{-1} when dosed by ABTS and between 11.9 and 85.7 μM eq. PG L^{-1} when evaluated by DPPH (Table 3).

AIF_{50} was correlated negatively with the phenol content of the fractions, whatever the method used (Spearman's test, Table 4), suggesting that most of the antioxidant activity in fractions was due to phenols. So, M100 Aq and M50 EA D2 are logically few active, whereas M100 EA C50 and M50 EA C50 are among the most active fractions. Values obtained for AIF_{50} by the three methods vary in the same way, indicating a good correspondence between electrochemistry, ABTS and DPPH (Table 4). The relationship between AIP_{50} and the phenol content, however, depended on the method used to assess the antioxidant activity, a discrepancy which could result from an increased variability due to phenol contents themselves. However, AIP_{50} values obtained by both ABTS and DPPH varied also in the same way, whereas voltammetry data did not fit with (Table 4).

4. Discussion

This work reports on both the extraction and the fractionation of phlorotannins in *A. nodosum*, based on both the polarity and the molecular size of the fractions. Contents were comparable to those obtained in previous reports on *Ascophyllum* or on closely related species [20,31,36–41]. Ethyl acetate fractions, particularly when restricted to polymers above 50 kDa, were the purest, whereas aqueous fractions and dialysates with molecules below 2 kDa contained less than 10–15% phenols.

In this study, three methods, ABTS, DPPH and electrochemistry, have been tested in parallel for the evaluation of radical-scavenging activities in phenol fractions. Interestingly, most of the works on the antioxidant capacities of phlorotannins involved so far either a single dosing technique or two (e.g. [31,42–45]). As suggested by previous authors however, the use of several assays is recommended to generate a more or less complete antioxidant profile, even though (or because) these assays do not give generally similar results for a given set of samples [46], due to both various chemical pathways [47] and different analytical strategies [48]. In addition, a method recently developed for the determination of the antioxidant capacity of flavonoids by cyclic voltammetry [35] has been applied for the first time to phlorotannin fractions, opening therefore new potentials for the qualitative characterization of algal

Table 4
Results of the Spearman's test between phenol contents and antioxidant activities measured by the three methods tested and expressed either as AIF_{50} or as AIP_{50}. Statistic numbers correspond to the correlation coefficients. Significant correlations at a 95% level are indicated in bold.

	Phenol contents %	AIF_{50} electro-chemistry	AIF_{50} ABTS	AIF_{50} DPPH	AIP_{50} electro-chemistry	AIP_{50} ABTS	AIP_{50} DPPH
Phenol contents %	1.0000	**−0.8252**	**−0.7692**	**−0.8881**	**0.7343**	−0.1119	**−0.6504**
AIF_{50} electrochemistry	**−0.8252**	1.0000	**0.8951**	**0.7692**	−0.2867	0.4126	**0.6434**
AIF_{50} ABTS	**−0.7692**	**0.8951**	1.0000	**0.8462**	−0.2378	**0.6504**	**0.8392**
AIF_{50} DPPH	**−0.8881**	**0.7692**	**0.8462**	1.0000	**−0.6224**	0.4126	**0.8811**
AIP_{50} electrochemistry	**0.7343**	−0.2867	−0.2378	**−0.6224**	1.0000	0.2797	−0.2797
AIP_{50} ABTS	−0.1119	0.4126	**0.6504**	0.4126	0.2797	1.0000	**0.6154**
AIP_{50} DPPH	**−0.6504**	**0.6434**	**0.8392**	**0.8811**	−0.2797	**0.6154**	1.0000

phenols. The main interest of this method is to generate superoxide radicals ($O2^-$), i.e. reactive oxygen species really involved in both stress and degenerative processes in cells and so allowing a more accurate evaluation of biological antioxidants [49,50]. Moreover, electrochemical methods differ strikingly from spectrophotometric assays like both DPPH and ABTS. The gap observed in the orders of magnitude between values obtained from the three assays results probably from both the nature and the electric charge of the radicals involved in each method: neutral DPPH• [34], positively charged ABTS• [51] and negative oxygen radical in the case of electrochemistry [35]. The accuracy of colorimetric methods is also regularly discussed (e.g. [52]) and eventually improved, including both DPPH [53] and ABTS [33]. Anyway, the good correspondence between values obtained by three different methods used here to determine AIF_{50} is uncommon in such studies.

In spite of some discrepancies between patterns followed by phenol-based antioxidant activities (AIP_{50}) of the various fractions purified from *A. nodosum*, some tendencies may be stressed out from our results. From AIF_{50} values, it appears that ethyl acetate fractions and concentrates containing molecules over 50 kDa are the most active. Fraction M100 EA C2 (molecules between 2 and 50 kDa) is more efficient than the corresponding fraction obtained from the crude methanol 50% extract, suggesting differences based on polarity, i.e. putatively on structural variations in substances involved in radical scavenging. The fact that fraction M50 EA D2 is the least active far below M100 EA D2 tends to reinforce that assertion. Activities calculated on a phenol basis (AIP_{50}) showed higher theoretical antioxidant capacities of phlorotannins with molecular sizes between 2 kDa and 50 kDa (at first in fraction M100 EA C2) and below 2 kDa (particularly in M100 EA D2). Once again, phenolic compounds isolated from more apolar extracts (M100) seem to be more efficient, except that fractions were not pure. Once re-expressed as AIP_{50} in the same units as in our study, results given by Cérantola et al. [44] after dosing by the DPPH method for poly-fucols (0.88 mM eq. PG L^{-1}) and poly-fuco-phlorethols (0.90 mM eq. PG L^{-1}) purified from *A. nodosum* are at the same order of magnitude, with better activities however. From their data, we can also infer that antioxidant capacities of our fractions are also slightly lower than those of both ascorbate (1.03 mM eq. PG L^{-1}) and phloroglucinol (1.12 mM eq. PG L^{-1}) and clearly below that of gallic acid (0.29 mM eq. PG L^{-1}).

In conclusion, we report here for the first time on the application of cyclic voltammetry for the functional qualification of seaweed extracts. The use of an electrochemical method, in addition to other techniques based on artificial radicals, should make possible a more realistic approach of the antioxidant capacity of the phenol pool in seaweeds.

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