Antioxidant and antitumoural activities of some Phaeophyta from Brittany coasts

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ABSTRACT

In this study, the antioxidant activity exhibited by crude extracts from 10 Phaeophyta species from Brittany coasts was assessed by three methods (DPPH, reducing activity and β-carotene–linoleic acid system), and their phenolic contents were examined. Antitumoural activities were determined by a cytotoxic assay with three different tumoural cells lines (Daudi, Jurkat and K562). Among the species under study, four Fucales, i.e. *Bifurcaria bifurcata*, *Cystoseira tamariscifolia*, *Fucus ceranoides* and *Halidrys siliquosa*, displayed a high antioxidant activity. Further to the fractionation of these crude extracts, a significant correlation was found in most fractions between the high antioxidant activity and the high phenolic content. Five species, i.e. *B. bifurcata*, *C. tamariscifolia*, *Desmarestia ligulata*, *Dictyota dichotoma* and *H. siliquosa*, exhibited strong cytotoxic activities against all tumoural cells.

1. Introduction

Over the last decades, products liable to promote health and well-being have aroused a huge interest among consumers and food industrialists. These food items are, nowadays, gathered under the generic name of functional food; in addition to their nutritional or energetic benefits, these items can be also worth for the physiology of humans by exerting an antihypertensive, antioxidant or anti-inflammatory effect (Herrero, Cifuentes, & Ibáñez, 2006). The beneficial action exerted by functional food is usually ascribed to a given functional component (e.g. vitamins, minerals or micronutrients such as ω-3 fatty acids), which is either missing in the analogous conventional food or present at lower concentrations (Plaza, Cifuentes, & Ibañez, 2008).

Due to their low content in lipids, high concentration in polysaccharides, natural richness in minerals, polyunsaturated fatty acids and vitamins as well as their content in bioactive molecules, e.g. phenolic compounds or more specifically phlorotannins, terpenes, alkaloids, (Kornprobst, 2005), marine algae are known to be a source of healthy food. Among them, microalgae have attracted considerable attention from functional food industrialists because of their high contents in polyunsaturated fatty acids, β-carotene and other pigments, sterols (Herrero et al., 2006). Macroalgae are mainly traded as food items in particular in Asia (e.g. sushi wrappings, seasonings, condiments and vegetables) and employed in

the phycocolloid industry. Over the last years, the development of innovative projects for the agri-food industry and/or pharmaceutical and para-pharmaceutical applications has been at the origin of a great expansion in the demand for macroalgae. Indeed, the potential offered by macroalgae in functional foods or ingredients industries is huge because of their exceptional richness in bioactive compounds liable to be endowed with antioxidant, antimicrobial, anti-inflammatory, antitumoural activities (Kornprobst, 2005; Smit, 2004).

Among the most relevant compounds found in the algae, antioxidants are probably the substances that have attracted major interest. Antioxidants are considered key-compounds in the fight against various diseases (e.g. cancer, chronic inflammation, atherosclerosis and cardiovascular disorder) and ageing processes (Kohen & Nyska, 2002). Moreover, the relevance of using antioxidants from natural sources has been considerably enhanced by consumer's preference for natural products and concerns about the toxic effects by synthetic antioxidants (Ito et al., 1986). Algae, as photosynthetic organisms, are exposed to a combination of light and high oxygen concentration at the origin of the formation of free radicals and other oxidative reagents. But, the awareness of the lack of structural damage in their organs has led the scientific community to consider that their protection against oxidation comes from their natural content, or production under stress, in antioxidant substances. Indeed, macroalgae are particularly rich in natural antioxidants, e.g. phlorotannins, ascorbic acid, tocopherols and carotenoids, and a literature search shows that most investigation has been devoted to the phlorotannins that play an essential role in the photoprotection of the brown algae.

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Another area of focus is the search for anticancer drugs since marine molecules have led to promising results in trials at different phases of cancer diseases (Mayer & Gustafson, 2006). Numerous macroalgae have shown potent cytotoxic activities (see reviews in Mayer & Gustafson, 2006; Smit, 2004), and certain authors have suggested to consume algae as a chemopreventive agent against several cancers (Yuan & Walsh, 2006). Dehydrothyrsiferol and halomon extracted from *Laurencia viridis* sp. *nov.* (Pec et al., 2003) and *Portieria hornemanii* (Egorin et al., 1997) respectively, have been tested in the preclinical phase. Concerning brown algae, polysaccharides (Aisa et al., 2005; Dias et al., 2005; Kwon & Nam, 2007) and terpenoids (Culioli et al., 2004; Duran, Zubia, Ortega, & Salva, 1997) are considered as promising bioactive molecules in the search for anticancer drugs.

With about 700 identified species (Dizerbo & Herpé, 2003), the coasts of Brittany (France) are acknowledged for their particular richness in macroalgae. Among them, only few species, mainly brown algae, have been investigated for their antioxidant capacities (Cerantola, Breton, Ar Gall, & Deslandes, 2006; Connan, Delisle, Deslandes, & Ar Gall, 2006; Connan, Deslandes, & Ar Gall, 2007; Le Lann, Jegou, & Stiger-Pouvreau, 2008), their anti-fouling (Hellio, Bremer, Pons, Le Gal, & Bourgougnon, 2000; Hellio et al., 2004; Plouguerné et al., 2008) and antitumoural (Moreau et al., 2006) activities. These studies have highlighted the great potential of brown macroalgae in the search for bioactive molecules.

The present study was aimed at assessing the antioxidative and antitumoural capabilities of 10 brown algae settled along the shoreline of Brittany. To gain more insight into antioxidant processes, the antioxidative activities of extracts were characterised by three biochemical methods (DPPH radical-scavenging activity, reducing activity and β-carotene–linoleic acid system), and their total phenolic contents were quantified. This led us to further select some crude extracts for fractionation in order to determine the antioxidant activity and total phenolic content of each fraction to analyse relationships between both parameters. A cytotoxic assay was also conducted with three different tumoural cells lines (Daudi, Jurkat and K562) to determine the antitumoural activities of the crude extracts.

2. Material and methods

2.1. Chemicals

Ascorbic acid, α-tocopherol, β-carotene, BHT (butylated hydroxytoluene) and BHA (butylated hydroxyanisole), DPPH (2,2-diphenyl-1-picryhydrazyl radical), Folin–Ciocalteu's phenol reagent, linoleic acid, phloroglucinol, potassium ferricyanide [$K_3Fe(CN)_6$], trichloroacetic acid and Tween-40 were purchased from Sigma–Aldrich Co. (France). The organic solvents (chloroform, dichloromethane, formol, ethanol and methanol) were from VWR (France); Fontainebleau sand was from Grosseron (France). The other chemicals used were of analytical grade.

2.2. Collection

Samples of 10 species of brown macroalgae were collected along the coasts of Brittany between 2006 and 2007 (Table 1). Once harvested, they were stored in plastic bags for transport to the laboratory. Voucher specimens of all species were pressed and stored in 4% formol for identification (Cabioc'h et al., 2006). All of the samples were washed thoroughly with freshwater to remove salts, sand and epiphytes, and then stored at −20 °C. Each macroalgal sample was lyophilised and pulverised into powder before extraction.

2.3. Preparation of algal extracts and fractions

The extraction was carried out with an Accelerated Solvent Extraction system (ASE 300) equipped with a solvent controller unit (Dionex, France) under optimised conditions. After mixing of 10 g of lyophilised samples with 10 g of Fontainebleau sand as dispersing agent, the resulting mixture was placed in 66 ml stainless steel extraction cell equipped, at its outlet, with a cellulose filter. A preliminary study was done to improve the extraction conditions (not published), then all extractions were performed with the optimised parameters: a mixture of dichloromethane methanol (1:1, v:v) at 75 °C and 1500 psi during 2 static 7 mn cycles. Each sample was extracted twice, then the cell was rinsed with solvent and purged with a flow of nitrogen. Then, the extracts were filtered with a grade 4 Whatman filter and concentrated to 10 ml under reduced pressure prior to storage at −20 °C.

The crude extracts were purified by Solid Phase Extraction (SPE) with the automated GX271-ASPEC instrument (Gilson, France). After conditioning with methanol (20 ml) and distilled water (20 ml) successively delivered at a flow rate of 6 ml/mn, the SPE cartridge was loaded with the crude extracts (50 mg) (flow rate: 1 ml mn^{-1}). After adsorption, fractionation was performed by stepwise elution (flow rate: 2 ml mn^{-1}) with 40 ml of each of the following solvents: distilled water (FI), 50% methanol (FII), 100% methanol (FIII), dichloromethane:methanol 50:50 (FIV) and 100% dichloromethane (FV). The SPE cartridges used were Strata C18-E 1000 mg/6 ml (Phenomenex, France). Each crude extract was purified four times to accumulate fractions in sufficient weight: the same elute fractions were combined and evaporated under reduced pressure at 40 °C. Each fraction was weighted, re-dissolved in the appropriate solvent and stored at −20 °C prior to biochemical analysis and assays.

2.4. Antioxidant assays

2.4.1. DPPH radical-scavenging activity

The DPPH radical-scavenging activity was determined by application of the method developed by Brand-Williams, Cuvelier and Berset (1995) and modified by Fukumoto and Mazza (2000). The fundamental principle of the DPPH method is the reduction of

Table 1
Information about the brown macroalgae under study.

Scientific name	Family	Place of collection	Date of collection
Alaria esculenta (Linnaeus) Greville	Alariaceae	Anse de Melon	May 2007
Asperococcus bullosus J.V. Lamouroux	Asperococcaceae	Caro	June 2007
Bifurcaria bifurcata R. Ross	Sargassaceae	Anse de Melon	September 2007
Cystoseira tamariscifolia (Hudson) Papenfuss	Sargassaceae	Anse de Melon	April 2007
Desmarestia ligulata (Stackhouse) J.V. Lamouroux	Desmarestiaceae	Anse de Melon	May 2007
Dictyota dichotoma (Hudson) J.V. Lamouroux	Dictyotaceae	Anse de Melon	May 2007
Fucus ceranoides Linnaeus	Fucaceae	Aber benoît	March 2006
Fucus serratus Linnaeus	Fucaceae	Anse de Melon	March 2007
Halidrys siliquosa (Linnaeus) Lyngbye	Sargassaceae	Anse de Melon	May 2007
Saccorhiza polyschides (Lightfoot) Batters	Phyllariaceae	Callot	June 2007

the DPPH radical in alcoholic solution by an H-donor antioxidant (AH) to form the non-radical form DPPH-H. In a 96-well microplate, 22 µl of each extract at various concentrations were mixed with 200 µl of a DPPH solution (25 mg l^{-1}) prepared daily. Because of the colour of the extracts, blanks had to be prepared by mixing 22 µl of extract at various concentrations with 200 µl of methanol. The reaction was allowed to develop for 2 h in the dark at room temperature, and then the absorbance was read at 515 nm with a multi-well spectrophotometer (Sunrise™, TECAN, France). The DPPH concentration in the reaction medium was calculated from a calibration curve ($n = 8$; $r = 0.99$) determined by the following linear regression: [DPPH] = (Abs − 0.0398)/0.0137 to further deduce the percentage of remaining DPPH (% DPPH). A curve of extract concentration against % DPPH was generated to estimate the concentration of extract needed to cause a 50% reduction of the initial DPPH concentration. This value is known as EC$_{50}$ (efficient concentration, also called oxidation index) and was expressed in terms of mg per ml. This assay was done in triplicate for each sample, and then the mean values were used to calculate the EC$_{50}$. Ascorbic acid, α-tocopherol, BHA and BHT were used as positive controls.

2.4.2. β-Carotene–linoleic acid system

The antioxidant activities of samples assayed by the β-carotene–linoleic acid system were measured according to the method described by Koleva, Van Beek, Linssen, Groot, and Evstatieva (2002) after slight modifications. This method is based on the loss of the yellow colour by β-carotene further to its reaction with the radicals formed by linoleic acid oxidation; the β-carotene bleaching is, thus, slowed down in the presence of antioxidants. After dissolution of β-carotene (1 mg) in 5 ml of chloroform, 1 ml of this solution was mixed with linoleic acid (25 µl) and Tween-40 (200 mg). After removal of chloroform by evaporation at 40 °C under vacuum, 50 ml of distilled water oxygenated by air-bubbling were added slowly to the semi-solid residue under vigorous stirring to form an emulsion, which was always prepared just before each experiment. A 96-well microplate was loaded with 50 µl per well of the samples under test or positive controls (α-tocopherol, BHA and BHT prepared in ethanol) and 200 µl of the emulsion. The ascorbic acid was not used as a positive control in this assay since it shows pro-oxidant behaviour in this system. Four final concentrations were tested (50, 100, 200 and 500 mg l^{-1}) and ethanol was used as blank. The absorbance value was read at 450 nm on a multi-well spectrophotometer (Sunrise™, TECAN, France) at addition of the emulsion considered as the starting time of the reaction ($t = 0$ mn). Then, the plate was covered with a film and stored at 50 °C to let the reaction develop for 3 h; the absorbance was measured every 30 mn. All of the determinations were made in triplicate. The antioxidant activity (AA) of the extracts was evaluated as the percentage of inhibition of the bleaching of β-carotene by using the following formula:

$$AA = [1 - (A_0 - A_t)/(A_0' - A_t')] \times 100,$$

where A_0 is the absorbance of the sample at $t = 0$ mn and A_0' is the one exhibited by the control, also at $t = 0$ min, A_t and A_t' are those by the sample and the control, respectively, at $t = 3$ h.

2.4.3. Reducing activity

The method by Kuda, Tsunekawa, Hishi, and Araki (2005) was used, after slight modifications, to assess the reducing activity of each extract. It relies on the evaluation of the total antioxidant capacity of a given extract from the redox potentials of the compounds. Aliquots of extracts (0.2 ml) at four different concentrations (50, 100, 200 and 500 mg l^{-1}) were mixed with phosphate buffer (0.2 ml, 0.2 M, pH 6.6) and potassium ferricyanide

[K$_3$Fe(CN)$_6$] (0.2 ml, 1%). After incubation at 50 °C for 30 min, the mixture was cooled down prior to the addition of 0.2 ml of trichloroacetic acid (10%). Then, aliquots (0.125 ml) of this mixture were transferred to a 96-well microplate before addition of 20 µl of 0.1% FeCl$_3 \cdot 6H_2O$ to each well. Any rise of the reaction mixture absorbance, read at 620 nm on a multi-well spectrophotometer (Sunrise™, TECAN, France), was indicative of an increase in reducing activity. Absorbance was then transformed into a percentage of inhibition through comparison to a blank (ethanol). This assay was carried out in triplicate for each sample and positive controls (ascorbic acid, α-tocopherol, BHA and BHT).

2.5. Total phenolic content

The total phenolic content of algal extracts was determined according to the Folin–Ciocalteu method (Sanoner, Guyot, Marnet, Molle, & Drilleau, 1999). The samples (100 µl) were mixed with 50 µl Folin–Ciocalteu reagent, 200 µl of 20% sodium carbonate solution and 650 µl of distilled water. Then, the mixture was allowed to stand at 70 °C in the dark for 10 min. After the production of a blue colour, the absorbance was read at 700 nm. The total content of phenolic compounds was expressed in % of dry weight (% dw) from a standard curve of phloroglucinol. This analysis was made in triplicate for each extract.

2.6. Cell culture

Daudi (Human Burkitt's lymphoma), Jurkat (Human leukaemic T cell lymphoblast) and K562 (Human chronic myelogenous leukaemia) cells were obtained from ECACC (European Collection of Cell Cultures) and grown on RPMI 1640 medium with L-glutamine (Lonza) supplemented with 10% of heat inactivated FBS (Cambrex), or 20% for Daudi cells. For the test, the cells were plated in triplicate at 5×10^4 cells per well in 200 µl onto 96-flat-bottomed well ELISA plates and incubated at 37 °C in 5% CO$_2$ for 24 h before exposure to the drug. The seaweed extracts were added at a concentration of 100 µg ml^{-1}. The stimulated cultures and the control ones (solvent alone) as well as the blank (cell-free medium) were all incubated for an additional 24 h period.

2.7. Antitumoural assays

The antitumoural activities of these crude extracts were investigated by the cytotoxic assay adapted from the method by Ishiyama and co-workers (1995). To assess the mitochondrial function, mitochondrial dehydrogenase (succinate-tetrazolium-reductase) activity was determined with the WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) colorimetric assay (Roche Diagnostics, France). The colorimetric assay is based on the cleavage of the tetrazolium salt, WST-1, to a water-soluble formazan-class dye by the mitochondrial succinate-tetrazolium reductase. There is a direct correlation between this reaction and the number of metabolically active cells. After 24 h of incubation, 10 µl of the formazan dye were added to each well prior to a 3 h incubation period at 37 °C. Absorbance was measured at 450 nm on a multi-well spectrophotometer (Infinite, TECAN, France). For each condition, the mean optical density (OD) of the three technical replicates per exposure condition was compared to the mean OD of the control (DMSO). This assay was made twice.

2.8. Statistical analysis

All of the statistical analyses were performed with Statistica 6 software. The data were tested for normality (Shapiro–Wilk's test) and subjected to the Bartlett test to check for the homogeneity of

variance. The antioxidant activities displayed by extracts were compared by one-way analysis of variance (ANOVA) after transformation of data when required. A post-hoc test (Tukey HSD) was carried out when the differences shown by data were significant ($p < 0.05$). The Student t-test was used to compare antitumoural activity of the extracts against the control at a significant level of 1%. The Pearson correlation coefficient (r) was also calculated ($p < 0.01$) to assess the strength of the linear relationship between two variables.

3. Results

3.1. Antioxidant assays and total phenolic content

3.1.1. Crude extracts

Fig. 1, Tables 2 and 3 show the main results of the assessment by the DPPH radical-scavenging activity, the reducing activity and the β-carotene–linoleic acid system, respectively, of the antioxidative activities displayed by the crude extracts under study. All of the species collected for this study showed DPPH radical-scavenging activities (Fig. 1). By exhibiting the lowest EC_{50} (0.21 ± 0.00 mg ml^{-1}), the crude extract from Halidrys siliquosa proved to be endowed with a very strong antioxidant activity. Moreover, Fucus ceranoides (0.46 ± 0.01 mg ml^{-1}), Cystoseira tamariscifolia (0.49 ± 0.01 mg ml^{-1}) and Bifurcaria bifurcata (0.56 ± 0.00 mg ml^{-1}) proved to be the most active (Fig. 1).

The reducing activity assay confirmed the results obtained by the DPPH assay: indeed, Table 2 shows that the highest reducing activity ($94.90 \pm 0.15\%$ at 500 mg l^{-1}) was displayed by the extract from H. siliquosa; it is worth noting that it is enhanced at higher concentrations of extract. Moreover, according to the statistical analysis, this reducing activity is equivalent to those displayed by any of the commercial antioxidants tested at the concentration of 500 mg l^{-1} and by α-tocopherol at lower concentrations (100 and 200 mg l^{-1}). As expected from the DPPH assay results, the crude extracts from C. tamariscifolia, B. bifurcata and F. ceranoides prove also to be endowed with high reducing activities ($91.83 \pm 0.32\%$, $90.97 \pm 0.01\%$ and $84.68 \pm 0.86\%$ at 500 mg l^{-1}, respectively). The negative correlation observed between DPPH radical-scavenging activities and the reducing activities measured in brown algae extracts was found to be significant ($r = -0.880$, p < 0.01).

Table 3 shows that the strong antioxidant activity of the H. siliquosa extract, deduced from the previous results, is confirmed by the β-carotene–linoleic acid system assay: the percentage inhibition of β-carotene bleaching is, indeed, the highest ($88.41 \pm 0.69\%$ at 500 mg l^{-1}). The antioxidant activity of the H. siliquosa extract is significantly equivalent to those of BHT (at the concentrations of 500, 200 and 100 mg l^{-1}) and BHA (at the concentrations of 500 and 200 mg l^{-1}). Concerning C. tamariscifolia and B. bifurcata, the finding of a high antioxidant activity ($78.55 \pm 1.37\%$ and $76.13 \pm 0.55\%$ at 500 mg l^{-1}, respectively) is consistent with the results of the DPPH and reducing activity assays. On the other hand, for the F. ceranoides extract, the results of the β-carotene–linoleic acid system assay are not in full agreement with those from the two other assays. Indeed, no significant correlation was found between either the DPPH radical-scavenging activities, or the reducing activities, and the antioxidant activities measured with the β-carotene–linoleic acid system ($r = -0.390$ and $r = 0.737$, respectively, p < 0.01). On the other hand, with the β-carotene–linoleic acid system, the crude extracts from Desmarestia ligulata and Dictyota dichotoma demonstrated pro-oxidant activities ($-41.94 \pm 0.71\%$ and $-39.17 \pm 1.77\%$ at 500 mg l^{-1}, respectively).

Table 4 highlights variations, among species, in the contents of extracts in total phenolic compounds, e.g. 0.96–10.91% dw for B. bifurcata and C. tamariscifolia, respectively. No significant correlations were found between the total phenolic content in a given crude extract and the antioxidant activity measured in the same extract by the DPPH method ($r = -0.399$, p < 0.01), or by the reducing activity ($r = 0.434$, $p < 0.01$) or by the β-carotene assay ($r = 0.299$, $p < 0.01$). However, it is worth noting that the highest phenolic contents ($10.91 \pm 0.07\%$ dw and $5.47 \pm 0.40\%$ dw, respectively) were found in the crude extracts from C. tamariscifolia and F. ceranoides both previously identified as displaying marked antioxidant activities.

3.1.2. Fractions of some crude extracts

To gain more insight into the relationships between reducing activity and total phenolic content, the crude extracts that displayed the highest reducing activities (B. bifurcata, C. tamariscifolia, F. ceranoides and H. siliquosa) were fractionated, and then each fraction was assayed. Fig. 2 highlights the percentage of antioxidant activity exhibited by each fraction. One should note that the
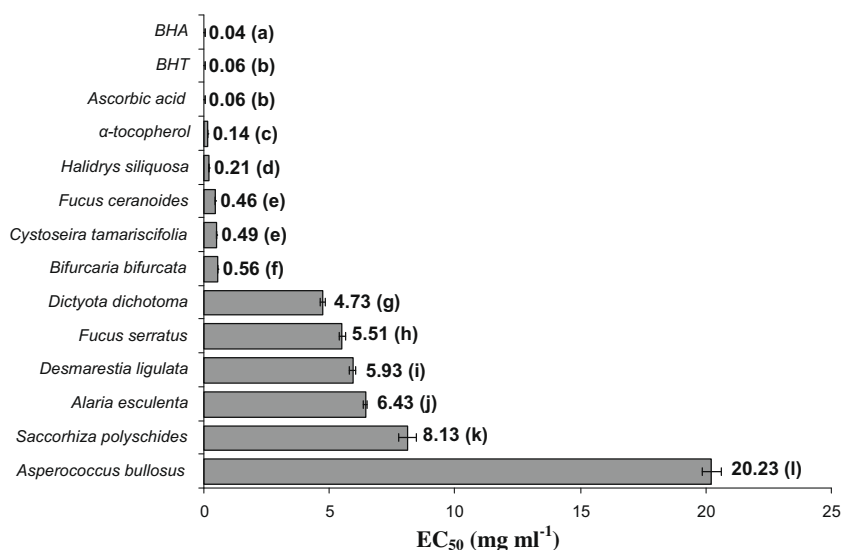

Fig. 1. DPPH radical-scavenging activities displayed by brown algae crude extracts and controls (ascorbic acid, BHA, BHT and α-tocopherol) and expressed as oxidation index, EC_{50} given in mg ml^{-1} (mean ± SD; $n = 3$). The bars represent the standard deviation. Significant differences determined by the Tukey HSD test ($p < 0.05$) are indicated by different letters (a–l).

Table 2
Reducing activity displayed by brown algae crude extracts at different concentrations (50, 100, 200 and 500 mg l^{-1}) and expressed as % of inhibition.

	50 mg l^{-1}	100 mg l^{-1}	200 mg l^{-1}	500 mg l^{-1}
Alaria esculenta	20.40 ± 1.43 (j)	38.71 ± 0.43 (g)	55.81 ± 0.59 (f)	76.64 ± 0.32 (d)
Asperococcus bullosus	8.43 ± 0.95 (l)	16.46 ± 1.57 (j)	26.90 ± 1.60 (j)	44.61 ± 1.89 (h)
Bifurcaria bifurcata	54.57 ± 0.69 (g)	69.56 ± 0.10 (e)	81.31 ± 0.20 (d)	90.97 ± 0.01 (b)
Cystoseira tamariscifolia	60.05 ± 0.69 (f)	73.22 ± 0.27 (d)	84.20 ± 0.27 (c)	91.83 ± 0.32 (b)
Desmarestia ligulata	16.26 ± 0.87 (k)	27.98 ± 0.64 (i)	45.90 ± 0.97 (h)	63.02 ± 0.93 (g)
Dictyota dichotoma	21.45 ± 1.33 (j)	27.59 ± 1.13 (i)	40.84 ± 0.88 (i)	61.95 ± 0.32 (g)
Fucus ceranoides	32.09 ± 1.90 (h)	42.74 ± 1.00 (f)	65.95 ± 1.28 (e)	84.68 ± 0.86 (c)
Fucus serratus	24.86 ± 0.71 (i)	35.03 ± 1.38 (h)	43.72 ± 0.80 (h)	69.19 ± 1.37 (f)
Halidrys siliquosa	72.40 ± 0.26 (e)	83.77 ± 0.15 (c)	90.71 ± 0.01 (b)	94.90 ± 0.15 (a)
Saccorhiza polyschides	20.00 ± 0.73 (j)	35.02 ± 1.46 (h)	49.14 ± 1.29 (g)	72.29 ± 0.45 (e)
BHA	87.42 ± 0.07 (b)	94.02 ± 0.08 (a)	94.17 ± 0.08 (a)	95.64 ± 0.02 (a)
BHT	84.63 ± 0.37 (c)	90.18 ± 0.17 (a)	93.65 ± 0.17 (a)	94.93 ± 0.11 (a)
Ascorbic acid	90.04 ± 0.32 (a)	92.91 ± 0.14 (a)	93.53 ± 0.09 (a)	94.87 ± 0.01 (a)
α-Tocopherol	77.02 ± 0.39 (d)	85.08 ± 0.16 (c)	90.30 ± 0.13 (b)	94.61 ± 0.04 (a)

Each value corresponds to the mean ± SD (n = 3). Significant differences determined by the Tukey HSD test ($p < 0.05$) are indicated by different letters (a–l).

Table 3
Antioxidant activities as % of inhibition in brown algae crude extracts at different concentrations (50, 100, 200 and 500 mg l^{-1}) assayed by the β-carotene–linoleic acid system.

	50 mg l^{-1}	100 mg l^{-1}	200 mg l^{-1}	500 mg l^{-1}
Alaria esculenta	24.63 ± 1.71 (e)	39.99 ± 2.77 (d)	52.45 ± 1.74 (e)	58.19 ± 2.68 (d)
Asperococcus bullosus	5.62 ± 0.94 (g)	7.94 ± 1.49 (g)	10.86 ± 1.56 (g)	5.16 ± 0.58 (f)
Bifurcaria bifurcata	2.73 ± 0.81 (g)	26.01 ± 1.34 (e)	60.14 ± 1.24 (d)	76.13 ± 0.55 (c)
Cystoseira tamariscifolia	38.79 ± 2.12 (d)	52.79 ± 1.63 (c)	71.07 ± 1.03 (c)	78.55 ± 1.37 (c)
Desmarestia ligulata	−28.11 ± 2.61 (h)	−28.24 ± 0.71 (h)	−29.96 ± 0.42 (h)	−41.94 ± 0.71 (g)
Dictyota dichotoma	−24.17 ± 1.72 (h)	−29.60 ± 1.84 (h)	−34.04 ± 2.59 (h)	−39.17 ± 1.77 (g)
Fucus ceranoides	4.29 ± 1.59 (g)	18.42 ± 2.93 (f)	26.34 ± 2.10 (f)	31.78 ± 2.26 (e)
Fucus serratus	16.85 ± 0.24 (f)	17.83 ± 1.55 (f)	27.76 ± 1.46 (f)	57.45 ± 0.41 (d)
Halidrys siliquosa	75.17 ± 0.90 (c)	76.04 ± 0.92 (b)	85.45 ± 0.73 (b)	88.41 ± 0.69 (b)
Saccorhiza polyschides	23.64 ± 0.49 (e)	37.06 ± 1.00 (d)	50.62 ± 1.14 (e)	60.42 ± 1.13 (d)
BHA	82.40 ± 1.92 (b)	87.18 ± 1.19 (a)	87.45 ± 1.20 (b)	90.45 ± 1.55 (b)
BHT	80.44 ± 1.41 (b)	80.70 ± 1.76 (b)	87.41 ± 0.88 (b)	91.06 ± 1.88 (b)
α-Tocopherol	89.01 ± 1.39 (a)	90.16 ± 0.18 (a)	93.42 ± 0.59 (a)	97.20 ± 0.54 (a)

Each value corresponds to the mean ± SD (n = 3). Significant differences determined by the Tukey HSD test ($p < 0.05$) are indicated by different letters (a–h).

Table 4
Total phenolic content of brown algae crude extracts (mean ± SD; n = 3) expressed in % of dry weight (dw).

Species	% dw
Alaria esculenta	2.03 ± 0.03
Asperococcus bullosus	1.11 ± 0.02
Bifurcaria bifurcata	0.96 ± 0.01
Cystoseira tamariscifolia	10.91 ± 0.07
Desmarestia ligulata	1.22 ± 0.10
Dictyota dichotoma	1.88 ± 0.02
Fucus ceranoides	5.47 ± 0.40
Fucus serratus	2.82 ± 0.23
Halidrys siliquosa	1.60 ± 0.01
Saccorhiza polyschides	1.66 ± 0.06

fraction showing the most elevated antioxidant activity depends on the species: indeed, the *F*I fraction (aqueous fraction) proved to be the most active in *B. bifurcata* (71.48 ± 0.39%) and *C. tamariscifolia* (79.32 ± 0.21%), whereas it was the *F*II (hydromethanolic fraction) in *F. ceranoides* (86.35 ± 0.06%) and *H. siliquosa* (83.98 ± 0.15%). In this last species, the methanolic fraction, *F*III, also displayed quite a high activity (76.66 ± 0.06%). Moreover, the statistical analysis showed that the reducing activities displayed by *F*II from the *F. ceranoides* crude extract and α-tocopherol were quite alike (86.35 ± 0.06% and 85.08 ± 0.16%, respectively). On the other hand, neither *F*IV (dichloromethane:methanol fraction), nor *F*V (dichloromethane fraction) showed significant activity. It is worth noting that the total phenolic contents were the highest in the fractions that exhibited the highest antioxidant activity: *F*I from *C. tamariscifolia* and *B. bifurcata* (25.40 ± 0.33% dw and

13.31 ± 0.36% dw, respectively), *F*II from *F. ceranoides* and *H. siliquosa* (41.91 ± 0.49% dw and 28.77 ± 0.25% dw, respectively). Moreover, a significant positive correlation ($r = 0.836$, $p < 0.01$) was found between the total phenolic contents and the antioxidant activities measured in every fraction (Fig. 3).

3.2. Antitumoural activities

The cytotoxic activities of the crude extracts were tested against three tumoural cell lines (Daudi, Jurkat and K562). Fig. 4 highlights that the crude extracts from *B. bifurcata*, *C. tamariscifolia*, *D. ligulata*, *D. dichotoma* and *H. siliquosa* present significant cytotoxic activities against the three tumoural cell lines ($p < 0.01$). One should note that the crude extract from *Alaria esculenta* also shows a significant reduction of the cell viability with Daudi (73.74 ± 4.14%, $p < 0.0001$) and Jurkat (61.01 ± 21.90%, $p = 0.0073$) cell lines, conversely to the crude extract from *F. ceranoides*, which exhibits a significant increase in Daudi cell viability (128.79 ± 15.62%, $p = 0.0063$).

4. Discussion

4.1. Antioxidant activities

In order to by-pass the inability of a one-dimensional test of antioxidant capacity to accurately mirror the *in vivo* complexity of interactions between antioxidants, in this study the antioxidative activities displayed by algal crude extracts were characterised by three simple, fast and reliable biochemical methods (Frankel & Meyer, 2000). DPPH radical-scavenging activity, reducing activity
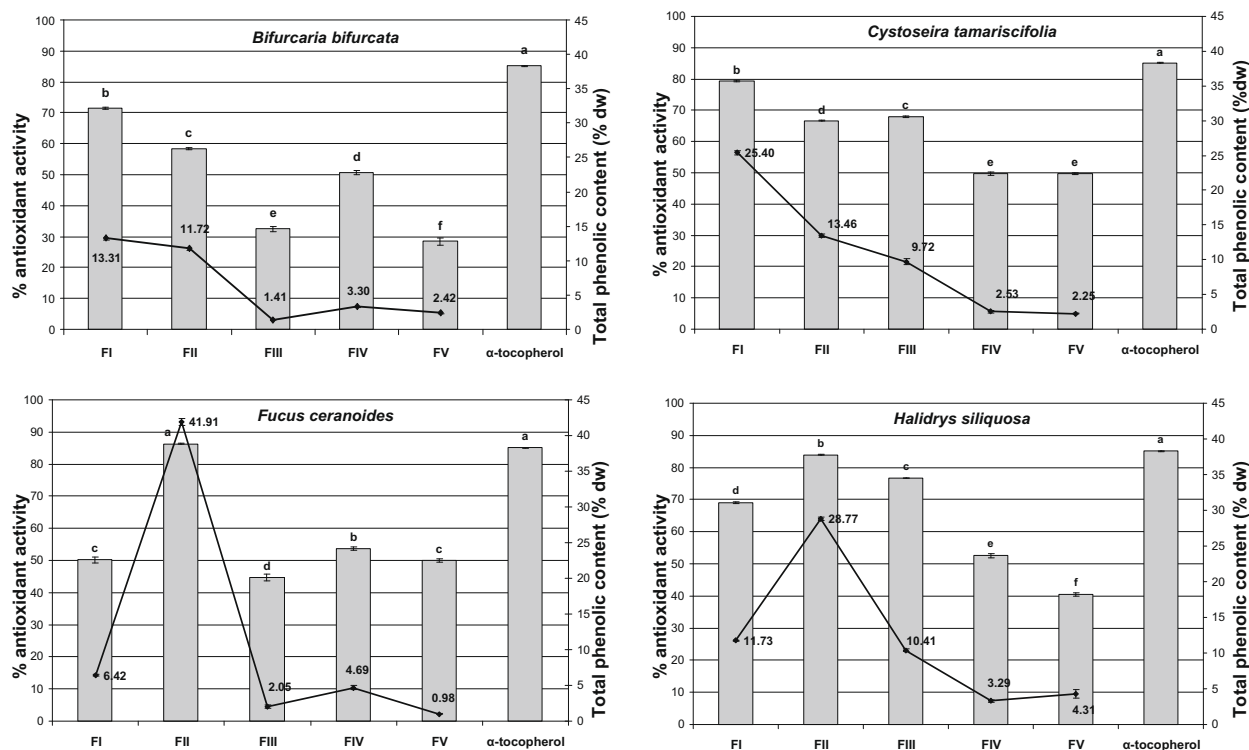
Fig. 2. Antioxidant activities (%, histograms, mean ± SD, *n* = 3) and total phenolic content (% dw, curve, mean ± SD, *n* = 5) of the different fractions (*FI*, *FII*, *FIII*, *FIV* and *FV*) from *Bifurcaria bifurcata*, *Cystoseira tamariscifolia*, *Fucus ceranoides* and *Halidrys siliquosa* crude extracts. Antioxidant activity was measured by the reducing activity method in 100 mg l⁻¹ fractions versus the positive control α-tocopherol. Significant differences between antioxidant activities determined by the Tukey HSD test (*p* < 0.05) are indicated by different letters (a–f).
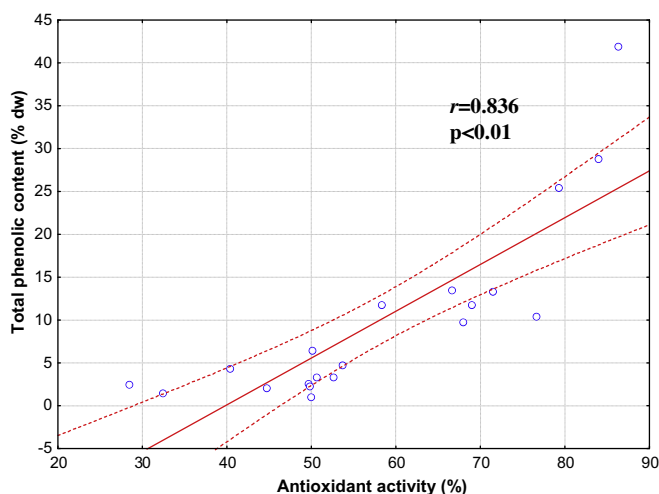

Fig. 3. Correlations between total phenolic content (% dw) and antioxidant activity (% reducing activity) measured in the fractions (*FI*, *FII*, *FIII*, *FIV* and *FV*) of the most active crude extracts (*Bifurcaria bifurcata*, *Cystoseira tamariscifolia*, *Fucus ceranoides* and *Halidrys siliquosa*). The dotted lines correspond to the 95% confidence limit. A significant Pearson correlation (*r*; *p* < 0.01) was observed.

and β-carotene–linoleic acid system assays proved to be relevant, here, in the assessment of the total antioxidant activity of the extracts. The finding of a significant correlation between the DPPH radical-scavenging activities and the reducing ones displayed by the extracts under study is explained by the fact that both assays rely on a mechanism of electron/hydrogen donation. In the β-carotene assay, the system is more complex, and the emulsified lipid in use introduces additional variables liable to affect the oxidation process; moreover, the antioxidant molecules can act as inhibitors

of lipid oxidation through various mechanisms, e.g. prevention of chain initiation, binding of transition metal ion catalysts peroxide decomposition, in addition to free radical trapping (Frankel & Meyer, 2000).

Among the species under study, *H. siliquosa* exhibited the highest antioxidant activity whatever the assay, and its antioxidant activity was equivalent to those displayed by the commercial antioxidant molecules under test in this study (ascorbic acid, α-tocopherol, BHA and BHT). To our knowledge, this study is the first one to provide evidence for the existence of high antioxidant activity in the brown alga, *H. siliquosa*. This species belongs to the Sargassaceae family, which has been the subject of extensive investigations because of its natural diversity in bioactive compounds and use as model system for chemotaxonomic studies (Kornprobst, 2005). The finding, in this study, of three species of the Sargassaceae family, i.e. *B. bifurcata*, *C. tamariscifolia* and *H. siliquosa*, among the four most active ones constitutes more evidence of the bioactive potential of this group. Moreover, the marked antioxidant activity exhibited by these extracts and their fractions was correlated with their high contents in phenolic compounds. This finding is in agreement with numerous literature data (e.g. Connan, Delisle, Deslandes, & Ar Gall, 2006; Kim et al., 2005; Le Lann et al., 2008; Zhang, Duan, Huang, Zhang, & Wang, 2007). Phenolic compounds are thought to protect the algal thallus against photodestruction by UV radiation and to exhibit radical-scavenging properties (Connan, Delisle, Deslandes, & Ar Gall, 2006). According to Frankel and Meyer (2000), the well-acknowledged structure-activity relationships of radical-scavenging phenolic antioxidants involve the ability to donate a phenolic hydrogen as well as the stabilisation of the resulting antioxidant radical through electron delocalisation and/or intramolecular hydrogen bonding or further oxidation. Some phenolic compounds have been isolated from *H. siliquosa* extracts (Kornprobst, 2005;
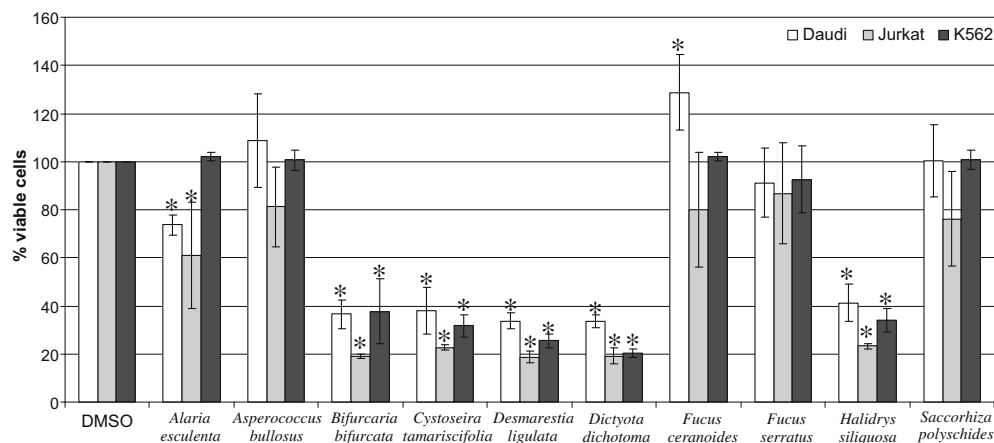
Fig. 4. Percentage of cell viability in comparison to a control (mean ± SD; *n* = 6). Three different tumourigenic cell lines (Daudi, Jurkat and K562) were exposed for 24 h to the brown algae crude extracts and to the control (DMSO; 100 µg ml^{-1}); *indicates significant differences (*p* < 0.01) compared with the control.

Ragan & Glombitza, 1986; Singh & Bharate, 2006), and Kornprobst (2005) suggested that some hydroquinols with oxygenated diterpene side-chains (tocopherol-like compounds) could act as antiradical or antioxidant molecules. Tetraprenyltoluquinol derivatives (tocopherol-like compounds) have been identified as the antioxidant molecule further to the observation of a marked antioxidant activity in *Cystoseira* species (Fisch, Böhm, Wright, & König, 2003; Foti, Piattelli, Amico, & Ruberto, 1994). Phenolic compounds extracted from *C. tamariscifolia* (Abdala-Diaz, Cabello-Pasini, Pérez-Rodriguez, Conde-Alvarez & Figueroa, 2006) and *B. bifurcata* (Connan, Deslandes, & Ar Gall, 2007) have also demonstrated photoprotection mechanisms against high solar irradiance through absorption of incident photons, or indirectly as a result of their antioxidant activity. The whole of these studies are in favour of the implication of phenolic compounds in the antioxidant activity of these extracts. However, concerning our study, further purifications are needed to isolate and identify the antioxidant molecules involved in the fractions from *B. bifurcata*, *C. tamariscifolia* and *H. siliquosa* that showed a marked antioxidant activity.

The crude extract from *F. ceranoides* also proved to be active, and its high phenolic content, especially in fraction II, could partly explain its high radical-scavenging activity. Few studies have been devoted to the isolation of phenolic compounds from this genus. *Fucus spiralis* is known to produce two types of polymeric phlorotannins of the fucol and fucophlorethol classes endowed with a high antioxidant activity (Cerantola et al., 2006). Moreover, some of these compounds (trifucol and fucophlorethol) have been isolated from *Fucus vesiculosus* (Singh & Bharate, 2006). Thus, one can assume that these families of phlorotannins are implicated in the antioxidative activities displayed, in the present study, by extracts from *F. ceranoides*. Furthermore, the lack of confirmation, by the β-carotene assay, of the strong antioxidant activity exhibited by the *F. ceranoides* extract suggests that the antioxidant mechanisms at play in this extract are mainly related to the trapping of free radicals. This result could also be explained by the interactions, through synergistic-, additive- or antagonistic-effects, between the emulsified medium used in the β-carotene assay and the complex composition of the crude extracts. With the β-carotene–linoleic acid system, the crude extracts from *D. ligulata* and *D. dichotoma* also showed a specific response (pro-oxidant activities) unobserved with the DDPH and reducing activity assays. Other authors (Le Tutour et al., 1998) have also mentioned a pro-oxidant activity in the *Laminaria digitata* extract, but no explanation was given. A prerequisite to the understanding of this pro-oxidant activity is the identification of the antioxidant compound. However, some antioxidants, e.g. ascorbic acid or α-tocopherol, can act as pro-oxidants at certain doses and under certain experimental conditions (Zhang & Omaye, 2001). Some hydrophilic polyphenolic compounds showed antioxidant activity in lecithin liposomes or low-density lipoprotein systems and pro-oxidant activity in oil-in-water emulsions (like the β-carotene emulsion used in this study) (Frankel & Meyer, 2000). This marked variation in antioxidant activity has been attributed to differences in their relative partition between phases in various lipid systems. Together these literature data underline that, in any assessment of natural antioxidants, the analysis of results must thoroughly consider the system and the analytical method in use. Though the use of complementary methods to assay antioxidant activity is very appealing to researchers, we personally think that measuring a specific antioxidant activity in both *in vitro* and *in vivo* biological systems would be more relevant in our future studies.

4.2. Antitumoural activities

This study highlighted the strong cytotoxicity against the human cancer cell lines Daudi, Jurkat and K562 by crude extracts from *B. bifurcaria*, *C. tamariscifolia*, *D. ligulata*, *D. dichotoma* and *H. siliquosa*. While confirming the high bioactivity of Sargassaceae, this screening provided the first evidence of cytotoxic activities by the *C. tamariscifolia* and *H. siliquosa* extracts. The demonstration of cytotoxic activity in *B. bifurcata* extracts (Culioli et al., 2004; Moreau et al., 2006) has led to the identification of an acyclic diterpene, bifurcariol, as the cytotoxic compound. Further to studies about cytotoxic activity by species of the *Cystoseira* genus, various diterpenes have been identified as the bioactive compounds in *C. crinita* (Fisch et al., 2003), *C. myrica* (Ayyad, Abdel-Halim, Shier, & Hoye, 2003) and *Cystoseira usneoides* (Urones et al., 1992). These reports suggest that diterpenes compounds could be responsible for the antitumoural activities measured in the Sargassaceae species collected in our study. Moreover, the finding, in extracts from these species, of cytotoxic effects together with strong antioxidant activity makes these species promising candidates for further investigations. As damage events are frequently correlated with oxidative stress, the prevalence of both properties in a single compound could be beneficial in terms of rational, preventive or therapeutic purposes. Since the preliminary results reported here sound really promising, it would be worth developing further studies on bio-guided fractionation and isolation of pure compounds.

Among the crude extracts under study, those from *D. ligulata* and *D. dichotoma* exhibited the strongest cytotoxic activities with all of the tested tumoural cell lines. This is in accordance with literature data about evidences of cytotoxic activities in *D. dichotoma*

(Duran, Zubia, Ortega, & Salva, 1997; Kolesnikova, Kalinovsky, Fedorov, Shubina, & Stonik, 2006), where diterpenes were identified as the cytotoxic compounds. In fact, the Dictyotaceae family has been extensively studied for its wide variety of bioactive diterpenes with marked biological activities (Kornprobst, 2005). Phenolic compounds such as chromenols have been identified as the cytotoxic molecules in *Desmarestia menziesii* (Davyt, Enz, Manta, Navarro, & Norte, 1997). It is thus worth wondering about the relationship between the cytotoxic activities of *D. ligulata* and *D. dichotoma* crude extracts and their pro-oxidant activities measured in the β-carotene assay. Indeed, oxidative stress is known to be implicated in the process of carcinogenesis (Halliwell, 2007). Any increase in the formation of reactive oxygen species (ROS) in the cells can contribute to the process of carcinogenesis through either direct genotoxic effects, or indirect ones *via* modification of signaling pathways leading to an altered expression of numerous genes. Further to their production by various biochemical reactions or as a by-product of oxygen metabolism in mitochondria, ROS can damage different types of cellular molecules such as proteins, lipids and nucleic acids (Kohen & Nyska, 2002). DNA mutation is considered as a crucial step in carcinogenesis, and the recording of more numerous oxidatively-derived DNA lesions in many tumours is in favour of the strong implication of such damage in the etiology of cancer (Cooke, Olinski, & Evans, 2006). Additional studies are needed to gain more insight into the mechanism of action of *D. ligulata* and *D. dichotoma* crude extracts through studies, for example, of its cell cycle impact, its ability to activate caspases, or mitochondrial and DNA damages.

On the contrary, the *F. ceranoides* crude extract exhibited pro-tumoural activity by increasing the cell viability of Daudi tumoural cell lines. The phenolic compounds of this extract likely involved in its radical-scavenging activity could also be implicated in this pro-tumoural activity. The ability of phenols to protect cells from oxidative stress has been demonstrated, but these compounds have a contradictory behaviour characterised by anti- and pro-tumoural activities according to their chemical structure, the system and conditions used in the study (Gomes et al., 2003). Indeed, it is believed that they can behave as either antioxidants or pro-oxidants and that the inhibitory potency displayed on the *in vitro* growth and proliferation of some malignant cells is strongly dependent on their structural characteristics (see review in Gomes et al., 2003). The present study suggests that the action of the bioactive molecules extracted from *F. ceranoides* (likely phenolic compounds) as either antioxidants or pro-oxidants depends on the experimental conditions and on the tumoural cell lines under test. In the future, it would be worth identifying the mechanisms of action and isolating the bioactive compounds in order to understand the pro-tumoural activity observed in Daudi cell lines.

5. Conclusions

This study constituted the largest screening of antioxidant and antitumoural activities in brown macroalgae from Brittany coasts achieved till now. It emphasised the great antioxidant potential of *H. siliquosa*, which was found to be equivalent to the antioxidant activity of all of the commercial antioxidant molecules assessed in the same study. Moreover, two other Sargassaceae species (*B. bifurcata* and *C. tamariscifolia*) and one Fucaceae (*F. ceranoides*) demonstrated a high antioxidant activity. The correlation usually found between marked radical-scavenging activities and a high total phenolic content is in favour of the involvement of phenolic compounds in the antioxidant mechanisms. Furthermore, the crude extracts from the Sargassaceae species under study and those from *D. ligulata* and *D. dichotoma* showed a strong cytotoxicity against human cancer cell lines. Further to these findings, it would be worth carrying out additional experiments in order to identify and characterise the bioactive compounds present in the most active extracts and get a better understanding of their mechanisms of action.

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