

Full Length Research Paper

# Antioxidant activity of Rhodophyceae extracts from Atlantic and Mediterranean Coasts of Morocco

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The evaluation of the antioxidant activity of ten aqueous and methanol extracts of the red seaweeds, *Pterosiphonia complanata*, *Boergeseniella thuyoides*, *Sphaerococcus coronopifolius*, *Asparagopsis armata*, *Halopitys incurvus*, *Hypnea musciformis*, *Gelidium spinulosum*, *Plocamium cartilagineum*, *Gelidium pulchellum* and *Ceramium rubrum* was realized through three different tests. Using the DPPH (2,2-diphényl-1-picrylhydrazyl) test, four methanol extracts allowed the transformation of DPPH radical in reduced form with an EC<sub>50</sub> between 96 and 862 µg.mL<sup>-1</sup>. With respect to the β-carotene test, 7 methanol extracts showed activity against peroxide radicals with an EC<sub>50</sub> between 9 and 176 µg.mL<sup>-1</sup>. In the deoxyribose test, the inhibition percentage of hydroxyl radicals varies between 25 and 68% for five aqueous extracts; the most important being the extract of *A. armata*.

**Key words:** Red algae, antioxidant activity, DPPH, Deoxyribose, β-carotene–linoleic acid system.

## INTRODUCTION

Seaweeds as natural sources with a high degree of bioavailability of trace elements are strongly advised for fast grown children and pregnant women. In contrast to their use as a source of food, marine algae are widely used in the life science as the source of compounds with diverse structural forms and biological activities (Athukorala et al., 2006; Bouhlal et al., 2010a; b; 2011). Seaweeds are considered to be a rich source of antioxidants (Cahyana et al., 1992; Devi et al., 2011; Kelman et al., 2012; Murugan and Iyer, 2012; Kim et al., 2012). Consequently, antioxidant activity is intensively focused due to the currently growing demand from the pharmaceutical industry where there is interest in anti-aging and anticarcinogenic natural bioactive compounds, which possess health benefits (Heo et al., 2005; Murugan and Iyer, 2012).

Reactive oxygen species (ROS) such as superoxide

anion (O<sub>2</sub><sup>-</sup>), hydroxyl radical (HO<sup>•</sup>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) are formed during aerobic life as a result of the metabolism of oxygen. DNA, cell membranes, proteins and other cellular constituents are target site of the degradation processes, and consequently induce different kinds of serious human diseases including atherosclerosis, rheumatoid arthritis, muscular dystrophy, cataracts, some neurological disorders and some types of cancer as well as aging (Kovatcheva et al., 2001; Ruberto et al., 2001; Ganesan et al., 2008; Kumar et al., 2008). Moreover, ROS are predominant cause of qualitative decay of foods, which lead to rancidity, toxicity and destruction of biomolecules important in physiologic metabolism (Heo et al., 2005). Furthermore, antioxidants from natural sources increase the shelf-life of foods. Therefore, consumption of antioxidant and addition of antioxidant in food materials protect the body as well as foods against these events (Schwarz et al., 2001; Ganesan et al., 2008).

Recently, the potential antioxidant compounds were identified as some pigments (e.g. fucoxanthin, astaxanthin, carotenoid) and polyphenols (e.g. phenolic

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**Table 1.** List of marine algae screened for antioxidant activity.

Seaweeds		Collection localities	Collection date
Orders	Scientific name		
Bonnemaisoniales	<i>Asparagopsis armata</i> Harvey C	Ksar sghir	19/07/2007
Ceramiales	<i>Ceramium rubrum</i> C. Agardh	Ksar sghir	31/07/2007
	<i>Halopitys incurvus</i> (Gomel) Kützinger	Sidi bouzid	02/08/2007
	<i>Boergeseniella thuyoides</i> (Harvey) Kylin C	Ksar sghir	31/07/2007
	<i>Pterosiphonia complanata</i> (Clemente) Falkenberg C	Belyounech	13/04/2006
Gelidiales	<i>Gelidium pulchellum</i> (Furner) Kützinger C	Ksar sghir	24/03/2007
	<i>Gelidium spinulosum</i> (C. Agardh) J. Agardh P	Sidi bouzid	18/08/2007
Gigartinales	<i>Hypnea musciformis</i> (Wulfen) J.V. Lamouroux	Ksar sghir	26/06/2007
	<i>Plocamium cartilagineum</i> (Linnaeus) P.S. Dixon C	Belyounech	19/07/2007
	<i>Sphaerococcus coronopifolius</i> Stackhouse	Belyounech	14/05/2006
		Belyounech	13/04/2006

acid, flavonoid, tannins) (Pangestuti and Kim, 2011). Those compounds are widely distributed in plants or seaweeds and are known to exhibit higher antioxidative activities. The activities have been reported through various methods of reactive oxygen species scavenging activity and the inhibition of lipid peroxidation (Yan et al., 1998; Athukorala et al., 2003a; Athukorala et al., 2003b; Heo et al., 2003; Siriwardhana et al., 2003; Siriwardhana et al., 2004; Heo et al., 2005).

The objective of the present study was to investigate the antioxidant properties of ten different Moroccan red seaweeds using three different reactive oxygen species scavenging assays such as DPPH free radical, Deoxyribose and assay with  $\beta$ -carotene–linoleic acid system.

## MATERIALS AND METHODS

### Chemicals

Butylated hydroxytoluene (BHT), Butylated hydroxy anisole (BHA),  $\alpha,\alpha$ -diphenyl- $\beta$ -picrylhy -drazyl (DPPH), Linoleic acid, Tween-40 (polyoxyethylene sorbitan monopalmitate),  $\beta$ -carotene, thiobarbituric acid (TBA), trichloroacetic acid (TCA), deoxyribose, ferric chloride, EDTA, hydrogen peroxide, ascorbic acid, D-mannitol were purchased from Sigma-Aldrich (France).

### Seaweed material

Seaweeds were collected by hand using Scuba diving or snorkeling (1 to 4 m depth) and preserved on ice until further processing. Ten species were sampled between 2006 and 2007 at various sites, along the Strait of Gibraltar (Ksar-sghir, Belyounech) and on the Atlantic coast (Sidi Bouzid) (Table 1). The taxonomic identification of species was done by experts in these fields, using standard literature and taxonomic keys. Voucher specimens of all species tested are deposited in the herbarium of our Laboratory of Applied

Algology-Mycology, Department of Biology, Faculty of Sciences, Abdelmalek Essâadi University, 93002 Tetouan, Morocco (Table 1). Two species belong to the Gelidiales order, three to Gigartinales order, four to Ceramiales order and one to Bonnemaisoniales.

### Preparation of extracts

The samples were collection, rinsed with sterile seawater to remove associated debris and necrotic parts. Epiphytes were removed from the algae and the surface microflora was removed by soaking the algal samples for ten minutes with 30% ethanol. The samples were shade dried, cut into small pieces and powdered in a mixer grinder. The powder obtained was preserved cold in - 12°C. Samples (5 g) were extracted with solvent methanol (100%) or with water for 8 h using a soxhlet apparatus. The resulting organic extracts were concentrated to dryness under reduced pressure at 30 to 35°C with a rotary evaporator and aqueous extracts were lyophilized. Each residue was weighed and stored in sealed vials in a freezer until being tested. All extracts were stored at (-4°C) (Ozdemir et al., 2004).

To extract polysaccharides from *Sphaerococcus coronopifolius* and *Boergeseniella thuyoides* (collected at Belyounech) and after the cleaning and the drying, the seaweed was depigmented with absolute ethanol and acetone. The polysaccharides from seaweed (20 g) were extracted in hot distilled water (1.5 L) at 80 °C for 4 h with magnetic stirring. Insoluble residues were eliminated by filtration and centrifugation (20 min, 30,000 g). The supernatant was poured into 2 volumes of absolute ethanol during one night at 4°C. The precipitate were recovered and washed by absolute acetone, dried overnight at 50°C, weighed and ground to a powder. The polysaccharide was redissolved in distilled water (Bourgougnon, 1994; Haslin et al., 2000).

### Antioxidant assays

#### Antioxidant assay for DPPH radical-scavenging activity

The scavenging effects of samples for DPPH radical were monitored according to the method of the previous report of Duan et al. (2006). Briefly, a 1 ml aliquot in 99% of methanol of test

**Table 2.** Evaluation of the antioxidant activity in the DPPH (EC<sub>50</sub>) of ten methanolic extracts.

Methanolic extracts	EC <sub>50</sub> (µg.mL <sup>-1</sup> )
BHA	8.38±0.09
BHT	11.43±0.17
<i>Pterosiphonia complanata</i>	96.07±0.10
<i>Boergeseniella thuyoides</i>	132.23±0.24
<i>Sphaerococcus coronopifolius</i>	224.12±0.28
<i>Asparagopsis armata</i>	862.45±0.11
<i>Halopitys incurvus</i>	>1000
<i>Hypnea musciformis</i>	>1000
<i>Gelidium spinulosum</i>	>1000
<i>Plocamium cartilagineum</i>	>1000
<i>Gelidium pulchellum</i>	>1000
<i>Ceramium rubrum</i>	>1000

EC<sub>50</sub>: effective concentration.

sample was added to 1.0 ml of 0.25 mM DPPH solution (in methanol). The mixture was agitated using a vortex for 1 min and then left to stand at room temperature for 30 min in the darkness, and its absorbance was read at 517 nm. The ability to scavenge the DPPH radical was calculated using the following equation:

$$I(\%) = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Where the A<sub>control</sub> is the absorbance of the control (DPPH solution without sample), the A<sub>sample</sub> is the absorbance of the test sample (DPPH solution plus test sample), and the synthetic antioxidants, BHT and BHA were used as positive controls.

#### Antioxidant assay with β-carotene–linoleic acid system

The antioxidant activities of marine algae using the β-carotene–linoleic acid system was measured as in the method as reported by Jayaprakasha et al. (2001) and Chew et al., (2008) with some modification. Briefly, 200 µl of a solution of β-carotene in chloroform (1 mg/ml) in a flask containing 20 mg of linoleic acid and 200 mg of Tween-40. The chloroform was removed by rotary evaporator under vacuum at 50°C for 5 min, and 50 ml distilled water were added slowly to the semi-solid residue with vigorous agitation to form an emulsion. The tubes of spectrophotometer were charged with 200 µl of test sample and 3.8 ml of the emulsion, and the absorbance was measured at 470 nm, immediately, against a blank consisting of the emulsion without β-carotene. The tubes with sample were conserved at room temperature (50°C), and the absorbance measurements were conducted again at 60 min intervals up to 470 min. All determinations were carried out in triplicate. The antioxidant activity (AA) of the extracts was evaluated in terms of bleaching of β-carotene using the following formula:

$$AA(\%) = 100 [(DR_c - DR_s) / DR_c]$$

with DR<sub>c</sub> = Degradation rate of the control and DR<sub>s</sub> = degradation rate of the test.

Degradation rate: DR = [ln (a/b)/T] with a = absorbance at t= 0 and b = absorbance at t= T.

#### Deoxyribose radical scavenging activity

**Deoxyribose assay:** Stock reagents were prepared before the assay as follows, (1) deoxyribose, 16.8 mmol/l, ca. 100 mg/45 ml;

(2) FeCl<sub>3</sub>, 300 mmol/l, EDTA, 1.2 mmol/l, then EDTA and iron (III) were mixed at the ratio of 1:1 to give stock FeCl<sub>3</sub>/EDTA solution; (3) H<sub>2</sub>O<sub>2</sub>, 16.8 mmol/l; (4) KH<sub>2</sub>PO<sub>4</sub>/KOH buffer, pH 7.4, 10 mmol/l; (5) Ascorbic acid, 0.6 mmol/l. After deoxyribose, FeCl<sub>3</sub>/EDTA, H<sub>2</sub>O<sub>2</sub>, KH<sub>2</sub>PO<sub>4</sub>/KOH buffer, and water extract were added one by one, each in 200 µl, into a 10 ml tube, ascorbic acid, 200 µl, was finally added to start the reaction (total volume was 1.2 ml). The reaction mixture was incubated at 37°C in a thermostatic water bath for 1 h. Then, 1 ml TBA solution (1% (w/v) thiobarbituric acid in 50 mM NaOH), and 1 ml TCA solution (2.8% (w/v) trichloroacetic acid), were added, and the tubes were incubated in another thermostatic water bath at 80°C up to 20 min (Yan et al., 1999).

After that, the tubes were removed and cooled; the absorbance was measured at 532 nm by a spectrophotometer. Mannitol was used as a positive control.

**Free radical scavenging activity calculation:** The free radical scavenging activity I(%) was calculated as follows:

$$I(\%) = [1 - (A_i - A_j) / A_c] \times 100$$

For the deoxyribose assay, A<sub>i</sub> = the absorbance of water extract in deoxyribose assay; A<sub>j</sub> = the absorbance of the same water extract mixed with 1 ml distilled water, 1 ml TBA solution and 1 ml TCA solution; A<sub>c</sub> = the absorbance of control solution (distilled water) in the assay system.

## RESULTS

### Antioxidant assay for DPPH radical-scavenging activity

DPPH is a useful reagent for investigating the free radical-scavenging activities of compounds. In DPPH test, the extracts were able to reduce the stable radical DPPH<sup>•</sup> to the yellow-coloured diphenylpicrylhydrazine. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH–H by the reaction (Shon et al., 2003; Duan et al., 2006).

According to the results (Table 2), we can consider that the methanol extracts of *Pterosiphonia complanata* and *B. thuyoides* have significant scavenging activity with EC<sub>50</sub> of 96 and 132 µg.mL<sup>-1</sup>, respectively.

In conclusion, among the ten methanol extracts tested, those of *P. complanata*, *B. thuyoides*, *S. coronopifolius* and *Asparagopsis armata* participated in the transformation of DPPH radical in reduced form with an EC<sub>50</sub> between 96 and 862 µg.mL<sup>-1</sup>, while no activity was showed by the extracts of *Halopitys incurvus*, *Hypnea musciformis*, *Gelidium spinulosum*, *Plocamium cartilagineum*, *Gelidium pulchellum*, *Ceramium rubrum* in 1 mg. mL<sup>-1</sup>.

### Antioxidant assay with β-carotene–linoleic acid system

The antioxidant activity of carotenoids is based on the radical adducts of carotenoids with free radical from

**Table 3.** Evaluation of antioxidant activity by  $\beta$ -carotene ( $EC_{50}$ ) with ten methanol extracts of red algae.

Methanolic extracts	$EC_{50}$ ( $\mu\text{g}\cdot\text{mL}^{-1}$ )
BHA	1.45 $\pm$ 0.7
BHT	4.51 $\pm$ 0.13
<i>Pterosiphonia complanata</i>	9.0 $\pm$ 0.39
<i>Gelidium pulchellum</i>	39.23 $\pm$ 0.25
<i>Halopitys incurvus</i>	51.09 $\pm$ 0.14
<i>Boergeseniella thuyoides</i>	68.21 $\pm$ 0.06
<i>Ceramium rubrum</i>	100.01 $\pm$ 0.23
<i>Gelidium spinulosum</i>	144.08 $\pm$ 0.15
<i>Hypnea musciformis</i>	176.05 $\pm$ 0.10
<i>Plocamium cartilagineum</i>	>1000
<i>Asparagopsis armata</i>	>1000
<i>Sphaerococcus coronopifolius</i>	>1000

$EC_{50}$ : effective concentration.

linoleic acid. The linoleic acid free radical attacks the highly unsaturated  $\beta$ -carotene models. The presence of different antioxidants can hinder the extent of  $\beta$ -carotene-bleaching by neutralizing the linoleate- and other free radicals formed in the system (Shon et al., 2003; Duan et al., 2006; Chew et al., 2008). The antioxidant activities of methanol extracts from ten species, positive control (BHT and BHA) are presented in Table 3. The extract of *P. complanata* presents a relatively important activity ( $EC_{50}$  of 9.0  $\mu\text{g}\cdot\text{mL}^{-1}$ ) in comparison with the values of BHT and BHA, followed by extracts from *G. pulchellum*, *H. incurvus*, *B. thuyoides*, *C. rubrum*, *G. spinulosum* and *H. musciformis*. While the extracts of *P. cartilagineum*, *A. armata* and *S. coronopifolius* present an  $EC_{50}$  superior to 1 mg.  $\text{mL}^{-1}$ .

In conclusion, in this test of  $\beta$ -carotene, seven extracts showed an activity towards the radicals peroxides with a  $EC_{50}$  between 9 and 176  $\mu\text{g}\cdot\text{mL}^{-1}$  and three extracts presented no efficiency in the range of concentrations used.

### Deoxyribose test

The evaluation of antioxidant activity in the deoxyribose test involves the hydroxyl radical the most active of reactive oxygen derivatives (Yan et al., 1999; Chandini et al., 2008). The effect of ten aqueous extracts (1 mg. $\text{mL}^{-1}$ ) and two polysaccharides on the radical scavenging of hydroxyl (OH) to prevent oxidative degradation of the substrate deoxyribose was determined (Figure 1). The highest percentage of inhibition was obtained by the aqueous extract of *A. armata* (68.76%), followed by that of *B. thuyoides* (PS) (35.05%), *P. cartilagineum* (28.84%), *S. coronopifolius* (PS) (25.88%), *P. complanata* (25.37%), *S. coronopifolius* (24.30%) and *B. thuyoides* (19.13%). It may be noted that the percentages

of inhibition of the extracts described above have values superior to the mannitol used as positive control that presents an inhibition of 18.37%.

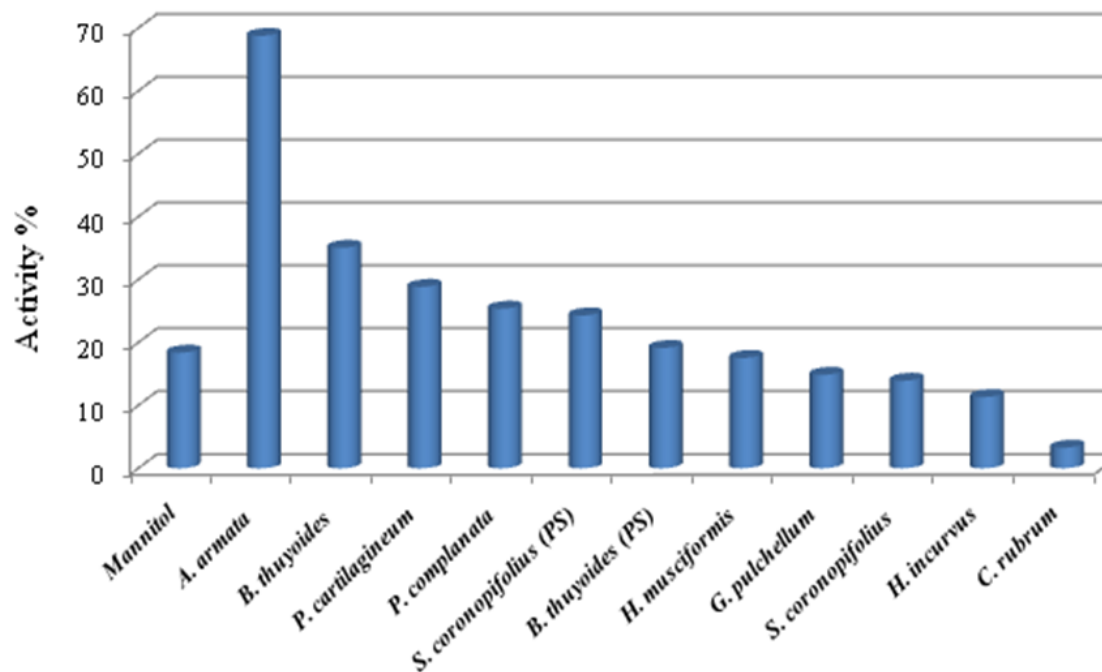
In this test of deoxyribose, there is liberation of the radicals of the hydroxyl group. The aqueous extracts of *A. armata*, *B. thuyoides*, *P. cartilagineum*, *P. complanata*, *S. coronopifolius* were involved in the inhibition of the formation of OH groups. Extracts of *H. musciformis*, *G. pulchellum*, *H. incurvus* showed an average percentage of inhibition and the two extracts of *C. rubrum* and *G. spinulosum* showed a lower percentage of inhibition towards this type of free radicals.

### DISCUSSION

Derivatives reactive oxygen (DRO) such as superoxide radical ( $\text{O}_2^-$ ), the hydroxyl radical (OH), the peroxide radical (ROO) and the radical nitric oxide (NO), attack biological molecules such as lipids, proteins, enzymes, DNA or RNA, causing tissue damage resulting in aging, atherosclerosis, and carcinogenesis (Valentao et al., 2002; Fisch et al., 2003; Nakamura et al., 2003; Shon et al., 2003; Chandini et al., 2008).

The antioxidants are effective in protecting the body against damage by reactive oxygen derivatives. There is an increasing interest in natural antioxidants because of health problems and toxicity of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), generally used in the food domain associated with lipids (Howell, 1986; Ito et al., 1986; Amarowicz et al., 2000). Many natural antioxidants have already been isolated from plants such as oleaginous seeds, cereals, vegetables, leaves, roots, spices and herbs (Wettasinghe and Shahidi, 1999; Shon et al., 2003). Among the most important natural antioxidants are the phenolic compounds, they are widely distributed in plants.

The plants contain various phenolic compounds, including simple phenolics, phenolic acids, anthocyanins, hydroxycinnamic acid derivatives, and flavonoids. All the phenolic classes present structural characteristics of free radical scavengers and have potential antioxidant in the food sector (Bandoniene and Murkovic, 2002; Devi et al., 2011; Lopez et al., 2011). However, natural antioxidants are not limited to the ground sources. Some algae are also considered a rich source of antioxidants (Lim et al., 2002). For example, chlorophylls, carotenoids, tocopherol derivatives such as vitamin E, and isoprenoids extracted from marine algae have shown their efficiency (Shahidi et al., 1992; Farombi et al., 2000; Kaur and Kapoor, 2001; Takamatsu et al., 2003; Canadanovic-Brunet et al., 2006). The antioxidant effect of natural phenolic compounds has previously been studied in relation to the prevention of coronary diseases and cancer, as well as for degenerative disorders relative to the age of brain (Gilani et al., 2000; Kahkonen et al., 2001; Stoclet et al., 2004; Cole et al., 2005; Fraga, 2007; Fusco et al., 2007;



**Figure 1.** Scavenging activity of hydroxyl radical (%) of ten aqueous extracts and two polysaccharides of red algae (PS: Polysaccharide).

Miyamoto et al., 2007; Stevenson and Hurst, 2007). As part of the screening of antioxidant activity of seaweed collected on the coast of Morocco, we studied ten methanol extracts of algae using the activity of DPPH radical trapping,  $\beta$ -carotene-linoleate assay and the deoxyribose test.

Species belonging to the family of Rhodomelaceae are known for their richness in phenolic compounds, especially bromophenols (De Carvalho and Roque, 2000; Zhao et al., 2004). In our study, the methanol extract of *P. complanata* (Rhodomelaceae) showed better results than the other species in the tests of DPPH ( $96.07 \mu\text{g}\cdot\text{mL}^{-1}$ ) and  $\beta$ -carotene ( $9 \mu\text{g}\cdot\text{mL}^{-1}$ ). In other studies, the crude extract of methanol: chloroform (2:1) and ethyl acetate-soluble fraction of *Polysiphonia urceolata* showed higher antioxidant activity than the control BHT in the DPPH analysis model and higher than standard antioxidants, gallic acid (GA) and ascorbic acid (ACAA) in the trial of  $\beta$ -carotene (Duan et al., 2006). Bromophenols isolated from *P. urceolata* were evaluated for their power and DPPH radical-scavenging and showed significant activity with  $\text{EC}_{50}$  values in the range of 9.67 to 21.90  $\mu\text{M}$ , compared to positive control (BHT with  $\text{EC}_{50}$  of 83.84  $\mu\text{M}$ ) (Li et al., 2007). The urceolatin of *P. urceolata* showed a DPPH radical-scavenging activity with an  $\text{EC}_{50}$  of 7.9  $\mu\text{M}$ , ten times higher than the positive control BHT (Li et al., 2008). The fractions of ethyl acetate obtained after separation of the methanol-chloroform (2:1) extract from *Rhodomela confervoides* gave a strong antioxidant activity in both antioxidants models tested, free radical

DPPH and the method of bleaching  $\beta$ -carotene (Wang et al., 2009). The aqueous, methanol and hexane extracts of *Digenea simplex* (Rhodomelaceae, Ceramiales) gave a percentage inhibition of DPPH radical of 42.3, 41.0, 21.5%, respectively (Al-Amoudi et al., 2009). With respect to *Acanthophora spicifera*, ethyl acetate fraction obtained from the methanol extract showed a strong antioxidant activity (equivalent to 32.01 mg ascorbic acid / g extract) while the fraction of petroleum ether showed an inhibition of 12% against the DPPH radical (Ganesan et al., 2008).

The antioxidant activity of extracts from the species belonging to the Areschougiaceae, Gracilariaceae and Palmariaceae families was shown. Indeed, the methanol extracts of *Euclima Kappaphycus* (Areschougiaceae, Gigartinales) and *Gracilaria edulis* (Gracilariaceae, Gracilariales) and their fractions of petroleum ether, ethyl acetate, dichloromethane, butanol and water showed a degradation oxidative deoxyribose with percentages of inhibition between 57.87 and 98.36% (Ganesan et al., 2008). The water extract of *P. complanata* (Plocamiaceae, Gigartinales) and *S. coronopifolius* (Sphaerococcaceae, Gigartinales) gave an antihydroxyl radical activity with percentages of inhibition of 25.37 and 24.30%, respectively. While the aqueous extract of *A. armata* (Bonnemaisoniaceae, Bonnemaisoniales) gave a strong degradation oxidative deoxyribose with percentage of inhibition of 68.76%. The methanol extract of *E. Kappaphycus* showed a percentage of 12% inhibition against the DPPH radical (Ganesan et al., 2008). The

reducing power and free radical scavenging by the extract of *E. Kappaphycus* are higher compared to standard antioxidants ( $\alpha$ -tocopherol). The methanol extract 50% *Kappaphycus alvarezzi* showed antioxidant activity of 37.8 mg / 100 g of a antioxidant capacity equivalent of ascorbic acid (Chew et al., 2008). The activity of DPPH radical scavenging by the ethanol extract of *K. alvarezzi* gave an  $EC_{50}$  of 3.03 mg.mL<sup>-1</sup>, while that of the aqueous extract of 4.76 mg.mL<sup>-1</sup> (Kumar et al., 2008). The activity of DPPH radical scavenging by the methanol extract of *S. coronopifolius* gave an  $EC_{50}$  of 224.12  $\mu$ g.mL<sup>-1</sup>, while that of the methanol extract of *H. musciformis* (Hypneaceae, Gigartinales) and *P. cartilagineum* (Plocamiaceae, Gigartinales) showed an  $EC_{50}$  greater than 1 mg.mL<sup>-1</sup>. The soluble fraction 1-butanol of *Palmaria palmata* (Palmariaceae, Palmariales) presented an  $EC_{50}$  of 12.5 mg.mL<sup>-1</sup> contre DPPH radical and a percentage inhibition of 18.2% in the test trapping of hydroxyl radical (OH) (Yuan et al., 2005).

Yan et al., (1999) studied the antioxidant activity of 27 species of seaweeds in particular: *Corallina pilulifera* (Corallinaceae, Corallinales), *Gelidium amansii* (Gelidiaceae, Gelidiales), *Ceramium boydenii* (Ceramiaceae, Ceramiales), *C. kondoi* (Ceramiaceae, Ceramiales), *Polysiphonia urceolata* (Rhodomelaceae, Ceramiales), *Rhodomela confervoides* (Rhodomelaceae, Ceramiales), *R. teres* (Rhodomelaceae, Ceramiales), *Gracilaria verucosa* (Gracilariaceae, Gracilariales) by DPPH and deoxyribose tests. In the first case, the chloroform, ethyl acetate, acetone and methanol extracts of *P. urceolata*, *Gelidium amansii* and *Rhodomela teres* present a high activity. The methanol appears to be the most efficient solvent for the extracts preparation (Yan et al., 1999). The methanol extract of *G. pulchellum*, *G. spinulosum* (Gelidiaceae, Gelidiales) and *C. rubrum* (Ceramiaceae, Ceramiales) showed and  $EC_{50}$  greater than 1 mg.mL<sup>-1</sup>. In the case of deoxyribose test, the aqueous extracts of these species gave an antihydroxyl radical activity ranging from 30 to 85% except for the extract of *Corallina pilulifera*, which showed no activity. The aqueous extract of *G. pulchellum* showed an average percentage of inhibition (14.86%) and the two extracts of *C. rubrum* (3.27%) and *G. spinulosum* (2.01%) showed a lower percentage of inhibition towards this type of free radicals.

Recently, a considerable number of studies have been reported on the antioxidant and anticancer activities with sulfated polysaccharides isolated from marine algae (Ruperez et al., 2002; Zhang et al., 2003; Gamal-Eldeen et al., 2009; Godard et al., 2009; Ananthi et al., 2010; Chattopadhyay et al., 2010; Costa et al., 2010; Zhang et al., 2010). The sulfated polysaccharides isolated from *Fucus vesiculosus*, *Laminaria japonica* and *Ecklonia Kuromi* have shown a good antioxidant activity (Hu et al., 2001). Moreover, the porphyran, a sulfated polysaccharide extracted from *Porphyra haitanesis* (Bangiaceae, Bangiales) delayed the aging process in

mice by increasing the amount of antioxidant enzymes and thereby reducing the risk of peroxidation lipid (Zhang et al., 2003; Zhang et al., 2010). Polysaccharides enhance the antioxidant activity because they exhibited the easy abstraction of anomeric hydrogen from the internal units of monosaccharides. The sulfated polysaccharides of *Gracilaria caudata* (Gracilariaceae, Gracilariales) gave inhibitions of the hydroxyl radical of 1.4, 2.4, 3.8, 8% for concentrations of 0.05, 0.1, 0.25, 0.5 mg.mL<sup>-1</sup>, respectively (Costa et al., 2010). Therefore, algal polysaccharides of *S. coronopifolius* and *B. thuyoides* also can be possible active principles by their antioxidant activity moderate.

## Conclusion

In our study, the best antioxidant activities were shown by aqueous extracts of *A. armata* and *B. thuyoides* with 68 and 35% of inhibition of the hydroxyl groups (OH.) and methanolic extract of *P. complanata* against the peroxide radicals with an  $EC_{50}$  of 9  $\mu$ g.mL<sup>-1</sup> and against the DPPH radical with an  $EC_{50}$  of 96  $\mu$ g.mL<sup>-1</sup>.

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