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Full Length Research Paper

Comparison of *in vitro* antioxidant activity of some selected seaweeds from Algerian West Coast

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***In vitro* antioxidant activity of methanolic (ME) and ethanolic (ET) extracts of six species of seaweeds (*Cystoseira stricta, Cystoseira compressa, Corallina elongata, Porphyra umbilicalis, Enteromorpha compressa* and *Ulva lactuca*) collected from Algerian West Coast was evaluated, using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay and reducing power assay. They were tested for total phenolic content. The highest phenolic content was observed in *C. compressa* [10.24 ± 0.09 mg gallic acid equivalent (GAE) g^{-1} dry weight (DW) in ME extract and 15.70 ± 0.72 mg GAE g^{-1} DW in ET extract]. In general, the ET seaweed extracts were the most effective fractions. In addition, *C. compressa* and *C. stricta* extracts showed the highest antioxidant activity in all assays and better than vitamin E. However, these extracts present lower DPPH radical scavenging activity and reducing power than butylhydroxytoluene (BHT), except at concentration of 1 mg mL^{-1}, in which it was similar. Therefore, *C. compressa* and *C. stricta* had a potential to be used as a natural antioxidant agent.**

Key words: Algerian, antioxidant activity, 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging, reducing power, seaweed.

INTRODUCTION

The marine flora and fauna constitute precious biological resources. The importance of these resources is due to the presence of marine organism having a wide range of metabolites which can be rare or even absent in animals, vegetables, mushrooms or microorganisms. For several years, a particular look has been concerned with the research for new substances of biotechnological interests. In the international pharmaceutical market and cosmetic,

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30% of active substance was developed from natural substances among which 10% was isolated from marine organisms (Ruiz, 2005).

Among these, seaweeds are an important source of bioactive compounds such as carotenoids, dietary fiber, protein, essential fatty acids, vitamins and minerals and many active substances like lipids, proteins, poly-saccharides and polyphenols (Meenakshi et al., 2011). Having many activities, anti-protozoan, antibacterial, anti-inflammatory, anticoagulative, antifungal, anti-free radical, antioxidant, etc (Hellio et al., 2004; Cox et al., 2010; Taboada et al., 2012) seem promising for the pharmaceutical, cosmetics and food industries.

An outbreak of interest is on the biological effects of natural antioxidants, included in the fight against oxidative stress that causes ageing, the release and progress of several diseases such as cancer, cardiovascular accidents, inflammatory diseases and neurodegenerative diseases (Favier, 2003). To prevent the toxic effects of oxygen, several species of seaweeds containing a wide specter of phytochemical substances, which are sources of natural antioxidants agents, such as phenolic acids, flavonoids and tannins, have the capacity to eliminate the reactive species of oxygen (RSO). They can thus minimize the oxidative damage caused by these RSOs in living cells and prevent oxidative degradation of food while supplying simultaneously remarkable nutritional advantages (Cornish and Garbary, 2010). These seaweeds also offer to industrial domain an interesting alternative to synthetic antioxidant substances as butylhydroxyanisol (BHA), butylhydroxytoluene (BHT) and propyl gallate (PG) which are reported carcinogenic (Safer and Al-Nughamish, 1999).

Algeria with its long coastal constitutes a rich but a useless source of marine algae. Indeed, there are few studies estimating the antioxidant potential of seaweeds in Algeria and even less industrial exploitation. These seaweeds constitute a pathway of economic development. In this regard, the aim of this study was to investigate the antioxidant activity of seaweed extracts of various species from Algerian West Coast: *Cystoseira stricta* (Montagne) Sauvageau, *Cystoseira compressa* (Esper) Gerloff & Nizamuddin, *Corallina elongata* Ellis & Solander, *Porphyra umbilicalis* Kützing, *Enteromorpha compressa* (Linnaeus) Nees, and *Ulva lactuca* Linnaeus, with the prospect of a valuation.

MATERIALS AND METHODS

Sample collection

Six species of fresh seaweeds were collected from Algerian West Coast: Division Pheaophyte: *C. stricta* and *C. compressa*; Division Rhodophyta: *C. elongata* and *P. umbilicalis*; and Division Chlorophyta: *E. compressa* and *U. lactuca*. These seaweeds were cleaned from epiphytes, salt and dried at room temperature (23 ± 2°C) for 72 h. The dried samples were powdered and stored at -20°C until used for further experiments.

Preparation of seaweeds extracts

Samples were extracted with two different solvents methanol (ME) and ethanol (ET), according to the method of Kelman et al. (2012) modified. Five grams of each sample was extracted with 100 ml of solvent for 24 h with mixing, at room temperature (25°C) and under dark condition. Resultant extracts were filtered and solvent removed under reduced pressure to yield dry material. Extracts weights were recorded and the yield expressed as percentage.

Determination of total phenolic content

Total phenolic content of each seaweed extract was quantified according to the method of Folin-Ciocalteu (Singleton and Rossi, 1965). A volume of 400 µl of each seaweed extract (at a concentration of 2 mg ml^{-1}) was added to 2 ml of the reactive of Folin-Ciocalteu (diluted 10 times). The mixture was allowed to incubate for 4 min at room temperature. Then, 1.6 ml of Na_2CO_3 (7.5 %) was added. Tubes were shaken and incubated for 2 h in darkness, at room temperature. Samples' absorbencies were read at 765 nm. Gallic acid was used as the standard for a calibration curve. The total phenolic contents of seaweed extracts were expressed as mg of gallic acid equivalents per gram of dry weight (mg GAE g^{-1} DW).

DPPH radical scavenging assay

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity of samples was performed according to the method of Yen and Chen (1995). Aliquot (2 ml) of test sample (at a concentration of 0.1, 1 and 2 mg ml^{-1}) was added to 2 ml of 0.16×10^{-3} mol L^{-1} DPPH methanolic solution. The mixture was vortexed for 1 min and then left to stand at room temperature for 30 min in the dark, and its absorbance was read at 517 nm. The ability to scavenge DPPH radical was calculated using the following equation:

$$\text{Scavenging effect (\%)} = [1 - (A_{sample} - A_{sample\ blank}) / A_{control}] \times 100$$

where $A_{control}$ is the absorbance of the control (DPPH solution without sample), A_{sample} is the absorbance of the test sample (DPPH solution plus test sample), and $A_{sample\ blank}$ is the absorbance of the sample (sample without DPPH solution). Then, the IC50 (concentration of 50% inhibition of the radical DPPH) was calculated. Vitamin E and BHT were used as positive control.

Reducing power

The reducing power was measured as described by Oyaizu (1986). 1 ml of each extract (at a concentration of 2 mg ml^{-1}) was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium hexacyanoferrate solution. After 30 min of incubation at 50°C, 2.5 ml of 10% trichloroacetic acid was added. The mixture was centrifuged at 3000 rpm, for 10 min. Finally, 2.5 ml of the upper layer were mixed with 2.5 ml of distilled water and 0.5 ml, 0, and 1% $FeCl_3$. The absorbance was measured at 700 nm. Vitamin E and BHT were used as positive controls.

Statistical analysis

All data were expressed as the mean ± standard deviation (SD) and are statistically compared by one-way variance analysis (ANOVA) and Tukey HSD test (for homogeneous variance) and by non-parametric variance analysis of Kruskal-Wallis and Mann-Withney U-test (for non homogeneous variance), after verification of variance homogeneity by Hartley test.

Table 1. Yield (%) and total phenolic content (mg GAE g^{-1} DW) of methanolic and ethanolic extracts.

Seaweed	Yield (%)		Total phenolic content (mg GAE g^{-1} DW)	
	ME extract	ET extract	ME extract	ET extract
Cystoseira stricta	8.41 ± 0.26[a]	3.81 ± 0.13[a]	8.24 ± 0.89[a]	4.63 ± 0.21[a]
Cystoseira compressa	20.26 ± 0.45[b]	8.89 ± 0.11[b]	10.24 ± 0.09[b]	15.70 ± 0.72[b]
Corallina elongata	2.53 ± 0.90[c]	1.70 ± 0.55[de]	4.58 ± 0.14[c]	3.09 ± 0.16[c]
Porphyra umbilicalis	5.19 ± 0.23[d]	2.55 ± 0.09[c]	3.80 ± 0.05[d]	4.56 ± 0.02[a]
Enteromorpha compressa	4.14 ± 0.20[e]	1.82 ± 0.42[d]	3.94 ± 0.28[cd]	3.62 ± 0.16[c]
Ulva lactuca	9.29 ± 0.16[f]	1.15 ± 0.24[e]	2.25 ± 0.05[e]	3.63 ± 0.06[c]

Each value is the mean ± standard deviation (n = 3). Different lowercase letters indicate significant difference between means ($p < 0.05$).

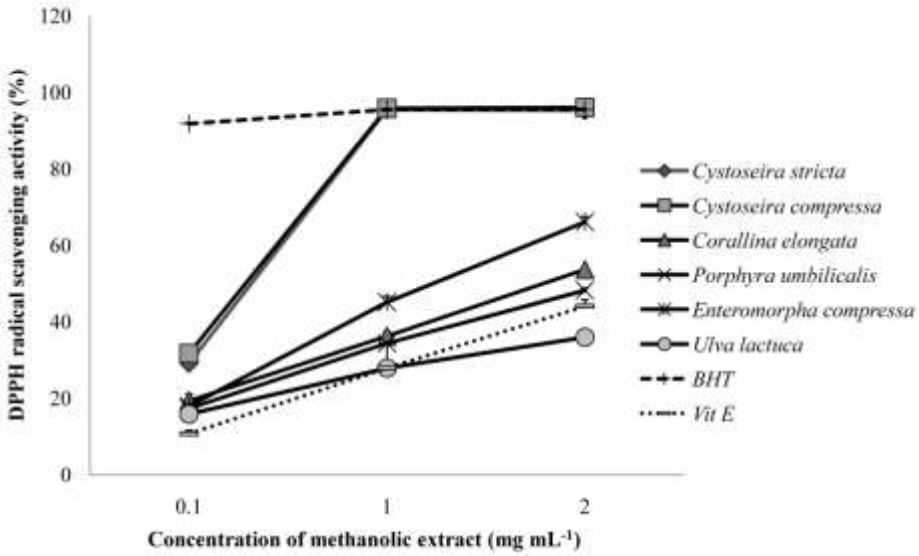

Figure 1. DPPH radical scavenging activity (%) of methanolic seaweeds extracts and positive control (BHT and Vit E), at various concentrations.

RESULTS

Extracts yield

Six species of seaweeds were extracted with methanol and ethanol. The yields of ME extracts recorded were superior to those of ET extracts ($p < 0.05$), except for *C. elongata* in which no effect of extraction solvent was shown (Table 1). A significantly higher yields were obtained in *C. compressa* (20.26% in ME extract and 8.89% in ET extract).

Total phenolic content

Phenolic contents of seaweeds extracts were evaluated and presented in Table 1. No significant effect of both organic solvents on the capacity of extraction of these compounds was recorded, except only in *E. compressa*. Ethanol can be considered as an excellent solvent of extraction ($p < 0.05$) in *C. compressa, P. umbilicalis* and *U. lactuca*. The brown seaweeds of *Cystoseira* genus showed higher phenolic contents, with the best amount in *C. compressa* (10.24 mg GAE g^{-1} DW in ME extract; 15.70 mg GAE g^{-1} DW in ET extract).

DPPH radical scavenging activity

Percentage of inhibition and IC50 of six seaweeds are presented in Figures 1 and 2 and Table 2. No significant effect of extraction solvents was recorded, except for ethanol, in *C. stricta, C. compressa, E. compressa* and *U. lactuca* at 0.1 mg ml^{-1} and in *P. umbilicalis* at 2 mg ml^{-1} ($p < 0.05$). Moreover, the extracts from *Cystoseira* genus showed the highest scavenging activity ($p < 0.05$) in both solvents (Figures 1 and 2). The ET extract of *C. stricta* recorded an IC50 of 0.1 mg ml^{-1} followed by *C. compressa*

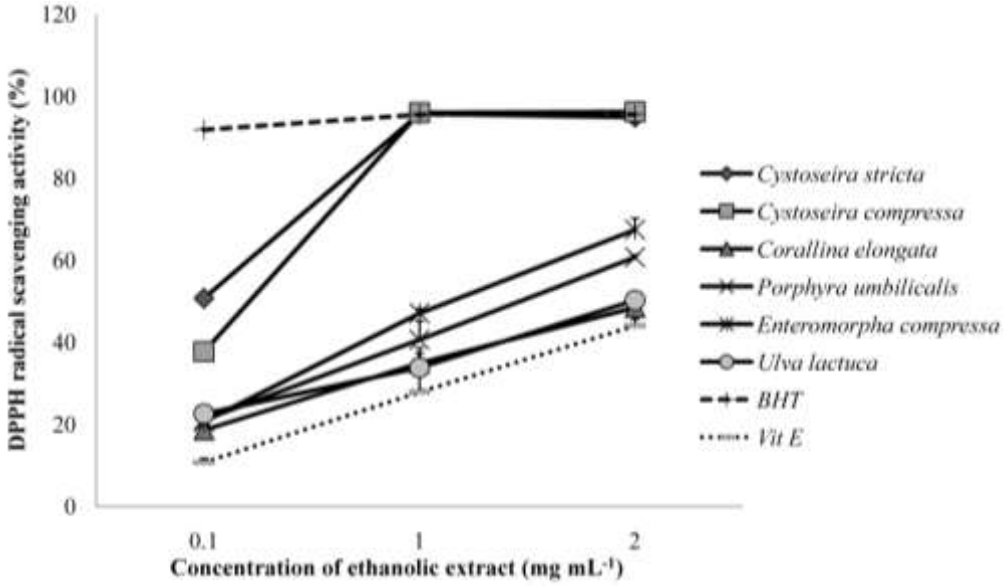

Figure 2. DPPH radical scavenging activity (%) of ethanolic seaweeds extracts and positive control (BHT and Vit E), at various concentrations.

Table 2. IC50 (mg mL^{-1}) of methanolic and ethanolic extracts.

Seaweed	IC50 (mg ml^{-1})	
	ME extract	ET extract
Cystoseira stricta	0.35	0.10
Cystoseira compressa	0.29	0.15
Corallina elongata	1.78	2.14
Porphyra umbilicalis	2.08	1.53
Enteromorpha compressa	1.32	1.26
Ulva lactuca	3.45	1.91
BHT	0.05	
Vit E	2.38	

with an IC50 of 0.15 mg ml^{-1} (Table 2). All seaweed extracts showed higher anti-radical activity than vitamin E and lower than BHT ($p < 0.05$), except *C. compressa* and *C. stricta* extracts at concentration of 1 and 2 mg m^{-1} that showed a similar percentage of inhibition of the radical DPPH than BHT.

Reducing power

Figure 3 presented results of reducing power. A significantly higher reducing activity was reported in ET extracts of all seaweeds ($p < 0.05$). Moreover, seaweeds of *Cystoseira* genus showed a maximum absorbance (OD) value in both organics extracts ($p < 0.05$): *C. compressa* (OD = 1.32) in ET extract and *C. stricta* (OD = 0.87) in ME extracts. The positive control BHT showed higher reducing power (OD = 5.18) than all seaweed samples and Vitamin E (OD = 0.079).

DISCUSSION

In the present study, the extraction yield in ME extracts was higher than ET extracts. These results are in agreement with those of Lopez et al. (2011), which showed that the yield of ME extract of brown alga was higher than that obtained with ethanol. Matanjun et al. (2008) noticed that ME extracts of red and green seaweeds had a higher yield, compared with those of diethylether and reported that more polar compounds were found in seaweed extracts and increasing solvent polarity increased the extraction yield (Boonchum et al., 2011).

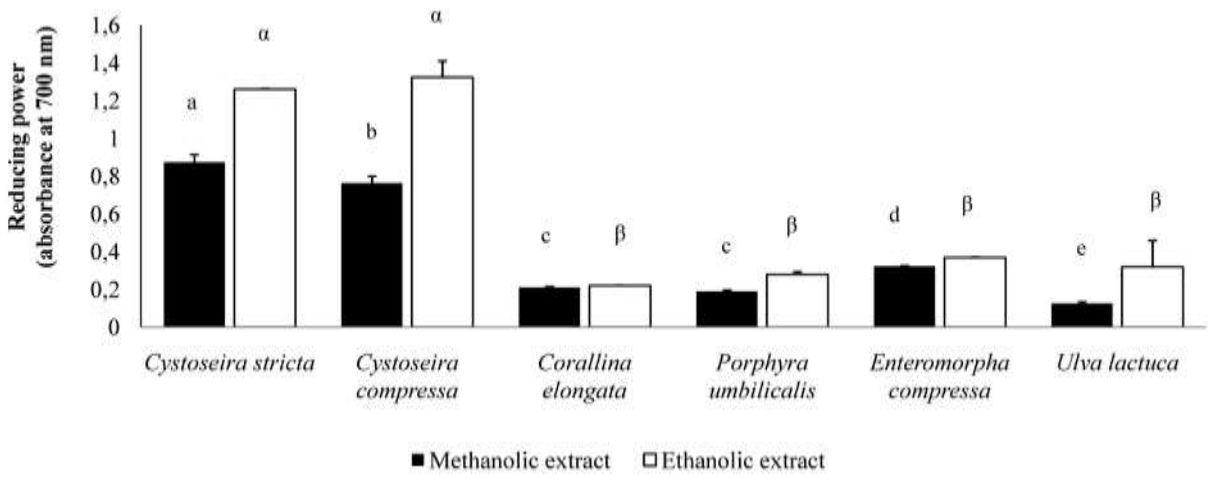

Figure 3. Reducing Power of methanolic and ethanolic seaweeds extracts (concentration of extract used = 2 mg mL^{-1}). Each value is the mean ± standard deviation (n = 3). Different lowercase letters indicate significant difference between means ($p < 0.05$).

Marine seaweed substances, especially polyphenols have antioxidant activity and the major active compounds are phlorotannins and fucoxanthin (Yan et al., 1999). In the polar organic solvents, the phenolic compounds are generally more soluble than in water. The recommended effective solvents are methanol, ethanol and acetone (Waterman and Mole, 1994). By polar solvents, phenolic compounds which were attached in sugars or proteins, saponines, glycosides, organic acids, salts, and in the mucus can be extracted (Cho et al., 2007). The most important contents in P.T were found in the brown seaweeds *Cystoseira* from Algerian West Coast. Matanjun et al. (2008) observed the same result. The brown seaweeds (*Dictyota dichotoma, Sargassum polycystum* and *Padina* species) contained higher phenolic content than the green (*Caulerpa lentillifera* and *Caulerpa racemosa*) and red seaweeds (*Eucheuma cottonii*, *Eucheuma spinosum* and *Halymenia durvillaei*).

Similar phenolic amounts to those observed were reported in *C. elongata* (4.43 ± 4.01 mg GAE g^{-1} DW) (Rico et al., 2012), *Porphyra tenera* (4.70 ± 0.60 mg GAE g^{-1} DW) (Machu et al., 2015), *Enteromorpha intestinalis* (2.65 ± 0.08 mg GAE g^{-1} DW), *U. lactuca* (2.36 ± 0.07 mg GAE g^{-1} DW) (Farvin and Jacobsen, 2013), *C. stricta* (10.14 ± 0.16 mg GAE g^{-1} DW) (Guezzen, 2014); *Cystoseira myrica* (10.08 ± 1.13 mg GAE g^{-1} DW) (Sadati et al., 2011), and *Cystoseira tamariscifolia* (10.91 ± 0.07 mg GAE g^{-1} DW) (Zubia et al., 2009).

Other works reported different phenolic contents. Ganesan et al. (2011) observed higher amount in *E. compressa* (7.76 ± 0.10 mg GAE g^{-1} DW); in *C. compressa,* Güner et al. (2015) reported 0.16 ± 0.08 mg GAE g^{-1} DW, and Mhadhebi et al. (2014), reported 61 ± 0.30 mg GAE g^{-1} DW. This difference is probably due to the very variable properties of every species, which are in connection with the extrinsic factors (irradiation, salinity, nutriments, season, site of sampling and its depth) and intrinsic factors (stage of development and reproduction of seaweed). Our results also indicate that the higher antioxidant activity was observed in extract with higher phenolic contents which are in agreement with Duan et al. (2006) and Boonchum et al. (2011).

Screening of antioxidant activity by DPPH radical scavenging activity was well used by authors because it can be used for various samples and a low amount of active substances can be detected (Sanchez-Moreno, 2002). Our results showed an anti-radical activity which is dependent-dose. In fact, Ismail and Tan (2002) and Cho et al. (2011) reported that the reduction of radical DPPH increased significantly according to the increase of concentrations of seaweeds extracts and positive controls. In general, results indicate that ethanol has a better effect than methanol on the anti-radical activity (Ismail and Tan, 2002). In agreement with Güner et al. (2015) and Mhadhebi et al. (2014), the genre *Cystoseira* revealed a strong anti-radical activity, at various concentrations. Parthiban et al. (2014) also reported that brown algae showed significantly higher phenolic content and antioxidant activities than the red and green seaweeds. *C. stricta* recorded strong IC50 of 0.1 mg ml^{-1} compared to those found in *Cystoseira crinita* (20 ± 0.5 µg ml^{-1}), *Cystoseira sedoides* (50.3 ± 0.1 µg ml^{-1}) and *C. compressa* (61 ± 0.3 µg ml^{-1}) (Mhadhebi et al., 2014). In addition, the capacity of all seaweed extracts to inhibit the DPPH radical is lower than that of positive control BHT (Ismail and Tan, 2002; Shanab et al., 2011), except, the anti-radical activity of *Cystoseira,* that remains comparable to that of BHT at 1 and 2 mg ml^{-1}. However, vitamin E showed an anti-radical activity lower than that of BHT and the various studied seaweeds. This is also

reported by Narasimhan et al. (2013). This suggests the use of this seaweeds species as a better source of polyphenols and it is more advantageous than vitamin E.

The brown algae of *Cystoseira* genus present a better reducing power, with an influence of the solvent of extraction. Luo et al. (2010) also reported the highest amount of reducing power in brown seaweeds. The ET extracts have a reducing power superior to that of methanolic extracts. Cho et al. (2010) also related that the most effective reducing power is obtained from ET extracts of the green algae *E. compressa* and *Capsosiphon fulvescens*. Ethanol is a solvent that is not toxic and more interesting for industrialists. Our results revealed also that green alga *E. compressa* had a capacity to reduce the iron higher than that of red seaweeds (*C. elongata* and *P. umbilicalis*). These results are in agreement with those of Zhang et al. (2007) and Narasimhan et al. (2013) which related that the reducing power of green seaweeds is superior to that of the red seaweed. In comparison with the positive control (BHT and vitamin E), our results indicate that the reducing capacity of our extracts is lower than that of the BHT and superior to that of the vitamin E. It was also reported by Shanab et al. (2011), who showed that the capacity to reduce the iron by BHT is higher than that of the green alga *E. compressa*. Ganesan et al. (2008) and Cho et al. (2010) observed that reducing capacity of the red seaweed *Euchema kappaphycus* and green seaweed *E. compressa* is higher or comparable to that of the vitamin E.

The BHT revealed an antioxidant capacity superior to those of the seaweeds. However, this synthetic antioxidant has proved toxic and carcinogenic (Safer and Al-Nughamish, 1999). So, it must be replaced by safe and little expensive natural antioxidants.

In conclusion, the brown algae *Cystoseira* collected from Algerian West Coast revealed the best anti-radical and reducing capacities, with higher phenolic content among the six seaweeds, in both solvents of extraction. This suggests that methanol and ethanol can be used for extraction of active compounds (in particular the total polyphenols); and preferentially ethanol, which would present less risk in industrial products. Also, it would be interesting to study *in vivo* antioxidant activity of this antioxidant-rich extracts which may be used as a dietary supplementary or as a natural antioxidant in food industries.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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