ISSN: 0975-833X

*Available online at http://www.journalcra.com*

*International Journal of Current Research*
*Vol. 5, Issue, 02, pp.271-275, February, 2013*

**INTERNATIONAL JOURNAL OF CURRENT RESEARCH**

**RESEARCH ARTICLE**

# Study of Oxidative stress responses in two Mediterranean Green Algae (Tunisian sea)

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**ARTICLE INFO**

***Article History:***
Received 15th November, 2012
Received in revised form
20th December, 2012
Accepted 21th January, 2013
Published online 14th February, 2013

***Key words:***

*Codium bursa* (Olivi) C. *Agard*, *Caulerpa racemosa var. Cylindracea* (Sonder), Carotenoids,
Lipid peroxidation,
Antioxidant enzvmes.

**ABSTRACT**

The aim of the present study was to compare the antioxidant system of two algae harvested from the Tunisian north costal region. Also we searched a potential resource of new antioxidant compounds, which can be used in health field or for food quality determination. Two Mediterranean, green and vigour algae: *Codium bursa* (Olivi) C. *Agardh* and *Caulerpa racemosa var. cylindracea* (Sonder) Verlaque, Huisman and Boudouresque were chosen for this purpose. The two species belonging to the Ulvaceae, grew in Sidi Raies a coastal region in Tunisia. Activities of antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT) and peroxidase (APX)]; carotenoïds as well as chlorophylls (a and b) and protein soluble contents were determined. Our result indicated that there are an important accumulation of carotenoids and chlorophylls; furthermore, the activities of antioxidant enzymes SOD, CAT and APX showed a positive correlation with antioxidant content in the two algae, revealing that they were resistant to the growth medium conditions. But in the other hand, all their antioxidants products and enzymatic activities were higher in *Codium bursa* than in *Caulerpa racemosa* means that *Codium bursa* had higher resistance to the medium thus it could be a good model for culture and production of healthy, useful, such as carotenoids.



## INTRODUCTION

Nowadays, one of the main areas of research is the extraction and characterization of new natural compounds with biological activity such as antioxidant. Antioxidants are important in food preservation and in the defense of living cells against oxidative damage (Plaza *et al*., 2008; Northanom and Yadad, 1999). They are capable of scavenging free radicals produced by oxidative stress which is thought to contribute to development to a wide range of diseases such as Alzheimer's one (Christen, 2000), Parkinson's one (Wood-Kaczmar *et al*., 2006). At cellular level oxidative stress is responsible of many disorders, for example, it has been shown to modify the damage proteins, carbohydrates and DNA (Halliwell and Gutteridge, 1990). In addition a high intake of antioxidant compounds might protect from aging, inflammation, stroke disease (Schwartz *et al*., 1996; Abouel -Enein *et al*., 2003), decrease the risk of cancer (Abe *et al*., 1999; El-Baz *et al*,. 2002; Skibola, 2004). They have been proposed by many authors and health food companies, as dietary supplements (Radimer *et al*., 2004; Rodriguez-Garcia and Guil-Guenor, 2008) for the prevention and treatment of a wide range of degenerative diseases and food preservation (Rodriguez-Garcia and Guil-Guenor, 2008).With natural antioxidants, cells are protected against oxidative stress by an interacting network of antioxidant enzymes (Sies, 1997; Davies, 1995). However, synthetic antioxidants were (i) rejected by consumer who did not appreciate synthetic dietary supplements and (ii) because they were thought to promote carcinogenesis (Namiki, 1990). Now, there is a current worldwide interest in finding new antioxidant from unexplored natural sources. Algae would be good sources, despite the fact that their antioxidant characteristics are poorly known. All studies on algae are promising and reported that algae can be considered as an ingredient for food production (Vonshak, 1997; Munteau, *et al*., 2007), due to their health-related antioxidants properties. Consequently algae have been increasingly required in pharmaceutical industry for their natural antioxidant products, used in cosmetic and medicine for their preventive roles. The aim of this paper is to investigate natural antioxidants produced in two vigorous macro-algae *(Codium bursa* and *Caulerpa ramocema)* collected in Mediterranean sea.

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## MATERIAL AND METHODS

Algae harvesting: Two green algae, *Codium bursa* (Olivi) C. *Agardh* and *Caulerpa racemosa var. cylindracea* (Sonder) Verlaque, Huisman and Boudouresque were gathered from Sidi Rais station; located in the bottom of Tunis Gulf (in Tunisia), situated at the south-western limit of the Cap Bon peninsula. It is mainly sandy and had low gradients; however, the bottom becomes rocky towards the northern boundary of the station. Although located in the open sea and widely exposed to prevailing winds from the north-west, the resort is relatively protected from sea's rough, by sea rocky extending cliffs of Cap Bon. The sampling site is located a few hundred meters from shore, outside the barrier reef, about 1.5 to 2.0 m deep (Figure 1). Many samples were collected, but only ten homogenate samples of each specie were used to realize different analysis.

Chlorophylls (a, b) and carotenoids were extracted in 80% acetone. The resulting suspension was centrifuged for 5 min at 3000 g. The pigment concentrations were calculated by equations allowing a simultaneous determination of Chl a, Chl b, and carotenoids in the same solution, according to Arnon, (1949). Lipid peroxide was determined by measuring the concentration of thiobarbituric acid-reactive substances (TBARS), as described by Alia *et al*. (1995). The leaves were homogenized in 5 % (w/v) trichloroacetic acid (TCA). After centrifugation, a sample of the supernatant was added to 20 % TCA containing 0.5 % (w/v) thiobarbituric acid (TBA). The mixture was incubated at 95 °C for 30 min. The concentration of TBARS was calculated using an extinction coefficient of 155 $mM^{-1}cm^{-1}$. Determination of protein soluble total content: Proteins extractions were carried out at 4° C. The plant tissue was reduced to powder in liquid nitrogen and extracted at a ratio 1:3 (w/v) fresh weight in 50 mM potassium phosphate buffer (pH 7) containing 1 mM EDTA, 5 mM cysteine and 5% (w/v) insoluble polyvinylpyrrolidone (PVP). For the Ascorbate peroxydase (APX) assay, 5 mM ascorbate was added to the extraction buffer. The homogenate was centrifuged at 14,000g for 30 min and the supernatant was used for enzyme assays. Protein content was determined spectrophotometrically at 595 nm as described by Bradford, (1976) using bovine serum albumin (BSA) as standard.

spectrophotometrically at 560 nm according to Beyer and Fridovich (1987), based on the inhibition of the photochemical reduction of nitro blue tetrazolium (NBT). One unit of enzyme activity was defined as the quantity of SOD required for 50% inhibition of NBT reduction. Ascorbate peroxidase (APX) (EC 1.11.1.11) activity was assayed in presence of ascorbate by following the decline in absorbance of the oxidized ascorbate at 290 nm, according to Chen and Asada (1989). Enzyme activity was calculated using the extinction coefficient of 2.8 mM $cm^{-1}$ for ascorbate. Statistics: One-way analysis of variances (ANOVA) and the Tukey´s test at p = 0.05 were used to test differences between two species. Values were expressed by means±SE.

## RESULTS

Carotenoids content was higher in *Cb* (61.02 ± $mg.g^{-1}FW$) than in *Cr* (20.418 ± $mg.g^{-1}FW$) (Fig 2). Also, Chla and Chlb were more accumulated in *Cb* than in *Cr* grown at the Mediterranean Sea (Fig 2). Variations of total protein content in *Cr* and *Cb* living in the Mediterranean Sea, were represented in Fig 3. The later indicated that protein content in *Cb* was higher than in *Cr* which exhibited 12.572 and 5.72 mg. $g^{-1}$ FW, respectively. Lipid peroxidation in both algae was determined. TBARS were significantly higher in *Cr* than in *Cb*


**Figure 1. Site location of harvesting algae**

### Enzymes assay

Catalase (CAT) (EC 1.11.1.6) activity was assayed according to Chaparro-Giraldo *et al*. (2000), by monitoring the decrease of $H_2O_2$ absorbance at 240 nm, Enzyme activity was evaluated using the extinction coefficient of 40 mM $cm^{-1}$ for $H_2O_2$. Superoxidase dismutase (SOD) (EC 1.15.1.1) activity was measured (Fig 4). Regarding the two species of algae, antioxidant enzymes (SOD, CAT and APX) were active. SOD activity was found to be important in both algae enabling the algae to protect themselves against oxidative damage. However it was noteworthy that in *Cb*, CAT, and SOD activities were higher than in *Cr* (Fig 5 and 6). The slight difference of APX activity in the algae was not significant (Fig 7).

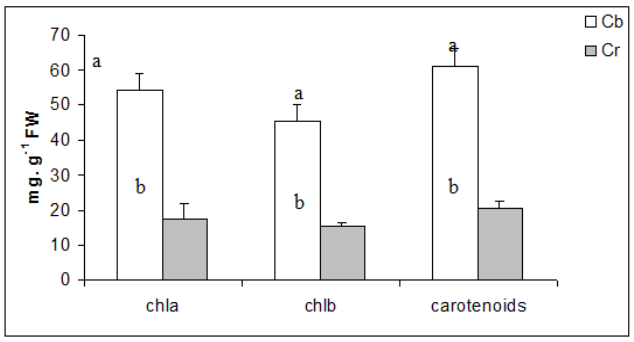


**Figure 2. Level of chlorophylls and carotenoids in Codium bursa (Olivi) C. Agardh and Caulerpa racemosa var. cylindracea harvested from Tunisian Golf. Barkes marked with different letters are significantly different at p=0.05 using Statistica, ANOVA.**

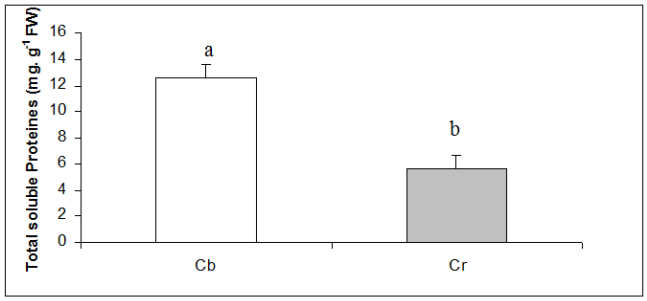


**Figure 3. Total soluble proteins content in *Codium bursa* (Olivi) C. *Agardh* and *Caulerpa racemosa var. cylindracea* harvested from Tunisian Golf. Barkes marked with different letters are significantly different at p=0.05 using Statistica, ANOVA.**

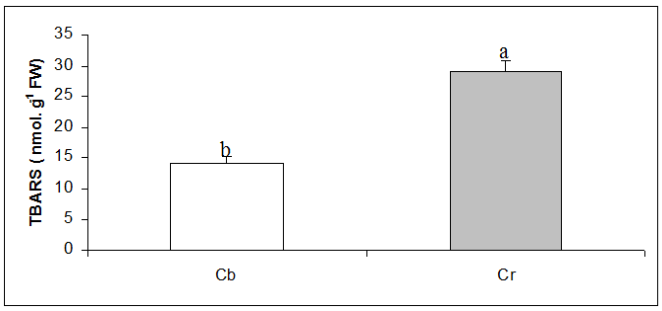


**Figure 4. Level of Lipid peroxidation as a measure of TBARS in *Codium bursa* (Olivi) C. *Agardh* and *Caulerpa racemosa var. cylindracea* harvested from Tunisian Golf. Barkes marked with different letters are significantly different at p=0.05 using Statistica, ANOVA.**

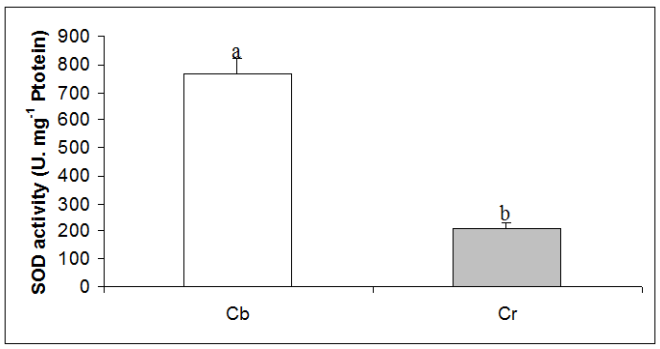


**Figure 5. Activity of SOD in *Codium bursa* (Olivi) C. *Agardh* and *Caulerpa racemosa var. cylindracea* harvested from Tunisian Golf. Barkes marked with different letters are significantly different at p=0.05 using Statistica, ANOVA.**

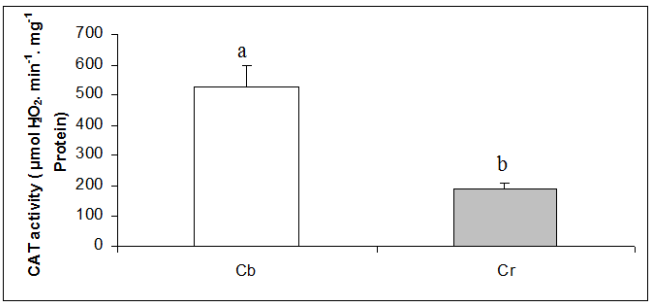


**Figure 6. Activity of CAT in *Codium bursa* (Olivi) C. *Agardh* and *Caulerpa racemosa var. cylindracea* harvested from Tunisian Golf. Barkes marked with different letters are significantly different at p=0.05 using Statistica, ANOVA.**

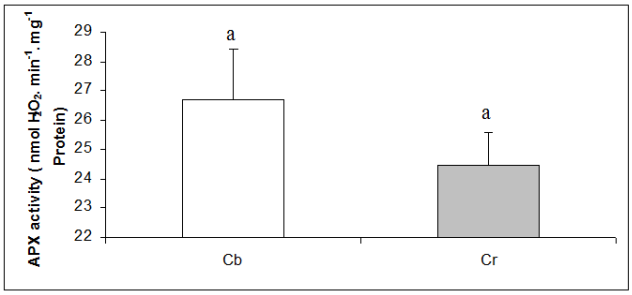


**Figure 7. Activity of APX in *Codium bursa* (Olivi) C. *Agardh* and *Caulerpa racemosa var. cylindracea* harvested from Tunisian Golf. Barkes marked with different letters are significantly different at p=0.05 using Statistica, ANOVA.**

## DISCUSSION

As photosynthetic organisms, algae play a key role in the productivity of oceans and constitute the basis of the marine food chain (Bold *et al*., 1985). Moreover, macro-algae are considered as the actual producers of some highly bioactive compounds found in marine resources (Ali and Gamel, 2010). In our data, we have chosen two Mediterranean macro-algae: *Codium bursa* and *Caulerpa ramocema* which accumulated large amount of carotenoids and chlorophylls (a and b). The correlation between accumulation of non-enzymatic antioxidant products (such as carotenoids) and resistance to oxidative damage is well known in plants (El-Baz *et al*., 2002; Paridas and Das, 2005). Beside antioxidants, the two algae showed important chlorophylls (a and b) contents, this response suggested that antioxidant system might participate in protecting talus biochemical structures against oxidative damages and minimizing the sensitivity of the photosynthetic machinery (Kaddour *et al*. 2011). Antioxidant defence system includes non-enzymatic antioxidant defence system such as carotenoids it may assume great importance in adaptative response to stress condition and may be considered as a power to cope this stress (Meneguzzo *et al*., 1999). However, we can say, that *Cb* exhibits the great quantity of carotenoids and was more resistant than *Cr* to growth medium conditions. According to Abe *et al*. (1999) the ability to accumulate simultaneously large quantities of carotenoids was recognized as safe. Since, in plants it was well known that they were efficient antioxidants protecting plants against oxidative damage. In human nutrition, carotenoids served as a major dietary source of retinol (Lachance, 1988). In addition to their provotamin A activity, carotenoids had a variety of other functions, including playing the role of light filters and prevented oxidative stress by diminishing light exposure (Stahl and Sies, 2003). According to Mayne (1996), an increased consumption of a diet rich in carotenoids was correlated with a reduced risk for several degenerative disorders, including various types of cancer, cardiovascular or ophthalmological diseases. The preventive effects have been associated with their antioxidant activity, protecting cells

and tissues from oxidative damage. Carotenoids also influenced cellular signalling and may trigger redox-sensitive regulatory pathways (Eichler *et al*., 2002).

Membrane destabilization is generally attributed to lipid peroxidation, due to an increased production of ROS (Jin *et al*., 2008b). Our results revealed that salt condition in Mediterranean sea may be involved in lipid peroxidation and membrane damage, of algae, which was obvious from the significantly higher TBARS content. However, we can suggest that *Cb* has a more important capacity to stabilize and protect the biomembrane protein and phospholipids from oxidative and peroxidative damage and loss of membrane integrity. Moreover, tenor of total soluble proteins content has shown more important in *Cb* than in *Cr,* was an additional argument confirming the previous suggestion. The parallel increase in superoxide dismutase (SOD; E.C. 1.15.1.1), ascorbate peroxidase (APX; E.C. 1.11.1.11) and catalase (CAT; E.C. 1.11.1.6) activities may suggest as mentioned by Gratão *et al*. 2005, Abdul-Jaleel *et al*. (2006), Valderrama *et al*. (2007), the existence of an effective scavenging mechanism to remove ROS. SOD, was also one of the principal antioxidant enzymes. It eliminated $O_2^{\cdot}$ transforming it into $H_2O_2$ and $O_2$ (Blokhina *et al*., 2003). Since $H_2O_2$ was toxic, it was removed by CAT and several classes of peroxidases like APX, which scavenged the $H_2O_2$ produced (Benavides *et al*., 2005; Gratão *et al*., 2005). In the two algae, as in the case of other plants described by Paridas and Das, (2005) and Silva *et al*., (2008); CAT and SOD have a combined action in minimising the effects of oxidative stress: their roles in the cell metabolism are complementary (Benavides *et al*., 2005). CAT was found more active in *Cb* than in *Cr*. However CAT led to better destruction of H2O2 in *Cb* than in *Cr*. CAT catalyzes the dismutation of H2O2 into H2O and O and has been found to increase under stress (Khan *et al*., 2007). However, CAT alone cannot destroy H2O2 because of its low affinity for $H_2O2$ and it needs the components of ascorbate-glutathione cycle for its successful removal. We can conclude that (i) The two Algae may constitute a potential resource of new antioxidant compounds, which can be used in the field of the health or of the quality of food. (ii) *Codium bursa* was more efficient than *Caulerpa ramocema* in developing enzymatic and non enzymatic antioxidative defences.

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