Ukrainian Journal of Ecology

*Ukrainian Journal of Ecology*, 2020, 10(4), 116-121, doi: 10.15421/2020_177

*ORIGINAL ARTICLE*

# Biochemical study of *Ulva lactuca* and *Cystoseira stricta* from Mostaganem coastline (Western Algeria)

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***Received: 07.08.2020. Accepted: 07.09.2020***

Our research includes biochemical study, evaluation of anti-oxidant activities and heavy metals contamination in Ulva lactuca and Cystoseira stricta collected at the site of Sidi El Adjel (Mostaganem). We registered a total phenol content in Ulva lactuca and Cystoseira stricta were 0.383 5.43 mg EAG/g MS respectively. The polyphenols contamination caused the significant antioxidant effects on DPPH. The yield of crude extract for petroleum ether were 2 and 8.8% in Ulva lactuca sp and Cystoceira stricta, whereas it were 7.9 and 2.07% for the methanol respectively. We noted the ability of studied algae extracts to reduce the DDPH. A low total lipid content was 4 and 5.5% in Cystoseira stricta and Ulva lactuca respectively, whereas the appreciable crude protein contents wad 21 and 30.6%. Chemical analyzes of our algae reveal levels estimated at 22% in Ulva lactuca sp, and 19.4% in cystoseira stricta. We estimated high zinc concentrations, which were 187.59 and 298.64 ppm. PS in Cystoseira stricta and Ulva lactuca respectively.

**Key words:** algae; *Cystoseira stricta*; *Ulva lactuca*; phenolic compounds; Sidi Ladjel beach; antioxidant power; toxicity.

## Introduction

The algae are the immense rich sources of metabolites including proteins, lipids, antioxidants like the polyphenols. The present work is oriented towards a recovery method that has a little exploitation in Algeria. Algae are very diverse and constitute a heterogeneous group to the extent that they do not all belong to the same evolutionary path but to very different phylogenetic groups, illustrated by large variations in their physiologies and metabolism, reflecting a great genetic diversity (Cabioc, 1992). They are used around the world in a broad spectrum (Fleurence,1999) on one hand as a source of food for their high nutritional values, justified by the presence of a varied and abundant mineral fraction, that constitutes an important contribution of macro-elements and trace elements in non-negligible quantity of protein, well balanced in general in amino acids, and a varied vitamin content where most of the vitamins are represented in a low lipid fraction. However, in some species that are rich in polyunsaturated fatty acids and on the other hand as a bio-indicator of their environment through their bio concentration capacities of various dissolved substances in their biotope. The objective of our study is to use these two species as a bio-indicators to evaluate the metallic contamination of their biotope and to collect some data on the antioxidant activities by the method of measuring the capacity of antioxidants to scavenge the radical DPPH.

## Materials and methods

Green algae (Chlorophyceae) are of very varied forms, uni- or multicellular (Fig. 1). Their plastids are colored in green by chlorophylls a and b, which are associated with carotenes and xanthophylls. They can be in ribbons or tubulars form and can reach a size of 20 to 30 cm (Wichard, 2015).

A B

**Fig. 1.** A. *Ulva lactuca* (green algae). B. *Cystoseira stricta* (brown algae)

*Cystoseira stricta*, which is collected at Sidi Ladjal beach, 50 km from Mostaganem city, located in the west of Algeria, coordinates (36°15'16.9"N 0°30'46.6"E), are tree-like, much branched, very bushy brown algae (Fig. 2).

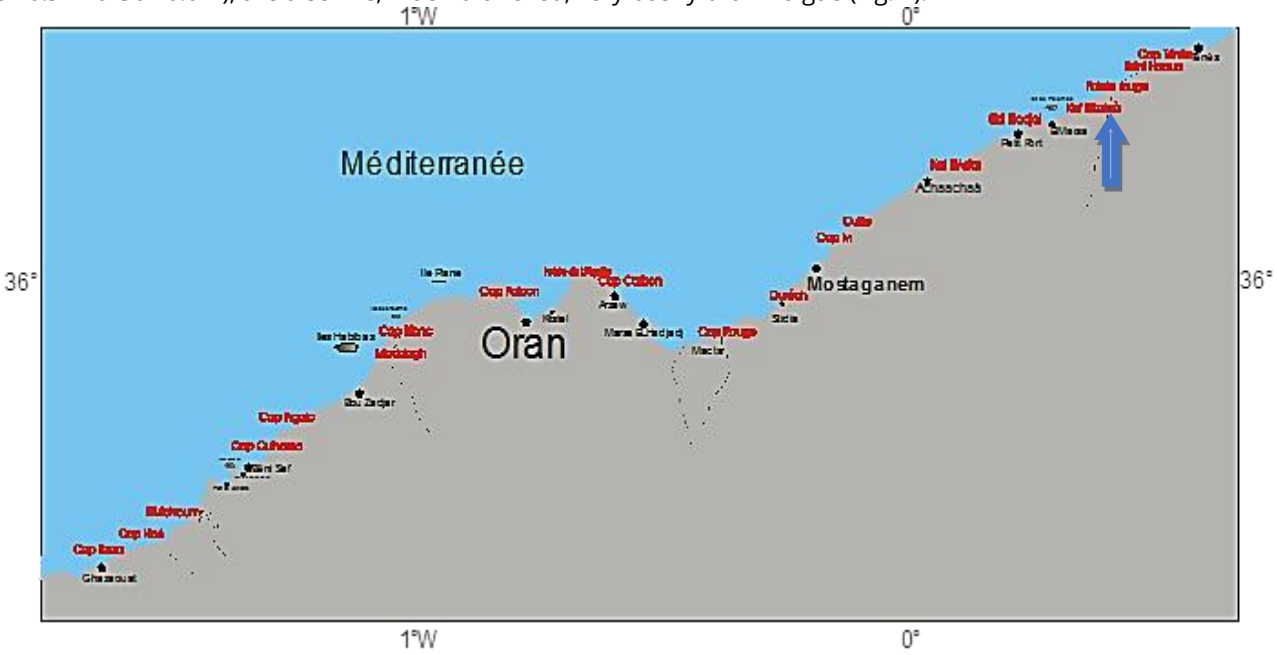



**Fig. 2**. Study area

Species identification was conducted at the Laboratory of Management and Valorization of Coastal Resources and Laboratory of Molecular Systematics. Speceies were treated by washing, cleaning under the running water, air-dried and protected from the light in order to preserve the algae biomass.

**Preparation of seaweed extracts.** We took 250 ml of each solvent (methanol and light petroleum), along with 50 g of the algae sample, and filter through Wattman paper. Then these extracts were collected and concentrated using a rotary evaporator and stored in smoked bottles at 4°C until further use.

**Quantitative and nutritional study**

The water content was evaluated by drying in an oven 40 °C for 24 hours. The measurement of the water content was calculated by the following formula:

$$\text{Water content (\%)} = \text{Fresh weight (FW)} - \text{Dry weight (DW)} / \text{Fresh weight (FW)} \times 100.$$

The raw ash content was determined by incineration of the product (LAB23 I-MET006-Raw ash v 11 2013-02-01-3/5). For the determination of total DPPH (2,2-Diphenyl-1-picrylhydrazyl) for two algae extracts, the DPPH free radical scavenging method allows us to study the antioxidant activity of the extracts (Benariba et al., 2013). The IC50 value is the concentration which ensures the 50% reduction of DPPH and it is determined graphically for each extract from the curve of the percentage reduction as a function of the concentration. The dosage of the polyphenols contained in the algae extracts was determined according to the method described by (Miliaukas et al., 2004) and the levels are measured in milligrams (mg) standard equivalent (gallic acid) per gram of algal material (mg EAG/g). The total lipids determination was based on the principle of cold extraction of the lipids by mixture of chloroform/methanol solvent (2/1; v/v) and adding 0.58 % of NaCI aqueous solution, which allows the separation of the phases. The determination of crude proteins was carried according to Kjeldahl (1883). For the determination of photosynthetic pigments, the extraction of photoreceptor pigments from leaf tissue was carried out by the Arnon method (1949). The chlorophyll (a), (b), (a + b) and carotenoid contents were measured in µg/g of MF.

**Ecotoxicological study**

A lethal dose test of *Artemia salina* LD50 was conducted on cysts of this crustacean with different concentrations of algae extracts (20 to 100 µg $ml^{-1}$). After 24 hours, the number of dead individuals is counted in the test boxes and the control; to define the LDc50 lethality dose of each solvent. For the extraction of heavy metals, the plant extracts are prepared by wet digestion. Heavy metals (Cu, Pb and Zn) are extracted with aqua regia (sulphonitric solution - hydrogen peroxide) and determined by atomic absorption spectrophotometry.

The yield was calculated using the following equation:

$$R = (\text{weight of extract} / \text{weight of dried algae}) * 100$$

The products have been tested at concentrations ranging from 20, 40, 60, 80 and 100 µg/mL

## Results

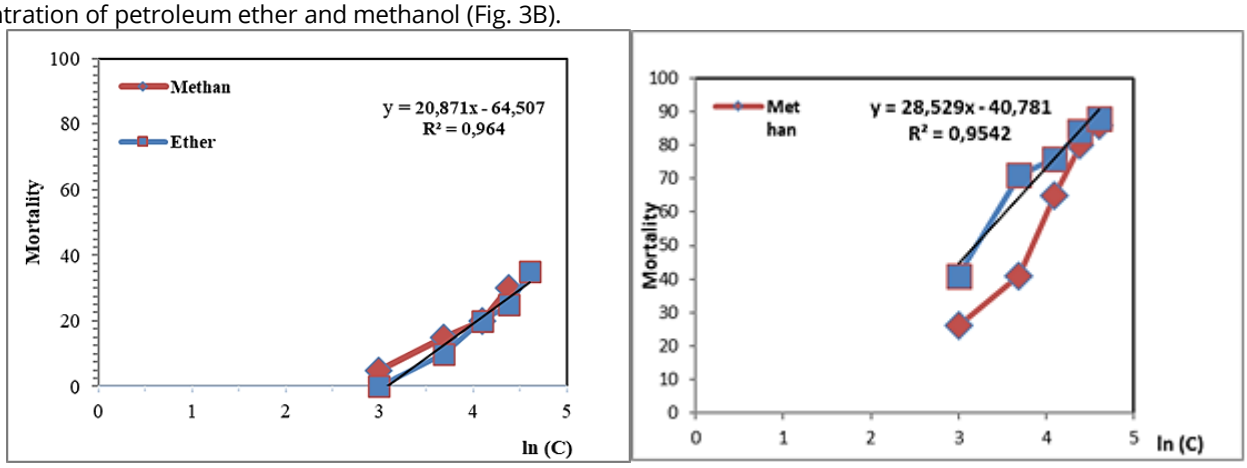
The water content in *Ulva lactuca sp* was medium (15.25%), and in *Cystoseira stricta* it was high (64.9%). The crude ash contents were 22% and 19.4% respectively. Our results are comparable to work for *Ulva lactuca*, with respect to the dry matter: 11% (Yaich et al., 2011) and 19.6% (Ortiz et al., 2006). The extraction of the secondary compounds after 24 hours of maceration from the two algae allowed calculating the yield of the expressed extract as a percentage for 20 g of crushed dried algae. The result

obtained for light petroleum is evaluated at 2% and 8.8% in *Ulva lactuca sp* and *Cystoceira stricta* respectively; while there are 7.9% and 2.07% for methanol respectively (Table 1).

**Table 1.** The different characteristics of the soxhlet method

| Species | Types of solvents | Number of cycles | Temperature | Yields |
|---|---|---|---|---|
| *Ulva lactuca* | Petroleum ether | 5 | 60 | 2 % |
| | Methanol | 2 | 57 | 7.9 % |
| *Cystoseira stricta* | Petroleum ether | 7 | 60 | 0,36% |
| | Methanol | 8 | 57 | 2,07% |

The obtained mortality results during these toxicity tests of the crude products in *Artemia salina* larvae as a function of the concentration and their logarithms are shown in Fig. 3 (A, B). The percentage of mortality of *Artemia salina* larvae exposed to algae extracts reveals that for each concentration subjected to brine and the percentage of mortality increases as a function of the concentration. The mortality percentage of *Artemia salina* larvae exposed to algae extracts reveals that for each concentration subjected to brine and the percentage of mortality increases as a function of the concentration. The results indicated that the extracts of the *Ulva lactucta* samples taken from the beach of Sidi Ladjel were not toxic to *Artemia salina* (Fig. 3A). While the crude extracts of *Cystoseira stricta* samples from Sidi Ladjel beach were toxic to *Artemia salina*, due to high concentration of petroleum ether and methanol (Fig. 3B).




A: *Ulva lactuca* extract B: *Cystoseira stricta* extract

**Fig. 3.** *Artemia salina* mortality at different algae extract concentrations.

The concentrations of *Ulva lactucta* showed more than 48% of Artemia mortality after 24 hours by the extracts of ether and methanol. However, *Cystoceira stricta* showed more than 80 and 85% Artemia mortality after 24 hours respectively by the same extracts. The toxicity of the extracts based on their LD50 was generally imposed as reported by Clarkson et al. (2004) and Meyer et al. (1982). We calculated the LD50 lethality dose from various solvent extracts (Table 2, Fig. 4.)

Table 2. LD50 values of algae extracts

| Species | Extract | $DL_{50}$ (µg/ml) |
|---|---|---|
| *Ulva lactuca* | Ether | 48 |
| | Methanol | 30 |
| *Cystoseira stricta sp* | Ether | 15.36 |
| | Methanol | 39.64 |

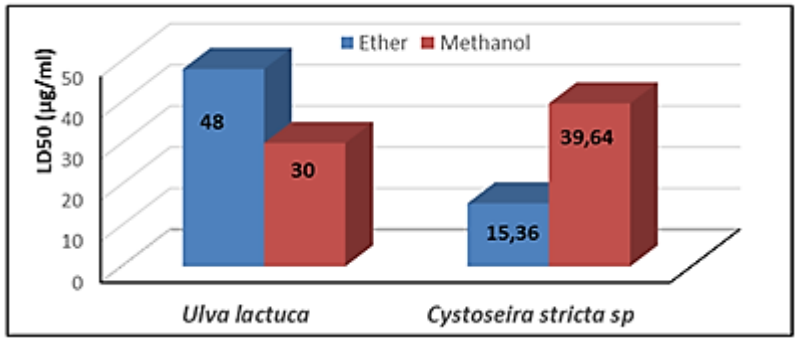



**Fig. 4.** LD50 of algae extracts

The toxicity of the extracts based on algae expressed as LD50 is generally valued either by comparison with Meyer or the Clarkson toxicity index. According to the Meyer toxicity index, the extracts with LD50 <1000 µg / ml are considered toxic, while extracts with LD50> 1000 µg / ml are considered to be non-toxic (Meyer et al, 1982). The results of the anti-free radical effect (anti-oxidant activity) of algae extracts are evaluated by tests based on the total anti-oxidant capacity and the DPPH free radical scavenging (Fig. 5). There is an increase in anti-radical activity proportional to the increase in the concentration of the extract

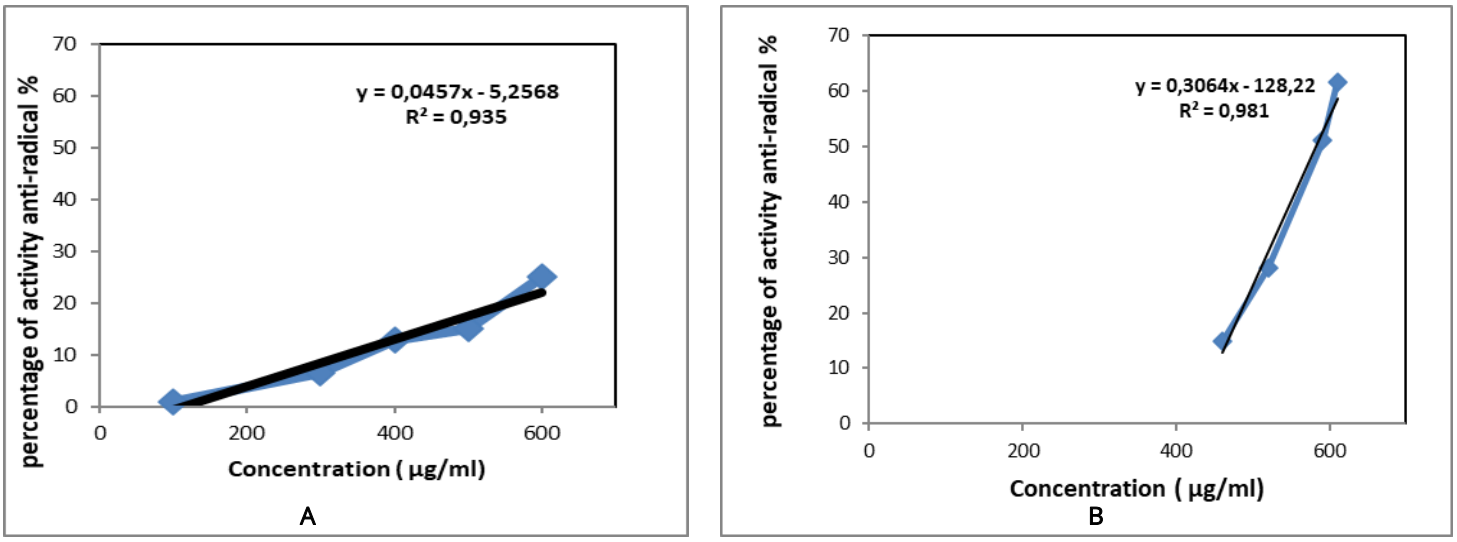



**Fig. 5.** Percentage inhibition of DPPH. **A.** Extract of *Ulva lactuca sp.* **B.** Extract of *Cystoseira stricta sp*

For the extract of *Ulva lactuca*, there is a low percentage reduction of DPPH (2%) at low concentration (100 µg/ml), whereas at moderately high concentrations (500 µg/ml), it shows 15 % of DPPH reduction. Regarding the extract of *Cystoceira stricta*, at low concentration (466 µg/ml), the extract shows a low percentage reduction in DPPH evaluated at 15.11%, while at moderately high concentrations (621 µg/ml), the extract exhibits higher DPPH reduction percentages which varies up to 61.14%, reflecting an anti-free radical effect. The total polyphenol content is determined from the equation of the linear regression of the calibration curve expressed in µg equivalent of gallic acid (mg EAG) per g of dry matter (DM) (Fig. 6).

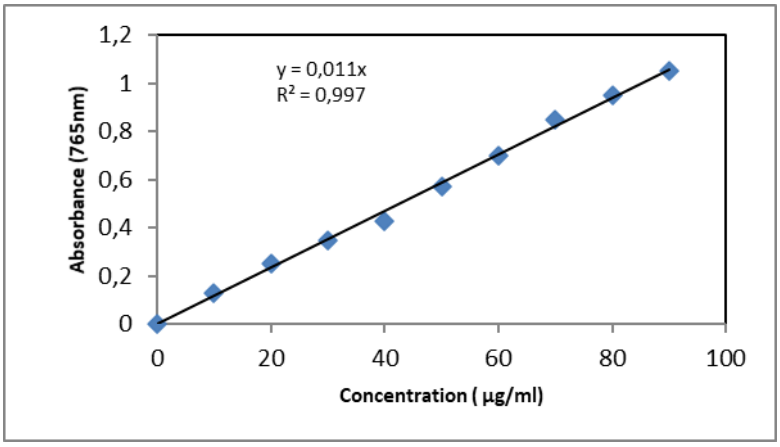



**Fig. 6.** Gallic acid calibration curve for the determination of total phenols

The content of polyphenols can serve as an important indicator of antioxidant capacity and be used as a preliminary selection for any product when it is intended as a natural source of antioxidants in functional foods (Mezghani et al., 2013; Viuda-Martos et al., 2011). The analysis of the poly-phenols shows that the extracts obtained from *Ulva lactuca* and *Cystoseira stricta* have total phenol contents of 0.383 mg EAG/g (DM) and 5.43 mg EAG/g (DM) respectively. The total phenol content of our extracts remains comparable with the work of (Sadati et al., 2011) and (Zubia et al., 2009) on the species *Cystoseira myrica* and *Cystoseira tamariscifolia* where the contents are 10.08±1.13 mg EAG/g (MS) and 10.91±0.07 mg EAG/g (MS), respectively. The lipid contents in algae vary between species, geographic location, season, temperature, salinity, light intensity, but also the interaction between these factors and finally the extraction method used (Yaich et al., 2011; Satpati, 2011; Sanchez-Machado et al., 2004 a, b). According to (Fleurence,1999), the protein content in marine algae in general varies between 10 and 21%, but also varies greatly between species (De Oliveira et al., 2009; Dawczynski et al., 2007), depending on the seasons and environmental conditions. Our results (Table 3) are close to those of (Makkar et al., 2016; Shuuluka et al., 2013; Arasaki, 1983 in Fleurence, 1999) which suggest protein contents up to 30% in green algae while that in brown algae have low levels of about 3 to 15% (DM).

Table 3. Total lipids and crude protein contents of studied algae

| Species | Total Lipids | Crude protein |
|---|---|---|
| *Ulva lactuca* | 5.5% | 30.6%. |
| *Cystoseira stricta* | 4% | 21% |

However, the industrially exploited green macroalgae (*Laminaria digitata*, *Ascophylum nodosum*, *Fucus vesiculosus* and *Himanthalia elongata*) have a lower protein content (15% DM) except for the species *Undaria pinnatifida* (Wakame) that have a variable protein level of 11 to 24% DM (Fleurence, 1999). The contents of chlorophylls a and b and the content of carotenoids are shown in Table 4, expressed in µg/g of MF.

Table 4. Chlorophyll (a), (b) and carotenoid content.

| Algae species | CHla | CHlb | CHla + CHlb | Cart |
|---|---|---|---|---|
| *Ulva lactuca* | 1.89 | 2.27 | 4.19 | -0,95 |
| *Cystoseira stricta* | 0,83 | 1,70 | 5,59 | -0,468 |

Our analysis revealed the presence of xenobiotics (Cu, Pb and Zn) in *Cystoseira stricta* (Table 5), which shows that zinc had the highest concentration (298.642 ppm. PS), which in fact is a non-toxic metal, but it can cause physiological disturbances at high concentrations. We also registered high level copper and lead (169.09 and 83.0967 ppm. P.S correspondingly).

Table 5. Average concentrations of heavy metals in µg/g PS of dry weight in studied algae

| Species | Zinc (Zn) | Copper (Co) | Lead (Ld) |
|---|---|---|---|
| *Ulva lactuca* | 298.64 | 169.09 | 83.09 |
| *Cystoseira stricta* | 187.59 | 109.65uld be | 97.0 |

Our results are almost similar to previous research. The metal contents in the tissues of algae, depending mainly on the differences in biological cycles and the conditions of the bioavailability of the metals, since algae accumulate Zn and Cu easily from seawater (Bennasser et al., 2000; Ho, 1988). Metal concentrations vary not only among algal species, but also within the same species from different sites. These variations are related to the tissue age, life cycle, and ambient metal concentrations. The influence of physicochemical speciation of dissolved metals, and the content of mineral salts and nutrients in ambient water on the zinc contents in marine algae should be studied.

## Conclusion

Our research includes biochemical study, evaluation of anti-oxidant activities and heavy metals contamination in *Ulva lactuca* and *Cystoseira stricta* collected at the site of Sidi El Adjel (Mostaganem). We registered a total phenol content in *Ulva lactuca* and *Cystoseira stricta* were 0.383 5.43 mg EAG/g MS respectively. The polyphenols contamination caused the significant antioxidant effects on DPPH. The yield of crude extract for petroleum ether were 2 and 8.8% in *Ulva lactuca sp* and *Cystoceira stricta*, whereas it were 7.9 and 2.07% for the methanol respectively. We noted the ability of studied algae extracts to reduce the DDPH.

A low total lipid content was 4 and 5.5% in *Cystoseira stricta* and *Ulva lactuca* respectively, whereas the appreciable crude protein contents wad 21 and 30.6%. Chemical analyzes of Artemia mortality levels caused by the algae estimated at 22% in *Ulva lactuca sp* and 19.4% in *Cystoseira stricta*. We estimated high zinc concentrations, which were 187.59 and 298.64 ppm. PS in *Cystoseira stricta* and *Ulva lactuca* respectively.

## Acknowledgments

We thank reviewers for their comments and suggestions. We are grateful to all persons, who contributed to the field and lab analysis. We thank the MESRS and the DGRSDT for supporting and funding this work

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*Citation:*

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