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Contribution to the Toxicological Study of the Brown Alga Cystoseira Stricta by Shrimp Brine Test

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Abstract

This study examines the eco-toxicological assessment of brown algae contamination in the western Mediterranean coast of Algeria at beach of Sidi Ladjal, Wilaya Mostaganem.

The toxicity of *Cystoseira stricta* forests has been estimated in Shrimp Brine (*Artemia salina*), LD_{50} lethality tests are widely used in research and applied toxicology; The responses to cyto-toxicity tests on algal extracts by solvents using a standardized method of Brine Shrimp are based on different concentrations. The LD_{50} values of the different extracts are obtained by different linear expressions. The results indicate that the raw extracts of the brown seaweed *Cystoseira stricta* samples from Sidi Ladjel beach are toxic to *Artemia salina,* These extracts gave different results for each concentration subjected to brine and the percentage of larval mortality increases with concentration. According to different Toxicity Assessment Criteria we were able to conclude that: The most toxic are those of chloroform and petroleum ether where ever Methanol and hexane extracts that are moderately toxic.

The study of the antioxidant power by the trapping of the radical DPPH, and the quantification of the total antioxidant capacity, reveals the presence of antioxidant properties for the studied extract; Also regarding the metal contamination, our alga has higher levels of zinc followed by copper and lead. All these results obtained are only a first steep in the search for biologically active natural substances from marine algae.

Keywords: antioxidant power, *Artemia salina,* beach sidi ladjal, *cystoseira stricta*, metal contamination, toxicity.

1. Introduction

Brown seaweeds are widespread because of their adaptability through reproduction and response to various ecological conditions.

This supposes that they secrete chemical defense substances, against the multiple dangers to which they are exposed (mobile predators, and invading micro-organisms).

The study of cyto-toxicity test responses on algal extracts by solvents was done using a standardized method of Brine Shrimp that is based on different concentrations.

This biological assay method using Artemia salina has been commonly used in eco-toxicological tests, it is sensitive, accurate and reliable for detecting toxic and bioactive compounds in plant extracts, but also the sample must be having exactly the same age and the same physiology (Parra et al., 2001; Lachumy et al., 2010).

Our goal is to detect the toxic or dangerous potential of our Cystoseira stricta algae sample by this Brine-Shrimp test, whose principle is a lethality assay of the practical system, to monitor the

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biological activities and the evaluation of the toxic potential. Brown algae contamination sampled at Sidi Ladjal Beach (Dahmani, 2014).

According to the study by P. Lavens, P. Sorgeloos who worked on the acetonic and hexanic extracts of the brown alga Cystoseira stricta harvested from the Algerian west coast (madrid beach), admits that it has a potency of antimicrobial activity that can possibly replace the less effective antibiotics and especially the antifungals where this species has shown a remarkable fungistatic effect during the test months (Lavens, Sorgeloos, 1996).

2. Materials and methods

The seaweed Cystoseira stricta sp was collected on 01/03/2017 at the beach of Khadra (Sidi Ladjal) willaya of Mostaganem in Algeria (see Figure 1-A), its botanical identification was carried out in situ (see Figure 01-B) and in the laboratory in order to be certain about our sample, then it is kept in a black plastic bag

A B

Fig. 1. A- Satellite view of the sampling site (Khadra commune). **B** – Algae Cystoseira stricta sp

Systematic position of the genus Cystoseira (source algaebase):

Field	Eukaryota
Reign	Chromista Cavalier-Smith
Branch	Heterokonta Cavalier-Smith
class	Phaeophyceae Kjellman
Order	Fucales Kylin
Family	Sargassaceae Kützing (incl Cystoseiraceae)
Genus	Cystoseira amentacea var.stricta C. Agardh

2.1. Extraction of the seaweed extract

Using the Soxhlet method and with four types of solvent, namely chloroform, methanol, petroleum ether and hexane The various characteristics (number of cycles, temperature and yield) were summarized in Table 1. 500 ml of each type of solvent are taken with 100 g of the algal sample, all the components are then filtered with Wattman Paper No. 1 filter. The extracts were then collected and concentrated using a rotary evaporator to finally calculate the yield of each extraction, the extracts collected are finally kept under low temperature until the moment of the test.

Table 1. The different characteristics of the soxhlet method

Types of solvents	Number of cycles	température	Yield
Chloroform	6	52	12,01%
Petroleum ether	7	60	0,36%
Méthanol	8	57	2,07%
Hexane	3	56	12,01%

2.2. Hatching of larvae

The cysts are imported by the aquaculture feed business Advenced solutions for animal rearing, the species of Artemia salina is of American origin from the salt lake of Utha (USA). In order to ensure

optimum cyst hatching, certain strict conditions must be respected P. Sorgeloos, namely: a temperature maintained between 25 and 28 °C and a salinity of 15 to 35 g/l with a pH at about 8.0 (Sorgeloos, 1986).

The cysts should be fed with an oxygen quantity of at least 2 mg/l, and a maximum cyst density not exceeding 2g/l to ensure a constant illumination of 1000 to 2000 lux.

The standard procedure employed is that of J. Dobbeileir, N. Adam, E. Bossuyt, E. Bruggrman, P. Sorgeloos, (Dobbeileir et al., 1980). It consists in incubating 250 mg of cysts in a cylindro-conical glass container containing 100 ml of filtered natural seawater (0.45 and 0.2 µm). The cysts should be kept in suspension by applying aeration to the bottom of the container (see Figure 2).

The temperature is maintained at 26°C, sometimes at 28°C using a thermostat, the young larvae are thus obtained after 24 hours and are obviously fed with yeast and milk powder (Benariba et al., 2013).

The collection of these young individuals is done with a plastic pipette for the study of the toxicity test.

Fig. 2. Breeding of Artemia salina

2.3. The lethal dose dose LD50 of Artemia salina

The tests were carried out in Petri dishes (see Figure 3), the extracts were diluted with 0.8 ml of dimethylsulfoxide (DMSO) at different concentrations (20 to 100 µg / ml-1) which filled with 39.2 ml of seawater, then 10 nauplii are added to each box.

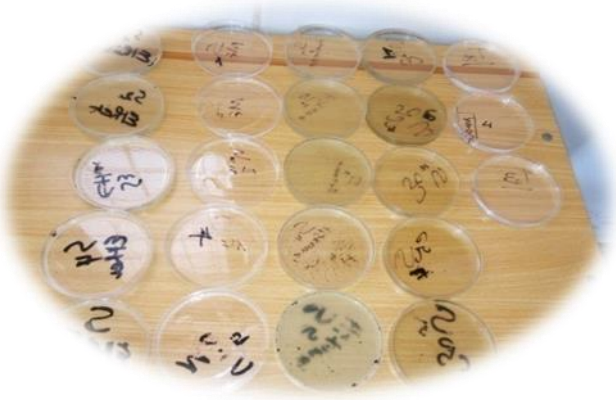

Fig. 3. Lethality test LD50

After 24 hours, we count the number of dead individuals in the test boxes and the control; beyond it is possible to define the lethal dose LD50.

In the case where the control contains dead larvae, the mortality percentage is corrected using the following formula:

% M = NLP / NLT * 100

With:

M: mortality percentage

NLP: number of dead larva in the presence of the product tested

NLT: number of dead larva in the presence of the control (solvent)

2.4. Evaluation of the antioxidant power :

The antioxidant activity of the extract of Cystoseira stricta sp was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Hoenig, 1978).

The absorbances measured at 517 nm are used to calculate the percentage inhibition of the DPPH radical, which is proportional to the anti-radical power of the sample.

• Preparation of DPPH at a concentration of 0.004 mg / ml in methanol,

• Preparation of extract in methanol at different concentrations (100, 200, 300, 400, 500 mg/ml);

• To 2 ml of the DPPH solution is added 0.014 mg for each extract at different concentrations;

• Preparation of white tube for each concentration: 50 ml of methanol and 0.014 of the corresponding extract;

• Preparation of negative tube control: 50 ml of methanol with 2ml of the DPPH solution,

• The white tube contains 2 ml of methanol,

• Incubation 30 min at room temperature and in the dark,

• Absorbance measurement at 517 nm

The results of the DPPH were expressed taking into account the average of three measurements obtained for the extract, the percentage reduction of the DPPH is calculated according to the following formula:

$$I\,\% = \frac{(\text{WHITE ABS}) - (\text{ABS EXTRACT})}{(\text{WHITE ABS})} \times 100$$

Knowing that :

DPPH (%): Percentage reduction of DPPH.

WHITE ABS: Absorption of white extract.

ABC EXTRACT: Extract Absorption

Extraction of heavy metals in marine plants

According to S.H. Hoenig, I. Kazap, J. Leibovici method which consists of wet digestion. Heavy metals (Cu, Pb and Zn) are extracted with aqua regia (sulphonitric solution – hydrogen peroxide). For that 1 ml of sulfuric acid, 3 ml of nitric acid, 3 ml of hydrogen peroxide at 30 volume, are added to 1g of the sample plants dried and crushed into fine particles (Hoenig, 1978).

The whole is heated at 75 ° C., until boiling for 15 minutes, after cooling the contents are filtered on filter paper at medium filtration speed in a 50 ml flask to its ml as required.

Automatically accompanied by a mineralization of whites, which consists of a regal water solution. It is from this test solution that flame atomic absorption spectrophotometry is performed.

a. Principle

It is an analytical method based on the exposure of the spectra of lines, allowing measure the metal elements. This method is based on the rule experimented by P. Clarkson, Y. Li, G. Richardson (Clarkson et al., 2004).

3. Results and discussion

Result of the extraction in different types of solvents according to the polarity and the number of cycles:

The result obtained in Figure 5 shows that the highest yields are those of Chloroform and Hexane with a level of 12.01 %, whereas Methanol and Ether of oil gives the lowest rates with respectively 2.07 % and 0.36 %.

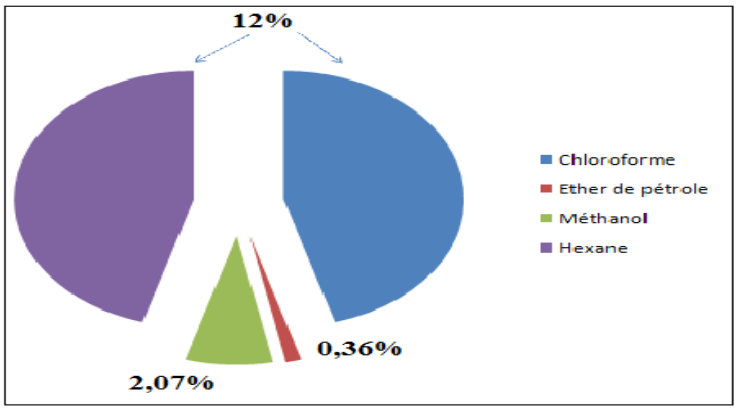

Fig. 4. Rates of return of different extracts

The calculation of the yield is done by the following equation:
R = (weight of extract / weight of dried seaweed) * 100
The products were tested at concentrations of 20, 40, 60, 80 and 100 µg / mL and the results obtained during these tests of toxicity of crude products in larvae of Artemia salina depending on the concentration and their Logarithms are summarized in Table 2 and illustrated in Figure 4.

Table 4. Result of Toxicity Activity

Cystoseira stricta (ug.ml-1) / Extrait	[c]	NLm	NLT	Nlc	%M	Ln[c]
Hexane	20 40 60 80 100	05 8 8.5 9.5 10	10 10 10 10 10	01 01 01 01 01	40 70 75 85 90	2.99 3.68 4.09 4.38 4.60
Petroleum ether	20 40 60 80 100	5 9 8.5 9.5 9.75	10 10 10 10 10	01 01 01 01 01	40 70 75 85 87.5	2.99 3.68 4.09 4.38 4.60
Chloroform	20 40 60 80 100	6 6.5 8 9.2 10	10 10 10 10 10	01 01 01 01 01	50 55 70 82 90	2.99 3.68 4.09 4.38 4.60
Méthanol	20 40 60 80 100	3.5 5 7.5 9 9.5	10 10 10 10 10	01 01 01 01 01	25 40 65 80 85	2.99 3.68 4.09 4.38 4.60

The results indicate that the raw extracts of the seaweed Cystoseira stricta samples from Sidi Ladjel beach are toxic to Artemia salina.

- The most toxic are those of chloroform and petroleum ether
- Methanol and hexane extracts are moderately toxic.

The extracts gave different results for each brine concentration and the percentage of larval mortality increases with concentration, which is clearly illustrated by the curves shown in Figure 5.

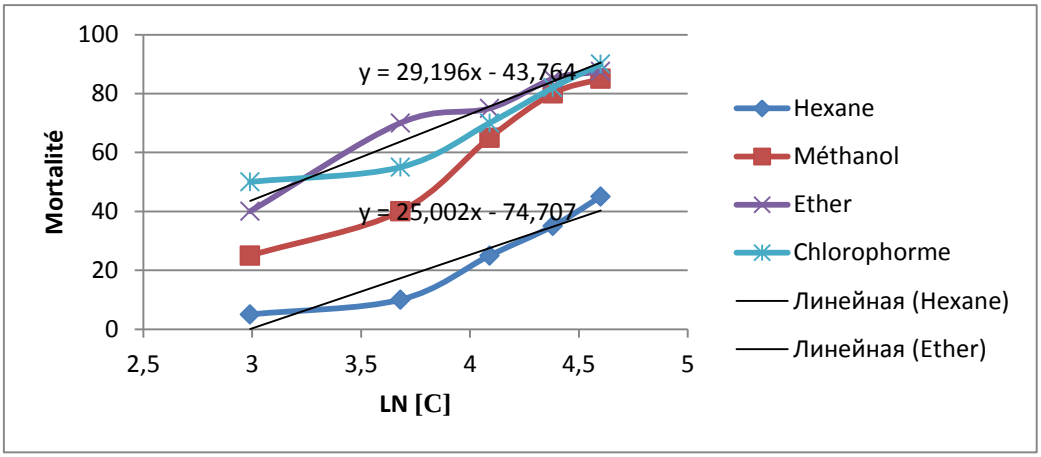

Fig. 5. Graph of the mortality at the different values of concentration of extracts by different types of solvents

Concentrations showed more than 80 % mortality of Artemia salina after 24 hours with ether, chloroform and methanol extracts (Figure 5).

These solvent extracts gave mortality rates of 10 % to 40 % for hexane, 25 % to 85 % for methanol, chloroform to give a mortality of 50 % to 90 % and finally the petroleum ether shows a rate of mortality rate ranging from 40 % to 90 %.

It can be seen from Figure 5 that the percentage mortality of Artemia salina increases after 24 hours of exposure to different concentrations of crude brown algae extracts, which was greater than 50 % for most components indicating that these extracts namely ether, methanol and chloroform are toxic.

It can be noted that:

These solvents have a selective effect and active ingredients extracted from the brown alga studied, for that the substance extracted from this alga has different effects on the larvae of Artemia salina, according to the types of solvents used during the extraction;

This variability of the biological activity results of brown algae extracts may depend on the chemical compound contents.

The toxicity criteria for the evaluation of Clarkson toxicity of plant extracts are classified as follows:

The toxicity of extracts based on this algae expressed in Dl50 is generally imposed on a valued comparison of P. Clarkson, Y. Li, G. Richardson Extracts with an LD50 greater than 1000 mg/ml are not toxic, the LD50 values of between 500 and 1000 µg/ml are weakly toxic and those with an LD50 of 100-500 µg/ml are then moderately toxic, while the extracts with an LD50 of 0-100 ug/ml are highly toxic (Clarkson et al., 2004).

Concerning our results, the values of the lethal dose "LD50" of the various solvent extracts obtained by the different linear expressions are displayed in Table 3 and illustrated by FIG.

Table 3. LD50 values of extracts of Cystoseira stricta sp

Species	Extrait	DL_{50} (µg/mL)
Cystoseira stricta Sp	Hexane	146.62
	Petroleum ether	15.36
	Chloroform	19.88
	Méthanol	39.64

According to the graph (Figure 6), the hexane extract exhibits a D150 cyto-toxic activity value of greater than 146.62 µg/ml which classifies it according to R. Merad, M. Reggabi, B. Alammir, S. Benali, R. Abetroun, M. Azzouz, D. Beaissa, as moderately toxic (Merad et al., 1991).

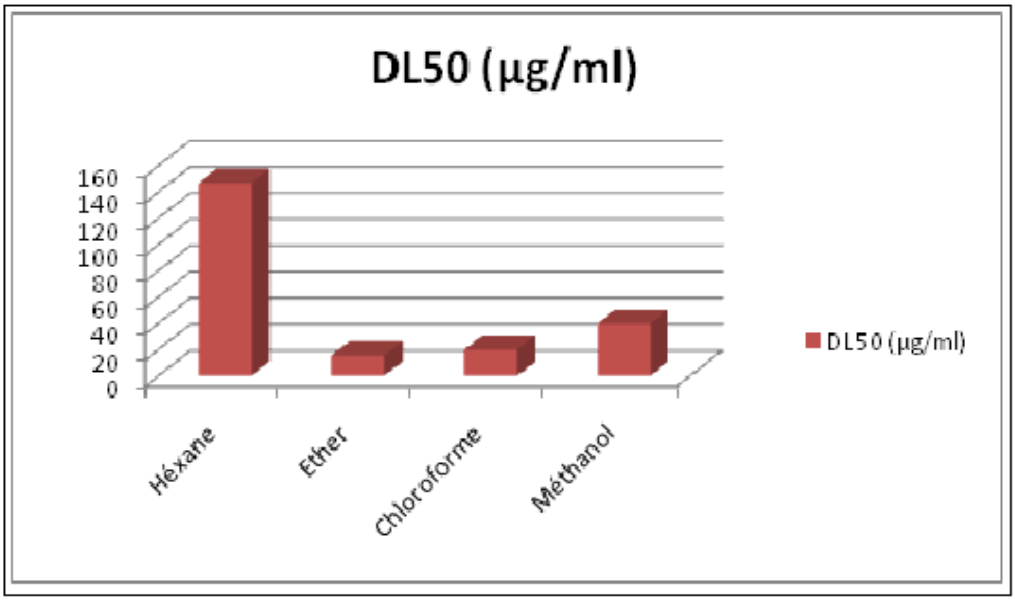

Fig. 6. Values of the lethal dose "LD50" of the different extracts

The hexane extract has a cyto-toxic activity value Dl50 greater than 146.62 µg/mL which classifies it according to M.H. Moshafi, F. Sharififar, G. Dehghan, A. Ameri as moderately toxic (Moshafi et al., 2009).

While other extracts of Cystoseira stricta are highly toxic to Artemia salina larvae with an LD50 of 15.36, 19.88 and 39.64 for ether, chloroform and methanol respectively.

In order to compare this toxicity noted with other products already tested, the results of other tests submitted to Artemia salina were used and summarized in Table 4.

Table 4. LD50 Results of Reference Products (Benkdad et al., 2011)

Product	DL_{50}(µg/mL)
Podophyllotoxin (alientary)	2.4
Digitalis (pharmaceutical)	77.2
Strychnine sulfate	515

These results show that our extracts are lower than strychnine sulfate (515 µg/mL) but are in the range of podophyllotoxin with 2.4 µg/mL and digitalis with 77.2 µg/mL.

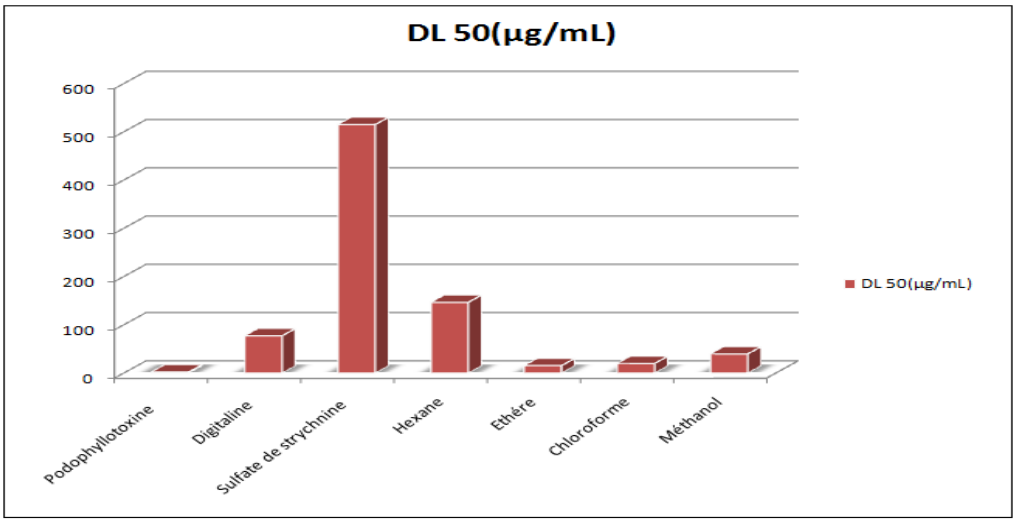

Fig. 7. Percent inhibition of DPPH by the extract of Cystoseira stricta sp.

Anti radical activity

The results of the anti-radical effect (antioxidant activity) of the extract of Cystoseira stricta sp are evaluated by tests based on the total antioxidant capacity and the trapping of the free radical DPPH that has been summarized in the Table 4 and represented by (Figure 5).

Table 4. The results of the anti-radical effect (antioxidant activity) of the extract of Cystoseira stricta sp are evaluated by tests based on the total antioxidant capacity and the trapping of the free radical DPPH

% of réduction of DPPH	61,14	50,93	28,15	15,11
Concentrations of the extract (mg/ml)	6,21	5,99	5,29	4,66

According to the results obtained there is an increase in anti-radical activity proportional to the increase in the concentration of the extract. At a low concentration of 4.66 mg/ml, the extracts have a low percentage of DPPH reduction estimated at 15.11 %. At moderately high concentrations, 6.21 mg/ml, the extract has higher percentages of DPPH reduction that vary up to 61.14 %.

From this result it is found that this high-dose extract (6.21 mg / ml) has an antiradical effect on DPPH.

Contamination by heavy metals

The analysis revealed the presence of xenobiotics (Cu, Pb and Zn) in the brown algae Cystoceira stricta (Table 6) shows that the most important contamination concerns relatively Zinc which is not considered as a toxic metal. However, a high concentration can cause physiological disturbances to the body.

Table 3. Average concentrations of heavy metals in µg/g dry weight in the brown seaweed Cystoceura stricta of Sidi Ladjal

Métals	Zinc (Zn)	Copper (Cu)	Lead (Pb)
Concentration found	187,589	109,648	97,0029

The comparison with the values of the literature allows us to note that the values obtained are close to those of polluted environments.

A. Kaimoussi, A. Mouzdahir, A. Saih found values between 6.96 and 66.8 µg/g in U. lactuca tissues, and between 16.21 and 147 µg/g in E. intestinalis tissues, arrived at a greater value, reaching a maximum limit of 337 µg/g dry weight, in U tissues. lactuca (Kaimoussi et al., 2004; Ho, 1988).

N. Favero, M.G. Frigo showed that algae accumulate Zn and Cu easily from seawater. But also according to the study of Benkdad et al. the metal contents in the tissues of the algae, mainly depending on the differences of the biological cycles and the conditions of the bioavailability of the metals (Favero, Frigo, 2002; Benkdad et al., 2011).

The comparison of the results obtained by our study with previous studies indicates the presence of high levels of zinc which is displayed with 187,589 µg/g. PS, followed by copper with 109.648 µg/g. PS and finally the lead that registers at a value of 97.0029 µg/g.

The concentrations of metals vary, not only among species of algae, but also within the same species from different sites.

That is due to abiotic or biotic factors, anthropogenic factors and distribution heterogeneous metals in the ecosystem. These variations are related to the age of the tissues, life cycle, ambient concentrations of metals other conditions environmental.

4. Conclusion

This study gave low LD50 values for the brown algae Cystoseira stricta sp obtained from the different extracts tested.

This toxic action is attributed to a cytogenetic and toxic compounds present in this alga, for this the study of the toxicity is essential in order to locate the limits of tolerance.

This study has shown in particular an abundance of this group of toxic compounds, whose main activity is antifungal, antimycotic, anti-inflammatory and anthelmintic is of particular interest in various pathologies.

As a result, the most toxic extract has the lowest LD50 and, by comparing the different lethal concentrations for the surfactants, it is quite possible to determine if the species is more sensitive and can to be affected by the pollution of its biotope.

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