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Seasonal Fluctuation of Cytotoxic Activity of Moroccan Marine Algae (El Jadida-Morocco)

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Khadija Oumaskour[1*], Najwa Hassou[1], Nabila Boujaber[1], Fatima Lakhdar[1], Omar Assobhei[2], Samira Etahiri[1]

[1] *Laboratory of Marine Biotechnology and Environment, Faculty of Science, University Chouaib Doukkali, BP 20, El Jadida, Morocco.*

[2] *University Sidi Mohamed Ben Abdellah, BP 2202, Fez-Morocco.*

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ABSTRACT

The present study was performed to screen out the extracts of marine algae form Atlantic coast of morocco and evaluate the seasonal variation in cytotoxic activity (Brine shrimp) of nine marine algae agains *Artemia salina*: *Bornetia secundiflora*, *Caulacanthus ustulatus*, *Gelidium sesquipedale (Rhodophyceae)*, *Ulva Lactuca*, *Codmium tomentosum*, *Enteromorpha compressa (Chlorophyceae)*, *Fucus spiralis*, *Sacchoriza polyschides*, *Laminaria ochroleuca (Phaeophyceae)*. The seasonal variation of cytotoxic activity of algae extracts was evaluated to determine the effect of the harvest period on cytotoxic activity. Results assumed that this activity is highly maximum for three classes of algae harvested in spring and summer. Red and brown marine algae were the most active in comparison with green algae. Study reveals that the best time to harvest marine algae to detect the cytotoxic activity is spring.

INTRODUCTION

The potential contribution of marine organisms to the discovery of new bioactive molecules is remarkably increasing [1-3], marine algae were reported to produce a wide variety of bioactive secondary metabolites as antimicrobial, antifeedant, antihelmintic, cytotoxic and anti-inflammatory agents [4]. Bioactive substances included alkaloids, polyketides, cyclic peptide, polysaccharide, phlorotannins, diterpenoids, sterols, quinones, lipids and glycerols [5]. Marine macro-algae are considered as the actual producers of some bioactive compounds with high activity [6]. Hence, they have drawn great attention recently [5,7-11].

Several cytotoxic compounds such as fucoidans, laminarians and terpenoids stated to possess anticancer, antitumor and antiproliferative properties are reported to be abundant in seaweeds[12]. These compounds could be further explored as novel leads to cancer chemoprevention and complementary chemotherapy and necessitates further investigation[13,14].

Several studies suggest firstly the role of ecological parameters resulting from climate factors on the biology and physiology of the species, especially on the production of secondary metabolites[15].

The variation of cytotoxic activity according to the period of harvest was realized to determine the period during which the algae presents a maximum of activity. For that, 9 species of marine algae belonging to the Chlorophyta (3 species), Phaeophyta (3 species) and Rhodophyta (3 species), were collected in period of winter, autumn, spring and summer, the extracts were prepared in dichloromethane/methanol and were tested for their cytotoxic activity against *Artemia salina* (Brine shrimp test) in order to search cytotoxic bioactive compounds and to detect the best time of harvest of marine algae.

MATERIALS AND METHODS

1.1. Algal materials:

Algae were collected freshly in period of winter, autumn, spring and summer from Sidi Bouzid coast (33°-33°16’09’’N, 8°30’-8°45’W). Samples were washed thoroughly with fresh water, transported to the laboratory and immediately rinsed with distilled water. Specimens were preserved in formalin (5%) and were identified by experts in these fields. The samples

were put in shade at ambient temperature, cut into small pieces and powdered in a mixer grinder then the powder was conserved for further experiments.

In this study nine algae were collected and were identified as:

Red macroalgae: *Bornetia secundiflora*, *Caulacanthus ustulatus*, *Gelidium sesquipedale.*

Green macroalgae: *Ulva lactuca*, *Codmium tomentosum*, *Enteromorpha compressa.*

Brown macroalgae: *Fucus spiralis*, *Sacchoriza polyschides*, *Laminaria ochroleuca.*

1.2. Extracts preparation :

The powder of each dried algae was extracted in dichloromethane/methanol (1:1) [16]. The resulting extracts were concentrated to dryness in a rotary evaporator under reduced pressure (at 45°C) until a crude extract is obtained and were conserved at 4°C.

1.3. Cytotoxic activity test :

1.3.1. Brine shrimp lethality bioassay :

Brine shrimp lethality test for larvae nauplii was used to determine the cytotoxicity of methanol/dichloromethane extracts of seaweeds[17]. The eggs of brine shrimp (*Artemia salina* Leach) were collected and hatched in an Erlenmeyer at 30ºC with constant oxygen supply. Two days were allowed to hatch and mature the nauplii. Stock solution of the extracted sample was prepared by dissolving 25, 50, 75, 100, 250 and 500 µg of extract in 4 µl of pure dimethyl sulfoxide (DMSO). 10 living nauplii were taken to each of the vial containing different concentrations of test sample with pipette Pasteur. Then, specific volumes of sample were transferred from the stock solution to the vials to get final sample concentration. In the control vials, same volumes of DMSO (as in the sample vials) were taken. After 24 hours the vials were observed and the number of nauplii survived in each vial was counted. As controls, *A. Salina* naupli were submitted to seawater and that containing 1% DMSO (100% survival).

The number of survivors was counted and the percentage of death was calculated. Larvae were considered dead when they did not exhibit any internal or external movement during several seconds of observation[18].

1.4. Statistical analysis:

All assays were done in triplicate. Statistical analyses were performed using the SPSS software version 17.0 (Statistical Package for the Social Sciences). Duncan test was used to identify significant relationships between seasons and cytotoxic activity. The data were analyzed by an analysis of variance and considered to be statistically significant at $P<0.05$.

RESULTS

The variation of cytotoxic activity according to the period of harvest was realized to determine the period during which algae presents a maximum activity. For that, nine marine algae were collected monthly over a period of winter, autumn, spring and summer and their dichloromethane/methanol extracts were tested for their cytotoxic activity.

For red algae, a high cytotoxic activity was observed in summer and spring with a percent death of brine shrimps greater than 80% compared to autumn and winter with a maximum activity is observed in spring, *G. ustulatus* and *G. sesquipedale* were the most active and exhibited a significant percent death of brine shrimp than *B. secundiflora* (Figure 1).

For brown algae, the variation of cytotoxic activity of this class of algae during the four seasons is similar to that observed for red algae, in spring, the activity is the strongest. The percent death of brine shrimps is 100% for *F. spiralis*, *L. ochroleuca* and *S. polyschides* followed by summer, winter, and autumn (Figure 2).

For green algae, cytotoxic activity varies for the four seasons, the percent death varies between 20% and 70%, the high activity was observed in the case of *E. compressa* (Figure 3).

The seasonal variation of cytotoxic activity for the three classes Rhodophyta, Phaeophyta and Chlorophyta, summarized in Figure 4, shows that this activity is highly maximum for 3 classes in spring followed by summer, winter and autumn.

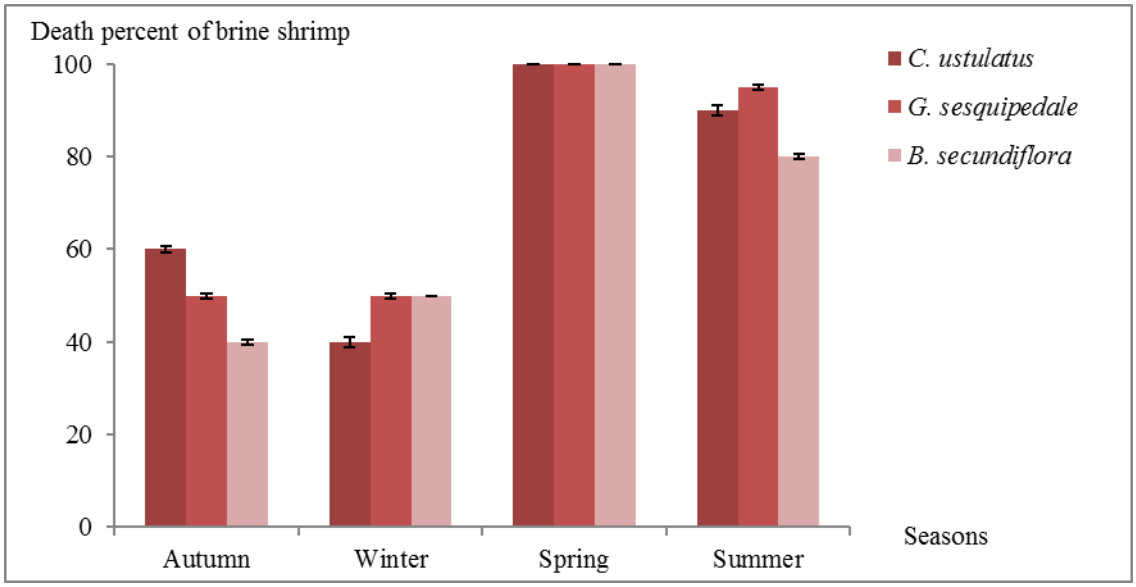

Figure (1): Seasonal variation of cytotoxic activity of red seaweeds extracts.

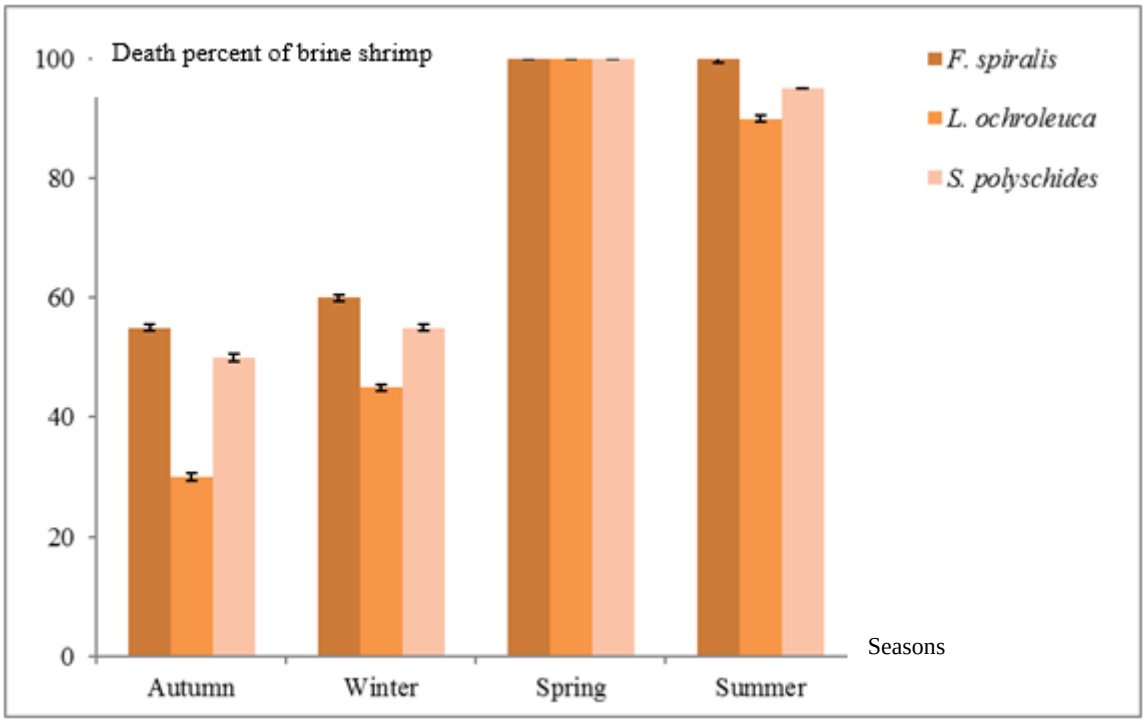

Figure (2): Seasonal variation of cytotoxic activity of brown seaweeds extracts

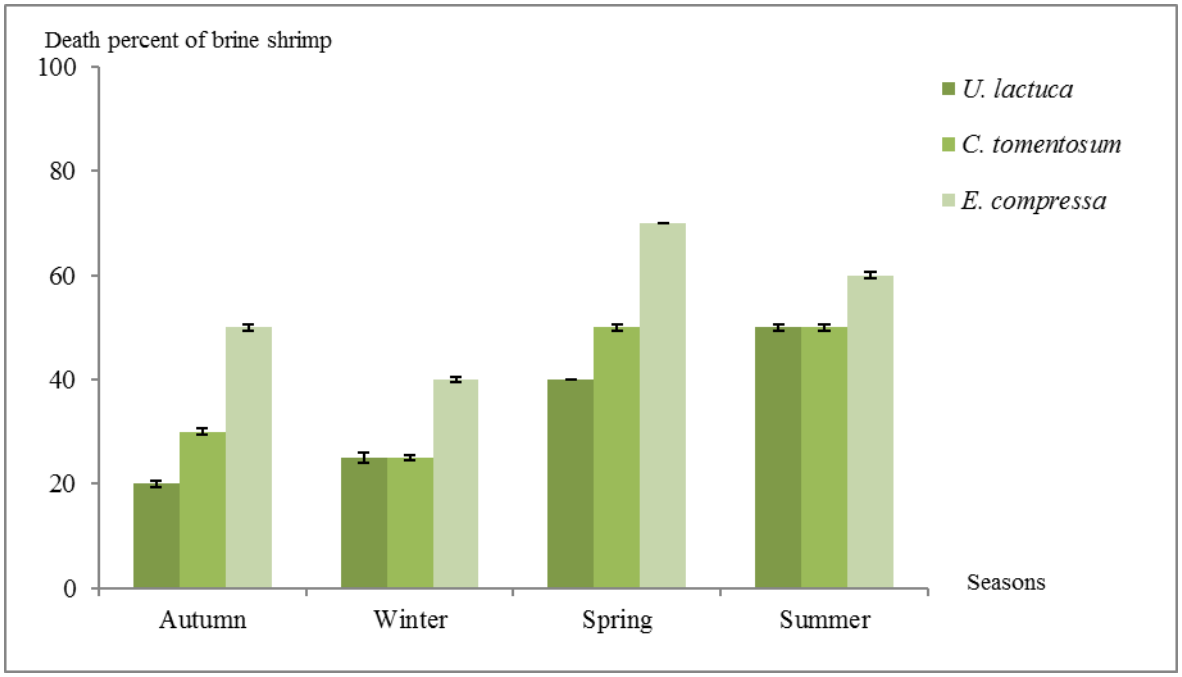

Figure (3): Seasonal variation of cytotoxic activity of green seaweeds extracts

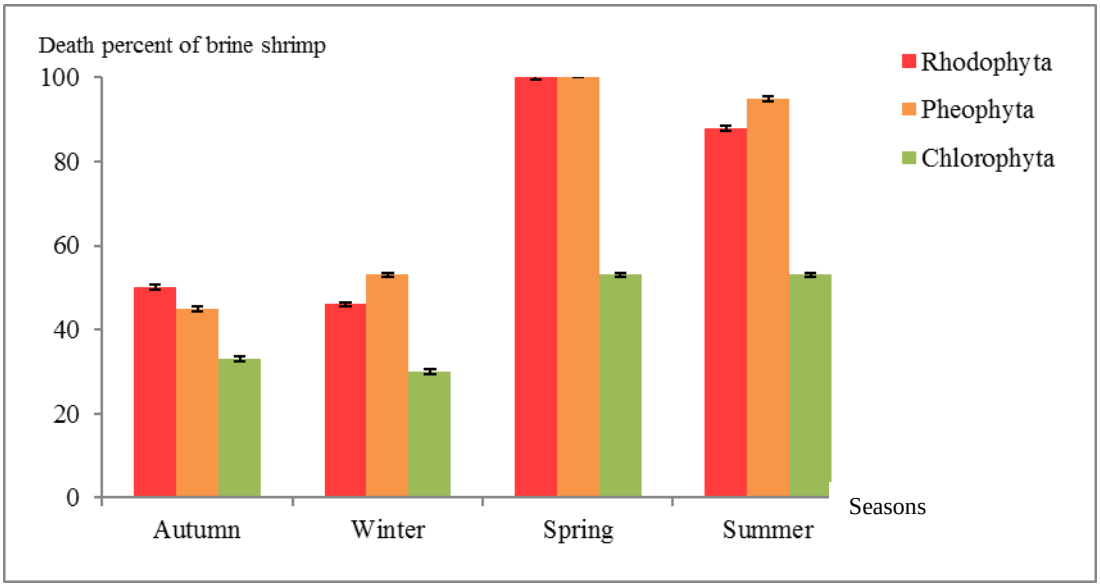

Figure (4): Seasonal variation of cytotoxic activity of three classes of marine algae.

In order to establish a relationship between the season and the variation of cytotoxic activity of seaweeds harvested from the coast of Sidi Bouzid, an ANOVA treatment was applied (Table 1).

Table (1): Comparison of seasonal averages of cytotoxic activity of three classes of marine algae during the four seasons (Duncan Test)

	Cytotoxic activity								
	Rhodophyta			Pheophyta			Chlorophyta		
Seasons	**Mean**	**± standard deviation**	**DuncanGroups**	**Mean**	**± standard deviation**	**Duncan Groups**	**Mean**	**± standard deviation**	**DuncanGroups**
Winter	47,33	1,94	**a**	53,66	2,35	**b**	29,67	3,46	**a**
Autumn	50,44	1,96	**a**	45,44	2,33	**a**	33,56	3,47	**a**
Summer	88,89	1,92	**b**	94,89	2,32	**c**	53,67	3,45	**b**
Spring	100,00	1,91	**c**	100,00	2,31	**c**	53,44	3,40	**b**

(a, b, c): Seasons having different letters for the variation of the cytotoxic activity showing significant differences at the 5% (multiple comparisons of means by the Duncan test)

The variability of cytotoxic activity is studied by analysis of variance with one factor (season) to appreciate the difference between the seasons. This analysis is performed by ANOVA tests.

Analysis of variance revealed significant effects ($P< 0.005$) of the seasonal factor on the cytotoxic activity of Rhodophyceae, chlorophyceae and Phaeophyceae.

Comparison of means by Duncan test allowed to the classification of four seasons for each algae class (Table 1). Thus, spring and summer seasons have significant maximum values (% death of Brine shrimps), while autumn and winter seasons show the lowest values. These results assumed that the best time to harvest marine algae to detect cytotoxic activity is spring.

This study demonstrates that seasonal fluctuations of cytotoxic activity among the three classes of algae due to variations in environmental factors such as temperature, concentration of dissolved oxygen and salinity. The algae harvested in spring are the most active; this season should be the period when these factors interact with each giving the best conditions for the development and growth of algae.

DISCUSSION

In literature, various works cite the effect of season on the variation of the biological activities. El Kouri et al. 2004 [19] reveal that the study of seasonal variation of antibacterial

and anti-inflammatory activities of fourteen marine algae showed that the maximum of activity was obtained between February and May.

The algae harvested in the spring are the most active, this season should be the period when different factors interact with each giving the best conditions for the development and growth of algae[20].

Macroalgae from Abu Qir coast in Alexandria-Egypt are potential sources of bioactive compounds. The production of these compounds could be affected by seasonal variation and should be investigated for natural antimicrobial properties[21].

Results obtained by Ismail-Ben Ali et al. 2010[22] showed that Dichloromethane/Methanol extracts of *Padina pavonica* collected from Cap Zebib (northern coast of Tunisia)demonstrated a large activity against Gram+, Gram- human and fish pathogens and warm season seems to be the approved period for *Padina pavonica* harvest for antibacterial compounds extraction.

Ainane et al. 2014[23,24] show that *Cystoseira tamariscifolia* and *Bifurcaria bifurcata* collected from the coast of morocco, have a cytotoxic activity against *Artemia salina* in Spring.

Other studies concerned the study of biological activities of medicinal plants revealed that the season has an effect on the variation of the biological activity (antimicrobial) and affects the chemical composition of secondary metabolites[25, 26].

Species of marine algae from Sidi Bouzid coast are a good source of bioactive molecules. Harvesting algae for use must be during the spring and must not exceed June because after this period the temperature and salinity increases and algae degenerate and become less active.

In the natural environment, light, temperature, mineral salts and water movements are essential ecological parameters in determining of the algae fertility, light and temperature are the origin of seasonal and spatial variation of algae flora, they act both on the growth of algae on their morphological characters. The temperature fluctuations which are essentially linked to the seasons constitute a determining factor of the seasonal cycle of marine flora.

In this context, it has been shown that the growth of algae is not identical throughout the year, it is maximum when the light conditions and temperature are favorable, it is the case in temperate regions where the fertility of a large number of species is highest in spring and autumn[27], however, some species are known for their fertility throughout the year. This phase of active growth and sexual maturity of the algae is the period of synthesis of secondary metabolites responsible for biological activities[15]. Therefore, the influence of environmental parameters on the biology and physiology of algae can also achieve the production of secondary metabolites.

CONCLUSION

Results obtained showed that seaweeds from the coast of El Jadida, possess a very good cytotoxic potential, for that, it should be considered as eventual source ofcytotoxic activity, and indicate that this activity varies according to the season. Spring season seems to be the suitable period for harvest for cytotoxic compounds extraction. Therefore, harvesting season affected the chemical composition as well as the biological activities of marine seaweeds. The suitable season for collection of seaweeds producing cytotoxic activity must be taken in consideration.

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