ORIGINAL ARTICLE

ANTIOXIDANT POTENTIAL OF HYPERSALINES MICROALGAE *Dunaliella spp.* ISOLATED FROM MOROCCAN SALTPONDS

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ABSTRACT

Background: Microalgae are organisms that use light as an energy source to fix CO_2, and today they are attracting a growing interest in the world. A multitude of scientific and technological locks deserve to be raised, at the crossroads of innovations between agro-ecological approach and techno-economic vision. In the context of the biotechnological valuation of microlagal species, we are interested in the study of a halotolerant species, *Dunaliella spp.*, which develops in salinas that are generally exploited in Morocco only for salt production, while Contain this microalga exploited for these bioactive molecules and its high antioxidant power. **Objective:** This study evaluate the antioxidant activity by the diphenyl-pycryl-hydrazyl (DPPH) test of different extracts (ethanol, acetone, methanol and chloroform) prepared from six native strains of the microalgal *Dunaliella spp*. isolated from six different Moroccan saltworks. **Methods:** To this end, algal cultures were maintained in a culture chamber at T=25±1°C under continuous white light and constant stirring for a period of 15 days and then the antioxidant power was estimated by the DPPH test. **Results:** Dunaliella extracts revealed an important antioxidant activity, especially for ethanolic extract in all the strains tested with a highest level of 83% observed for the strain (DUN4) isolated from Tislatine saltpond in Boujdour region. **Conclusion:** this study highlated the important antioxidant activity of the majority of microalgal extracts of Dunaliella. These findings motivate the need to study more closely the virtues of these microalgae, particularly the determination of their active biomolecules.
Keywords: *Microalgae, bioactive molecule, antioxidant activity, Dunaliella spp., DPPH.*

1. INTRODUCTION

Today, the global community is confronted with a multitude of interdependent challenges; Ranging from the impacts of the financial and economic crisis to increased vulnerability to global climate change. At the same time, it must reconcile the need to meet the urgent alimentary and nutritional needs of an ever-expanding population with limited natural resources. Indeed, the emergence of socio-economic niches that could offer an unavoidable alternative in terms of energy, agro-food and health to cope with the major development challenges was inevitable. Innovative use of natural products; High value-added products that are renewable and environmentally friendly, such as microalgal biomass, is one of the promising niches.

Microalgae are single-celled photosynthetic organisms that play a key role in ecosystems by the production of biomass and oxygen through photosynthesis. They are at the origin of the regulation and balance of the atmospheric composition (fixation of carbon dioxide and release of oxygen).

Despite a few species, the micro-algae have not yet reached their level of industrial maturity, so Morocco is therefore obliged to undertake in the valorization of this immense wealth, the use of which arouses a growing interest. Thanks to their metabolic power, some microalgae appear as standard bearers of the development of bioprocesses. Several applications have been developed in several industrial sectors such as biodiesel production, CO_2 capture from pollutant emissions, bioremediation and treatment of contaminated waters (especially by trace metals) and production of biomolecules with high added value.

In the context of the biotechnological valorization of microalgal species, we are interested in the study of a halotolerant species, *Dunaliella spp.* which develops in salt-works that are generally mined in Morocco only for salt production, while

they constitute an conducive ecosystem to this microalga very appreciated for its battery of bioactive molecules including anti-oxidants such as carotenes. Indeed, to circumvent the damages of oxidative stress caused by the combined action of salinity and high solar radiation, the microalga *Dunaliella spp.* modulates its metabolism towards an active production of β-carotene, which explains the reddish appearance of the cultures of this microalga [1].

On the other hand, β-carotene is recognized by its important antioxidant properties, manifested by the trapping of free radicals and the inhibition of their negative action on all the vital processes of the organisms. Thus, their stabilizing effect of membrane lipids and their preventive action on cancers is asserted [2,3,4].

This work consists in studying the biological activity by evaluating the in vitro antioxidant potential of some organic extracts (ethanol, acetone, methanol and chloroform) prepared from six native strains of the microalgae *Dunaliella spp*. isolated from six different Moroccan salt ponds.

2. MATERIALS AND METHODS

2.1 Conditions of culture and kinetic growth of microalgal strains:

The halotolerant chlorophyceae *Dunaliella spp.* were sampled from six salt production tables in Morocco ; in marine saline (Tazgha) and in continentals (Zima, Idao Iaza, Tislatine, Oum dbaa and Azla) (Figure 1). The microalgal strains are then isolated by two methods of cell isolation (successive dilution and on plates). The six isolated strains were cultured on F/2 Guillard culture medium [5] over a period of 15 days under continuous white light and constant stirring at 25±1°C in a culture room.

The studied strains of *Dunaliella spp.* according their sampling locality are as following : DUN1 isolated from Zima saline in the region of Chamaia (Latitude 32,076127. Longitude -8, 652444°), DUN2 from the saline of Azla (Latitude 31.160723°. Longitude -9.705030°) and DUN3 from the saline of IdaoIaza (Latitude 31.145431° .Longitude -9.737920°) located both in the region of Essaouira; DUN4 from Tislatine in Boujdour region (Latitude 26,6905316°. Longitude -13, 5562407°), DUN5 from the Tazgha saline in Akhfenir (Latitude 27, 9477594°. Longitude -12,2852711°)and DUN6 from OumDbâa saline in Tarfaya region (Latitude 27,577745°.Longitude –12,9563731°) of Morocco.

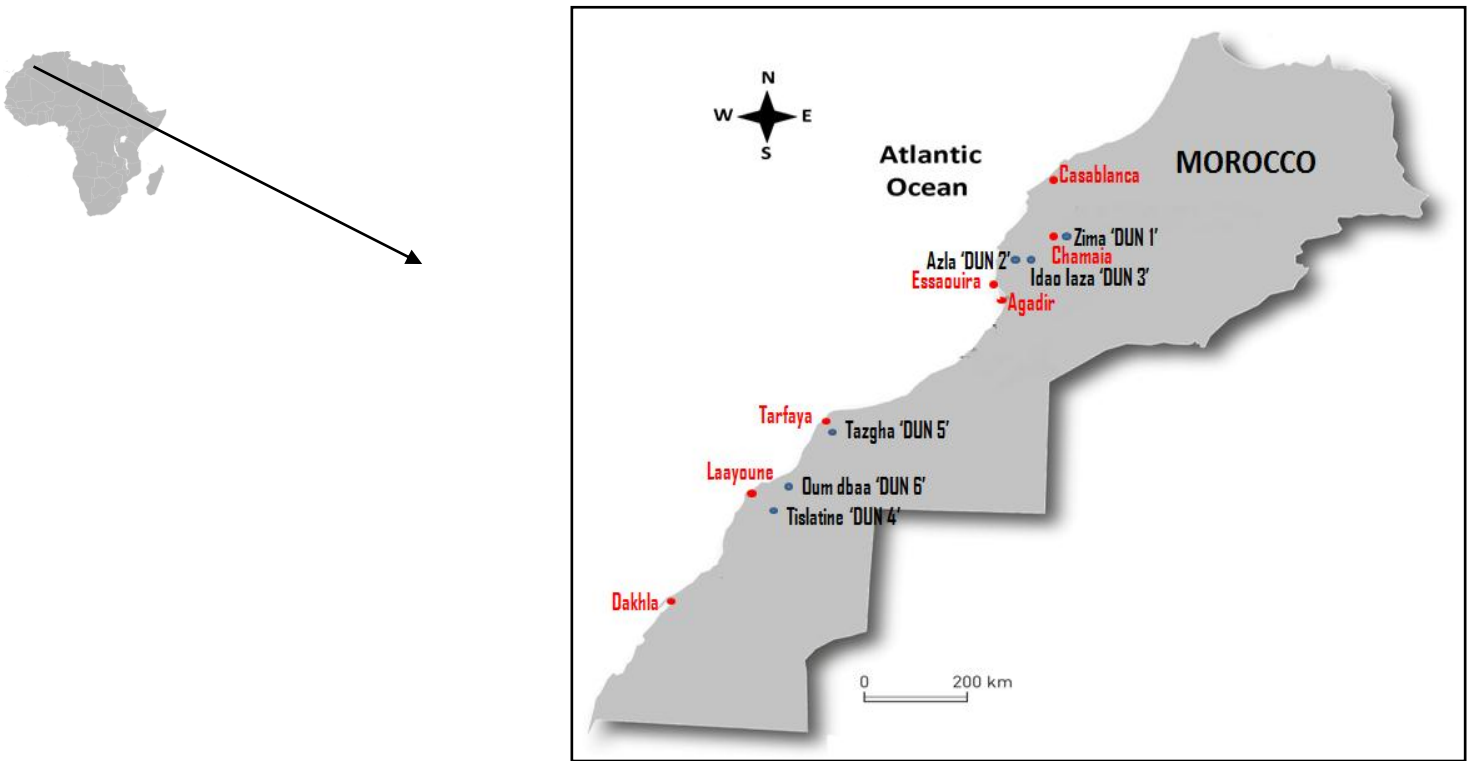

Figure 1: The map presents the geographical localization of microalgal sampling saline.

2.2. Preparation of extracts and testing of antioxidant activity:

In order to study the antiradical activity of the various extracts of microlalgae of the genus Dunaliella, we have opted for the method which uses DPPH (diphenyl-pycryl-hydrazyl) as a relatively stable free radical, according to the protocol described by [6]. In this test, antioxidants reduce di-phenyl-picryl-hydrazyl having a violet color to a yellow compound whose color intensity is inversely proportional to the ability of the antioxidants present in the medium to give protons.

The antioxidant activity of the *Dunaliella spp.* extracts with respect to the DPPH radical was evaluated with the aid of a spectrophotometer following the reduction of this radical which is accompanied by its passage of the violet color (DPPH•) to the yellow color (DPPH-H) measurable by following the variations of absorbance at 517 nm (Figure 2). This reduction capacity is determined by a reduction in the absorbance induced by antiradical substances [7].

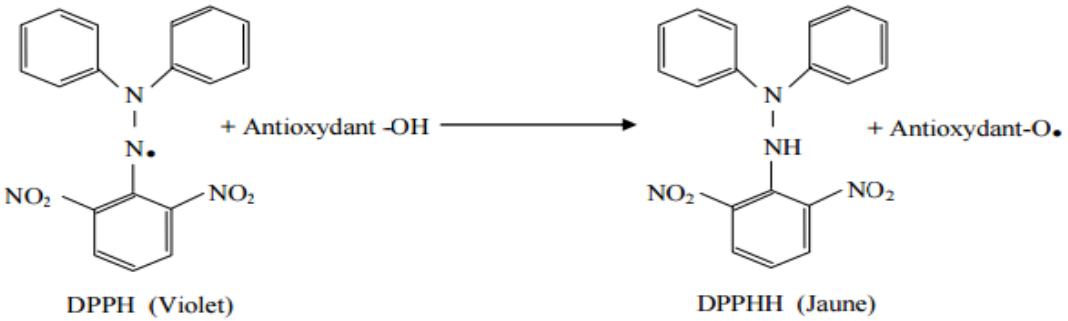

Figure 2: The figure presents the reaction of an antioxidant with the free radical DPPH• (2, 2 DiPhenyl-1-Picryl-Hydrazyl).

The free radical scavenging activity of acetone, methanol, ethanol and chloroform was evaluated using cell biomass of microalgae strains.

Briefly, DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) solution (Figure 2) was prepared by dissolving 0.004 g of DPPH in 100 ml of methanol [8,9]. 1 ml of the microalgae suspension was centrifuged at 10000 rpm at 4°C for 15 minutes and then the pellet was extracted with 1 ml of each of the above solvents and stirred at a sustained rate. The extract is left at 4°C for 4 hours and 2 ml of the DPPH solution are added. Thereafter, the mixture was incubated for 30 min at room temperature in the dark. A blank sample (absolute solvent) was also taken as a control. The absorbance at 517 nm of the extract was determined by spectrophotometry. The antioxidant activity was calculated based on the inhibition of free radical DPPH in percent according to the formula:

Percentage (%) of inhibition of DPPH activity = [(A blank - A extracted) / A blank] x 100 (1)

[10,11,6]

Where, A Blank = absorbance of DPPH,
A extracted = absorbance of the test sample.

2.3. Statistical analysis:

The graphic illustrations were made using the Microsoft Excel program. In addition, the statistical analysis was based on the classification in homogeneous groups carried out by the DUNCAN test ($p > 0.05$).

3. RESULTS

The six strains of microscopic algae of continental and marine origin are extracted in four different solvents. It should be noted that microalgae of the genus Dunaliella have very high levels of β-carotene (provitamin A), a natural antioxidant. The DPPH radical trapping activity (%) of the various extracts of these microalgae is shown in Figure 3. All these microalgae extracts possess the DPPH scavenging ability to varying degrees, this DPPH scavenge test was used to investigate the antioxidant potential of each solvent extract.

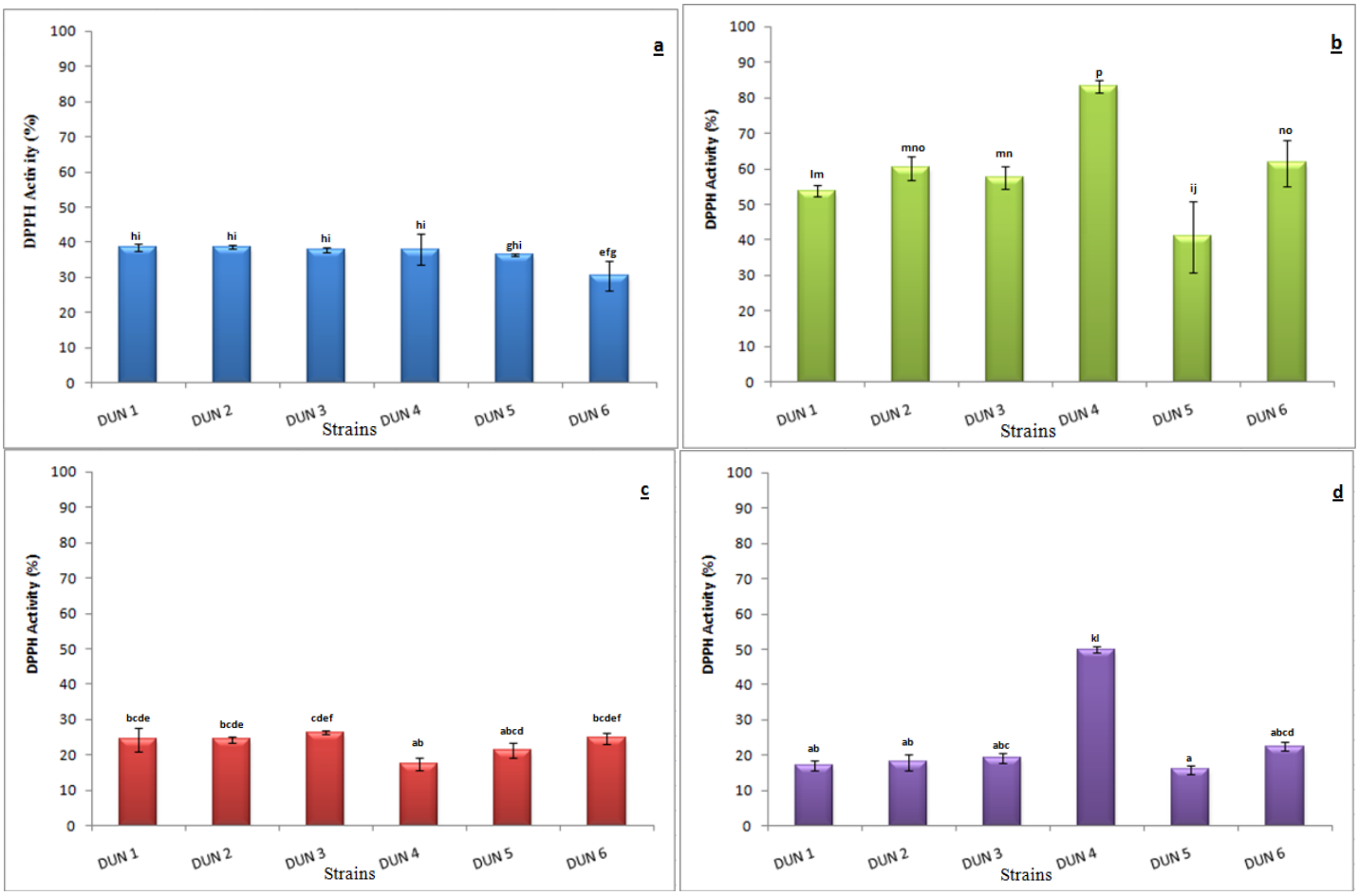

Figure 3: Antioxidant activity as DPPH reaction of extracts of six microalgal strains of Dunaliella : **a** : Acetone extracts, **b** : Ethanolic extracts, **c** : Methanolic extracts, and **d** : Chloroformic extracts

We see, as shown in figure 3, that all the extracts have an anti-radical effect against DPPH with different degrees. Indeed, the ethanolic fractions of the studied samples showed clearly an antioxidant activity exceeding 50% (ranging from 40,90% to 83,18 %) explained by their high capacity of trapping the DPPH radical. The ethanolic extract of strain DUN 4 (83.18%) gave the best activity followed by strain DUN 6 (61.58%). The lowest activity corresponds to the DUN5 strain (40.90%) for the same solvent.

Extraction with acetone shows that the best DPPH inhibitions of the extracts of the microalgal strains belong to the two strains DUN 1 and DUN 2 (38.65%); this antioxidant activity is relatively high compared to the other strains. The lowest activity returned to strain DUN 6 with a percentage of 30.43%.
The percentage of the antioxidant activity does not exceed 27% for the methanol extracts. The highest antioxidant activity amounts to the extract of the strain DUN 3 with a percentage of 26.37%. The lowest value is the extract of strain DUN 4 with 17.40%.

The chloroform extracts also show antioxidant activities which remain fairly low and hardly exceed 25% for all strains. Nevertheless, the strain DUN 4 stands out with a percentage inhibition of DPPH of 49.93%. It is noted that the continental strain DUN4 has a better antioxidant capacity with respect to the free radical DPPH for the extracts with ethanol, acetone and chloroform.

4. DISCUSSION

According to Chabert and al., (2011), *Dunaliella spp.* is known for its antioxidant properties thanks to a concentration of 5% dry matter of β-carotene, which suggests a potential for development and commercialization [12]. Indeed, the antioxidant power of the extracts of *Dunaliella spp.* showed maximum activity in the ethanol extracts; followed by extracts with acetone, methanol and chloroform, respectively. DPPH is considered to be one of the best reagents for examining free radical scavenging activities [13]. The ethanolic extract of all microalgal strains of the genus Dunaliella showed maximum inhibition of DPPH, particularly in strain DUN 4 with a highest percentage of 83.18%. Except in methanolic extract, this strain recorded high antioxidant capacity in all the extracts tested. The extract with acetone of

Dunaliella spp. showed that the best trapping activity of the DPPH radicals (38.65%) is noted in strain DUN 1 and DUN 2. On the other hand, the methanolic and chloroform extracts did not exhibit antioxidant activities of interest to the free radical DPPH compared to those tested.

Our previous studies have showed that Dunaliella is capable to produce large amounts of carotenoids that are recognized as essential source of antioxidant [14]. These findings are perfectly consistent with this screening study, which would explain the significant antioxidant activity of the studied extracts, in particular the ethanolic one. Similar results are reported by Tran et al., (2014) on the antioxidant capacity of different ethanolic extracts of Dunaliella strains, which reached 32% [6]. Similarly, the work of Cakmak et al., (2014) reported that the ethanolic extract of the Dunaliella strain showed a maximum inhibition of the order of 68.88% [15]. The results of our tests showed maxima of inhibition which remained better than those reported in the literature (83.18%).

In addition, our results do not fully agree with those of other authors. The work of Rajendran et al., (2014) revealed that the acetone extracts had the highest inhibition (93%), followed by methanol extract (44%), and chloroform (18%) [16]. While the lowest results are obtained for the ethanol extracts (15%). The work of Hemalatha et al., (2013) show that the methanol extracts of Dunaliella recorded a maximum antioxidant activity of 17.66% and that the acetone extract revealed an activity of 12.62% [17]. Moreover, these activities are still lower than those recorded in our work with maximum contents for methanol extract and acetone of 26.37% and 38.65%, respectively.

Sivakumar et al., (2011) and Uma et al., (2011) observed that methanolic extracts have the greatest potential for antioxidant activity in various species of green microalgae [18,19]. The maximum DPPH radical scavenging activity was found in the methanol extract of the *Isochrysis galbana* microalgae with an activity of 34.18%.

Otherwise, each extract of Dunaliella strain shows distinct antioxidant activity, this can be explained by the nature and physiology of each strain and its tendency to produce antioxidants and therefore its ability to trap free radicals.

5. CONCLUSION

This study of the antioxidant activity of the extracts of Dunaliella species showed that all extracts possesses a pronounced antioxidant activity. However, ethanolic extracts of Dunaliella species Seems to have the most important antioxidant power including free radical scarvenging activity. Indeed, extracts of the microalgae isolated from the saline of Tislatine showed a promising antioxidant potential on all the solvents which requires a more in-depth study.
Moreover, the results obtained showed the antioxidant potential of the extracts of the local Moroccan microalgae determined by the DPPH method, and demonstrate their good antioxidant activity. Indeed, these microalgae contain carotenoids; antioxidants that contribute very effectively to the prevention of diseases. In addition, current findings encourage further study and identification of these active principles in these hypersaline microalgae and test them for a variety of beneficial chemo-preventive effects.

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