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Research Article

Antioxidant Activity and Total Phenolic Content of the Red Alga *Halopitys incurvus* Harvested from El Jadida Coast (Morocco)

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ABSTRACT

This work aims to research and to highlight the antioxidant activity in the alga *Halopitys incurvus* (Rhodomelaceae) harvested from El Jadida coast (Morocco). After having set up an adapted protocol of extraction by different solvent polarities (Chloroform/Methanol (2:1, V:V), Chloroform, and Isopropanol/water (7:3, V: V), the antioxidant activity was evaluated by two complementary techniques: on thin layer chromatography (TLC) plate and spectrophotometry using the free radical DPPH (2, 2-diphenyl-1-picrylhydrayl). The tests were validated by comparison with reference antioxidant substances (ascorbic acid and δ-tocopherol).The preliminary screening of the extracts on TLC plate allowed to target the scavenger activity of the DPPH radical in the various prepared extracts. The evaluation of the scavenging power of the extracts against DPPH by spectrophotometry confirmed the results of the first screening and shows that the extracts resulting from *H. incurvus* have a real antioxidant activity with EC_{50} value of 0.154, 0.150 and 1.320 respectively in the crude extract (Chloroform/Methanol), and in the Chloroformic extract and in the Isopropanolic extract compared to the EC_{50} value of δ-tocopherol (0.260). Moreover, *H. incurvus* proved rich in total phenolics and the content of these products varies according to the nature of the studied extract. The results of this work show that the used method allowed to highlight the presence of antioxidant properties of interest in the alga *Halopitys incurvus.*

Keywords: *Halopitys incurvus*, Morocco, extraction, antioxidant activity, DPPH, phenolic compounds.

INTRODUCTION

Oxidative stress is the cause of multiple dysfunctions in the cellular machinery. The resulting cellular damage can be found in many diseases (diabetes, cancer, atherosclerosis ...). The natural antioxidants are very useful[1]. They protect the cells of the body against damage caused by oxidative stress and strengthen the immune system[2,3,4]. These natural compounds include vitamins (ascorbic acid and its derivatives, tocopherols of vegetable origin), phenolic compounds and other plant source compounds. Algae are plant organisms that live in varying environmental conditions. These conditions induce algae to produce a wide variety of secondary metabolites, some of which are characterized by their biological activities and can act at different levels. Many compounds derived from algae are antioxidants that could neutralize the active forms of oxygen. These compounds include: sulfated polysaccharides[4,5,6], pigments[2,7,8], vitamins[9,10] and phenolic compounds[11,12].

Having as a research focus the obtaining of new natural antioxidant substances, this study aims to evaluate the antioxidant activity in different extracts prepared from the red alga *Halopitys incurvus* collected on the coast of El Jadida (Morocco).

MATERIEL AND METHODS

Algal material

Halopitys incurvus (Hudson) Batters 1902 (Rhodomelaceae) was harvested manually during April 2013 on the coast of Sidi Bouzid, El Jadida (33 ° 09'-33 ° 16"N, 8 ° 30'-8 ° 45'W). In the laboratory, the studied seaweed (figure 1) was sorted and rinsed with running water, then with distilled water. The sorted sample was frozen at -80 °C and freeze-dried using a lyophilizer (Free Zone Plus 2.5 liters).

Preparation of extracts

Crude extract

The lyophilized sample was ground in a mortar and the powder obtained was extracted in a Chloroform /Methanol mixture (2:1, V: V) for 8h at ambient temperature and with constant stirring. After filtration, the residue was extracted a second time for 4 hours under the same conditions as previously done and filtered. The two extracts were assembled and concentrated under vacuum using a rotary evaporator (Büchi Rotavapor R-3000) at reduced pressure and at a temperature of ≤45 °C. The concentrated extracts were reduced to powder by lyophilization and the obtained powders were stored in a desiccator.

Chloroformic extract and Isopropanolic extract

The freeze-dried seaweed powder was delipidated with hexane for 6 h in a Soxhlet extractor. The residue **I** was recovered and then underwent a second Chloroformic extraction for 6 h. The Chloroformic extract was concentrated under vacuum and lyophilized to be stored in

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Figure 1: *Halopitys incurvus.*

a desiccator. The residue **II** underwent a third extraction with Isopropanol / water (7:3, V:V) for 6 h. The Isopropanolic extract was concentrated under vacuum and then lyophilized and stored in a desiccator.

Determination of total phenolic content

The total phenolic contents were determined by the Folin-ciocalteu method described by Luis *et al.*[13] in the various extracts obtained from *Halopitys incurvus.* A volume of 50 µl of the methanolic solution of alga extract (5 mg / ml) was mixed with 450 µl of distilled water and 2.5 ml of Folin-Ciocalteu reagent (0.2 N). The mixtures were left to stand for 5 min, and then 2 ml of aqueous Na_2CO_3 (75 g / l) were added to the reaction medium. After incubation of the reaction mixtures (2 h /25 °C), the total phenolic compounds were determined at 765 nm.

A calibration curve was carried out under the same experimental conditions as stated above using a concentration range (0-400 mg / l) of a methanolic solution of gallic acid (Sigma-Aldrich). The total phenolic contents of the extracts were graphically determined and expressed in terms of equivalents of gallic acid (mg/g dry extract).

Evaluation of antioxidant activity

On TLC plate

The technique used to evaluate the antioxidant activity was inspired by the method of Takao *et al.* [14]. It was carried out on a thin layer chromatography plate using the free radical DPPH (Sigma-Aldrich).

The powder of each extract was solubilized in methanol (Fluka). Aliquots of each sample (2, 4 and 8 µl) were deposited on a silica gel plate (CCM Sil G25 UV 254 mm-Marcherey-Nagel, 5x20 cm, ep. 0.25 mm) with a RINGCAPS micropipettes (Hirschmann DIN / ISO7750). A methanolic solution of DPPH ($6\text{-}10^{-4}$M) was uniformly vaporized on the plate in the absence of light. The plate was read at specific time intervals and images were produced using a scanner (HP Deskjet 2050A) at the end of each period.

The detection of the antioxidant activity was carried out by comparison with reference controls: ascorbic acid and (+) δ-Tocopherol (Sigma-Aldrich).

By spectrophotometry

The evaluation of the antioxidant activity was realized by spectrophotometric assay using DPPH as a free radical according to the method of Brand-williams *et al.* [15]. Different concentrations of each extract were prepared in Methanol. The kinetics of disappearance of the DPPH was followed according to the time and the concentration of the various extracts until reaching a plateau. The antioxidant activity was evaluated by determining the EC_{50}, and this one corresponding to the amount of the tested extract necessary to reduce by 50% the amount of DPPH present in the reaction medium. The remaining DPPH concentration was determined from a calibration curve with the equation:

$$\mathbf{Abs_{517nm} = 0.0885*[concentration_{DPPH}] + 0.0094}$$

RESULTS AND DISCUSSION

Total phenolic content

The phenolic compounds contained in the extracts were performed by the Folin-Ciocalteu method adapted by Luis *et al.*[13]. The phenolic content was determined by equivalence to the gallic acid (EAG) realized in the same conditions ($R^2 = 0.981$). The results of this assay are shown in figure 2.

The obtained results show that the alga *Halopitys incurvus* is rich in phenolic compounds. In fact, the obtained content in the crude extract is of 11.20% (112 mg ± 0.62 EAG / g of dry extract). This content is greater in the Chloroformic extract of the delipidated algae powder and reaches 30.36% (303±4.21 mg EAG / g of dry extract). However,

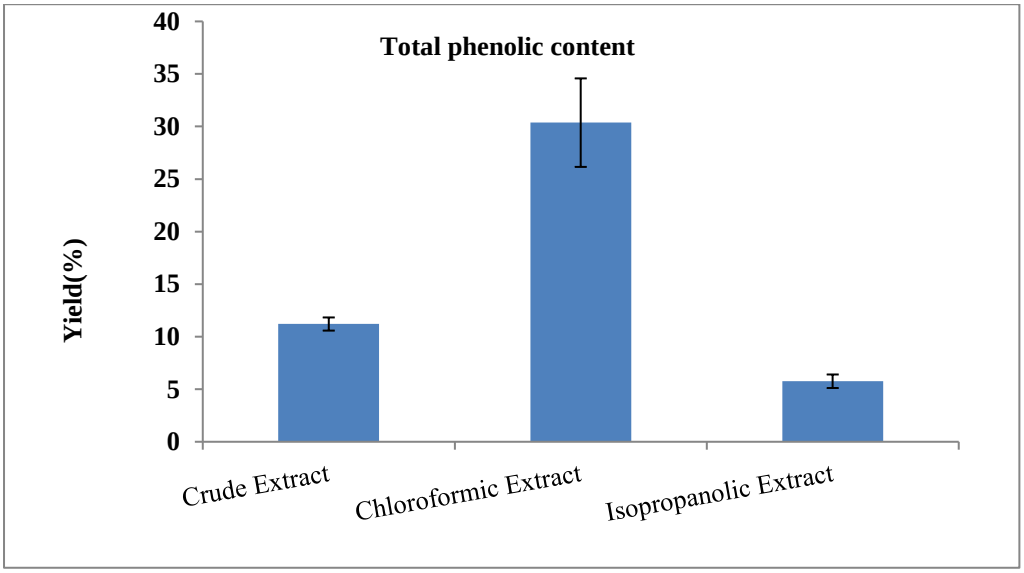

Figure 2: The yield of total phenolic content (%) of the different extracts.

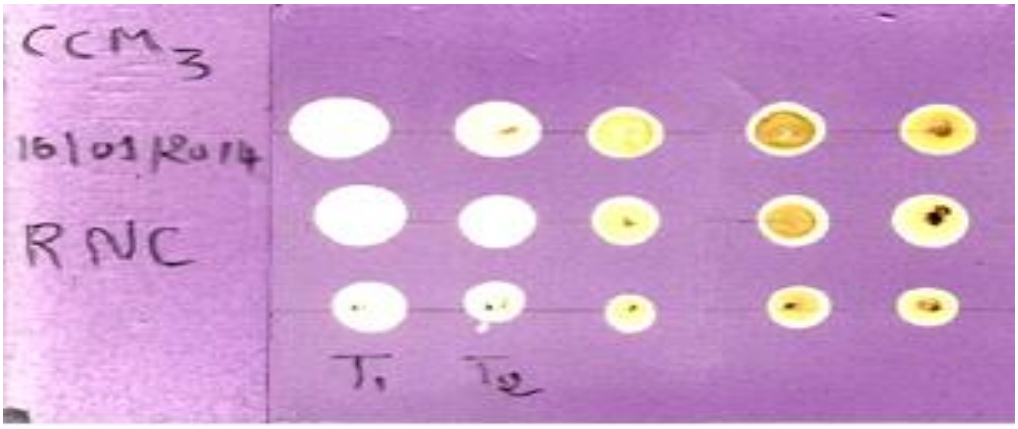

Figure 3: TLC seaweed extracts and controls (T1 and T2) after 15 min of reaction with DPPH.
T1: Ascorbic Acid; T2: Tocopherol; 1: Crude Extract; 2: Chloroformic Extract; 3: Isopropanolic Extract;
A : 2 µl, B : 4 µl et C : 8 µl

Table 1: EC_{50} of various extracts of Halopitys incurvus compared to δ-Tocopherol.

Tested extract	EC_{50}
Crude extract	0.154
Chloroformic extract	0.150
Isopropanolic extract	1.320
δ-Tocopherol	0.260

the Isopropanolic extract presents a phenolic content in the order of 5.76% (57±0.64 mg EAG / g of dry extract). The determination of the total phenolic compounds of the aqueous extract of *Halopitys incurvus* (subcritical water extraction at 200 °C) realized by Plaza *et al.*[16] showed a content in the order of 41.78 ± 8.15 mg EAG / g extract. Among the phenolic compounds found in marine algae bromophenol is the main constituent with high levels in various algal species[17]. Moreover, in *Halopitys incurvus*, a bromophenol was isolated from acidified ethanolic extracts[18].

Evaluation of the Antioxidant Activity

On TLC plate

The various prepared extracts were screened for their antioxidant activity. The result of the DPPH test on silica plate of the 3 studied extracts is shown in figure 3.

The antioxidant activity of the extracts is estimated by the discoloration of the spots of the extracts deposits in comparison with the spots of the control deposits. The analysis of the plate shows a clear discoloration in the deposits of the various extracts resulting from *Halopitys incurvus*. The reaction is very rapid in all the prepared extracts. The DPPH was degraded from the first minutes of reaction.

Moreover, the discoloration of the spots, which reflects the reaction with the DPPH, appears to be dependent on the concentration of the deposited extract (2 µl, 4 µl and 8 µl from the bottom to the top of the plate). Thus, the three tested extracts react positively with DPPH and seem to possess an antioxidant activity.

The method employed in this screening was inspired by that used by Takao *et al.* [14] and has been slightly modified to adapt to the needs of our study. Indeed, unlike these authors, our aim is to carry out a qualitative study on different extracts at the same time, the detection is done

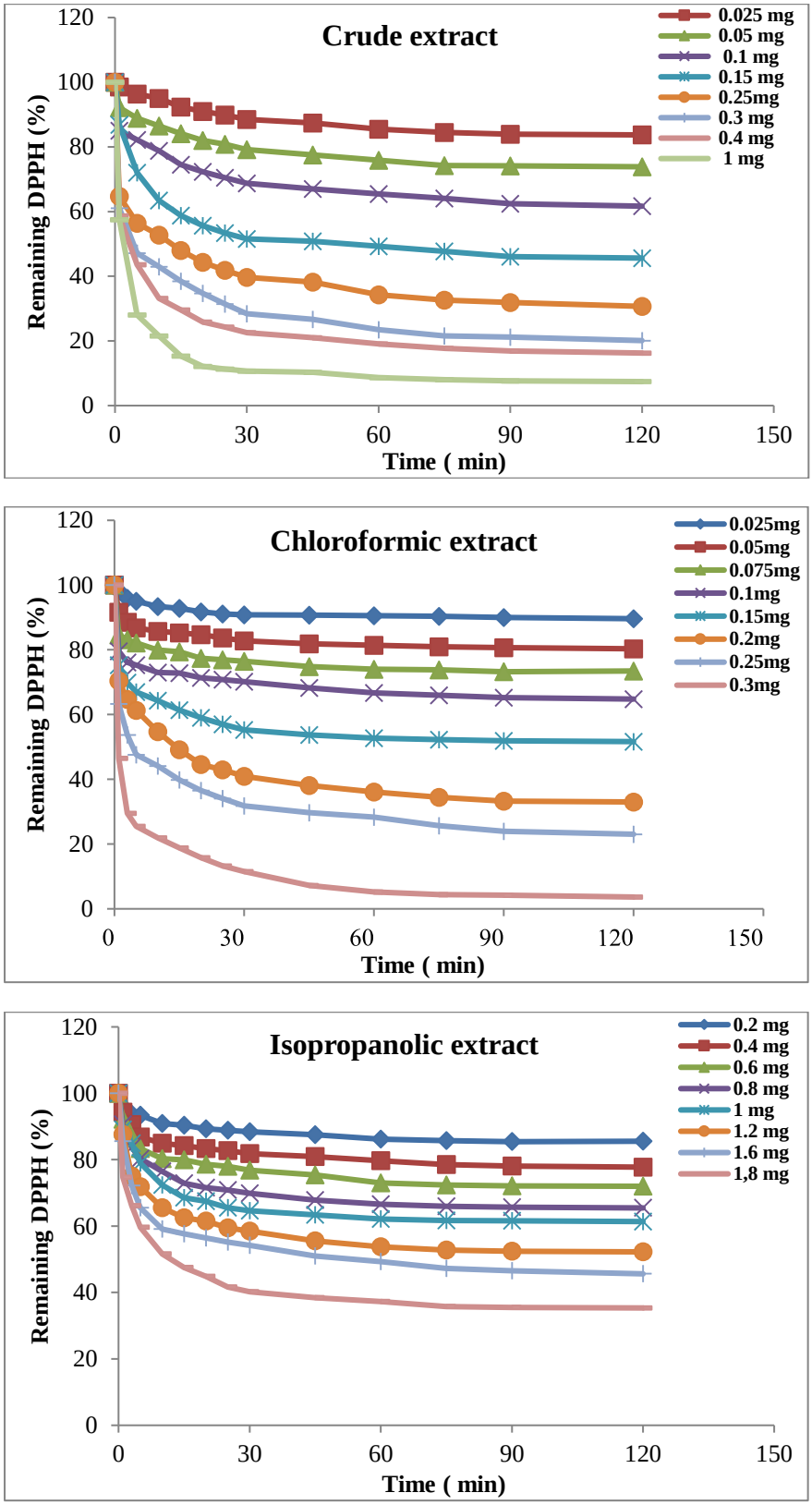

Figure 4: Percentage of DPPH remaining according to the time and the concentrations of different extracts of *Halopitys incurvus*.

without migration. This method is based on a visual analysis of the discoloration of the spots at the site of the deposition of the extracts after spraying of the DPPH. In addition, the extract is deposited three times at three different concentrations, which minimizes any false reading of the plate.

By spectrophotometry

The second screening by spectrophotometry is performed in order to confirm or invalidate the results of the previous screening and thus constitutes a logical sequel of the screening on the TLC plate.

Before analyzing the antioxidant activity of the extracts, the verification and the control of the used DPPH were firstly performed and showed a maximum absorbance peak at 517 nm. This result is similar to that reported in several studies[15, 19, 20], where the absorption peak for DPPH is between 510 and 520 min.

The DPPH degradation kinetics in the presence of different concentrations from each extract is followed by calculating the percentage of DPPH remaining in the reaction medium (figure 4).

In the crude extract the analyzed concentrations range from 0.025 to 1 mg / ml. At the low concentration of extract the percentage of DPPH remaining after 120 minutes of reaction is 83.70%. This percentage decreases progressively according to the increase in the extract concentration in the medium to reach the value of 7.41% in the presence of 1 mg / ml at 120 min.

In the presence of the Chloroformic extract the degradation of the DPPH present in the medium is more and more important according to the concentration and the time. At low concentration (0.025 mg / ml) the percentage of DPPH remaining in the medium reaction at the end of 120 minutes of reaction is of the order of 89.49%. It decreases progressively (increasing degradation of the DPPH) to reach a value of 3.92% in the presence of the extract at 0.3 mg / ml. At the crude extract the reaction obtained with 0.3 mg / ml results in a degradation of nearly 20%.

At the Isopropanol extract, obtained by sequential extraction, the test was performed on a range of concentrations from 0.2 to 1.8 mg / ml. The percentage of the remaining DPPH varies from 85.52% to 35.34% for the lowest and highest tested concentration respectively.

The EC_{50} of different *Halopitys incurvus* extracts are determined from these curves and using the equation of the calibration curve of DPPH ($R^2 = 0.982$). The values of the various obtained EC_{50} are summarized in table 1. These EC_{50} are compared with that of δ-Tocopherol, an antioxidant reference.

The antioxidant activity of a compound is higher, while its EC_{50} is low. In *Halopitys incurvus*, the various studied extracts reveal each a power to capture the free radicals, the most interesting of which are recorded in the crude extract and the Chloroformic extract with EC_{50} of the order of 0.154 and 0.150 respectively. These EC_{50} are very interesting in comparison with that of δ-tocopherol which is of the order of 0.260. In the work of Brand-williams *et al.*[15] the EC_{50} determined for δ-tocopherol is 0.250, an EC_{50} very comparable to that obtained in our study, which confirms our results.

The presence of the activity at the three studied extracts led us to suggest that this species may contain several antioxidant products. Indeed, the study of Zbakh *et al.*[21] performed on the Acetone / MeOH (1: 1) extract of the *Halopitys incurvus* causes a reduction of 55% of the ABTS radical (400 µg / ml). This confronts the hypothesis that this species contains various antioxidant products.

The phenolic compounds are widely known for their antioxidant activities. The quantitative analysis of these compounds in our extracts showed a concordance between the level of phenolic compounds and the antioxidant activity of these extracts. This may lead us to believe that the observed activity would be largely due to these metabolites. Indeed, in natural substances, particularly of algal origin, a large number of phenolic compounds are known for their antioxidant properties[11,12,22,23,24,25].

CONCLUSION

This study was conducted in order to demonstrate the presence of antioxidant activity in *Halopitys incurvus.* The results show that the various prepared extracts have real and important antioxidant properties, especially in the crude and Chloroformic extract. This activity is corresponding with the richness of these extracts in total phenolic compounds. The crude extract and the Chloroformic extract of *Halopitys incurvus* were highly antioxidant compared to δ-tocopherol. This qualifies the alga *Halopitys incurvus* as a potential candidate to be a true source in the field of nutraceuticals. These results are interesting because it must be emphasized that tocopherol is a pure molecule known as antioxidant whereas our samples are crude extracts (mixture of several molecules).

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