INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

Cytotoxic and antioxidant activities of the red seaweed *Halopithys incurva*

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ABSTRACT

Marine algae have proven to be an important source of novel bioactive compounds, which can be used for the manufacture of pharmaceutical drugs. The main aim of this study was to assess the in vitro antitumoral and antioxidant potentials of the extract of red seaweed *Halopithys incurva* collected from the Mediterranean coast of Morocco. The effect on the cytotoxic and antioxidant activities of algal extract was examined by sulforhodamine-B (SRB) and ABTS free radical decolorization assays, respectively. The results indicated that the extract of *H. incurva* showed cytotoxic and antiproliferative activity against HT-29 human colon cancer cells. In addition, based on the capacity of the seaweed to scavenge the ABTS radical cation, we demonstrated that *H. incurva* extract presented a moderate antioxidant activity. These results suggest that the marine alga *H. incurva* could be a new alternative to produce antioxidative and cytotoxic drugs.

Keywords: Seaweed, *Halopithys incurva*, antioxidant, cytotoxicity.

INTRODUCTION

Over the last decades, the search for new drugs designed for anticancer chemotherapy, as well as the discovery of novel targets for such treatment have aroused a huge interest among scientific groups and pharmaceutical industrialists. The marine environment is an exceptional source of bioactive natural products with unique structural and chemical characteristics, which are very distinctive from those of the terrestrial environment. Among marine organisms, seaweeds have proven to be a very rich source of markedly potent compounds in the biomedical area, which show great potential as anti-inflammatory, antimicrobial, antiviral, and anti-tumoral drugs [1, 2, 3, 4]. Recent studies have shown that the metabolites isolated from marine algae represent nearly 22% of marine natural products described to date [5]. Indeed, seaweeds are known for their richness in vitamins, minerals, trace elements, proteins, iodine and bromine. They also contain bioactive substances like polysaccharides, terpenes, lipids, sterols, fatty acids, phenolic compounds, phytochromes, pigments, sugar, and alcohols [6, 7, 4, 8, 9]. They are the source of many useful products in medicine and pharmaceutical industries [10]. The Moroccan coast extends over 3500 Km with two fronts, Atlantic and Mediterranean. They are characterized by algal biodiversity and constitute a reserve of bioactive natural marine products.

Halopithys incurva, is a red seaweed extensively distributed in the Mediterranean and Atlantic seas of Morocco. Chemical analyses of the species, revealed its richness in bromophenols [11]. However, little information about *H. incurva* biological active compounds is available. Some studies have reported that the extracts of *H. incurva* showed powerful antibacterial, antiviral, anti-inflammatory and antiplasmodial activities [12, 13].

Therefore, the major goal of our study was to demonstrate the antitumoral activity of the red seaweed *H. incurva* collected from the Mediterranean coast of Morocco. A cytotoxic assay (SRB) was conducted with the human colorectal adenocarcinoma cell line HT-29. Then, we attempted to screen the antioxidant activity of the crude extract from the macroalgae using ABTS assay.

MATERIALS AND METHODS

Chemicals and reagents

Ethanol, acetone and methanol were obtained from Merck (Darmstadt, Germany). 2, 2-azino-bis (3-ethylbenzothia-zoline-6-sulfonic acid) diammonium salt (ABTS) and 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Potassium persulfate were procured from Fluka Chemical Co. (Buchs, Swizerland) and sulforhodamine-B (SRB) was purchased from Sigma-Aldrich (Taufkirchen, Germany).

Seaweed material

Samples of *Halopithys incurva* (Hudson) Batters 1902 (Phylum Rhodophyta, class Florideophyceae, order Ceramiales, family Rhodomelaceae) were collected by hand from the northern Mediterranean coast of Morocco, during the summer of 2007 (Ksar-sghir 35°50'52.58"N, 5o33'39.04"W). The algae specimens were washed with sterile seawater, and epiphytes, the associated debris and necrotic parts were removed. Voucher specimens of all tested species were deposited in the herbarium of the Laboratory of Applied Algology-Mycology, Department of Biology, Faculty of Sciences, Abdelmalek Essaâdi University, 93002 Tetouan, Morocco.

Preparation of extract

Shade-dried specimens of *H. incurva* (150 g) were ground and extracted with acetone/MeOH (1:1, 2L). After filtration, the solution was then evaporated under reduced pressure to obtain a residue, which was partitioned between acetone and ethanol. The organic layer was evaporated to dryness to give an extract. The residue was weighed and stored in sealed vials in a freezer at -4°C until being tested. Before biological screening, organic extracts were dissolved in 2% dimethylsulphoxide (DMSO).

Cell culture

HT-29 human colon carcinoma cells were obtained from ATCC (American Type Culture Collection) and cultured in McCoy's medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin and 100 µg/ml streptomycin. The cells were then incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2.

Sulforhodamine B assay

The growth of HT-29 cancer cell lines was measured using the sulforhodamine B (SRB) assay [12]. Cells were seeded at their optimal cell density (5000 cells/well) into a 96-well microtiter plate and were incubated overnight to allow cell attachment. They were then treated with various concentrations of *C. usneoides* extract (6.25, 12.5, 25, 50 and 100 µg/ml). Plates were incubated at 37°C, 5% CO_2 and 100% humidity for 48 and 72 hours. The cells were fixed with TCA by gently adding 50 µl TCA (50%) to each well for 1 h at 4°C, and processed as described in the literature [14].

ABTS radical scavenging activity

The antioxidant activity was examined by ABTS free radical decolorization assay described by Re *et al.*, 1999 [15] with a slight modification.

ABTS was dissolved in water to a 2.9 mM concentration. ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stock solution with 0.98 mM potassium persulfate (final concentration) and kept in the dark at room temperature for 18 h before use. For the study of the algal extract, the solution was diluted with ethanol to obtain the absorbance of 0.7 ± 0.2 units at 734 nm. To determine the scavenging activity, 100 µl ABTS reagent was added to 90 µL of EtOH and 10 µl of different concentrations of seaweed extract. The absorbance, monitored for 6 min, was measured spectrophotometrically at 734 nm and equilibrated at 30°C using a microtitre plate reader. Trolox was used as reference standard and the percentage inhibition of the sample was calculated by the following equation:

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] \times 100$$

A_0 expresses the absorbance of control; A_1 expresses the absorbance of the tested seaweed extract. The ABTS radical anion-scavenging assay was expressed as Trolox equivalent (TE).

RESULTS AND DISCUSSION

The SRB assay was used to investigate the potential cytotoxic effects of *H. incurva* extract on HT-29 cell line, where the cells were treated at increasing concentrations up to 200 µg/ml for 48 and 72 hours. *H. incurva* extract exhibited a dose- and time-dependent inhibitory effect on the HT-29 cancer cell growth (IC50 value were 88.26 µg/ml and 77.62 µg/ml, respectively after 48 and 72 h of incubation). As seen in Figure 1, increased concentration of *H. incurva* extract (0-200 µg/ml) markedly suppressed proliferation of HT-29 cells. At the low/moderate dosage, the algal extract exhibits a weak activity; whereas at higher concentrations, strong cytotoxic activity is observed.

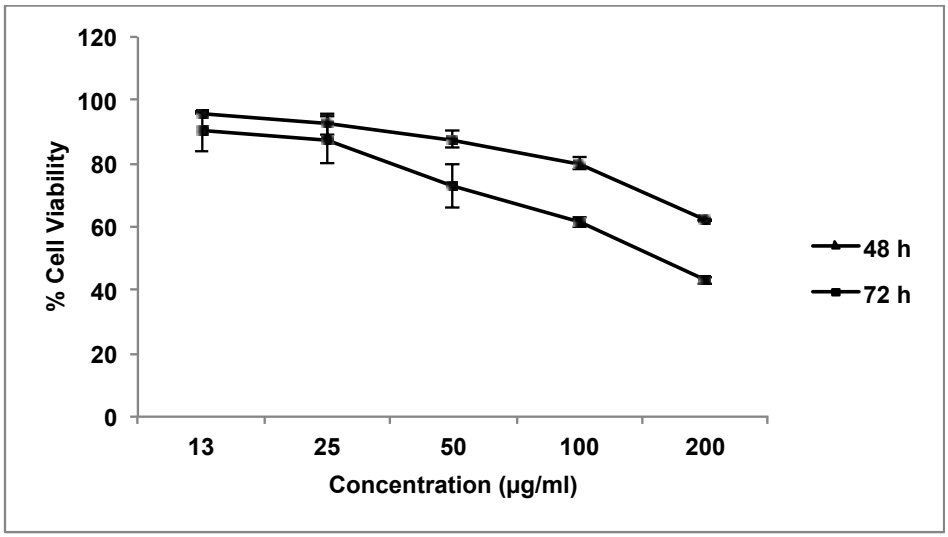

Figure 1.
Anti-proliferative activity of *H. incurva* against HT-29 colon cancer cells. Effect of extract treatment on the growth of HT-29 cells after 48h and 72h of incubation. Cells were treated with varying concentrations of *H. incurva* extract (12.5, 25, 50, 100 and 200 µg/ml) for 48 and 72 h. Cell viability was determined by SRB assay. Data are expressed as mean ± S.D. (n = 6).

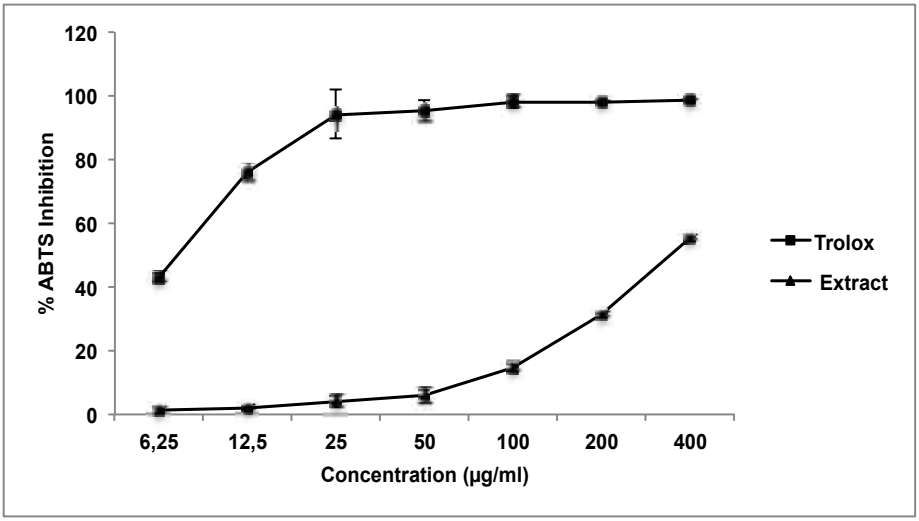

Figure 2.
The effects of *H. incurva* extract on the inhibition of the $ABTS^{\bullet+}$ compared to Trolox. Values are the mean ± SD of three determinations.

The present study demonstrated for the first time, to the best of our knowledge, that the extract of *H. incurva* exhibits antiproliferative activity against HT-29 colon cancer cells. However, only very few researches on the antiproliferative potential of *H. incurva* have been reported until know. It has been demonstrated that the proliferation of RAW macrophages was not affected by the addition of the *H. incurva* polysaccharides (0 to 100 µg/mL) to the culture medium [16].

Antioxidant capacity of *H. incurva* extract was measured by using the ABTS assay. The radical

scavenging activity of *H. incurva*, along with standard Trolox was shown in Figure 2. The seaweed extract was able to act against $ABTS^{\bullet+}$ radical and the activity was lower than that of standard compound. In addition, the reaction of the sample in this study with ABTS radicals was concentration-dependent. The percentage of inhibition was 98% and 55% for the Trolox and *H. incurva* extract, respectively, at the concentration of 400 µg/mL.

Recent studies have revealed that the species of the family Rhodomelaceae contain an important richness of bromophenols as phenolic compounds [17, 18].

Previous reports have revealed that the red alga *H. incurva* has interesting antioxidant properties [19, 20]. Therefore, Plaza *et al.*, 2010 reported highest content on total phenol (41.78 ± 8.15 mg gallic acid/g extract) in the natural extract obtained after subcritical water extraction at 200 °C, of *H. incurva* [19]. Those phenolic compounds are regarded for their important dietary roles as antioxidants and chemo preventive agents [21]. Our findings for antioxidant activity of *H. incurva* are in contrast with a previous report that showed that this seaweed demonstrated no activity against the DPPH radical with an EC50 > 1000 µg/mL [20]. However, the same study, in the ß-carotene assay, showed an activity towards the radicals peroxides with an EC50 of 51.09 ± 0.14 µg/mL. Thus, the values detected here for *H. incurva* activity are extremely interesting, leading us to further testing of different antioxidant assays to determine their possible antioxidant mechanisms.

CONCLUSION

The current study showed that *H. incurva* could be utilized as a source of natural pharmaceutical products as their crude extract exhibit antitumoral and antioxidant activities. More work is required to further characterize the specific chemical, which is responsible for their cytotoxic activity and pharmacological profile.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

ACKNOWLEDGEMENTS

The authors are grateful to Spanish Agency of International Cooperation and Development of the Foreign Ministry (AECID), Spain for providing the financial assistance for the work.

REFERENCES

1. Zbakh H, Chiheb H, Bouziane H, Motilva VS, Riadi H, Antibacterial activity of benthic marine algae extracts from the Mediterranean coast of Morocco. J. Microbiol. Biotechnol. Food Sci. 2012; 2 (1): 219-228.
2. Zbakh H, Chiheb I, Motilva V, Riadi H, Antibacterial, cytotoxic and antioxidant potentials of *Cladophora prolifera* (Roth) Kutzing Collected from the Mediterranean Coast of Morocco. AJPCT. 2014; 2 (10): 1187-1199.
3. De los Reyes C, Zbakh H, Motilva V, Zubía E, Antioxidant and anti-inflammatory meroterpenoids from the brown alga *Cystoseira usneoides*. J. Nat. Prod. 2013; 76 (4): 621-629.
4. Bouhlal R, Haslin C, Chermann JC, Colliec-Jouault S, Sinquin C, Simon G, Cerantola S, Riadi H, Bourgougnon N, Antiviral activities of sulfated polysaccharides isolated from *Sphaerococcus coronopifolius* (Rhodophytha, Gigartinales) and *Boergeseniella thuyoides* (Rhodophyta, Ceramiales). Marine Drugs. 2011; 9 (7): 1187-1209.
5. Cosandey C, Bigot SS, Dacharry M, Gille E, Laganier R, Salvador P, Les eaux courantes : Géographie et environnement. Collection Belin Sup Géographie. 2003; 240 pp.
6. Bhacuni DS, Rawat DS, Bioactive Marine Natural Products. Springer/Anamaya Publishers. 2005; 382 pp.
7. Li K, Li XM, Ji NY, Wang BG, Natural bromophenols from the marine red alga *Polysiphonia urceolata* (Rhodomelaceae): Structural elucidation and DPPH radical-scavenging activity. Bioorg. Med. Chem. 2007; 15 (21): 6627-6631.
8. Priyadharshini S, Bragadeeswaran S, Prabhu K, Ran SS, Antimicrobial and hemolytic activity of seaweed extracts *Ulva fasciata* (Delile 1813) from Mandapam, Southeast coast of India. Asian Pac. J. Trop. Biomed. 2011; 1: S38-S39.
9. Li YX, Wu HX, Xu Y, Shao CL, Wang CY, Qian PY, Antifouling Activity of Secondary Metabolites Isolated from Chinese Marine Organisms. Mar. Biotechnol. 2013; 15 (5): 552-558.
10. Raghukumar S, Marine biotechnology – an approach based on components, levels and players. Indian J. Geo. Mar. Sci. 2011; 40 (5): 609-619.
11. de Nanteuil G, Mast Agli P, A bromophenol in the red alga *Halopitys incurvus*. Phytochemistry. 1981; 20 (7): 1750-1751.
12. Oumaskour K, Boujaber N, Etahiri S, Assobhei O, Anti-inflammatory and antimicrobial activities of twenty-three marine red algae from the coast of sidi bouzid (el jadida-morocco). Int J Pharm Pharm Sci. 2013; 5 (3): 145-149.
13. Spavieri J, Allmendinger A, Kaiser M, Itoe MA, Blunden G, Mota MM, Tasdemir D, Assessment of Dual Life Stage Antiplasmodial Activity of

British Seaweeds. Marine Drugs. 2013; 11: 4019-4034.

14. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR, New colorimetric cytotoxicity assay for anticancer-drug screening. J. Natl. Cancer Inst. 1990; 82 (13): 1107-1112.
15. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C, Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 1999; 26 (9-10): 1231-1237.
16. Díaz RTA, Chabrillón M, Cabello-Pasini A, Gómez-Pinchetti JL, Figueroa FL, Characterization of polysaccharides from *Hypnea spinella* (Gigartinales) and *Halopithys incurva* (Ceramiales) and their effect on RAW 264.7 macrophage activity. J. Appl. Phycol. 2011; 23 (3): 523-528.
17. De Carvalho LR, Roque NF, Halogenated and/or sulfated phenols from marine macroalgae. Quim. Nova. 2000; 23 (6): 757-764.
18. Zhao J, Fan X, Wang S, Li S, Shang S, Yang Y, Xu N, Lu Y, Shi J, Bromophenol derivatives from the red alga *Rhodomela confervoides*. J. Nat. Prod. 2004; 67 (6): 1032-1035.
19. Plaza M, Amigo-Benavent M, del Castillo MD, Ibáñez E, Herrero M, Facts about the formation of new antioxidants in natural samples after subcritical water extraction. Food Research International. 2010; 43 (10): 2341-2348.
20. Bouhlal R, Riadi H, Bourgougnon N, Antioxidant activity of Rhodophyceae extracts from Atlantic and Mediterranean Coasts of Morocco. African Journal of Plant Science. 2013; 7 (3): 110-117.
21. Bravo L, Polyphenols: chemistry, dietary source, metabolism, and nutritional significance. Nutr. Rev. 1998; 56 (11): 317-333.