International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 11, Issue 3, 2019

Original Article

SCREENING OF THE ANTIOXIDANT ACTIVITY OF CRUDE EXTRACTS IN 86 ALGAE SPECIES FROM EL JADIDA COAST (MOROCCO)

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Received: 21 Jul 2018 Revised and Accepted: 22 Jan 2019

ABSTRACT

Objective: This work aimed to screen the antioxidant activity of marine macroalgae from the Moroccan Atlantic coast (region of El Jadida).

Methods: Evaluation of the antioxidant activity of different collected species, lyophilized and extracted with a solvent mixture chloroform/methanol (2/1; v/v) was conducted according to two techniques, first by thin layer chromatography (tlc) then by spectrophotometry, using a free radical 2,2-diphenyl-1-picrylhydrazyl (dpph). The sampling on a distance of 110 km allowed to harvest 86 algal species (16 brown algae, 47 red algae, 14 green algae and 9 algae being identified).

Results: The analysis by thin layer chromatography reveals an antioxidant activity in nearly half of harvested algal species (52.32 %). This activity varies depending on the concentration of the extract and in function of incubation time in the presence of dpph. The monitoring of the kinetics of degradation of dpph by spectrophotometer in the presence of extracts which were active by tlc allowed to confirm the results and select the most active algal species based on the percentage of remaining dpph in the medium after 120 min of reaction: *Fucus spiralis* (17.02 %), *Cyctoseira ericoides* (12.16 %) (Phaeophyceae), and *Gracilaria multipartita* (36%), *Halopitys incurvus* (5%) (Rhodophyceae).

Conclusion: The results show that the methodology adopted in this work is reliable and can be used for rapid screening of antioxidant property in plants and the species: *Fucus spiralis, Cyctoseira ericoides, Gracilaria multipartita,* and *Halopitys incurvus* can be a promising source of natural compounds endowed with high antioxidant potential.

Keywords: Marine algae, Morocco, Extracts, Antioxidant activity, Dpph

DOI: http://dx.doi.org/10.22159/ijpps.2019v11i3.28666

INTRODUCTION

Since ancient times, marine algae have been used as food for livestock, fertilizers in agriculture, or as a source of medical agents. They are also widely used in traditional Chinese medicine to their pharmacological activities [1]. The algae are considered as a source of bioactive compounds. They are able to produce a large variety of secondary metabolites characterized by a large spectrum of biological activities and that can act at different levels [2, 3]. In addition to their use as a food source, especially in Asian countries [4], many algal compounds were studied for their variable activities as well as in health and environment fields [5]. The marine algae are considered an important source of bioactive substances such as antiviral [6], antioxidant [7], antitumoral [8, 9], antibacterial [10], anti-inflammatory, anticoagulant and antibiotic agents [11].

The intensive research has been conducted on the antioxidant activity due to the increasing demand for most food and pharmaceutical industries to develop bioactive natural compounds against aging and against cancer [7, 12-22]. These works led to the isolation of antioxidant molecules of very different natures: tocopherols [23], phenolic compounds [24], carotenoids and sterols [18] and sulfated polysaccharides [25, 26].

The Moroccan coast presents an undeniable richness in terms of diversity and quantity of macroalgae. Many of these algae are studied to assess different biological properties like antimalarial [27], antibacterial [28], antimicrobial [28], antiviral [30], anti-inflammatory [31], and antifungal activities [32]. However, the realized researches on the antioxidant activity of macroalgae from the Moroccan Atlantic coast remain to our knowledge generally modest [33, 34].

This study is done in the framework of the valorization of the Moroccan marine natural resources; it aims at the screening of antioxidant activity in the marine algae from the coast of El Jadida city. It is part of a project that aimed at the isolation and characterization of bioactive products from marine algae potentially usable in therapeutic and cosmetic fields.

MATERIALS AND METHODS

Algal materials and extraction

The algae inhabiting the Atlantic coast on a distance of 110 km, from Sidi Bounaim village (33 ° 22'37.2 "N 8 ° 14'56.7" W) to the city of Oualidia (32 ° 46'16.9 "N 9 ° 00'01.9" W) (fig. 1) were collected at low tide during several field outings (April 2013).

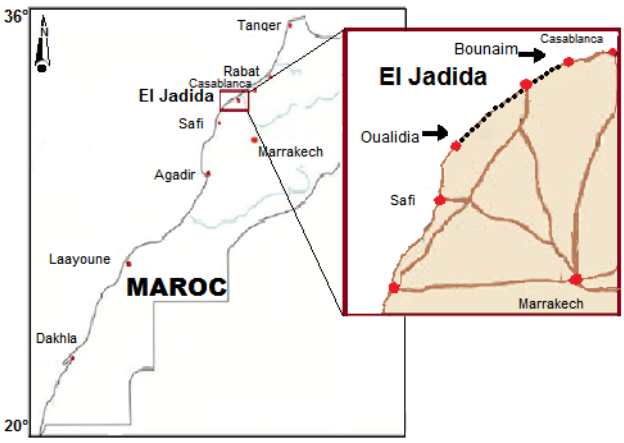

Fig. 1: Localization of collection sites

The algae were harvested manually, cleaned at first on site by sea water. In the laboratory, the algae were sorted and rinsed with running water and then with distilled water. After taxonomic identification, the specimens (table 1) were photographed and the voucher specimens were deposited in the laboratory of Biotechnology and Valorization of Plant Resources: Algae and Plants, Faculty of Sciences El Jadida, Morocco. The sorted samples were frozen at-80 °C and freeze-dried using a lyophilizer (Free Zone Plus 2.5 liters).

Table 1: List of collected algae on the moroccan atlantic coast from sidi bounaim to oualidia city

Species of seaweed	Family	Order	Collection localities
Brown algae (Phaeophyceae)			
Bifurcaria bifurcata	Sargassaceae	Fucales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Cladostephus verticillatus	Cladostephaceae	Spacelariales	Moulay Abdellah(33 °11'56.1"N 8 °35'32.4"W)
Colpomenia sinuosa	Scytosiphonaceae	Ectocarpales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Cystoseira myriophylloides	Sargassaceae	Fucales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Cystoseira ericoides	Sargassaceae	Fucales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Cystoseira sp	Sargassaceae	Fucales	Sidi Bounaim (33 °22'37.2"N 8 °14'56.7"W)
Dictyopteris mambranacea	Dictyotaceae	Dictyotales	Sidi Bounaim (33 °22'37.2"N 8 °14'56.7"W)
Dictyopteris sp	Dictyotaceae	Dictyotales	Sidi Bounaim (33 °22'37.2"N 8 °14'56.7"W)
Dictyota dichotoma	Dictyotaceae	Dictyotales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Fucus spiralis	Fucaceae	Fucales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Halopteris scoparia	Stypocaulaceae	Spacelariales	Sidi Bounaim (33 °22'37.2"N 8 °14'56.7"W)
Laminaria digitata	Laminariaceae	Laminariales	Sid Daoui (33 °15'42.3"N 8 °30'10.9"W)
Padina pavonica	Dictyotaceae	Dictyotales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Saccorhiza polyschides	Phyllariaceae	Tilopteridales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Sargassum muticum	Sargassaceae	Fucales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Sargassum vulgare	Sargassaceae	Fucales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Red algae (Rhodophyceae)			
Asparagopsis armata	Bonnemaisoniaceae	Bonnemaisoniales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Bornetia secundiflora	Ceramiaceae	Ceramiales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Callithamnion tetricum	Callithamniaceae	Ceramiales	Mazagan (33 °18'03.5"N 8 °21'55.9"W)
Callithamnion sp	Callithamniaceae	Ceramiales	Oualidia (32 °46'16.9"N 9 °00'01.9"W)
Caulacanthus ustulatus	Caulacanthaceae	Gelidiales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Ceramium flabelligerum	Ceramiaceae	Ceramiales	Harass (33 °14'39.6"N 8 °28'27.0"W)
Ceramium sp1	Ceramiaceae	Ceramiales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Ceramium sp2	Ceramiaceae	Ceramiales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Chondria coerulescens	Rhodomelaceae	Ceramiales	Mazagan (33 °18'03.5"N 8 °21'55.9"W)
Chondria dasyphylla	Rhodomelaceae	Ceramiales	Jorf (33 °08'32.0"N 8 °36'51.9"W)
Corallina sp	Corallinaceae	Corallinales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Falkenbergia rufolanosa	Bonnemaisoniaceae	Bonnemaisoniales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Gelidium crinal	Gelidiaceae	Gelidiales	Harass (33 °14'39.6"N 8 °28'27.0"W)
Gelidium latifolium	Gelidiaceae	Gelidiales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Geliduim pulchelum	Gelidiaceae	Gelidiales	Sid Daoui (33 °15'42.3"N 8 °30'10.9"W)
Gelidium sesquipedale	Gelidiaceae	Gelidiales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Gelidium spinulosum	Gelidiaceae	Gelidiales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Gelidium sp1	Gelidiaceae	Gelidiales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Gelidium sp 2	Gelidiaceae	Gelidiales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Gelidium sp 3	Gelidiaceae	Gelidiales	Sid Daoui (33 °15'42.3"N 8 °30'10.9"W)
Gigartina acicularis	Gigartinaceae	Gigartinales	Sidi Abed (33 °03'11.6"N 8 °41'18.1"W)
Gigartina teedii	Gigartinaceae	Gigartinales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Gracilaria cervicornis	Gracilariaceae	Gracilariales	Sidi Abed (33 °03'11.6"N 8 °41'18.1"W)
Gracilaria conferta	Gracilariaceae	Gracilariales	Harass (33 °14'39.6"N 8 °28'27.0"W)
Gracilaria confervoides	Gracilariaceae	Gracilariales	Sidi Abed (33 °03'11.6"N 8 °41'18.1"W)
Gracilaria multipartita	Gracilariaceae	Gracilariales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Gracilaria sp	Gracilariaceae	Gracilariales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Grateloupia filicina	Halymeniaceae	Halymeniales	Sidi Bounaim (33 °22'37.2"N 8 °14'56.7"W)
Gymnogongrus griffithsiae	Phyllophoraceae	Gigartinales	Herchan (32 °52'12.8"N 8 °52'22.0"W)
Gymnogongrus norvegicus	Phyllophoraceae	Gigartinales	Sid Daoui (33 °15'42.3"N 8 °30'10.9"W)
Gymnogongrus patens	Phyllophoraceae	Gigartinales	Herchan (32 °52'12.8"N 8 °52'22.0"W)
Halopitys incurvus	Rhodomelaceae	Ceramiales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Halurus equisetifolius	Wrangeliaceae	Ceramiales	Sidi Bounaim (33 °22'37.2"N 8 °14'56.7"W)
Hypnea musciformis	Cystocloniaceae	Gigartinales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Jania longifurca	Corallinaceae	Corallinales	Moulay Abdellah(33 °11'56.1"N 8 °35'32.4"W)
Laurencia pinnatifida	Rhodomelaceae	Ceramiales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Laurencia sp	Rhodomelaceae	Ceramiales	Sidi Bounaim (33 °22'37.2"N 8 °14'56.7"W)
Myriogramme costata	Delesseriaceae	Ceramiales	Sidi Abed (33 °03'11.6"N 8 °41'18.1"W)
Plocamium coccineum	Plocamiaceae	Plocamiales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Plocamium sp1	Plocamiaceae	Plocamiales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Plocamium sp2	Plocamiaceae	Plocamiales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Pterocladia capillacea	Pterocladiaceae	Gelidiales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Pterocladia sp	Pterocladiaceae	Gelidiales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Rhodymenia sp	Rhodymeniaceae	Rhodymeniales	Sidi Abed (33 °03'11.6"N 8 °41'18.1"W)
Scinaia furcellata	Scinaiaceae	Nemoniales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Sphaerococcus coronopifolius	Sphaerococcaceae	Sphaerococcales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Sphaerococcus sp	Sphaerococcaceae	Sphaerococcales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Green algae (Chlorophyceae)			
Bryopsis balbisiana	Bryopsidaceae	Bryopsidales	Jorf (33 °08'32.0"N 8 °36'51.9"W)
Cladophora ramossissima	Cladophoraceae	Cladophorales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)
Cladophora sp	Cladophoraceae	Cladophorales	Harass (33 °14'39.6"N 8 °28'27.0"W)
Codium adhaerens	Codiaceae	Bryopsidales	Sidi Bouzid (33 °09'-33 °16'N, 8 °30'-8 °45'W)

Codium elongatum	Codiaceae	Bryopsidales	Sidi Bouzid (33 °09’-33 °16’N, 8 °30’-8 °45’W)
Enteromorpha intestinalis	Ulvaceae	Ulvales	Sidi Bouzid (33 °09’-33 °16’N, 8 °30’-8 °45’W)
Enteromorpha ramulosa	Ulvaceae	Ulvales	Sidi Bouzid (33 °09’-33 °16’N, 8 °30’-8 °45’W)
Enteromorpha sp	Ulvaceae	Ulvales	Sidi Bouzid (33 °09’-33 °16’N, 8 °30’-8 °45’W)
Valonia macrophysa	Valoniaceae	Cladophorales	Sidi Bouzid (33 °09’-33 °16’N, 8 °30’-8 °45’W)
Valonia utricularis	Valoniaceae	Cladophorales	Oualidia (32 °46'16.9"N 9 °00'01.9"W)
Ulva fasciata	Ulvaceae	Ulvales	Sidi Bounaim (33 °22'37.2"N 8 °14'56.7"W)
Ulva lactuca	Ulvaceae	Ulvales	Sidi Bouzid (33 °09’-33 °16’N, 8 °30’-8 °45’W)
Ulva rigida	Ulvaceae	Ulvales	Sidi Bouzid (33 °09’-33 °16’N, 8 °30’-8 °45’W)
Ulva sp	Ulvaceae	Ulvales	Sid Daoui (33 °15'42.3"N 8 °30'10.9"W)
Algae being identified			
Esp N. I.1	-	-	Sidi Bounaim (33 °22'37.2"N 8 °14'56.7"W)
Esp N. I.2	-	-	Sidi Bounaim (33 °22'37.2"N 8 °14'56.7"W)
Esp N. I.3	-	-	Sidi Bounaim (33 °22'37.2"N 8 °14'56.7"W)
Esp N. I.5	-	-	Sidi Bounaim (33 °22'37.2"N 8 °14'56.7"W)
Esp N. I.6	-	-	Sid Daoui (33 °15'42.3"N 8 °30'10.9"W)
Esp N. I.7	-	-	Moulay Abdellah(33 °11'56.1"N 8 °35'32.4"W)
Esp N. I.8.	-	-	Oualidia (32 °46'16.9"N 9 °00'01.9"W)
Esp N. I.9	-	-	Oualidia (32 °46'16.9"N 9 °00'01.9"W)
Esp N. I.10	-	-	Oualidia (32 °46'16.9"N 9 °00'01.9"W)

Preparation of extracts

A part of dried samples was milled into powder manually in the mortar and the obtained powder (1 g) was extracted with a solvent mixture chloroform/methanol (2/1; v/v) under continuous agitation during 1h at room temperature.

After filtration, the samples were extracted a second time with the same new solvent under ultrasound (Transonic-420) for 15 min and filtered. The two extracts were assembled and concentrated under reduced pressure (temperature≤45 °C) using a rotary evaporator (Büchi Rotavapor R-3000). The concentrated samples were reduced to powder by lyophilization and the obtained powders were stored in a desiccator.

Evaluation of antioxidant activity by tlc plate

The chemical assay used to evaluate the antioxidant activity was developed in the laboratory; it is inspired by the method of Takao et al. [35]. The evaluation of antioxidant activity was realized by the technique of thin layer chromatography using the free radical dpph (Sigma-Aldrich).

The powder of each extract was dissolved in methanol (Fluka) using ultrasound for 5 min. Aliquots of each sample (2, 4 and 8 µl) were deposited on a silica gel plate (tlc Sil G25 UV 254 mm-Marcherey-Nagel, 10 x 20 cm, ep. 0.25 mm)using a micropipettes RINGCAPS (Hirschmann DIN/ISO7750). A methanol solution of dpph 6.10-4 mol/l was sprayed uniformly on the plate in shelter from the light. After development, the plates were read at the well determined time intervals. The images were made using a scanner (HP Deskjet 2050A) at the end of each period. The screening of antioxidant activity was realized by comparing two controls: ascorbic acid and (+) δ-Tocopherol (Sigma-Aldrich).

The antioxidant activity of the extracts was estimated by the fading of spots compared to controls spots.

Evaluation of the antioxidant activity by spectrophotometry

The antioxidant activity of the algae extracts which were reacted positively on tlc plate is evaluated by spectrophotometry based on the method described by Brand Williams et al. [36]. The antioxidant activity of different algae was evaluated by measuring the dpph degradation kinetics in the presence of obtained extracts at 5 mg/ml. The results were compared to the profile of dpph alone or in the presence of δ-tocopherol. A methanolic solution of dpph at 6.10-5 mol/l was prepared in advance and stored at 4 °C in the dark. The powders of crude extracts were dissolved in methanol. 0.1 ml of the extract solution was mixed with 3.9 ml of methanolic dpph. The reaction mixture was stirred vigorously with a vortex. The absorbances at 517 nm were measured at regular time intervals: 0 min, 1 min, 5 min, and every 15 min until reached a plateau. The negative control was prepared of 0.1 ml of methanol and 3.9 ml of dpph.

RESULTS

The harvested algae of the different sites on the coast of El Jadida show an important algal richness. This richness has characterized by the abundance of red algae compared to the brown and green algae. This distribution varies from site to site depending on coastal nature and in the function of human activities.

Screening of antioxidant activity on TLC plate

The preliminary screening of antioxidant activity in different algal extracts by tlc was based on the discoloration of dpph in the spots of deposits. The more deposit is discolored more antioxidant activity is important. The observed discoloration in the ascorbic acid spot and (+) δ-Tocophérol represents the reference.

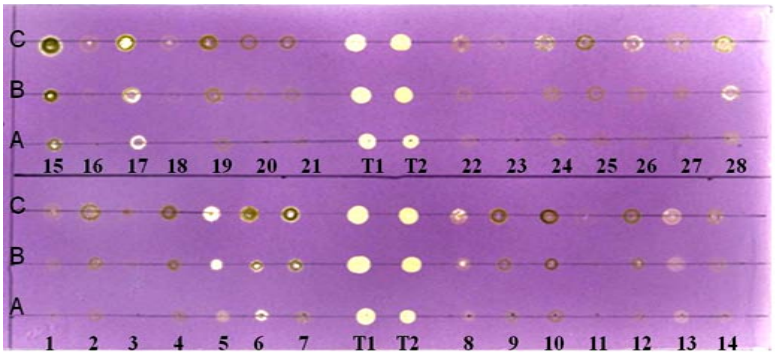

Fig. 2: Chromatogram of the algae extracts and controls after 120 min of reaction with dpph 1: *E. intestinalis sp1*, 2: *E. intestinalis sp2*, 3: *F. rufolanosa*, 4: *C. ramosissima*, 5: *F. spiralis sp1*, 6: *F. spiralis sp2*, 7: *G. acicularis*, T_1: Tocopherol, T_2: Ascorbic Acid, 8: *C. myriophylloides*, 9: *P. capillacea*, 10: *U. lactuca*, 11: *E. ramulosa*, 12: *G. spinulosum*, 13: *B. bifurcata*, 14: *G. multipartita sp1*, 15: *G. multipartita sp2*, 16: *G. teedii*, 17: *C. ericoides*, 18: *H. musciformis*, 19: *D. dichotoma*, 20: *C. adhaerens*, 21: *G. sesquipedale*, 22: *P. coccineum*, 23: *S. coronopifolius*, 24: *Corallina sp*, 25: *V. utricularis*, 26: *C. sinuosa*, 27: *C. elongatum* and 28: *P. pavonica*, A: 2 µl, B: 4 µl et C: 8 µl

The analysis of the plate (fig. 2) reveals different reactions with dpph:

-Extracts that do not show any reaction with dpph (complete absence of discoloration): *Falkenbergia rufolanosa, Sphaerococcus coronopifolius, Enteromorpha ramulosa*.

-Extracts whose reaction was rapid, discoloration was visible from the first minutes: *Gelidium sesquipedale, Cystoseira ericoides*, *Fucus spiralis*, *Dictyota dichotoma.*

-Extracts in which the reaction was slow, fading appears only belatedly: *Cystoseira myriophylloides, Plocamium coccineum, Pterocladia capillacea,* and *Esp N. I.1.*

The chromatograms also show that the reaction with dpph, when it takes place, varies depending on the concentration of the deposited extract and the discoloration was more intense from bottom to upward of the tlc plate.

The analysis of several obtained chromatograms showed in some seaweed extracts that the reaction with dpph is increasingly important depending on incubation time (table 2).

All brown algae tested react positively with dpph and seem to possess antioxidant activity. In this class, the first batch of algae (64.28 %): *Bifurcaria bifurcata, Colpomenia sinuosa, Cyctoseira ericoides, Cystoseira sp, Dictyota dichotoma, Fucus spiralis, Saccorhiza polyschides, Sargassum muticum,* and *Sargassum vulgare* induce degradation of the dpph in the first 15 min.

Table 2: TLC screening of antioxidant activity in different seaweed extracts at 15 min and 120 min of reaction with dpph

Seaweed species	Extraction yield (%)	Reaction with dpph according to the incubation time	
		15 min	120 min
Brown algae (Phaeophyceae)			
Bifurcaria bifurcata	0.62	+	+
Cladostephus verticillatus	0.39	NT	NT
Colpomenia sinuosa	0.75	+	+
Cystoseira myriophylloides	4.22	-	+
Cystoseira ericoides	7.00	+	+
Cystoseira sp	1.10	+	+
Dictyopteris membranacea	3.87	-	+
Dictyopteris sp	ND	NT	NT
Dictyota dichotoma	6.72	+	+
Fucus spiralis	5.47	+	+
Halopteris scoparia	0.13	-	+
Laminaria digitata	ND	-	+
Padina pavonica	9.07	-	+
Saccorhiza polyschides	3.64	+	+
Sargassum muticum	3.11	+	+
Sargassum vulgare	7.09	+	+
Red algae (Rhodophyceae)			
Asparagopsis armata	4.49	-	+
Bornetia secundiflora	6.36	-	+
Callithamnion tetricum	1.43	-	-
Callithamnion sp	1.63	-	-
Caulacanthus ustulatus	4.61	+	+
Ceramium flabelligerum	1.06	-	+
Ceramium sp1	ND	-	+
Ceramium sp2	1.50	-	-
Chondria coerulescens	1.22	-	-
Chondria dasyphylla	1.79	-	+
Corallina sp	1.38	+	+
Falkenbergia rufolanosa	ND	-	-
Gelidium crinal	ND	NT	NT
Gelidium latifolium	1.43	+	+
Geliduim pulchelum	ND	NT	NT
Gelidium sesquipedale	2.92	+	+
Gelidium spinulosum	5.16	-	+
Gelidium sp1	0.67	+	+
Gelidium sp 2	1.86	+	+
Gelidium sp 3	ND	+	+
Gigartina acicularis	0.74	+	+
Gigartina teedii	3.17	-	-
Gracilaria cervicornis	ND	-	+
Gracilaria sp	ND	-	+
Gracilaria multipartita	0.78	+	+
Gracilaria conferta	0.87	-	+
Gracilaria confervoides	2.21	-	-
Grateloupia filicina	1.60	-	-
Gymnogongrus griffithsiae	1.12	-	-
Gymnogongrus norvegicus	1.76	-	-
Gymnogongrus patens	6.27	-	-
Halopitys incurvus	4.61	+	+
Halurus equisetifoluis	ND	NT	NT
Hypnea musciformis	5.31	-	-
Jania longifurca	1.35	-	-
Laurencia pinnatifida	6.16	+	+
Laurencia sp	2.27	NT	NT
Myriogramme costata	0.56	NT	NT

Plocamium coccineum	3.26	-	+
Plocamium sp1	4.58	-	+
Plocamium sp2	4.43	-	+
Pterocladia capillacea	1.16	-	+
Pterocladia sp	0.65	-	-
Rhodymenia sp	0.21	-	+
Scinaia furcellata	ND	+	+
Sphaerococcus coronopifolius	ND	-	-
Sphaerococcus sp	1.52	-	-
Green algae (Chlorophyceae)			
Bryopsis balbisiana	1.83	-	+
Cladophora ramossissima	7.79	+	+
Cladophora Sp	ND	-	-
Codium adhaerens	ND	-	-
Codium elongatum	1.18	-	-
Enteromorpha intestinalis	0.76	+	+
Enteromorpha ramulosa	ND	-	-
Enteromorpha sp	ND	-	-
Ulva fasciata	3.51	-	-
Ulva lactuca	ND	+	+
Ulva rigida	1.11	-	-
Ulva Sp	2.10	-	-
Valonia utricularis	4.93	-	-
Valonia macrophysa	ND	NT	NT
Algae being identified			
Esp N. I.1	0.43	-	+
Esp N. I.2	2.15	-	+
Esp N. I.3	ND	-	-
Esp N. I.5	ND	NT	NT
Esp N. I.6	2.82	NT	NT
Esp N. I.7	4.34	NT	NT
Esp N. I.8.	ND	NT	NT
Esp N. I.9	ND	NT	NT
Esp N. I.10	1.49	NT	NT

ND: not determined, NT: not tested, (-): No degradation of dpph in the spot of deposited extract, (+): Degradation of dpph in the spot of deposited extract

The second batch of algae (35.72 %) composed of *Dictyopteris membranacea, Halopteris scoparia, Laminaria digitata,* and *Padina pavonica* shows a slower reaction with the radical dpph, the spots are visible after an hour of incubation.

For the Rhodophyceae, of the 42 tested extracts, only 25 showed a positive reaction with dpph. 12 extracts (28.57 %) were reacted during the first 15 min, which is the case of *Caulacanthus ustulatus, Corallina sp, Gelidium latifolium, Gelidium sesquipedale, Gelidium sp1*, *Gelidium sp2*, *Gelidium sp3*, *Gigartina acicularis, Gracilaria multipartita*, *Halopitys incurvus, Laurencia pinnatifida,* and *Scinia furcellata.*

The algae *Asparagopsis armata*, *Bornetia secundiflora*, *Ceramium flabelligerum*, *Ceramium sp1*, *Gelidium spinulosum*, *Gracilaria cervicornis*, *Gracilaria conferta*, *Gracilaria sp*, *Pterocladia capillacea*, *Plocamium coccineum*, *Plocamium sp1*, *Plocamium sp2,* and *Rhodymenia sp*, were induced belatedly the discoloration of the dpph radical. For other algae tested, no reaction with dpph was detected after 120 min of incubation.

Moreover, a strong dpph discoloration was observed in the first 15 min in the presence of *Halopitys incurvus* extract, it was strongly accentuated after 120 min.

Of the 13 tested Chlorophyceae, the result shows the presence of the antioxidant activity in *Bryopsis balbisiana*, *Cladophora ramossissima*, *Enteromorpha intestinalis,* and *Ulva lactuca.*

For *Bryopsis balbisiana*, the degradation of dpph was observed beyond the first 15 min. The species of *Cladophora sp, Codium adhaerens*, *Codium elongatum*, *Enteromorpha ramulosa*, *Enteromorpha sp, Ulva fasciata*, *Ulva rigida*, *Ulva sp, and Valonia utricularis* show no activity with dpph.

Evaluation of the antioxidant activity by spectrophotometry

The antioxidant activity was also evaluated by spectrophotometer in the extracts of Rhodophyceae, Chlorophyceae, and Phaeophyceae. This second work step is performed on the only extracts which have reacted positively with dpph by tlc technique.

The dpph degradation kinetics in the presence of extracts from different species of Phaeophyceae is represented in fig. 3. At 120 min of incubation, all the tested algal extracts cause degradation of the free radical dpph. The percentage of remaining dpph in the medium varies depending on the tested extract. It is particularly greatly reduced in the presence of the *Fucus spiralis* extract (17.02 %) and *Cystoseira ericoides* extract (12.16 %).

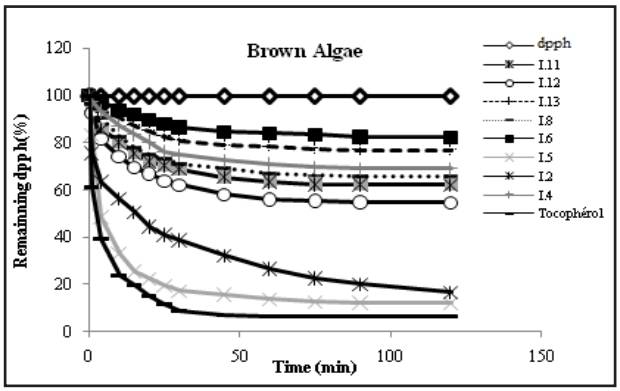

Fig. 3: Kinetic degradation of dpph (%) alone, in the presence of Brown algae extracts or of tocopherol according to the time (min) I.11: *Sargassum muticum,* I.12: *Cystoseira sp,* I.13: *Dictyopteris membranacea,* I.8: *Padina pavonica,* I.6: *Dictyota dichotoma,* I.5: *Cyctoseira ericoides,* I.2: *Fucus spiralis sp1* and I.4: *Bifurcaria bifurcata*

In addition, in the presence of *Cystoseira ericoides* extract, more than 50% of dpph was degraded in the first 5 min.

For the other tested algae: *Bifurcaria bifurcata, Cystoseira sp, Dictyopteris membranacea, Dictyota dichotoma Padina pavonica* and

Sargassum muticum the dpph degradation is not negligible, the percentage of remaining dpph in the reaction medium at 120 min varies from 82.43 % (*Dictyota dichotoma)* to 54.87% (*Cystoseira sp).*

The tested Rhodophyceae also cause degradation of the dpph (fig. 4). The results show a modest degradation in the presence of the *Corallina sp, Gigartina (Gigartina acicularis, Gigartina teedii), Gelidium (Gelidium latifolium, Gelidium sp1, Gelidium sp 2),* and *Pterocladia capillacea* extracts. In fact, at 120 min of incubation, the percentage of remaining dpph in the medium varies from 69.53 % to 95.66 %. However, in the presence of *Gracilaria multipartita* or *Halopitys incurvus* extracts, dpph is highly degraded. For *Gracilaria multipartita*, nearly 62.78 % dpph is degraded at 120 min.

In the presence of *Halopitys incurvus* extract, the degradation of dpph is very important and very rapid; nearly 80% of dpph was degraded in the first 5 min of incubation. At 30 min, the remaining dpph in the reaction medium does not exceed 6.65 %.

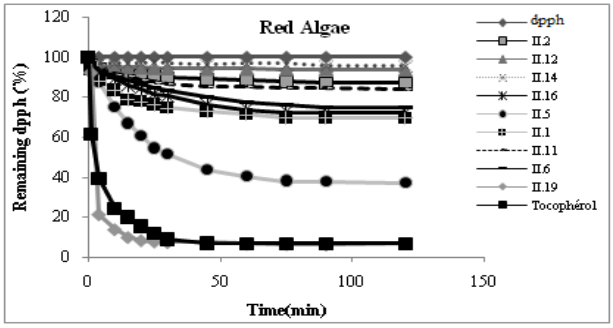

Fig. 4: Kinetic degradation of dpph (%) alone, in the presence of Red algae extracts or of tocopherol according to the time (min) II.2: *Pterocladia capillacea,* II.12: *Gelidium sp1,* II.14: *Gelidium latifolium,* II.16: *Gelidium sp 2,* II.5: *Gracilaria multipartita sp2,* II.1: *Gigartina acicularis,* II.11: *Corallina sp,* II.6: *Gigartina teedii* and II.19: *Halopitys incurvus*

For Chlorophyceae, the crude tested extracts revealed a moderate antioxidant activity. The results in fig. 5 show a low degradation of dpph (5 to 20 %) in the presence of an algal extract of *Codium adherens* (9.94 %), *Valonia utricularis* (14.01 %), *Ulva lactuca* (17.39 %), and *Codium elongatum* (22.38 %).

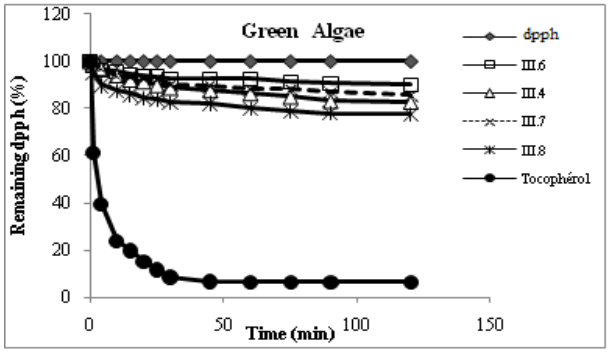

Fig. 5: Kinetic degradation of dpph (%) alone, in the presence of green algae extracts or of tocopherol according to the time (min) III.6: *Codium adherens,* III.4: *Ulva lactuca,* III.7: *Valonia utricularis et* III.8: *Codium elongatum*

DISCUSSION

The Moroccan coast is rich in marine flora, nowadays, 612 species have been recorded: 102 Chlorophyceae, 131 Phaeophyceae, and 379 Rodophyceae [37].

The Moroccan Atlantic coast is particularly rich in algal biodiversity with a considerable species resource of economic importance. For this, Morocco is among the world's largest producers of agar or carrageenan [38] and the major part of algae used in this processing industry comes from the region of El Jadida [39]. This demand goes globally on increasing due to the development of new biotechnological applications.

The results of our sampling show that the coast of the region of El Jadida presents a significant algal biodiversity. This algal richness is dominated by red algae. These results are consistent with previous work realized by Hanif *et al.* [40] in this region.

Along the coast, the spatial distribution varies considerably between sites, sometimes because of pollution from inland waters of various types (industrial or domestic wastewaters, leachate of agricultural land).

Several methods were used to test the antioxidant activity in the macroalgae [4, 12, 41-43]. In this work, we have developed a method based on the works of Takao *et al.* [35] and Brand Williams *et al.* [36] who used the dpph assay.

dpph is a stable free radical widely used as a means to estimate the radical scavenging activity of natural antioxidant substances in algae [17, 44] or in the algal products [11, 25-26] by reason of its stability, the simplicity of the method and of the reproducibility of results.

The results obtained during the screening of antioxidant activity by tlc plate in the tested algae show that all tested Phaeophyceae possess antioxidant activity. These results are consistent with those reported for various brown algae harvested on different sites and using the free radical dpph: three species of *Sargassum* [12], *Colpomenia sinuosa* [7], *Cystoseira sp, Dictyota dichotoma, Saccorhiza polyschides* [17], *Colpomenia sinuosa, Dictyota sp* [45], *Bifurcaria bifurcata, Dictyopteris membranacea, Dictyota dichotoma, Fucus spiralis, Laminaria digitata, Sargassum muticum, Sargassum vulgare* [44] *Sargassum vulgare* [11], *Fucus spiralis, Laminaria digitata and Sargassum muticum* [22].

Of the 42 tested species of Rhodophyceae, nearly two thirds (58.5%) reacted positively against dpph during this first screening by tlc. Several studies describe the presence of antioxidant activity in red algae. For *Chondria Coerulescens*, *Chondria dasyphylla*, *Gracilaria confervoides*, *Grateloupia filicina*, *Hypnea musciformis, Plocamium coccineum*, *Plocamium sp1*, *Pterocladia capillacea* and *Sphaerococcus coronopifolius,* our findings are entirely consistent with those of Martins *et al.* [45], Meenakshi *et al.* [46] and Bouhlal *et al.* [34].

For the Green algae tested (13 species), only *Bryopsis balbisiana*, *Cladophora ramosissima*, *Enteromorpha intestinalis* and *Ulva lactuca* have shown a presence of antioxidant activity, in the three last species, the reaction is early with dpph and is more accentuated at 120 min. For *Enteromorpha ramulosa* and *Ulva fasciata* no activity was show. A similar result was mentioned in other works [45, 46, 53]. However, for *Ulva lactuca,* an important activity is observed in the first 15 min as opposed to the results of Martins *et al.* [46] who reported a low activity against the dpph.

The antioxidant activity of the harvested algae is also evaluated by spectrophotometry using the dpph radical, only the algal extracts have shown activity by tlc technique are evaluated in order to confirm or refute the results of the previous screening.

Three extracts show a strong antioxidant activity: *Cystoseira myriophylloides, Fucus spiralis* and *Cyctoseira ericoides.* Therefore, the percentage of remaining dpph in the medium after 120 min of incubation is respectively 35.07, 17.02 and 12.16%. A high activity was also highlighted in the species of *Fucus spiralis* by Farvin and Jacobsen [22].

Ruberto *et al.* [47] showed strong activity in 8 species of Mediterranean *Cystoseira* other than *Cyctoseira ericoides* and *Cystoseira myriophylloides.* In our study *Cystoseira ericoides* has proven the most active brown seaweed; the results show degradation of more than 60% of dpph in the medium as soon as the first 10 min.

In the species of Rhodophyceae, the results show a modest antioxidant activity in most of the tested extracts except that of

Gracilaria multipartita and *Halopitys incurvus.* In *Gracilaria* nearly two-thirds of dpph present in the reaction medium are degraded after 120 min of incubation. Heo *et al.* [41] and Martins *et al.* [45], showed the presence of antioxidant activity in extracts of *Gracilaria* collected from the Brazilian coasts and the Icelandic coasts.

The *Halopitys incurvus* extract shows a strong antioxidant activity. A strong degradation of the dpph was observed in the first minutes. The dpph degradation kinetic in the presence of the extract is very comparable to that in the presence of tocopherol. This result is in opposition to those of Bouhlal *et al.* [34], who emphasized no activity in the methanol extract of this species.

The analyzed extracts of Chlorophyceae species showed a modest antioxidant activity. Martins *et al.* [45] also report a low activity of the *Ulva lactuca* extract and *Codium* extract against the dpph. This low activity in the extract of *Ulva lactuca* was also obtained by Meenakshi *et al.* [46] and Farvin and Jacobsen [22].

By these methodological approaches, we have been able to highlight the presence of the antioxidant activity in 45 algae extracts from a screening of 86 species of algae harvested from El Jadida Coast. Some of these algae have been studied and their antioxidant activity has been proven; *Fucus spiralis* [21-22, 48-51], *Cystoseira ericoides* [17, 21, 50], *Colpomenia sinuosa* [7, 16, 19], *Padina pavonia* [21], *Dictyota dichotoma* [16-18, 22], *Bifurcaria bifurcata* [12, 16], *Halopitys incurvus* [52], *Gelidium sesquipedale* [51], S*argassum vulgare* [11, 14-15, 21, 52], *Asparagopsis armata* and *Saccorhiza polyschides* [21]. These studies confirm the results of our work on these algal species.

In this work, the obtained results for the various tested extracts were compared to tocopherol, a reference antioxidant molecule. These results remain interesting because it must be emphasized that the tocopherol is a known pure antioxidant molecule, while our samples are crude extracts (a mixture of several molecules). Moreover, it can be suggested that the fractionation operations and purification of these extracts could give results where the antioxidant activity would be more promising.

CONCLUSION

To our knowledge, this study is the largest screening of the antioxidant activity of macroalgae in the Moroccan Atlantic coast (region of El Jadida) at the present time.

In addition to the algal richness biodiversity represented by the three groups Phaeophyceae, Rhodophyceae, and Chlorophyceae, our study highlights a strong activity in *Cyctoseira ericoides*, Cystoseira *myriophylloides, Fucus spiralis* (Phaeophyceae) and in *Gracilaria multipartita* and *Halopitys incurvus* (Rhodophyceae). Moreover, the strong activity found in the extract of *Cystoseira ericoides,* and that of *Halopitys incurvus* is obtained for the first time on species living in the Moroccan coasts. Additionally, the methodological approach used in this study is reliable and can be used for screening the antioxidant activity on a wide range of plant extracts from the marine or terrestrial natural source.

Our results show the importance of marine algae as a potential source of active compounds, principally, antioxidant substances. These can have a major importance in the development of new drugs.

ACKNOWLEDGMENT

We are immensely grateful to Mr Mifdal Mohamed for his linguistic support.

AUTHORS CONTRIBUTIONS

The authors declare that they contributed to this article according to the information mentioned below:

- F. CHIBI (PhD student): Realization of the experiments: sampling, extractions and tests, contribute to the manuscript preparation.

- H. RCHID (professor): Sampling, extractions and tests, correction and final shaping

- W. ARSALANE (professor): Identification of seaweed

- R. NMILA (professor, PhD supervisor): Sampling, extractions and tests and in assisting the manuscript preparation.

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest

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