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Characterization of Phenolic Compounds of *Ulva rigida* (Chlorophycae) and Its Antioxidant Activity

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SM, DC and JH designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SM and IB performed the experiments. Authors SM, MA and MB managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Ulva rigida is a worldwide distributed green alga and is commenly used for human nutrition. Extracts of this seaweed were shown anti-hypercholestierinemic, antioxidant and anti-hyperglycemic activities. The antioxidant effect was often ascribed to the presence of a huge amount of polyphenols. The aim of this study was to characterise by high-performance liquid chromatography-electrospray ionisation mass spectrometry (HPLC-ESI-MS) the phenolic molecules present in extracts obtained from *U. rigida*. The antioxidant activities of different extracts were evaluated in vitro by DPPH assay and on HeLa cells culture.

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Keywords: Ulva rigida; LC/MS; phenolic compounds; antioxidant; cytotoxicity.

1. INTRODUCTION

Ulva rigida is a very common green marine seaweed distributed worldwide that is commonly used as a popular food ingredient in Asian countries as well as in North and South America [1,2]. The high protein, lipid, mineral and vitamin content of marine *U. rigida* have encouraged its extensive use as a dietary supplement for humans and animals and as an organic fertilizer [3,4]. Moreover, *U. rigida* has shown biological activities that are related to the presence of polyphenols [3,5,6], polysaccharides [3], terpenoids [3], fatty acids [3,5] and vitamins [3]. Several studies have demonstrated its anti-hyperglycemic [7,8], anti-hypercholestierinemic [7,8], anti-bacterial [5], anti-genotoxic [5], antioxidant [9] and immunostimulating activities [10,11]. In particular, *U. rigida* has received much attention as novel sources of natural antioxidants. Previous investigation on the phytochemistry suggested that *U. rigida* extracts produced large amounts of phenolic compounds [5,6]. However, until now there is no report focused in the chemical identification of those molecules. The aim of this study was to identify by High-performance liquid chromatography-electrospray ionisation mass spectrometry (HPLC-ESI-MS) the main phenolic molecules present in the different extracts obtained from *U. rigida* in order to evaluate the correspondant phenolic profile. The evaluation of the free radical-scavenging properties, cytotoxicity and cytoprotective action of *U. rigida* extracts are also investigated. These data will offer a strong framework for new discoveries, particularly the pharmaceutical, cosmetic and agri-food processing industries.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Foetal bovine serum, L-glutamine, RPMI 1640, penicillin-streptomycin solution, phosphate-buffered saline (Gibco-BRL, France); dimethyl sulfoxide (DMSO) (Sigma, France); 5-6-chloromethyl 2',7'-dichlorodihydrofluorescein diacetate (Molecular probes, USA); Hydrogen peroxide (H_2O_2) (Pharmaghreb, Tunisia).

2.2 Algal material

Ulva rigida was collected from a shore of Ras-Djebel region in Tunisia. The algae were successively washed with water. The sample was botanically identified and a voucher specimen was deposited in the Laboratory of Functional Neurophysiology and Pathology (LFNP. URA 02).

2.3 Algal Extract Preparation

A fresh sample of *U. rigida* (1 kg) was mixed with 4 L of distilled water and sonicated. The mixture was then ground with an electric mortar and pressed. The resultant liquids (4.8 L) were pooled and subjected to chloroform and ethyl acetate extractions. The obtained extracts (chloroform, ethyl acetate, water) were concentrated with a rotary evaporator, freeze dried and stored until use. Approximately 15 ml of water extract was hydrolysed by H_2SO_4 (2 M, 8 h, 100°C) to allow glycoside separation. The obtained hydrolysate was neutralised, filtered and concentrated under vacuum. The methanol extract was obtained by using air-dried powdered *U. rigida* (5 g). The obtained solution was filtered through Whatman No. 1 paper and then evaporated at reduced pressure by rotary evaporator.

2.4 Total Phenolic Determination

The total phenolic contents in different extracts (ethyl acetate, methanol, water, hydrolysis water) were determined by the Folin-Ciocalteu method [12]. The total phenolic content was expressed as gallic acid equivalents (GAE) (mg/g of dry weight).

2.5 DPPH Free-radical Scavenging Activity

The effect of the extracts on DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals scavenging was estimated using a spectrometric method [13]. The test compound concentration providing 50% inhibition (IC_{50} expressed in $\mu g.mL^{-1}$) was calculated from the graph of the inhibition percentage plotted against the extract concentration. Butylated hydroxytoluene (BHT) was used as the positive control.

2.6 Cell Culture and Treatment

HeLa cells were maintained in RPMI medium containing 2 mM L-glutamine, 10% Foetal Bovine Serum (FBS) and 100 U/ml of antibiotic solution in a humidified 5% CO_2 incubator at 37°C. The cells were grown in 24-well microplates until 70-

80 % confluence. Different concentrations (250, 350, 400, 500 and 1000 µg/ml) of the water extract of *U. rigida* and/or H_2O_2 (10, 100, 1000 and 10 000 µM) were added to the cells and incubated for 4 h.

2.7 Cytotoxicity Assay

5-6-chloromethyl 2',7'-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA) was used as an intracellular esterase substrate to indicate cell integrity[14]. The CM-H_2DCFDA assay solution was freshly made by adding 15 µl of CM-H_2DCFDA stock solution (5 mg/ml DMSO, -20℃) to 1700 µl of RPMI. The culture medium was removed from the microplate wells, and the cells were incubated with CM-H_2DCFDA solution for 8 min at 37℃ in the dark. After the incubation period, the solution was aspirated, and the cells were rinsed with PBS (phosphate-buffered saline) at 37℃; then, the cells were lysed in lysis buffer (Tris-HCl). Fluorescence was measured using microplate reader ($\lambda_{excitation}$= 480 nm, $\lambda_{emission}$= 528 nm) and was normalised to control cells levels, which were set at 100% fluorescence. H_2O_2 was used as a positive control for cyotoxicity.

2.8 LC-MS Analysis

The LC–MS/MS experiments were carried out with an Agilent 1100 LC system. For the chromatographic separation a Zorbax 300Å Extend-C-18 Column (2.1 x 150 mm) was used. The column was held at 95% solvent A (0.1% formic acid in water) and 5% solvent B (0.1% formic acid in ACN) for 1 min, followed by an 11 min step gradient from 5% B to 100% B, then kept for 4 min with 100% B. Finally, elution was achieved with a linear gradient from 100% B to 5% B in 2 min. For MS experiments, the capillary voltage was set to 3.5 kV for electrospray ionisation with positive ion polarity.

2.9 Statistical Analysis

The data are presented as the mean ± S.D (standard deviation) and were evaluated using Student's *t*-test.

3. RESULTS AND DISCUSSION

3.1 Total Phenol Contents

Several studies of the *Ulva* species revealed that they are good dietary sources of antioxidants [15]. Thus, we evaluated the levels of total phenolic compounds in extracts of *U. rigida*. As shown in Table 1, the ethyl acetate and hydrolised water extracts showed the highest total phenolic contents (582.93±0.8 and 457.12±4.8 mg g^{-1}, respectively). These data are consistent with previous studies indicated that *U. rigida* extracts produced large amounts of phenolic compounds [5,6,16]. Overall, the results showed that phenolic content of all the extracts was quite high.

3.2 LC-MS/MS Analysis

LC-MS/MS analyses were performed to characterise the major phenolic compounds contained in *U. rigida* extracts. Several phlorotannins and phenolic acids (peak marked with number in Fig. 1) were tentatively identified using mass spectrometry and compared with literature data. The MS study of the ions allowed the detection of compound 1 with protonated molecular ion ($[M+H]^+$) at (*m/z* 127) (Table 2). This compound corresponds to phloroglucinol with a fragment at *m/z* 108, which is due to the loss of one molecule of water (-18). The compound **3** with ($[M+H]^+$) at *m/z* 499, composed by four phloroglucinol units, was also observed, and is tentatively identified as fucodiphloroethol (Table 2, Fig. 1). This tetramers showed a fragmentation pattern with losses of one molecule of water (- 18, *m/z* 481), three methyl (- 42, *m/z* 439) and six methyl (- 84, *m/z* 355), successively. Such phlorotannins characterization were demonstrated in Fucales extracts [17,18].

Table 1. Total phenolic contents of *U. rigida* extracts

Extract	Total phenols (mg GAE g^{-1})
Ethyl acetate	582.93±0.8
Methanol	272.08±0.2
Water	323.7±1.5
Hydrolyzed water	457.12±4.8

*The data are expressed as milligram of gallic acid per gram dry extract. Phenolic contents of ethyl acetate and hydrolyzed water extracts are significantly different from those of methanol and water extracts (*** P < 0.001). Values are the mean ± S.D. (Standard deviation) (n=3).*

In a similar manner, compound 5 with ($[M+H]^+$) at *m/z* 747 can be a fucophloroethols derivatives composed of six units of phloroglucinol. The fragmentation pattern showed losses of one molecule of water (-18, *m/z* 729), two molecules of water (-36, *m/z* 711), phloroglucinol and two

molecules of water (*m/z* 585, -126 -36) and one molecule of phloroglucinol, two molecules of water and methyl (*m/z* 571, - 126 – 36 – 14). A low-intensity product ion was detected (*m/z* 220) which is likely a result of the cleavage of benzene ring structure (Table 2). These results are similar with those obtained in brown seaweeds [19]. The compound 7, ($[M+H]^+$ at *m/z* 743) was identified as dieckol molecules composed of six units of phloroglucinol, with ion peaks observed at *m/z* 329, 311 and 227. This might be due to the loss of three phloroglucinol, two water molecules, and one molecule water and six methyl, respectively (Table 2). The compound 8 with $[M+H]^+$ at *m/z* 375 and product ion peaks observed at *m/z* 357, 339, 249 and 235 led us to assume that the compound correspond to fucophlorethol composed by three

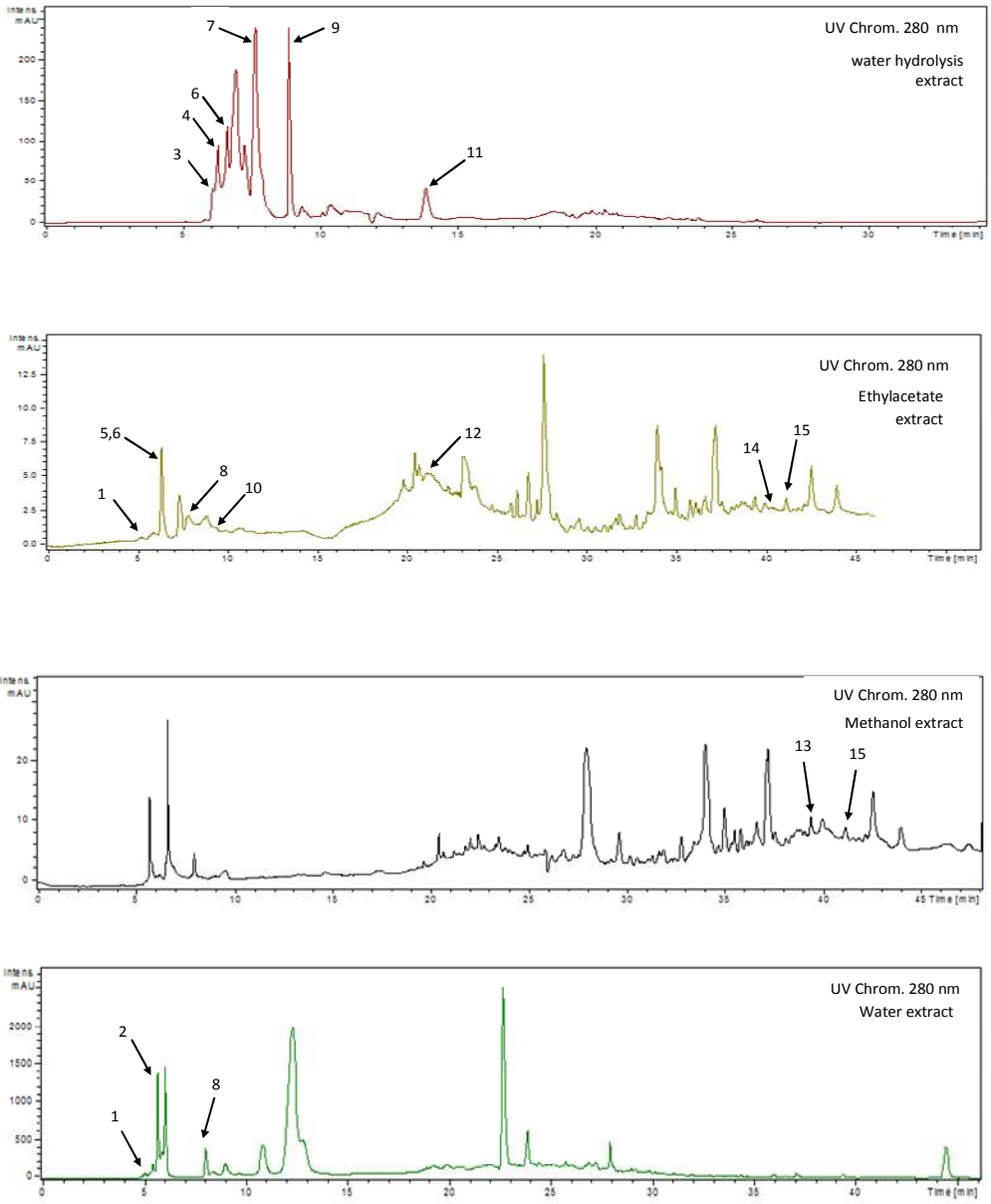

Fig. 1. UV chromatograms of the *U. rigida* extracts recorded at 280 nm

Peaks marked with numbers were identified as in Table 2

Table 2. Identification of polyphenols in *U. rigida* extracts

N°	Compound	RT(min)	Extract	UV λmax (mn)	$[M-H]^-$/ $[M-H]^+$	Major ESI fragments
1	Phloroglucinol	5.1	a, b	268	/127	108
2	Feruloyl-hexose	5.5	b	326	355	355, 193, 135
3	Fucodiphloroethol	6	c	270	499	481,439,355
4	Vanillic acid	6.4	c	219, 261	167	_
5	Fucophloroethols derivatives	6.4	a	280	747	729, 711, 585, 571, 220
6	Quinin acid	6.6	a, c	272	/193	_
7	Dieckol	7.5	c	272	/743	329,311,227
8	Fucophloroethol	8	a, b	273	/375	357,339,249,235
9	Syringic acid	8.7	c	218, 276	197	_
10	phloroeckol	9.3	a	273	/497	479, 451, 386, 368, 258
11	Dihydroxybenzoic acid	14	a, c	271	/155	_
12	Phenylethanol	21	a, c	270	/123	_
13	Dioxinodehydroeckol	39.1	d	275	/371	329, 311, 227
14	Eckol	40.4	a	274	/373	331, 313, 295
15	Diphloroethohydroxycarmalol	41.3	a, d	272	/513	495, 327, 301, 257

phloroglucinol units (Table 2), which is probably due to the loss of one molecule of water, two molecules of water, one molecule of phloroglucinol and one molecule of phloroglucinol and methyl, respectively. Such fragmentation patterns are identified in phlorotannins molecules obtained from *Fucus* brown algae [18,20]. The compound 10 at *m/z* 497 can be a phloroeckol molecule and showed a fragmentation pattern with losses of one water (*m/z* 479, –18), one water and two methyl (*m/z* 451, - 18 - 28) and other fragments at m/*z* 386, 368 and 258 (Table 2). The compound 13 at *m/z* 371 correspond to dioxinodehydroeckol. The product ions are *m/z* 329 corresponding to a loss of three methyl (- 42), 311 (- 18) and six methyl (- 84, *m/z* 227), succesively. The compound 14 at *m/z* 373 is suggested to be a polyphenolic compound composed by three phloroglucinol units, possibly a eckol. The fragmentation pattern is *m/z* 331 (- 42, three methyl), 313 (- 18, one water) and 295 (- 18, one water), successively. The compound 15 at *m/z* 513 is identified as diphloroethohydroxycarmalol, the product ions are successively, *m/z* 495 (- 18 one water), 327 (- 168, twelve methyl) and other low-intensity product ions (*m/z* 301 and 257 ions) which were probably a result of the cleavage of benzene ring structure. Moreover, the chromatogram of *U. rigida* extracts showed the presence of quinic acid and phenolic acids such as dihydroxybenzoic, quinic, siringic, vanillic acids, phenylethanol and feruloyl-hexose which are the main phenolic acids found in *U. rigida* (Table 2, Fig. 1). The present results indicated for the first time the identification of phlorotannins in Chlorophyta species. These compounds have been reported to occur in brown marine algae and in several families of Angiosperms [21].

3.3 Free-radical Scavenging Activity

The radical-scavenging activity of four *Ulva rigida* extracts were evaluated by DPPH assay based on their ability to quench radicals. The IC_{50} values for radical scavenging showed that the ethyl acetate extract had the highest radical scavenging activity with an IC_{50} value of 0.18 µg mL^{-1} followed by the hydrolysed water (0.21 µg mL^{-1}), water (0.25 µg mL^{-1}) and methanol (0.41 µg mL^{-1}) extracts (Fig. 2). As shown in Fig. 2, the antioxidant activities of the ethyl acetate and the hydrolysed water extracts were comparable to that of butylated hydroxytoluene (BHT), which was used as positive reference. Furthermore, the total phenolic compounds showed a positive correlation with the radical-scavenging activity results suggesting that phenolic components constitute the major molecules acting as free radical terminators. These findings are consistent with previous reports that evaluated the antioxidant capacity of *U. rigida* alga [5,6,16].

3.4 Cytotoxicity and Cytoprotective Effects

To investigate the cytotoxic effect of *U. rigida*, HeLa cells were treated with concentrations of alga water extract (0 to 1000 µg mL^{-1}) for 4 h or

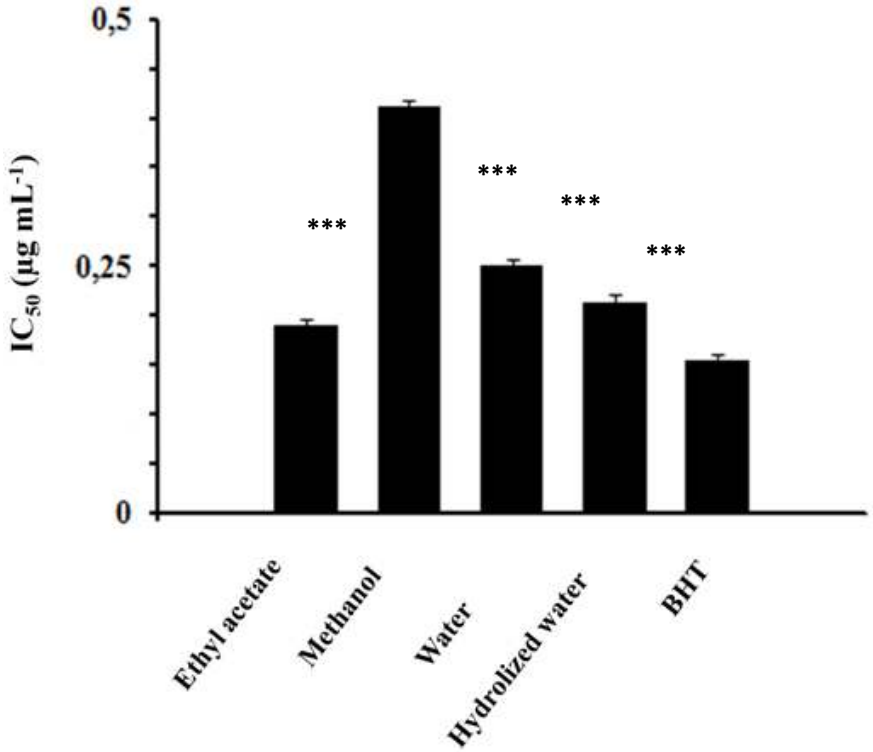

Fig. 2. Radical scavenging activities (IC_{50}) of studied *U. rigida* extracts
*Asterisk indicates a statistically significant difference, (*** P < 0.001)*

24 h and then subjected to a cell viability assay. The results clearly indicate that no significant cell death occured in either algal dose, and the *U. rigida* extract was not toxic even after continuous exposure for a 24 h period (Fig. 3). Moreover, the cytoprotective action of the *U. rigida* water extract was tested in H_2O_2 - induced cell death. Exposure to H_2O_2 markedly reduced cell viability in a dose-dependent manner, and over 80% of the cell population was dead after 4 h treatment with 10 000 µM H_2O_2. However, the co-exposure of cells with H_2O_2 and *U. rigida* water extract resulted in an increased percentage of viable cells. As shown in Fig. 3, *U. rigida* extract (400 µg/ml) prevented H_2O_2-induced damage, restoring cell viabilitiy to 74.12 (100 µM of H_2O_2), 47.1 (1000 µM of H_2O_2) and 20% (10 000 µM of H_2O_2) versus 45.17, 15.2 and 4.14%. These results indicated that the *U. rigida* extract was not toxic by it self and protects HeLa cells from cytotoxicity and the deleterious effect of H_2O_2-mediated oxidative damage. These data are in agreement with our previous study [16,22]. The high polyphenol content found in *U. rigida* extracts could be partly responsible for the antioxidant power reported here. Phenolic acids and phlorotannins such as phloroglucinol, eckol, bieckol, dioxinodehydroeckol, and diphloroethohydroxycarmalol are reported to exhibit strong DPPH and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activities [19,23]. The antioxidant activities of some phenolic acids and phlorotannins were also investigated in cellular models. In particular, phlorofucofuroeckol, dieckol, diphlorethohydroxycarmalol and dihydrobenzoic acids reduced the level of intracellular reactive oxygen species (ROS) [17-20,24,25]. The phloroglucinol molecule was found to scavenge ROS and increased the catalase-antioxidant enzyme activity [26]. Several reports have shown that phenolic acids (hydroxybenzoic, syringic, salicylic, etc.), phloroglucinol, phlorofucoeckol, eckol and bieckol markedly reduced lipid peroxidation, a hallmark of oxidative stress mediated through the free radicals produced in the cell [19,27]. Finally, other studies showed that most phlorotannins possess a remarkable ability to protect cells from death trigged by oxidative stress and prevent damage to biomolecules like DNA [28,29].

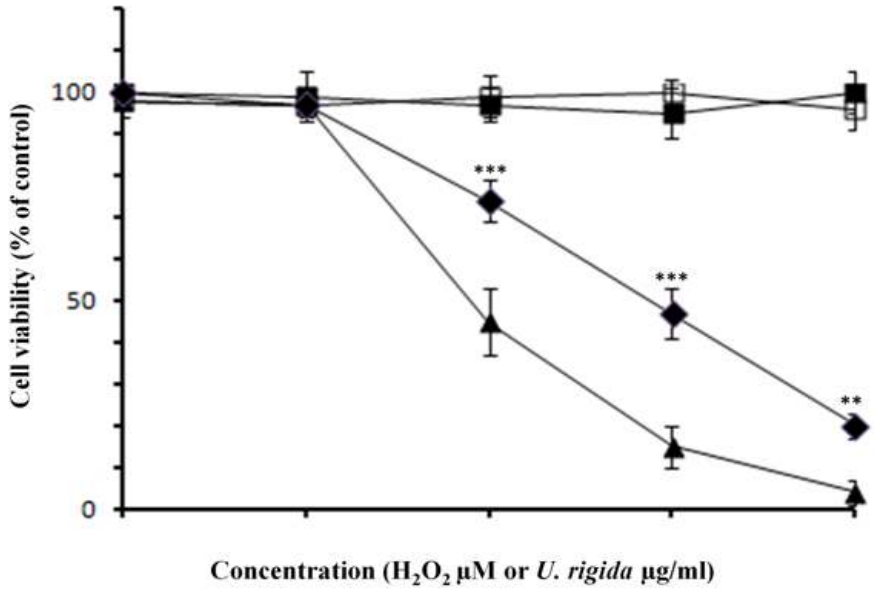

Fig. 3. Cytotoxicity and cytoprotective effects of *U. rigida* water extract on H_2O_2-induced oxidative cell damage

*The cells were treated with U. rigida water extract at 250, 350, 500 and 1000 µg mL^{-1} for 4 h ( ) or 24 h ( )( ) represents cells treated with H_2O_2 at 10, 100, 1000 and 10 000 µM. ( ) represents cells co-treated with H_2O_2 at 10, 100, 1000 and 10 000 µM and 400 µg mL^{-1} of U. rigida water extract and incubated for 4 h. Data are presented as the mean ± SEM (n=4). Asterisk indicates a statistically significant difference, where ** P < 0.01 and *** P < 0.001*

4. CONCLUSION

This paper reports the characterization of the phenolic compounds of *U. rigida* extracts for the first time. On the other hand this preliminary work is the first report mentioned the identification of phlorotannins in green seaweed. In addition, the different extracts exhibited large phenolic contents and potent antioxidant activity. Therefore, *U. rigida* extracts enriched in phenolic molecules may provide a promising source of natural antioxidants, applicable in the pharmaceutical, food and cosmetic industries. However, more studies are needed involving NMR identification and investigation on *Ulva* species from several origins, in attempt to establish a chemical fingerprint of *U. rigida* phenols.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Shalaby EA. Algae as promising organisms for environment and health. Plant Signal Behav. 2011;6:1338-1350.
2. Maskarzu M, Nakazoe JI. Production and use of marine algae in Japan. JPN AGR RES Q. 2001;35:281-290.
3. Satpati GG, Pal R. Biochemical composition and lipid characterization of marine green alga *Ulva rigida*-a nutritional approch. JABU. 2011;2:10-13.
4. Taboada C, Millan R, Miguez I. Evaluation of the marine alga *Ulva rigida* as a food supplement: Effect of intake on intestinal hepatic and renal activities in rats. J Med Food. 2011;14:161-166.
5. Trigui M, Gasmi L, Zouari I, Tounsi S. Seasonal variation in phenolic composition antibacterial and antioxidant activities of *Ulva rigida* (Chlorophyta) and assessment of antiacetylcholinesterase potentiel. J App Phycol. 2012;25:319-328.

6. Yildiz G, Celikler S, Vatan O, Dere S. Determination of the anti-oxidative capacity and bioactive compounds in green seaweed *Ulva rigida* C. Agardh. Int J Food Prop. 2012;15:1182-1189.
7. Taboada C, Millan R, Miguez I. Composition nutritional aspects and effect on serum parameters of marine algae *Ulva rigida*. J Sci Food Agr. 2010;90:445-449.
8. Celikler S, Tas S, Vatan O, Ziyanok-Ayvalik S, Yildiz G, Bilaloglu R. Anti-hyperglycemic and antigenotoxic potential of *Ulva rigida* ethanolic extract in the experimental diabetes mellitus. Food Chem Toxicol. 2009;47:1837-1840.
9. Goddard MK, Décordé K, Ventura E, Soteras G, Baccou JC, Cristol JP, Rouanet JM. Polysaccharides from the green alga *Ulva rigida* improve the antioxidant status and prevent fatty streak lesions in the high cholesterol fed hamster, an animal model of nutritionally-induced atherosclerosis. Food Chem. 2009;115:176-180.
10. Leiro JM, Castro R, Arranz JA, Lamas J. Immunomodulating activities of acidic sulphated polysaccharides obtained from the seaweed *Ulva rigida* C. Agardh. Int Immunopharmaco. 2007;7:879-888.
11. Castro R, Piazzon MC, Zarra I, Leiro J, Noya M, Lamas J. Stimulation of turbot phagocytes by *Ulva rigida C. Agardh* polysaccharides. Aquaculture. 2006;254: 9-20.
12. Wolfe K, Wu X, Liu RH. Antioxidant activity of apple peels. J. Agric Food Chem. 2003;51:609-614.
13. Dina A, Begoña RLM, José Igancio RS, Leandro J, Fadil B, Djebbar A. Antioxidant potential, cytotoxic activity and phenolic content of *Clematis flammula* leaf extracts. J Med Plants Res. 2011;5:589-598.
14. Anja ST, Cheryl S, Kristin JPS. Application of Alamar blue/5-carboxy fluorescein diacetate acetoxymethyl ester as a noninvasive cell viability assay in primary hepatocytes from rainbow trout. Anaytic Biochem. 2005;344:76-85.
15. Garcia-Casal MN, Ramirez J, Leets I, Pereira AC, Quiroga MF. Antioxidant capacity, polyphenol content and iron bioavailability from algae (*Ulva* sp., *Sargassum* sp. and *Porphyra* sp.) in human subjects. Br J Nutr. 2009;101:79-85.
16. Mezghani S, Bourguiba I, Hfaiedh I, Amri M. Antioxidant potential of *Ulva rigida* extracts: Protection of HeLa cells against H_2O_2 cytotoxicity. Biol Bull. 2013; 225:1-7.
17. Lee SH, Jeon YH. Anti-diabetic effects of brown algae derived phlorotannins, marine polyphenols through diverse mechanisms. Fitoterapia. 2013;86:129-136.
18. Ferreres F, Lopes G, Gil-Izquierdo A, Andrade PB, Sousa C, Mouga T, Valentao P. Phlorotannin extracts from fucales characterized by HPLC-DAD-ESI-MS^n: Approches to hyaluronidase inhibitory capacity and antioxidant properties. Mar Drugs. 2012;10:2766-2781.
19. Balboa EM, Conde E, Moure A, Falqué E, Dorminguez H. *In vitro* antioxidant properties of crude extracts and compounds from brown algae. Food Chem. 2013;38:1764-1785.
20. Wang T, Jonsdottir R, Liu H, Gu L, Kristinsson HG, Raghavan S, Olafsdottir G. Antioxidant capacities of phlorotannins extracted from the brown algae *Fucus vesiculosus*. J. Agric. Food Chem. 2012;60:5874-5883.
21. Pal Singh I, Bharate SP. Phloroglucinol compounds of natural origin. Nat Prod Rep. 2006;23:558-591.
22. Mezghani M, N'guessan P, Carrier A, Amri M. The ethanol precipitate of *Ulva rigida* protects HeLa cells from hydrogen peroxide-induced apoptosis. J Food and Biochem. 2015;39:48-54.
23. Sroka Z, Cisowski W. Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. Food Chem Toxicolo. 2003;41:753-758.
24. Kang HS, Chung HY, Kim JY, Son BW, Jung HA and Choi JS. Inhibitory phlorotannins from the edible brown alga *Ecklonia stolonifera* on total reactive oxygen species (ROS) generation. Arch Pharmacol Res. 2004;27:194-198.
25. Miranda MS, Sato S, Mancini-Filho J. Antioxidant activity of the microalga *Chlorella vulgaris* cultured on special conditions. Bollettino Chimico Farmaceuticol. 2001;140:165-168.
26. Kang KA, Lee KH, Chae S, Zhang R, Jung MS, Ham YM, Baik JS, Lee NH, Hyun JW. Cytoprotective effect of phloroglucinol on oxidative stress induced cell damage via catalase activation. J Cell Biochem. 2006;97:609-620.
27. Abd El-Baky H, El Baz FK, El-Baroty GS. Production of phenolic compounds from *Spirulina maxima* microalgae and its protective effects *in vitro* toward

hepatotoxicity model. Afr J Pharma Pharmacol. 2009;3:133-139.

28. Heo SJ, Jeon YJ. Evaluation of diphlorethohydroxycarmalol isolated from *Ishige okamurae* for radical scavneging activity and its protective effect against H_2O_2-induced cell damage. Process Biochem. 2009;44:412-418.

29. Ahn GN, Kim KN, Cha SH, Song CB, Lee J, Heo MS, Yeo IK, Lee NH, Jee YH, Kim JS, Heu MS, Jeon YJ. Antioxidant activities of phlorotannins purified from *Ecklonia cava* on free radical scavenging using ESR and H_2O_2-mediated DNA damage. Europ Food Res Technolo. 2007;226:71-79.

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