

Antioxidant and gastroprotective activities of polysaccharides from the Tunisian brown algae (Cystoseira sedoides)

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Abstract: Fucoidan and sodium alginate were extracted from the Tunisian brown seaweed *Cystoseira sedoides*, with good yields. The samples were characterized by colorimetric, spectroscopic (FTIR) and chromatographic (SEC/MALS/VD/DRI) methods. The quantitative estimation of the mannuronic acid/guluronic acid ratio (M/G ratio) of alginate was performed by FT-IR spectroscopy. It has been found that sodium alginate from *C. sedoides* contain more mannuronic acid (63%) than guluronic acid (37%). The isolated fucoidan was examined for *in vitro* antioxidant properties using various antioxidant assays. The sulfated polysaccharide exhibited important DPPH radical-scavenging activity (77% inhibition at a concentration of 5 mg/mL) and a considerable ferric reducing potential. An effective chelating activity was also recorded for the extracted fucoidan. Furthermore, the gastroprotective activity was determined for sodium alginate using HCl/EtOH induced gastric ulcers in rats. With a dose of 50 mg/kg, sodium alginate from *C. sedoides* showed a significant decrease (87%) in the intensity of gastric mucosal damages compared to a control group.

Keywords: Cystoseira sedoides, Polysaccharides, SEC/MALS/VD/DRI, Antioxidant activity, Gastroprotective activity.

Résumé : Les fucoidanes et les alginates de sodium ont été extraits à partir de l'algue brune Tunisienne *Cystoseira sedoides* avec des bons rendements. Les échantillons ont été caractérisés par des techniques colorimétriques, spectroscopique (IRTF) et chromatographique (CES/DDMA/DV/IR). Le rapport M/G de l'alginate de sodium a été estimé par IRTF montrant que celui-ci est plus riche en acide mannuronique (63%) qu'en acide guluronique (37%). L'activité antioxydante *in vitro* des fucoidanes isolés a été évaluée moyennant plusieurs tests. Nous avons montré que ce polysaccharide sulfaté possède un pouvoir anti-radicalaire important (77% d'inhibition de DPPH à une concentration de 5mg/ml), ainsi qu'un pouvoir réducteur du fer considérable. Une activité de chélation efficace a également été enregistrée pour le fucoidane extrait. De plus, l'activité gastroprotectrice d'alginate de sodium a été déterminée en utilisant le modèle d'ulcère gastrique induit par HCl/EtOH chez le rat. Avec une dose de 50 mg/kg, l'alginate de sodium issu de *Cystoseira sedoides* a montré une diminution significative (87%) de l'intensité des ulcères de la muqueuse gastrique par rapport à un groupe témoin.

Mots-clés : *Cystoseira sedoides*, Polysaccharides, CES/DDMA/DV/IR, Activité antioxydante, Activité gastroprotective.

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INTRODUCTION

Seaweed polysaccharides are highly active substances which have natural valuable applications. The most well-known polysaccharides of brown algae are alginates and fucoidans; Alginate is a linear anionic copolymer of β -D-mannuronic acid (M) and α -L-guluronic acid (G) (1-4)-linked residues. After partial hydrolysis, it was shown that this polysaccharide is formed of three different blocks: two types of homopolymeric sequences, D-mannuronic acid blocks (MM) and L-guluronic acid blocks (GG), and additionally, heteropolymeric sequences of M and G (MG blocks) [1,2]. It has been experimentally demonstrated that both the G content and the G block length of the alginate molecule contribute greatly to its gel-forming ability and gel strength [3], but the importance of the MG blocks in the gel formation was also emphasized in previous studies [4]. Otherwise, the composition sequential structure and thereby the and functionality of alginates vary according to season, age of population, species and geographic location. Alginates, especially water-soluble sodium alginates, are widely applied in the food and pharmaceutical industries due to their gel-forming properties in the presence of divalent cations such as Ca^{2+} [5-7].

On the other hand, fucoidans have been extensively studied due to their diverse biological activities. They are potent anticoagulant [8,9], antitumor [10] and antioxidant [11] agents. In addition, they can protect the gastric mucosa against the proteolytic activity of gastric juice [12]. The structure of algal sulfated fucans is complex. Heterogeneous and non defined regular polymers have been observed. They consist of (1-2) and/or (1-3) and/or (1-4) linked fucosyl backbones that are substituted at C-2 and/or C-4 with sulfate groups [13]. The chemical composition and structure of fucoidans are very diverse and depend on the algal species, season of harvest, age of the seaweed, local climatic conditions and the extraction procedure [14].

Tunisia is characterized by an algal diversity due to its geographical location and rich topography. However, the characterization of polysaccharides extracted from Tunisian seaweed has not been fully established to date with the exception of some research in this field [15].

In previous research we have demonstrated that fucoidans isolated from three species of the genus Cystoseira (*Cystoseira compressa*, *Cystoseira* *crinita* and *Cystoseira sedoides*) have interesting anti-radical, anti-inflammatory and gastroprotective activities [16]. These findings motivated us to improve yields and biological properties by trying another extraction method of fucoidans and to extend this study on sodium alginates.

We describe herein the extraction, physicochemical characterization and biological evaluation of fucoidan and sodium alginate from the Tunisian brown seaweed (*Cystoseira sedoides*).

MATERIAL AND METHODS

1. Plant material

The brown alga C. sedoides was harvested from the coastal region of Monastir (Tunisia), in June 2007, at a depth between 1 and 3 m. The seaweed species has been identified and authenticated by the National Institute of Marine Sciences and Technologies (Salambôo, Tunisia). The freshly collected seaweed fronds were initially, washed in seawater to remove the macroscopic epiphytes and other extraneous matter, and then rinsed in distilled water. Finally, the specimen was shade dried and coarsely powdered. The C. sedoides powder was then depigmented and defatted as follows. It was firstly extracted with acetone at 25°C with mechanical stirring for 24 hours. The supernatant was removed by filtration and the algal residue undergoes a second ethanol extraction (80%) at room temperature for 24 hours under mechanical stirring, the solution is then centrifuged. The recovered pellet was extracted with ethanol at 78° C for 24 hours with stirring.

2. Extraction of polysaccharides

Fucoidans and alginates were extracted according to the method adapted by Ponce et al. [17]. 100g of depigmented powders were treated with 800 mL of 2% aqueous solution of CaCl₂ during 7 hours, at 70°C. The pH is adjusted to 2 with a concentrated solution of HCl. After centrifugation, the supernatant enclosing the fucoidans was dialyzed against deionized water using dialysis tubing with molecular weight cut off 7 KDa and then lyophilized. While, the recovered pellet is solubilized in 2% Na₂CO₃ solution, at 70°C to finally obtain sodium alginates. The latter were recuperated by centrifugation, dialyzed and then lyophilized.

3. Chemical analyzes

The crude seaweed analyses (proteins, ash and lipids) were carried out following AOAC official



methods [18]. Total sugar content was determined according to the method of Dubois et al. [19], using galactose as standard. Uronic acid was estimated in a modified carbazole method using D-glucuronic acid as standard [20], the content of L-fucose units in fucoidans was determined by a colorimetric assay with L-cysteine [21]. Sulfate content was turbidimetrically evaluated [22] after a hydrolysis of polysaccharides in 2M HCl at 100°C for 2h. FTIR were performed for the extracted polysaccharides in KBr pellets (1mg)polysaccharide in 100 mg KBr). The spectra were recorded on a Perkin Elmer 1600 FTIR spectrometer from 400 to 4000 cm^{-1} .

4. Molecular weight determination

Samples analysis was performed using size exclusion chromatography (SEC) equipped with a triple detection: multi-angle light scattering (MALS) (Down HELEOS II, Wyatt Technology, Ca, USA), viscometer detector (VD) (Viscostar II, Wyatt Technology, Ca, USA) and differential refractive index (DRI) (RID 10 A Shimadzu, Japan). The SEC system consists of a pump (LC10 Ai Shimadzu, Japan) at a flow rate of 0.5 mL/min and two columns OHPAK SB 804 and 806 HQ. The samples were dissolved in the eluent (LiNO₃) 0.1 mol/L) at 2 g/L. The dissolution was carried out by stirring at 380 rpm for 24 h at room temperature. 3 mL solutions were filtered through membrane 0.45 µ (regenerated cellulose) before injection.

The analyzes were performed by a data processing Zimm [23] "order 1" using angles from (from 34.8° to 142.8°). The corresponding value of dn/dc, in our case is about 0.15 mL/g, the typical value for a polysaccharide [24]. The Astra 6.0.1.7 software package is set to collect and extrapolate data with the aim to obtain for each elution volume the molecular weight and the gyration radius. With an integration of the peak, we calculated the number (Mn) and weight (Mw) average molecular weight and the z-average gyration radius.

The differential viscometer detector permits to obtain for each elution fraction the intrinsic viscosity. An integration of the peak gives the average intrinsic viscosity, which allowed us to obtain the average hydrodynamic volume (V_h) using the Einstein - Simha equation:

$$V_h = [\eta] M / \nu NA$$

Where N_A is Avogadro's number, M is the molar mass, $[\eta]$ is the intrinsic viscosity (g/mL), and v is a conformational parameter that takes the value of 2.5 in the case of a spherical conformation.

5. Antioxidant activity of fucoidan (FCS)

5.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The free radical scavenging effect of FCS was estimated according to the method of Qiao et al. [25]. Briefly 1 mL of the sample was mixed with 0.2 mL of methanolic solution of DPPH (400 μ M), and then 2.0 mL of water was added. The reaction mixture was vortexed and incubated for 30 min at room temperature. The absorbance of the solution was measured at 517 nm. Ascorbic acid (AA) was used as standard. The inhibitory percentage of DPPH was calculated using the following equation:

DPPH Scavenging effect (%) = (1- (Abs _{sample}/Abs _{control})) x 100

5.2. Reducing power

The reducing power was determined according to the method of Oyaizu [26]. 1 mL of FCS solution at different concentrations (1–10 mg/mL) was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide, and the mixture was incubated at 50 °C for 20 min. Thereafter, 2.5 mL of 10% trichloroacetic acid was added and the mixture was centrifuged for 10 min. The upper layer (5 mL) was mixed with 5 mL of deionized water and 1 mL of 0.1% ferric chloride, and the absorbance was measured at 700 nm against a blank. A higher absorbance indicates a higher reducing power. AA was used for comparison. *5.3. Chelating ability on ferrous ions*

Chelating ability was determined according to the method of Dinis et al. [27]. 1 mL of FCS at different concentrations (1–10 mg/mL) was mixed with 3.7 mL of methanol and 0.1 mL of 2 mM ferrous chloride. The reaction was initiated by the addition of 0.2 mL of 5 mM ferrozine. After 10 min at room temperature, the absorbance of the mixture was determined at 562 nm against a blank. A lower absorbance indicates a higher chelating ability. Citric acid (CA) and EDTA were used for comparison. The percentage of inhibition of ferrozine-Fe²⁺ complex formation was calculated using the following equation:



Species	Ash (%)	Proteins (%)	Lipids (%)	Sugars ^a (%)
Cystoseira sedoides	28	0.5	3.1	68.4
Ascophyllum nodosum ^b	22.5	1.2	1.2	96.6
Saccharina longicruris ^b	27.7	12.4	2.1	57.8

Table I: Initial composition of crude seaweed given in % of dry weight, in comparison with those of others species

^{*a*}: Sugars content were obtained by difference, since no official method exists.

^b Data from Rioux et al. [30]

Metal chelating effect (%) = (1-(Abs _{sample}/Abs _{control})) x 100

6. Gastroprotective activity of sodium alginate (ACS)

- Animals

The activity test was performed according to the guidelines established by the European Union on Animal Care (CCE Council 86/609). Wistar rats (200-250 g) of both sexes purchased from Pasteur Institute (Tunis, Tunisia) were used. They were housed in groups of eight to ten animals in plastic cages at 20-25°C and maintained on a standard pellet diet with free access to water. Animals were fasted for 24 h before the experiment.

7. Experimental protocol

The gastroprotective activity of ACS was studied in HCl/EtOH induced gastric ulcer [28]. Rats were divided into different groups, fasted for 24 h prior receiving an intraperitoneal (i.p.) injection of ACS (25 or 50 mg/kg). Two other groups received Ranitidine (60 mg/kg, i.p.) and Omeprazole (30 mg/ kg, i.p.) as reference drugs. After 30 min, all groups were orally treated with 1 mL/100 g of 150 mM HCl/EtOH (40:60, v/v) solution for gastric ulcer induction. Animals were sacrificed 1 h after the administration of ulcerogenic agent; their stomach were excised and opened along the great curvature, washed and stretched on cork plates. The surface was examined to detect the presence of lesions and to measure their extent. The summative length of the lesions along the stomach was recorded (mm) as lesion index.

8. Statistical analysis

Statistical analyses were performed with SPSS ver.2.0, professional edition using ANOVA analysis. Differences were considered significant at p < 0.05.

RESULTS AND DISCUSSION

The chemical composition of crude seaweed is presented in (Table I). The high proportion of minerals observed in *C.sedoides* can be explained by the presence of inorganic salt in the water absorbed by the seaweed or by the association of cations with algal polysaccharides [29]. The obtained amounts of ash, proteins and lipids are consistent with those obtained by Rioux et al for other species [30].

1. Chemical analyzes of extracted polysaccharides *1.1.* Fucoidans

The main concern in the isolation procedures of fucoidans was to avoid their contamination with

Table II: Extraction yield and carbohydrates analysis of the extracted polysaccharides

Samples	Yield [*] (%)	Total sugar (%)	Uronic acids (%)	Sulphates (%)	Fucose (%)	SO ₃ / Fucose
FCS	4.2	51.3	7.6	15.5	17.6	0.9
ACS	11	69.3	-	-	-	-

*Yield of extraction is given in % of dry weight.





Figure 1: Infrared spectrum of the FCS.

other polysaccharides, like laminaran and especially alginic acid. The hot extraction, in the presence of $CaCl_2$ separates the insoluble calcium alginate from the soluble fucoidans. The yield and the chemical composition of the sulfated polysaccharides isolated from *C. sedoides* are presented in (Table II.). The extraction yield of FCS was 4.2 % (w/w) (on the basis of dried defatted seaweed weight). The isolated fucoidan consisted of total sugar (51.3%), uronic acids (7.6%), sulfates (15.5%) and fucose (17.6%). The sulfate-fucose ratio was about 0.9, this

may suggest that there is only one sulfate group per unit of fucose.

The FT-IR spectrum of FCS is shown in (Figure 1). The sample displayed characteristic absorption bands of sulfated polysaccharides [31]. The well-defined peak at 1251 cm⁻¹ was attributed to the presence of sulphate ester groups (S=O) [32] and the sharp band at 822 cm⁻¹ (C-S-O) suggested that the majority of sulphate groups occupy positions 2 and/or 3 (equatorial positions) [33]. Further absorption peaks are given in (Table III).

SEC/MALS/VD/DRI experiments were carried out in 0.1 mol/L LiNO₃ to determine molecular weights and size information of biopolymers studied. Mn, Mw, polydispersity index (Đ), (R_h) and [η] are summarized in (Table III).The molecular weight of fucoidans extracted from *C. sedoides* was about 280000 g/mol. Moreover, this fraction is characterized by its heterogeneity and low viscosity which suggest a compact spherical conformation.

1.2. Sodium alginate

Sodium alginate was isolated from the brown alga *C. sedoides* with a yield of 11%. This result was higher than that registered for *Cystoseira* barbata (9.9%) [15] and *Dictyota caribaea* (7.4%)

Samples	Wave number (cm ⁻¹)	Peak assignments	
FCS	3414	O-H assoc. stretching vibration	
	2924	C-H stretching vibration	
	1729	(C=O) stretching vibration of O-acetyl groups	
	1630	O-C-O asymmetric stretching vibration of carboxylate	
	1418	asymmetrical bending vibration of CH ₃	
	1251	S=O stretching vibration	
	822	C–O–S vibration	
ACS	ACS 3451 O-H assoc. stretching v		
	2927	C-H stretching vibration	
	1611	O-C-O asymmetric stretching vibration of carboxylate	
	1418	C-OH deformation vibration	
	1100	C-O stretching vibration of pyranosyl ring	
	953	C-O stretching vibration of uronic acid residues	
	887	(C1-H) deformation vibration of β -mannuronic acid	

Table III: The most diagnostic peaks in the IR spectra of extracted polysaccharides (FCS and ACS)

Did (0.1 mole Envo3)					
Samples	Mn (g/mol)	Mw (g/mol)	Ð (Mw/Mn)	$R_{\rm H}$ (nm)	[η] (mL/g)
FCS	45000	280000	6.2	7.2	27
ACS	64000	140000	2.2	9.6	51

Table IV: Average macromolecular characteristics of the extracted polysaccharides determined by SEC/MALS/VD/ DRI (0.1 mol/L LiNO₃)

[34]. Otherwise, it is much lower compared to the yields reported by Rioux et al. [30] (*Ascophyllum nodosum* (24%), *Fucus vesiculosus* (16%) and *Saccharina longicruris* (20%)). However, the extraction yields crucially depend on the algal species and the extraction method.

Assay of uronic acids was performed and the result confirms that the extracted polysaccharide was a polymer of uronic acids.

The FT-IR spectrum of ACS is presented in (Figure 2). The characteristic bands are summa-rized in (Table III). The sample displayed a broad stretching intense characteristic band at 3451 cm⁻¹ attributable to the stretching vibration of hydroxyl group and a weak band at 2927cm⁻¹ due to (C-H) stretching vibrations. When the proton is displaced by a monovalent ion (sodium), band appear at approximately 1600 and 1400 cm⁻¹, respectively, and are assigned to asymmetric and symmetric stretching vibration of free carboxyl group of sodium alginate [35]. The same spectrum shows a band at 953 cm^{-1} , which was assigned to the C-O stretching vibration of uronic acid residues [36], and another one at 887 cm⁻¹ attributable to the (C1-H) deformation vibration of β -mannuronic acid residues [37].



Figure 2: Infrared spectrum of the ACS.

Futhermore, FT-IR spectroscopy has proven being useful for quantitative estimation of the M/G ratio of alginates. Pereira et al. [35] has reported that the ratio of absorption band intensities at approximately 1100 and 1025 cm⁻¹, which were attributed to mannuronic and guluronic units, respectively, gave a fairly good estimation of the M/G ratio.

Alginates with low M/G ratio (<1) correspond to higher values of guluronic (G) than mannuronic acid blocks (M) forming strong and rigid gels, whereas high M/G ratio (>1) is related to low values of guluronic acid blocks producing soft and elastic gels [38]. This alginate heterogeneity provides the versatility for many food and non food industrial applications.

The M/G ratio of ACS, from the relative intensity ratio of the 1100 and 1027 cm⁻¹ bands, was 1.67. So the extracted alginate is composed of 63% mannuronic acid and 37% of guluronic acid.

Mn and Mw were determined for ACS as 64000 g/mol and 140000 g/mol, respectively (Table III). These values are in good agreement with the results recorded by Turquois and Gloria [39] with a molecular weight between 115000 and 321000 g/ mol for commercial alginates. The intrinsic viscosity ($[\eta] = 51 \text{ mL/g}$) and the hydrodynamic radius ($R_{\rm H}$ = 9.6 nm) obtained for the extracted sodium alginate suggest a random coil conformation. Besides, the polydispersity index (Đ) was 2.2, indicating that this polymer was homogeneous and that there is not much degradation during the extraction-purification steps adopted. From the data obtained, both the weightaverage degree of polymerization and the numberaverage degree of polymerization were determined as $DP_w = 707$ and $DP_n = 323$.

2. Antioxidant activity of FCS

2.1. DPPH radical scavenging assay

DPPH has been widely used as a free radical to examine reducing substances and is a useful

reagent for investigating the free radical scavenging activities of compounds [40]. The free radical scavenging activity of FCS was tested through the DPPH method and the results were compared with ascorbic acid (AA) used as positive control. As shown in (Figure 3A), the sample exhibited a concentration-dependent antiradical activity. AA showed a higher degree of free radical scavenging activity than the tested fucoidan at the same concentrations used. At 5 mg/mL, FCS exerted a significant free radical scavenging activity (77%), but remains lower than that of AA (92%) at the same concentration. In fact, the DPPH scavenging activity has been reported to be related to several mechanisms including chain inhibition, prevention of continued hydrogen abstraction, donating hydrogen, and radical scavenging [41]. In our opinion, FCS showed proton-donating ability, served as free radical inhibitors or scavengers, and possibly acted as primary antioxidants.

2.2. Reducing power

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Compounds with reducing power are electron donors that can reduce the oxidized intermediates of lipid peroxidation processes and thereby act as primary and secondary antioxidants [42]. Accordingly; reducing power assay was an important method to test drugs of antioxidant activity. In the reaction system, antioxidant components in samples cause reduction of Fe^{3+} /ferricyanide complex to the Fe^{2+} form, and Fe^{2+} can be monitored by measuring the formation of Prussian blue at 700 nm. As reported in (Figure 3B), the reducing power of FCS increased with increasing concentrations (1-10 mg/ mL). At 10 mg/mL, this polysaccharide exerted a significant reducing activity (OD at 700 nm = 1.6). However, this activity is lower than the reducing activity of AA at concentrations between 1 and 10 mg/mL. The obtained results demons-trated that FCS can act as electron donors and can react with free radicals to convert them to more stable products and thereby terminate radical chain reactions.

2.3. Chelating ability on ferrous ions

Transition metals have been implicated in the propagation of the radical chain reaction of lipid peroxidation. Chelating agents may inhibit lipid oxidation by stabilizing transition metals. Ferrozine can quantitatively form complexes with Fe^{2+} . In the presence of chelating agents, the

complex formation is disrupted, resulting in a decrease in the red color of the complex. Measurement of color reduction allows, therefore, estimating the metal chelating activity of the coexisting chelator [43]. As shown in (Figure 3C), the chelating activity of FCS increased in a dosedependent manner, while at all concentrations used, the activities were lower than citric acid and

EDTA. At a concentration of 10 mg/mL, the

Each value is presented as mean \pm SD (n = 3).

(A) DPPH radical scavenging activity

(AA: ascorbic acid as reference).

(B) Fe^{2+} reducing power (AA as reference).

(C) Ferrous ion-chelating activity (CA: citric acid as reference; EDTA as reference).





Yang et al. [44] demonstrated that the chelating activity of fucoidans is closely related to their sulfation content. Indeed it has been reported that the sulfation of polysaccharides increases their chelating activities. Results revealed that the FCS had an effective capacity for iron binding. It also suggests that its action as antioxidant might be related to its iron-binding capacity [45].

3. The Effect of ACS on Ethanol-Induced Gastric Damage

Gastric ulcer is a chronic disease that affects a considerable number of people worldwide. The proton-pump inhibitors (PPI) are the most commonly prescribed class of drugs to treat heartburn and acid-related disorders. However, like all drugs, PPIs have adverse effects, such as increasing the potential risk of malabsorption of minerals in the body, like calcium and magnesium, in addition to the increased risk of infections. Moreover, long-term use of these drugs may be accompanied with inefficiency of different drug regimens and even with inadequacy. Thus, it is urgent to find protective gastrointestinal agents that they are safer and more effective. Several experimental and clinical studies have shown that phycocolloids with interesting physical properties have a strong anti-ulcer effect against gastric lesions in rats.

In this study, the gastroprotective activity of sodium ACS was studied in HCl/EtOH induced gastric ulcer. Oral administration of the damaging agent to the control group clearly produced a mucosal damage characterized by multiple hemorrhage red bands of different sizes along the long axis of the glandular stomach.

As presented in (Table V), ACS showed dose related gastroprotective activity. At doses of 25 and 50 mg/kg, it significantly inhibited gastric ulcer induced by the necrotizing agent HCl/EtOH compared to control group. The percentages of inhibition of ulcer were 75% and 78%, respectively. These percentages are higher than those of both reference drugs Omeprazole (66.45%) and Ranitidine (50.58%). These results suggest that alginate acts on the pH of gastric juice: its alkaline pH replaces the acidic pH of gastric juice.

Samples	Dose (mg/kg)	Average lesion (mm)	Ulcer inhi- bition (%)
Control	-	52 ± 2.5	-
Omeprazole (reference)	30	17.66 ± 2.4	66.45± 4.6
Ranitidine (reference)	60	28.16± 4.8	50.58± 8.4
105	25	13.16±2.4***	75± 4.7
лсь	50	6.83 ± 2.6 ***	87± 5

Table V: Results of antiulcerogenic activity of sodium alginates isolated from *C. sedoides* on gastric ulcer induced by HCl/ethanol solution.

Data are expressed as mean \pm s.e.m. (n = 6). ***p < 0.001.

CONCLUSION

Fucoidan and sodium alginate were isolated from Tunisian brown seaweed *C.sedoides*, with good yields. The samples were characterized by chromatographic (SEC/MALS/VD/DRI), spectroscopic (FTIR) and colorimetric methods; the extracted fucoidan FCS consists of total sugar (51.3%), uronic acid (7.6%), fucose (17.6%) and sulfates (15.5%). Its average molar weight was equal to 280000 g/mol. The value of the M/G ratio of alginate was estimated by FTIR. Our findings showed that alginate contain more mannuronic acid (63%) than guluronic acid (37%), such alginate is used to form elastic gels.

Besides, the current study demonstrated that FCS exerted considerable antioxidant action involving several antioxidant mechanisms, including, metal ion chelating and hydrogen or electron donation. The sulfate and/or acetyl groups may be responsible for the overall antioxidant activity of FCS. As for sodium alginate, it showed an interesting gastroprotective effect using HCl/ EtOH induced gastric ulcers.

REFERENCES

- J. S. Yang, Y. J. Xie, W. He, *Carbohyd. Polym.*, 2011, 84, 33.
- [2] M. Fertah, A. Belfkira, El.M. Dahmane, M. Taourirte, F. Brouillette, *Arab. J. Chem.*, doi:10.1016/j.arabjc.2014.05.003.
- [3] X. X. Liu, L. Y. Qian, T. Shu, Z. Tong, *Polymer*, 2003, 44(2), 407.



- I. Donati, S. Holtan, Y. A. Mørch, M. Borgogna, M. Dentini, *Biomacromolecules*, 2005, 6(2), 1031.
- [5] I. A. Brownlee, A. Allen, J. P. Pearson, P. W. Dettmar, M. E. Havler, M. R. Atherton, E. Onsøyen, *Crit. Rev. Food Sci.*, **2005**, *45(6)*, 497.
- [6] E. Josef, M. Zilberman, H. Bianco-Peled, Acta Biomaterialia., 2010, 6(12), 4642.
- [7] Y. Li, M. Hu, Y. Du, H. Xiao, D. J. McClements, Food Hydrocolloid., 2011, 25(1), 122.
- [8] T. Nagumo, T. Nishino, Fucan Sulfates and Their Anticoagulant Activities, pp. 545–574, In Polysaccharides in Medicinal Applications, S. Dumitriu editor, Marcel Dekker, New York, 1996.
- [9] L. Chevolota, A. Foucaulta, F. Chaubetb, N. Kervarecc, C. Sinquina, A. M. Fisherd, C. Boisson -Vidal, *Carbohyd. Res.*, **1999**, *319*, 154.
- [10] H. Itoh, H. Noda, H. Amano, C. Zhuang, T. Mizuno, H. Ito, *Anticancer Res.*, **1993**, *13*, 2045.
- [11] V. Pandian, V. Noormohamed, T. Ganapathy, *Chin. J. Nat. Med.*, **2012**, *10(6)*, 421.
- [12] H. Shibata, I. Kimura-Takagi, M. Nagaoka, S. Hashimoto, R. Aiyama, M. Iha, S. Ueyama, T. Yokokura, *BioFactors*, 2000, 11, 235.
- [13] O. Berteau, B. Mulloy, *Glycobiology.*, **2003**, *6*, 29.
- [14] J. H. Fitton, D. N. Stringer, S. S. Karpiniec, Mar. Drugs, 2015, 13(9), 5920.
- [15] S. Sellimi, I. Younes, H.B. Ayed, H. Maalej, V. Montero, M. Rinaudo, M. Dahia, T. Mechichi, M. Hajji, M. Nasri, *Int. J. Biol. Macromol.*, 2015, 72, 1358.
- [16] H. H. Ammar, S. Lajili, R. Ben Said, D. Le Cerf4,
 A. Bouraoui, H. Majdoub, DARU J. Pharm. Sci.,
 2015, 23(1), 1.
- [17] N. M. Ponce, C. A. Pujol, E. B. Damonte, M. L. Flores, C. A. *Carbohyd. Res.*, **2003**, *338*, 153.
- [18] AOAC (1990). Officials Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Washington, DC.
- [19] L. You, Q. Gao, M. Feng, B. Yang, J. Ren, L. Gu, C. Cui, M. Zhao, *Food Chem.*, **2013**, *138*, 2242.
- [20] T.T.T. Thanh, V. T. T. Tran, Y. Yuguchi, L. M. Bui, T. T. Nguyen, *Mar. Drugs.*, **2013**, *11(7)*, 2431.
- [21] P.Saboural, F. Chaubet, F. Rouzet, F. Al-Shoukr, R. Ben Azzouna, N. Bouchemal, L. Picton, L. Louedec, M. Maire, L. Rolland, G. Potier, D. Le Guludec, D. Letourneur, C. Chauvierre, *Mar. Drugs*, 2014, *12(9)*, 4851.
- [22] T. Eluvakkal, N. Shanthi, M. Murugan, K. Arunkumar, Indian J. Nat. Prod. Resour., 2015, 5 (3), 249.

- [23] H. Majdoub, M.S. Roudesli, L. Picton, D. Le Cerf, G. Muller, M. Grisel, *Carbohyd. Polym.*, 2001, 46, 69.
- [24] H. Majdoub, S. Roudesli, A. Deratani, *Polym. Int.*, 2001, 50, 552.
- [25] D. L. Qiao, C. L. Ke, B. Hu, J. Luoa, H. Yea, Y. Suna, X. Yana, X. Zeng, *Carbohyd. Polym.*, 2009, 78, 199.
- [26] M. Oyaizu, Jap. J. Nutr., 1986, 44, 307.
- [27] T. C. P. Dinis, V. M. C. Madeira, L. M. Almeida, *Arch. Biochem. Biophys.*, **1994**, *315*, 161.
- [28] N. Hara, S. Okabe, Folia Pharmacol. Jpn., 1985, 85, 443.
- [29] M. Lahaye, J. Sci. Food Agr., 1991, 54, 587.
- [30] L. E. Rioux, S. L. Turgeon, M. Beaulieu, *Carbohyd. Polym.*, **2007**, *69*, 530.
- [31] F. Cabassi, B. Casu, A.S. Perlin, *Carbohydr. Res.*, 1978, 63, 1.
- [32] Y. Lijour, E. Gentric, E. Deslandes, J. Guezennec, *Anal. Biochem.*, **1994**, *220*, 244.
- [33] M. F. Marais, J. P. Joseleau, Carbohyd. Res., 2001, 336, 155.
- [34] V. Garcia-Rios, E. Rios-Leal, D. Robledo, Y. Freile-Pelegrin, *Phycol. Res.*, 2012, 60, 305.
- [35] L. Pereira, A. Sousa, H. Coelho, A. M. Amado, P. J. Ribeiro-Claro, *Biomol. Eng.*, 2003, 20, 223.
- [36] N. P. Chandía, B. Matsuhiro, E. Mejías, A. Moenne, J. Appl. Phycol., 2004, 16, 12
 7.
- [37] M. Mathlouthi, J. L. Koening, *Adv. Carbohyd. Chem. Bi.*, **1986**, *44*, 87.
- [38] J. I. Murillo-Alvarez, G. Hernandez-Carmona, J. Appl. Phycol., 2007, 19, 545.
- [39] T. Turquois, H. Gloria, J. Agric. Food Chem., 2000, 48, 5455.
- [40] X. J. Duan, W. W. Zhang, X. M. Li, B. G. Wang, Food Chem., 2006, 95, 37.
- [41] H. Yu, X. Liu, R. Xing, S. Liu, C. Li, P. Li, Bioorg. Med. Chem. Lett., 2005, 15, 2659.
- [42] X. Du, H. Mu, S Zhou, Y. Zhang, X. Zhu, Int. J. Biol. Macromol., 2013, 62, 691.
- [43] L. S. Costa, G. P. Fidelis, S. L. Cordeiro, R. M. Oliveira, D. A. Sabry, R. B. Câmara, L. T. Nobre, M. S. Costa, J. Almeida-Lima, E. H. Farias, E. L. Leite, H. A. Rocha, *Biomed. Pharmacother.*, 2010, 64, 21.
- [44] X. B. Yang, X. D. Gao, F. Han, R. X. Tan. Biochim. Biophys. Acta., 2005, 1725, 120.
- [45] A. Zhang, N. Xiao, P. He, P. Sun, Int. J. Biol. Macromol., 2011, 49, 1092.