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Chemical and Biological Properties of Sodium Alginates Isolated from Tow Brown Algae *Dictyopteris Membranaceae* and *Padina Pavonica*

Monia Deghrigue Abid[1,*], Sirine Lajili[1], Hiba Hadj Ammar[2], Dora Cherif[1], Nejeh Eltaief[1], Hatem Majdoub[2], Abderrahman Bouraoui[1]

[1]Laboratoire de Développement des Médicaments, Faculté de Pharmacie de Monastir, Université de Monastir, 5000, Monastir, Tunisia

[2]Laboratoire des Interfaces et des Matériaux Avancés (LIMA), Faculté des Sciences de Monastir, Université de Monastir, 5019, Monastir, Tunisia

*Corresponding author: Monia Deghrigue Abid (monia.deghrigue@laposte.net)

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Abstract Polysaccharides are known to have interesting biological activities. To date polysaccharides extracted from Tunisian seaweed have not been fully studied. In this paper we tried to isolate sodium alginate from two brown algae and evaluate their biological activities. Two brown seaweeds *Dictyopteris membranaceae* and *Padina pavonica* were treated with selective solvents to extract sodium alginate. Analyses were performed to determine their IR spectra, uronic acid's content and biological properties (antioxidant and gastroprotective activities). Results showed that sodium alginate extracted from *D. membranaceae* contained 65% of uronic acid while this extracted from *P. pavonica* contained only 9% of uronic acid. These polysaccharides showed also variation in the structure and the activities. Sodium alginate extracted from *D. membranaceae* had the highest antioxidant activity with ED_{50} of 20 µg/ml in the DPPH test. Additionally, this polysaccharide had the most important gastroprotective activity with a percent of 97% at dose 50mg/kg. Our finding suggested that sodium alginates extracted from *D. membranaceae* and *P. pavonica* could be used as a natural source of antioxidant and gastroprotective agents.

Keywords: *Sodium alginates, Brown seaweeds, DPPH, Uronic acid*

1. Introduction

Gastric ulcers associated with ethanol consumption remain a major health problem. In effect, excessive ethanol ingestion has been revealed to produce gastric damage characterized by mucous membrane edema, sub epithelial haemorrhages and inflammatory cells infiltration [1]. Free radical generation has been considered as a crucial step in ethanol induced mucosal damage [2]. Therefore, administration of antioxidants could reduce the severity of gastric ulcer. Recently, marine algae have been the subject of a lot of research in order to obtain compounds able to inhibit gastric damage. Several studies reported that marine algae are rich sources of structurally diverse bioactive compounds with valuable pharmaceutical and biomedical benefits [3]. Recent research has pointed out that sulfated polysaccharides isolated from brown algae possess antitumor, anti-mutagenic, hypoglycemic, antiviral and anti-inflammatory activities [4,5]. Among them, alginates, linear anionic polymers were commercially used as food additives (thickening, stabilizers and gelling agents), textile printing, and in cosmetics and

pharmaceutical areas [6]. Alginates are mainly composed by two monomeric units: β -D- mannuronic acid (M) and α- L-glucuronic acid (G) linked by a 1,4- glycosidic bond [7]. The structure and properties of alginates depends on the M/G ratio varying with the seaweed species [8]. Generally, alginates could be found as sodium, magnesium or calcium salts. However, the alginate of greatest industrial importance is the sodium salt [9]. Therefore, due to their biodegrability and low toxicity, our research group is interested to isolate natural bioactive polymers from Tunisian coasts. In this paper, we will focus our attention in the chemical and biological (antioxidant and gastroprotective) properties of sodium alginates, sulfated polysaccharides, purified from the Mediterranean seaweeds *Dictyopteris membranaceae* and *Padina pavonica*.

2. Materials and Methods

2.1. Sample collection

Dictyopteris membranaceae and *Padina pavonica* were collected from the Mediterranean Sea in various areas of the coastal region of Monastir (Tunisia), in June 2011, at a depth between 1 and 2 m. Identification of specimens was carried out in the National Institute of Marine Sciences and Technologies (Salamboo, Tunisia).

2.2. Extraction of sodium alginates

50 g of each brown algae powder was macerated with methanol and dichloromethane (1:1, v/v) for 48h three times. The obtained organic extract was concentrated to solvent free by evaporation in a rotating evaporator (Buchi, B- 480) at 40 ℃. After, a sequential extraction of seaweed's powders was carried out with petroleum ether then acetone in a soxhlet apparatus to remove lipophilic pigments and low molecular weight proteins. Depigmented dried seaweeds were treated three times with 2% aqueous solution of $CaCl_2$ during 3 hours, in order to precipitate calcium alginates. The precipitate was treated with aqueous solution of Na_2CO_3 (1M) for 2h and was filtered. Sodium alginates were precipitated by the addition of ethanol then were lyophilized [10,11].

2.3. Chemical composition

Uronic acids were determined using carbazole method [12] and glucuronic acid as standard. FTIR were performed 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}.

2.4. DPPH radical scavenging activity

The antioxidant activity of sodium alginates of *Dictyopteris membranaceae* and *Padina pavonica* were evaluated using the stable radical DPPH, according to the method of Kim et al. [13]. One milliliter of diluted sample (1mg/ml) was added to 1 ml of the ethanolic DPPH solution. The mixture was then shaken and allowed to stand at room temperature in the dark. After 30 min, the decrease in absorbance at 517 nm was measured against a blank (ethanol solution) by using a UV–Vis spectrophotometer. A mixture consisting of 1 ml of ethanol and 1 ml of DPPH solution was used as the control. The radical-scavenging activity of test samples, expressed as percentage inhibition of DPPH, was calculated according to the formula: % inhibition = $[(A_B \_ A_A)/A_B] \times 100$, where A_B and A_A are the absorbance values of the control and of the test sample, respectively. The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage plotted against test samples concentration. DPPH radical-scavenging activity of sodium alginates isolated from *Dictyopteris membranaceae* and *Padina pavonica* were compared with ascorbic acid used as standard.

2.5. Pharmacology

2.5.1. Animals

Wistar rats of either sex, weighing 150-180 g were purchased from Pasteur Institute (Tunis, Tunisia). Housing conditions and in vivo experiments were approved according to the guidelines established by the European Union on Animal Care (CCE Council 86/609).

2.5.2. Gastroprotective activity

The gastroprotective activity of sodium alginates of *Dictyopteris membranaceae* and *Padina pavonica* was evaluated by the HCl/EtOH method which induced gastric ulcer [14]. Rats fasted for 24 h were divided into eight groups. The control group received an intraperitoneal dose of saline solution (NaCl 9g/l, 2.5 ml/kg), the test groups sodium alginates of each alga (25 and 50 mg/kg, i.p.), the reference groups received ranitidine (60 mg/kg, i.p.) and omeprazol (30 mg/kg, i.p.) as reference drugs. After 30 min, 1ml/100g of 150 mM HCl/EtOH solution were orally given to all groups. Animals were sacrificed 1 h after ulcerogenic agent administration and their stomachs were removed and opened along the great curvature for ulcer lesions estimation. The lesion index defined as the summative length of the lesions along the stomach was determined.

2.6. Statistical analysis

Data are presented as the mean ± standard error of the mean (s.e.m). Statistical analysis was performed using Student's *t*-test. The significance of difference was considered to include values of $P<0.05$.

3. Results and Discussion

3.1. Chemical analysis

Our results revealed that *P. pavonica* is rich of sodium alginate with an extraction yield of 66.72 %. However, *D. membranaceae* showed an extraction yield of only 18.93 %. In addition, the amount of uronic acid varied between the two algae. While sodium alginate isolated from *D. membranaceae* had an amount of uronic acid of 65.6%, uronic acid present in sodium alginate from *P. pavonica* was only 9.7 % (Table 1). These results were in agreement with other seaweed specie (*Saccharina longicruris*) where the amount of uronic acid was 7.5% [15].

Table 1. Yields of extraction of *D. membranaceae* and *P. pavonica* sodium alginates and percentages of uronic acid

Sample		Yield* (%)	Uronic acid (%)
Sodium alginate	*D. membranaceae*	18.93	65.6
	P. pavonica	66.72	9.7

*Yields of extraction given in % of dry weight.

The FTIR spectrums of the isolated polysaccharides showed typical absorption bands of sodium alginate. Their exact absorption peaks are given in Table 2. The intensity of the bands at 3440-3437 cm^{-1} assigned to hydrogen bounded O-H stretching vibration, the weak signal of the bands at 2940-2925 cm^{-1} is due to CH stretching vibration, and the stretching vibration of C=O is centered at 1684-1642 cm^{-1}. The bands at 1461-1447 cm^{-1} were attributed to the C-O stretching frequency and the absorption at 1062-1039 cm^{-1} corresponded to the C-O-C frequency of the glycosidic bonds [16].

Table 2. The most diagnostic peaks in the IR spectra of extracted polysaccharides

Sodium alginates		Assignment
D. membranaceae	***P. pavonica***	
3440 cm^{-1}	3437 cm^{-1}	O-H stretching vibration
2925 cm^{-1}	2940 cm^{-1}	C-H stretching vibration
1642 cm^{-1}	1684 cm^{-1}	C=O stretching vibration
1447 cm^{-1}	1461 cm^{-1}	C-O stretching vibration
1039 cm^{-1}	1062 cm^{-1}	C-O-C stretching vibration

3.2. DPPH radical scavenging activity

DPPH is a stable free radical. The presence of antioxidant agents could be revealed by the decrease of the intensity of purple color typical of the free DPPH radical [17].

Sodium alginates purified from *D. membranaceae* and *P. pavonica* were very potent radical scavengers. They were able to reduce the stable radical DPPH to the yellow colored diphenylprilhydrazine and the EC50 defined as the concentration of sample at which the inhibition percentage reaches 50% were calculated and presented in (Table 3). Sodium alginate from *D. membranaceae* showed significant DPPH radical scavenging activity with an EC50 value of 20 µg/ml. In addition, sodium alginate purified from *P. pavonica* demonstrates also significant antioxidant activity with EC50 value of 22 µg/ml. These scavenging activities were found significantly similar to the activity of ascorbic acid (EC50=16 µg/ml), under the same experimental conditions (Figure 1).

Table 3. EC_{50} values of sodium alginates extracted from *D. membranaceae* and *P. pavonica* in radical scavenging activity

	Ascorbic acid	*D. membranaceae*	*P. pavonica*
EC_{50}* µg/ml	16 ±0.1	20 ±0.1	22 ±0.1

Values are expressed as mean ± S.D of triplicate measurement.

*EC_{50} means the concentration of sample that can decrease DPPH concentration by 50%.

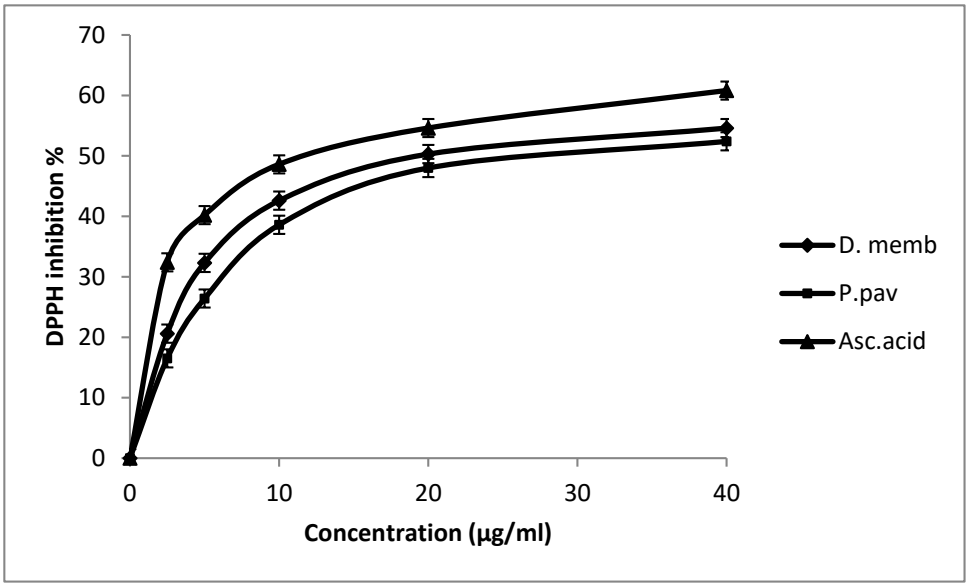

Figure 1. Free radical-scavenging activity of *D. membranaceae* (D.memb) and *P. pavonica* (P.pav) sodium alginates and ascorbic acid (Asc.acid) on DPPH.

3.3. Gastroprotective activity

In the recent years, a search has focused to identify new anti-ulcer drugs from natural sources. In the present study, the gastroprotective activity of sodium alginates isolated from *D. membranaceae* and *P. pavonica* were evaluated by gastric lesions induced by oral administration of HCl/EtOH in rats. As described by Salim [18], ethanol disturbs gastric secretory activity and depletes gastric mucus. In addition, the necrotizing effect of ethanol is associated with the production of free radicals and severe hemorrhage [19]. HCl causes gastric mucosal damage [20]. The administration of HCl/EtOH to untreated rats produced gastric ulcer with lesion index of 62.4 mm. Pretreatment of rats by *D. membranaceae* and *P. pavonica* sodium alginates produced significant decrease in the intensity of gastric mucosal damage in a dose dependent manner . *D. membranaceae* sodium alginate showed the highest activity. The lesion index was inhibited by 85.38 and 97% at doses of 25 and 50 mg/kg, respectively. Sodium alginate from *P. pavonica* at the doses of 25 and 50 mg/kg produced significant protective effect against gastric damage induced by the necrotizing agent HCl/EtOH. The percentages of inhibition of gastric lesions ranged from 80.62% at the dose 25 mg/kg to 88.24 % at the dose 50 mg/kg. These results were compared to the gastroprotective activity of two reference ulcer drugs ranitidine, histamine H_2 receptor antagonist, and omeprazole, a proton pump inhibitor [21]. The percentages of inhibition of gastric lesions of *D. membranaceae* and *P.*

pavonica sodium alginates depassed the activity of ranitidine (66%) while these percentages approached the percentage of omeprazole (84.37%) (Table 4).

Table 4. Effect of *D. membranaceae* and *P. pavonica* sodium alginates , and of reference drugs (ranitidine and omeprazol) on gastric ulcer induced by HCl/ethanol in rats

Treatment	Dose (mg/kg)	Ulcer index (mm)	Ulcer inhibition (%)
Vehicle	-	62.4 ±2.3	-
***D. membranaceae* sodium alginate**	25	9.12 ±2.24**	85.38
	50	1.83 ±2.58**	97.07
***P. pavonica* sodium alginate**	25	12.1 ±2.48**	80.62
	50	7.33 ±2,52**	88.24
Ranitidine	60	21.2 ±2.3**	66.02
Omeprazol	30	9.75 ±1,62**	84.37

Values are expressed as mean ± SEM; n=6 animals. **P<0.001.

Some reports on the gastroprotective effect of polysaccharides from brown algae are published [22]. Rajaonarivony et al,[23] demonstrated that alginates isolated from the brown algae have the ability to form viscous solutions and gels. Therefore, the gastroprotective activity of sodium alginates purified from *D. membranaceae* and *P. pavonica* may be mediated via its gel-formation property which causes the adherence to the epithelial cells and the protection of the gastric mucosa. Furthermore, the role of reactive oxygen species (ROS) has been involved also in the pathogenesis of experimental gastric lesions induced by ethanol [24]. Interestingly, *D. membranaceae* and *P. pavonica* sodium alginates were found to possess an antioxidant activity when tested in DPPH assay. It is therefore possible that the gastroprotective activity of sodium alginates purified from *D. membranaceae* and *P. pavonica* is mediated via its antioxidant activity.

5. Conclusion

The chemical study of sodium alginates extracted from the two brown seaweeds *D. membranaceae* and *P. pavonica* demonstrated difference of the content of uronic acid between the two algae. Also the biological evaluation showed that sodium alginate purified from *D. membranaceae* was more potent than this extracted from *P. pavonica*. More investigations are needed to understand the mechanism of action of these polysaccharides in the ulcer disease.

Conflict of interest

The authors declare no conflict of interest.

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