Antimicrobial, antioxidant, cytotoxic and anticholinesterase activities of water-soluble polysaccharides extracted from microalgae *Isochrysis galbana* and N*annochloropsis oculata*

MHAMMED BEN HAFSA[1,2]*, MANEL BEN ISMAIL[3], MARIEM GARRAB[3], RAIES ALY[1], JONATHAN GAGNON[2] and KARIM NAGHMOUCHI[1]

[1]*Laboratoire des Microorganismes et Biomolécules Actives (LMBA), Faculté des Sciences de Tunis, Université El-Manar II 2092 El-Manar-II, Tunis, Tunisia,* [2]*Département de biologie, chimie et géographie, Université du Québec à Rimouski, 300 allée des Ursulines, Rimouski, Québec, G5L 3A1, Canada and* [3]*Laboratoire de Microbiologie, Faculté de Médecine, Université de Monastir, Monastir 5000, Tunisia*

Abstract: The present work is carried out to evaluate potential applications of aqueous extracts of two microalgae *Isochrysis galbana* (PEA) and *Nannochloropsis oculata* (PEB) containing mainly polysaccharides. The monosaccharide composition of microalgal extracts was determined. GC–MS analyses after derivatization show that glucose is the major compound in both microalgae PEA (56.88 %) and PEB (68.23 %). Mannitol (38.8 %) and inositol (20.32 %) are respectively the second major compounds in PEA and PEB. Silylation of monosaccharides allows the determination of sorbitol that attained 3.38 % in PEB. The determination of antioxidant, antimicrobial and cytotoxic properties were also analyzed. Antioxidant activity was evaluated from the DPPH scavenging activity. PEA and PEB show a concentration dependent DPPH·radical scavenging activity. At concentration of 10 mg/mL, both PEA and PEB exhibit an antioxidant activity of 41.45 and 59.07 %, respectively. PEB and PEA are able to inhibit the growth of Gram-negative bacteria, Gram-positive bacteria and three *Candida* species. Cytotoxic activity was evaluated on human HeLa cervical cancer cells. HeLa cell proliferation was totally inhibited after treatment with PEA and PEB (1 mg/mL) and the inhibition was dose dependent (from 0.031 to 1 mg/mL). Their anticholinesterase activity was also investigated against butyrylcholinesterase enzymes. These polysaccharides possess interesting antimicrobial, anticancer and anticholinesterase activities that could represent an additional value for these microalgal products.

Keywords: algae; DPPH; cytotoxic activity; antimicrobial activity; polysaccharides; GC-MS.

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INTRODUCTION

Algae represent a large diversity of species that are estimated from 40 000 to 10 million where the majority are microalgae.[1] Microalgae are eucaryotic photosynthetic organisms that play a key role in aquatic ecosystems and account for approximately 40 % of the global photosynthesis.[2] They possess some different morphological, physiological, and genetic traits that confer the ability to produce several biologically active metabolites.[3] Microalgae can yield a large pool of biomolecules with biological activities, such as carotenoids, phycobilins, polyunsaturated fatty acids, proteins, polysaccharides, vitamins, and sterols among other chemicals.[4] These microalgal molecules can possess several health benefits and therefore be used in many sectors such as nutraceutical, pharmaceutical and functional foods.

Besides, polysaccharides are polymeric carbohydrates, formed by repeating units joined together by glycosidic bonds. Recently, they have widely been investigated due to their chemical and biological activities.[5] Polysaccharides present a large diversity of structures attributable to their variety in composition, substitutions and glycosidic bonds. Polysaccharides isolated from plants, fungi, yeasts and algae have attracted considerable attention for their biological activities in biochemistry and medicine.[6] They exhibit a wide range of biological activities such as anti-inflammatory, antioxidant, antitumor, anticoagulant, antithrombotic, antimetastic, antiviral, antimicrobial and immunostimulatory.[7–10] Laminarin and fucoidan are polysaccharides isolated from cell walls of brown seaweeds that possess immunomodulatory, antitumor, antiviral and antioxidant activities.[11]

Isochrysis galbana and *Nannochloropsis oculata* are two marine microalgae that are produced industrially for aquaculture. They are important food source and feed additive that were widely used especially in the aquaculture industry.[12] *N. oculata* has been reported to reduce blood pressure on hypertensive rats.[13] *I. galbana* is well-known for its nutritional quality and to be a good source of lipids that can be used as a substitution of fish oils in a healthy human diet.[14,15] Some promising curative effects were also reported including weight loss and reduction of glucose, triacylglycerol and cholesterol levels in diabetic rats.[16] Moreover compounds from these microalgae exhibit interesting bioactivities like antibacterial, anti-inflammatory, anti-algal, antifungal, analgesic, and antioxidant activities.[17–19]

Herein, we report the extraction of water-soluble polysaccharides in two microalgae pastes, *I. galbana* (PEA) and *N. oculata* (PEB), their composition in monosaccharides, the evaluation of their cytotoxicity against a cancer cell line and the antimicrobial activities against Gram-positive bacteria, Gram-negative bacteria and *Candida* strains. Finally, this study also presents the antioxidant and anticholinesterase activities of these polysaccharidic extracts.

EXPERIMENTAL

Culture conditions and samples

Microalgae pastes, *I. galbana* (clone T-iso) and *N. oculata* CCMP-1325, were obtained from NutrOcean (Rimouski, Canada). Briefly, microalgae came from NCMA-CCMP cultures and they were produced semi-continuous during two months (partial harvests and dilutions at 24 or 48 h). Microalgae were grown in airlift cylindrical photobioreactors. The temperature and salinity were 22±2 °C and 28±3 ‰, respectively. Irradiance was 140±20 µE (µmol $m^{-2} s^{-1}$) on the reactor surface. The photoperiod was always light (24 h light and 0 h dark). Culture medium (f/2 without silicate) was sterilized (UV and ultrafiltration) before to be used.

Extraction of water soluble polysaccharides

Each freeze-dried microalgae paste (20 g each) was extracted three times with 200 mL of methanol, the first time during 48 h and the two latter extractions during 24 h. The resulting microalgae pastes were then extracted twice with 200 mL of deionized water during 72 h and 24 h. Microalgae aqueous extracts were combined and were freeze-dried. The yields were determined.

Total sugar, proteins and sulfate measurement

Total sugar content in the aqueous extracts was determined by a modified phenol––sulfuric acid method based on literature.[20] Briefly, a mixture of 0.5 mL of sample and 0.5 mL of 5 % aqueous phenol solution was treated with 2.5 mL of concentrated sulfuric acid. The mixture was stirred during 30 min. The absorption was measured at 490 nm and glucose was used as external standard. Sulfate content was determined using barium chloride/gelatin method with some modifications.[21] Concisely, the aqueous extracts (0.2 mL) were treated with trichloroacetic acid (3.8 mL) followed by the addition of 1.0 mL of barium chloride/gelatin. The mixture was stirred during 20 min. The absorbance was read at 360 nm and potassium sulfate was used as external standard.

Protein contents were measured from nitrogen percentage obtained by combustion elemental analysis. The percentage of crude protein (CP) in samples was calculated by multiplying the nitrogen percentage (N) by a conversion factor using the following equation:[22]
$$CP= N×6.25$$

Hydrolysis and silylation of polysacharidic extracts

Hydrolysis of extracts was carried out according to Yang *et al.* with some minor modifications.[23] Freeze-dried aqueous extracts (10 mg) were hydrolyzed with 10 mL of aqueous trifluoroacetic acid (2 M, TFA) at 120 °C during 8 h. The solution was evaporated to dryness with a nitrogen flow. Samples were reacted with 0.2 mL of *N,O*-bis(trimethyl-silyl)trifluoroacetamide containing 1% of chlorotrimethylsilane in anhydrous pyridine (0.2 mL) during 3 h at 70 °C. The resulting solution was evaporated with a nitrogen flow. The solid was then extracted with *n*-hexane (2.00 mL) prior to monosaccharide analysis.[24]

Monosaccharide analysis

The silylated monosaccharide samples were analyzed using a Hewlett-Packard 6890 gas chromatograph (GC) equipped with a DB-5 capillary column (30 m×0.25 mm×0.25 µm film thickness) coupled to a mass spectrometer (MS, Micromass Platform II) operated to the electron impact mode (70 eV). The temperature of the injector was 300 °C. The column temperature was set at 80 °C during 5 min, then increased at a rate of 4 °C min^{-1} to 290 °C, and was then maintained isothermally for 20 min. The carrier gas was helium at a constant flow rate of 1.2 mL min^{-1}. Arabinose was used as internal standard. A volume of 1 µL of

sample was splitless injected. Chromatograms were analyzed with the MSD ChemStation E.02.02.1431 software. Assignation of chromatographic peaks was achieved with the NIST mass spectra search program (version 2.0d).[24]

DPPH assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma) radical scavenging activity was measured according to the literature.[25] Microalgae polysaccharidic extracts were dissolved in 10 mL of distilled water to a final concentration of 100 µg/mL. Two milliliters of 0.2 mM DPPH in ethanol were added to 1 mL of each microalgal polysaccharidic solutions (PEA and PEB). The absorbance was measured at 517 nm after 20 min of incubation at 25 °C. Distilled water was used as the control. Percentage of inhibition was determined according to the following formula: DPPH radical scavenging activity (%) = $100(A_c–A_s)/A_c$, where A_c is the absorbance value of the control group and A_s is the absorbance value of the group treated with the extract.

Vitamin E (Sigma Aldrich) was used as positive control and all measurements were performed in triplicate. The percentage inhibition of free radical activity was plotted against the concentration of polysaccharidic extracts and the concentration for 50% of inhibition (IC_{50}) was determined.

Antimicrobial activity

Microorganisms and culture conditions. PEA or PEB were tested against Gram-positive cocci (*Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 25923) and Gram-negative bacilli (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853). The antifungal effects of polysaccharidic extracts from *I. galbana* or *N. oculata* were also tested against a range of pathogenic reference yeasts (*Candida albicans* ATCC 90028, *C. glabrata* ATCC 90030, *C. kreusei* ATCC 6258 and *C. parapsilosis* ATCC 22019). Bacteria or *Candida* species were grown in nutrient broth and incubated aerobically without shaking for 24 h at 37 °C and sub-cultures were realized at least three times at 24 h intervals prior to tests. All microorganisms tested were provided from the laboratory of Parasitology–Mycology and the laboratory of Bacteriology of Monastir (Tunisia).

MIC *determination*. The minimum inhibitory concentration (*MIC*) of PEA and PEB was determined from a microdilution assay as described in literature.[26] PEA and PEB stock solutions were prepared by dissolution of 10 mg of PEA or PEB in 2 mL of 10 % dimethyl sulfoxide (DMSO, Sigma-Aldrich). After an overnight incubation, broth cultures were adjusted to yield approximately to 1×10^6 CFU/mL of bacteria or fungus. A sample of each extract (200 µL) was added to four wells of the first column of each plate and then diluted with DMSO (10 %) solution (dilution factor (1:1)) up to the well number eight of first column. Each well was then inoculated with 50 µL of bacteria or *Candida* species and microplates were incubated during 24 h at 37 °C. Controls (wells inoculated with the tested culture without polysaccharides extracts) and blanks (wells containing uninoculated broth with polysaccharide extracts) were run on each microplate. Imipenem and vancomycin were used as positive control for bacteria strains and fluconazole was used for *Candida* species. All antibiotics and antimycotic (Sigma–Aldrich) were tested at a final concentration of about 1 mg/mL. The M*IC* was the lowest concentration of tested agent giving the complete inhibition of growth (*i.e.*, optical density equal to *OD* of the blank). The microplate assays were repeated at least three times for each polysaccharide extract and the MIC was the average of three independent experiments.

Cytotoxic activity. The human HeLa cervical cancer cell line was obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and cultured in a humidified atmosphere at 37 °C in 5 % CO_2. RPMI 1640 (Sigma-Aldrich) supplemented with 10 % fetal calf serum, 1 % (w/v) glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin was used for HeLa cell cultures. Cell viability cytotoxicity was measured using an MTT (3--(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay with slight modifications.[27] HeLa cells ($5×10^3$) were seeded into wells with 100 μL of growth medium and incubated at 37 °C for 24 h. Cells were treated with polysaccharide extracts (0.031 to 1 mg/mL) and incubated for 48 h at 37 °C. After that, 10 μL of MTT (5 mg/mL) was added to each well and microplates were incubated for an additional 2 h. Then, the medium was dissolved with 100 μL of DMSO and the absorbance (A) was measured at 550 nm by a BioTek microplate reader. This assay was conducted in triplicate as a cell viability index. The percentages of cell growth were calculated as follow: Cell proliferation (%) = $100(A_s–A_c)/A_c$, where A_c is the absorbance value of the control group and A_s is the absorbance value of the group treated with sample.

Anticholinesterase activity. The anticholinesterase activity was determined by colorimetry using a Cholinesterase Kit (Chronolab, Spain).[28] PEA and PEB stock solutions were prepared by dissolution 20 mg of PEA or PEB in 2 mL of 10 % DMSO (Sigma–Aldrich). Human Plasma was provided from the Biochemistry–Toxicology Laboratory, University Hospital "Fattouma Bourguiba" of Monastir (Tunisia) and used as a source of butyrylcholinesterase (BChE). PEA or PEB (500 μL, 10 mg/mL) was added to 500 μL of plasma and the mixture was incubated at 37 °C during 5, 10, 15, 20 and 30 min. BChE activity was measured by COBAS INTEGRA® 400 (Roche diagnostics). The control (plasma and distilled water) was treated under the same conditions. The anticholinesterase activity was calculated by the same formula as for DPPH radical scavenging activity. All assays were carried out in triplicate.

RESULTS AND DISCUSSION

Total sugar, proteins, and sulfate composition

Figure 1 shows the composition in sugars, proteins and sulfate of the microalgae aqueous extracts. Total sugar content in aqueous extracts of *I. galbana* and *N. oculata* were 86.9±0.8 % (22.8 % of total dried matter) and 59±0.1 % (4.1 % of total dried matter), respectively. The sulfate groups represented respectively 7.9±1.2 % and 6.2±0.1 % of *I. galbana* and *N. oculata* extracts. Sulfate bands were confirmed by infrared spectroscopy (data not shown). The percentage of proteins in the microalgae aqueous extracts were respectively of 5.2±0.17 % and 21.0±0.2 % for *I. galbana* and *N. oculata*. According to literature, carbohydrates represented around 13 % of dry matter of *I. galbana*.[29] Brown showed that *N. oculata* is composed by 35 % of proteins and 7.8 % of carbohydrates.[30] However, Picardo *et al.* reported that carbohydrates represent 29.4 % when grown at 25 °C (22 °C in our study).[31] Many studies reported that chemical composition as carbohydrates, proteins and lipids in *N. oculata* and *I. galbana* was dependent of the environmental growing conditions like salinity, light intensity, nitrogen content, photoperiod, and stage of harvest.[26,27,32]
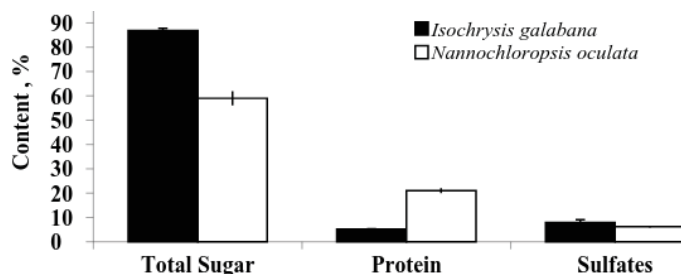
Fig. 1. Total sugar, proteins and sulfate content in water-soluble polysaccharidic extracts of *Isochrysis galbana* and *Nannochloropsis oculata.* All assays were carried out in triplicate.

Monosaccharide analysis

Polysaccharides from microalgae were hydrolyzed with TFA into monosaccharides, which were further trimethylsilylated to obtain volatile compounds for GC–MS analyses. Table I presents the monosaccharide composition in microalgal polysaccharidic extracts. Glucose was the major component in *I. galbana* and *N. oculata* extracts with 56.9 and 68.3 %, respectively. Mannitol (38.8 %) and inositol (20.32 %) were respectively the second major compounds in PEA and PEB (Fig. 2).
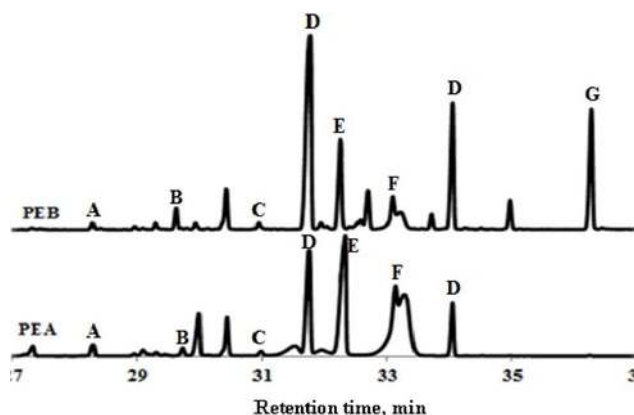

Fig. 2. Monosaccharide chromatogram of polysaccharidic extracts of *Isochrysis galbana* (PEA) and *Nannochloropsis oculata* (PEB) determined by trimethylsilylation method. A: xylose; B: mannose; C: galactose; D: glucose; E: sorbitol; F: mannitol; G: inositol.

Mannitol represented a percentage of 5.8 % of the *N. oculata* extract and inositol was absent in the *I. galbana* extract. Chu *et al.*, using trimethylsilylation method, observed 1.35 % of mannitol in *I. galbana*.[33] Xylose, mannose and galactose were minor constituents of the both PEA and PEB microalgae extracts (< 2 %). Glucose, galactose, mannose and xylose have been reported in various proportions in *I. galbana* extracts.[34] Brown reported that glucose was the major

sugar in *N. oculata* and *I. galbana,* which corresponds to 68.2 and 70.3 %, respectively.[30] During exponential and stationary growth phases of *I. galbana*, the percentage of glucose reached 60 and 80 %, respectively.[33] In our study, the percentages of sorbitol in *I. galbana* and *N. oculata* were respectively of 1.02 and 3.38 %. Sadovskaya *et al.* and Brown reported that sorbitol was not observed in *I. galbana* and *N. oculata* using the alditol acetate method.[30,34] Templeton *et al.* mentioned that it was impossible to distinguish between neutral sugar (glucose) and reduced sugar (sorbitol) in the original mixture with the alditol acetate derivatization method.[35]

TABLE I. Monosaccharides composition (%) of *Isochrysis galbana* and *Nannochloropsis oculata* aqueous extracts determined by the trimethylsilylation method

Component	Algae		Retention time, min
	Nannochloropsis oculata	*Isochrysis galbana*	
Xylose	0.90	0.43	28.253
Mannose	1.03	1.26	29.827
Galactose	0.96	1.73	31.049
Glucose	68.23	56.88	31.591 and 34.044
Mannitol	5.78	38.74	33.044
D-Sorbitol	3.38	1.02	32.392
Inositol	20.32	–	36.200

DPPH radical scavenging activity

DPPH is a free radical compound that has been widely used to evaluate the ability of antioxidant to scavenge radicals.[36] Fig. 3 shows the scavenging power of DPPH radicals by PEA and PEB. The antioxidant capacities of both PEA and PEB were dose dependent. The inhibition percentages of PEB and PEA (at 1 mg/mL) were 24.79±0.05 % and 15.71±0.03 %, respectively. At the maximal concentration of 10 mg/mL, PEB (59.28±0.04 %; IC_{50} = 4.2 mg/mL) showed a higher antioxidant activity compared to PEA (41.45±0.03 %, IC_{50} > 10 mg/mL). This difference of activity could be explained by the presence of proteins and highly branched polymers. Vitamin E (0.12 mg/mL) had a significant higher antioxidant capacity of 90.312±0.005 % (IC_{50} = 0.040 mg/mL), compared to PEA (9.74±0.003 %) and PEB (16.14±0.005 %). Custódio *et al.* indicated that organic extracts from *N. oculata* had also antioxidant properties with IC_{50} values between 4.93 and 7.31 %.[37] Moreover, Balavigneswaran *et al.* reported that an ethanol soluble polysaccharidic extract from *I. galbana* was active against DPPH (almost 40 %) at 10 mg/mL.[38]

The reducing properties are generally associated with the presence of reductones which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. Reductones are reported to react with some precursors of peroxide, thus preventing peroxide formation.[39] Carboxyl

groups may play an important role in scavenging radicals, possibly due to the higher hydrogen donation ability of carboxyl groups than hydroxyl groups, proteins and sulfate groups.[36] The low percentage of sulfate in PEA and PEB (7.9 and 6.21 %, respectively) could explain the moderate activity against DPPH radicals. The antioxidant activities depend on polysaccharides molecular weight, degree of ramification, monosaccharide composition, sulfate content and configuration.[40–42] The influence of sulfate content on the antioxidant activity depends rather on the origin of polysaccharides. For example, the polysaccharides from *Ulva fasciata* and other macro and microalgae with low sulfate content demonstrated a strong antioxidative power, while the antioxidant activity observed in polysaccharides from *Enteromorpha linza* and other seaweeds showed to be dependent of sulfate content. Furthermore, highly sulfated polysaccharides were shown to have an enhanced scavenging power, this property being also dependent on the sulfate distribution pattern.[43]
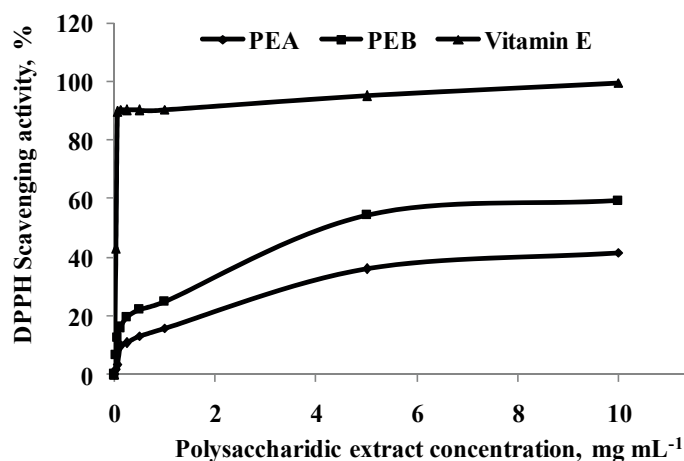

Fig. 3. DPPH scavenging power of polysaccharidic extracts of *Isochrysis galbana* (PEA) and *Nannochloropsis oculata* (PEB). Vitamin E was tested as positive control. All assays were carried out in triplicate.

PEA and PEB antimicrobial activity

Table II shows the *MIC* of polysaccharidic extracts (PEA and PEB) from microalgae *I. galbana* and *N. oculata*. All tested bacterial strains were sensitive to PEA and PEB. Results show that Gram-negative bacteria were more sensitive to PEA than Gram-positive bacteria. MICs of PEA against *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Enterococcus faecalis* were 1250, 1870 and 3750 μg/mL, respectively. *MIC*s of PEB against *E. coli* and *P. aeruginosa* were 2500 and 1870 μg/mL, respectively. On the other hand, the same author showed that methanolic extract is not active against multiresistant

Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative (*P. aeruginosa* and *Klebsiella pneumomiae*) pathogens. Bruce *et al.* reported that acetone extract of *I. galbana* was active against *S. aureus* and *Micrococcus* sp. with corresponding inhibition zone diameters of 10 and 15 mm, respectively.[44]

TABLE II. Antimicrobial activity of polysaccharidic extracts of *Isochrysis galbana* (PEA) and *Nannochloropsis oculata* (PEB) against Gram-positive bacteria, Gram-negative bacteria and *Candida* strains. Imipenem, vancomycin and fluconazole were used as positive controls. Minimum inhibitory concentration, µg mL^{-1}, was the average of three independent replications; NA: no activity; ND: not determined

Fluconazole	Vancomycin	Imipenem	PEB	PEA	Bacterium
			Gram-positive bacteria		
ND	62.5	NA	2500	3750	*Enterococcus faecalis*
ND	3.9	NA	3750	3750	*Staphylococcus aureus*
			Gram-negative bacteria		
ND	1.95	NA	2500	1250	*Escherichia coli*
ND	NA	1.95	1870	1870	*Pseudomonas aeruginosa*
			Candida yeasts		
NA	ND	ND	NA	NA	*Candida albicans*
NA	ND	ND	100	117	*Candida glabrata*
15.62	ND	ND	118	117	*Candida parapsilosis*
15.62	ND	ND	80	60	*Candida krusei*

For the antifungal activity, *Candida krusei* shows a higher sensitivity to PEA (60 µg/mL) and PEB (80 µg/mL) than other *Candida* species (Table II). *C. parapsilosis* was inhibited by PEB and fluconazole with MIC values of 118 and 15.62 µg/mL, respectively. No inhibitory activity against *C. albicans* for both PEA and PEB was detected. *C. glabrata* was resistant to fluconazole (1 mg/mL) and appeared to be sensitive to PEA or PEB, with respective *MIC*s of 117 and 100 µg/mL. The mechanisms involved in antimicrobial activity of polysaccharides extracts are worthy of further investigations.[45] Polysaccharides influence the cytoplasm permeability, the DNA decomposition after a polysaccharide/DNA binding, and the denaturation of essential bacterial proteins.[46] On the other hand, the activity against microorganisms can be related to the bacterial membrane composition, resistance capacity of yeasts, polysaccharides structure, degree of ramification and degree of sulfation. Goy *et al.* reported that polysaccharides inhibited the fungi growth by reacting with enzymes in hyphae.[47]

Cytotoxic activity

Figure 4 shows that cell proliferation decreases with increasing of PEA and PEB content. The proliferating cells reached 42.7 and 13.8 % at PEA concentrations of 31.25 and 500 µg/mL, respectively. After a treatment with PEB (125 and 250 µg/mL), the cell proliferation percentages were 51.5 and 38.36 %, respectively. Both PEA and PEB inhibited HeLa cell proliferation at a final concentration of 1

mg/mL. Remarkably, HeLa cell proliferations were more abundant with *N. oculata* aqueous extract (PEB) than with *I. galbana* extract (PEA) at different concentrations of extracts. The cell proliferation percentages of PEA and PEB at the minimal studied concentration (31 μg/mL) were respectively of 44 and 59 %. Sadovskaya *et al.* showed that the polysaccharide extracts from *I. galbana* inhibited U937 human leukemic monocyte lymphoma cell proliferation (30 % at 100 μg/mL) and consequently have potential antitumor activity.[34] Atasever-Arslan *et al.* reported that essential oils from *N. oculata* extract (at 500 μg/mL) caused K562 cell lines cytotoxicity (human chronic myeloid leukemia cell line) of 45.64 %.[48] Polysaccharides, especially sulfate polysaccharides, could affect the proliferation, differentiation, apoptosis and metastasis of tumor cells.[49] They bound the proteins like growth factors and inhibit the growth of tumors.[4,50] Inhibition of the cell proliferation may be mediated by the chemical properties of sulfated polysaccharides and the species of tumor cells.[49] Another mechanism of antiproliferative effect is to block the G1 phase.[49] Sulfated polysaccharides isolated from the filtrate of marine *Pseudomonas* sp. culture induced the apoptosis of human leukaemic cells.[50] Fucoidan induced apoptosis in human lymphoma HS-Sultan cell lines, which is accompanied by the activation of caspase-3 and down-regulation of extracellular signal-regulated kinase pathway.[51] The sulfated heteropolysaccharides isolated from red alga *Schizymenia dubyi* can induce the terminal maturation of non-small bronchopulmonary carcinoma cells and arrest cells in the G1 phase.[49]
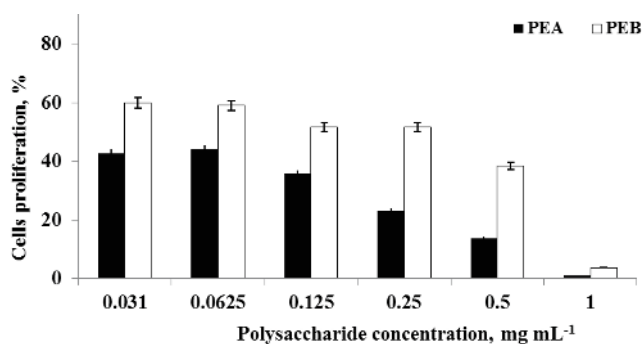

Fig. 4. Percentage of cell proliferation in presence of polysaccharidic extracts of *Isochrysis galbana* (PEA) and *Nannochloropsis oculata* (PEB). Error bars represent the standard deviation calculated from duplicate experiments.

Anticholinesterase activity

Alzheimer's disease is a deadly neurodegenerative disease with progressive character and has become a major health problem especially in industrialized countries where the life expectancy is higher. It is also a common form of dementia especially among the elder population in which irreversible neuronal loss and

abnormal behavioral changes are evident in this disease.[52] Antioxidant extracts from plants play an important role in the prevention of Alzheimer's disease.[53] In addition, reports indicated a correlation between antioxidant power and the anticholinesterase activity.[54] The use of antioxidants may reduce the Alzheimer's disease progression and minimize the neuronal degeneration by inhibition of the acetylcholinesterase enzyme.[55] Treatments of the Alzheimer's disease include disease-modifying treatments, psychotropic agents and especially the cholinesterase inhibitors, which block the hydrolysis of two chemical neurotransmitters, *i.e*., acetylcholine and butyrylcholine (by butylcholineesterase, BChE).[52] However, most of these drugs have side effects such as liver damage and bradycardia. Synthetic antioxidants also caused liver damage and carcinogenesis in rats, that stimulated scientists to find new natural and harmless antioxidants, as well as anticholinesterase compounds.[55] Figure 5 shows the effects of PEA or PEB (10 mg/mL) on anticholinesterase activity at different incubation times (5 to 30 min). Remarkably, time-dependent inhibition of butyrylcholinesterase was observed after PEA and PEB treatments. PEB was more active than PEA (Fig. 5). For PEA, the percentage of BChE inhibition after 5 and 30 min were respectively of 1.25±0.25 % and 7.30±0.48 %. After 30 min of PEB treatment, the BChE inhibition reached a maximum of 11.53±0.12 %. Anticholinesterase activities of polysaccharides were not well studied. No significant evidence has been proven that they were specifically active toward the Alzheimer's disease. But many polysaccharides could have regenerative properties and functions as memory and learning enhancers.[56] Asker *et al.* suggested that polysaccharides isolated from *Bacillus* sp. may be a good natural source for Alzheimer's disease therapy.[56] On the other hand, Custódio *et al.* evaluated the BChE activity of *N. oculata* organics and water extracts.[37] Maximum inhibition (21 %) was
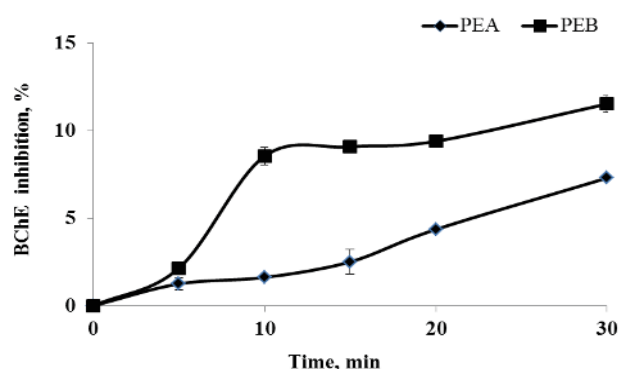

Fig. 5. Effect of polysaccharidic extracts from *Isochrysis galbana* (PEA) and *Nannochloropsis oculata* (PEB) on anticholinesterase activity. The results were expressed as butyrylcholinesterase inhibition percentage (%). Error bars represent the standard deviation calculated from triplicate experiments.

observed after treatment with *N. oculata* aqueous extract at the maximal concentration of 0.5 mg/mL. Custódio *et al.* indicated that aqueous extract from *Isochrysis galbana* possessed an anticholinesterase activity (IC_{50} of 0.11 mg/mL).[57]

CONCLUSIONS

I. galbana and *N. oculata* are used widely in aquaculture for feeding and pathogens prevention. PEA and PEB possessed important functional properties such as antioxidant, antimicrobial, anticholinesterase and antiproliferation activities, demonstrating the important value of these microalgae. *I. galbana* and *N. oculata* can be further tested for their nutritional and medical human applications. The mode of action of polysaccharidic extracts on pathogenic bacteria or fungi constitutes also an important field of study for future works.

ИЗВОД
АНТИМИКРОБНА, АНТИОКСИДАТИВНА, ЦИТОТОКСИЧНА И АНТИХОЛИНЕСТЕРАЗНА АКТИВНОСТ ПОЛИСАХАРИДА МИКРОАЛГИ *Isochrysis galbana* И *Nannochloropsis oculata* РАСТВОРНИХ У ВОДИ

МHAMMED BEN HAFSA[1,2], MANEL BEN ISMAIL[3], MARIEM GARRAB[3], RAIES ALY[1], JONATHAN GAGNON[2] и KARIM NAGHMOUCHI[1]

[1]*Laboratoire des Microorganismes et Biomolécules Actives (LMBA), Faculté des Sciences de Tunis, Université El-Manar II 2092 El-Manar-II, Tunis, Tunisia,* [2]*Département de biologie, chimie et géographie, Université du Québec à Rimouski, 300 allée des Ursulines, Rimouski, Québec, G5L 3A1, Canada u* [3]*Laboratoire de Microbiologie, Faculté de Médecine, Université de Monastir, Monastir 5000, Tunisia*

У овом раду је испитана могућност примене водених екстраката микроалги *Isochrysis galbana* (PEA) и *Nannochloropsis oculata* (PEB), који садрже претежно полисахариде. Одређен је садржај моносахарида у екстрактима. GC–MS анализа након дериватизације је показала да је главни састојак обе микроалге глукоза: у PEA 56,88 % и у PEB 68,23 %. Манитол (38,80 %) и инозитол (20,32 %) су следећи по заступљености у PEA односно PEB. Силиловањем моносахарида је утврђено да сорбитола има 3,38 % у PEB. Даље су анализиране антиоксидативне, антимикробне и цитотоксичне особине екстраката. Антиоксидативна активност је утврђивана DPPH методом и зависила је од концентрације. При концентрацији екстракта од 10 mg/mL, антиоксидативна активност PEA и PEB је била 41,45 %, односно 59,07 %. Екстракти су били способни да инхибирају раст Грам негативних и Грам позитивних бактерија, као и три врсте гљиве *Candida*. Цитотоксична активност је процењена на хуманим HeLa ћелијама тумора грлића материце. Пролиферација HeLa ћелија је била потпуно инхибирана третманом PEA и PEB екстрактима у концентрацији 1 mg/mL, а инхибиција је зависила од дозе у опсегу 0,03 до 1 mg/mL. Антихолинестеразна активност је потврђена спрам бутирилхолинестеразе. Због својих активности, полисахариди наведених микроалги могу имати додатну примену осим нутритивне.

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