**Evaluation of the antimicrobial, antioxidant, cytotoxic and anticholinesterase activities of water-soluble polysaccharides extracted from microalgae *Isochrysis galbana* and *Nannochloropsis oculata***

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*Abstract:*The present work is carried out to determine the monosaccharide composition of polysaccharidic extracts from *Isochrysis galbana* (PEA) and *Nannochloropsis oculata* (PEB) and to evaluate their antioxidant, antimicrobial and cytotoxic properties. GC-MS spectroscopy analysis shows that glucose is the major compound in both microalgae PEA (56.88%) and PEB (68.23%). Mannitol (38.8%) and inositol (20.32%) are the second major compound in PEA and PEB, respectively. PEA and PEB showed a concentration dependent DPPH·radical scavenging activity. At concentration of 10 mg/mL, both PEA and PEB exhibited antioxidant activitiesof about 41.45 % and 59.07 %, respectively. PEB and PEA are able to inhibit growthof Gram negative bacteria, Gram positive bacteria, and three Candida species. The inhibitory effects on humain HeLa cervical cancer cells proliferation (from 0.031 to 1 mg/mL) were also dose dependent. HeLa cells proliferation were totally inhibited after treatment with PEA and PEB (1mg/mL). Their anticholinesterase activity was investigated against butyrylcholinesterase enzymes. This naturally substances showing considerable anticancer and anticholinesterase activities as well as antimicrobial properties could represent an additional value in feed supplements in aquacultures for many aquatic animals, to prevent disease and to decrease the addition of antibiotics.

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

RUNNING TITLE: ACTIVITY OF MICROALGAE FROM NUTROCEAN

 INTRODUCTION

Algae present a big diversity species are estimated from 40000 to 10 million, the majority of them are microalgae.1 Microalgae are photosynthetic organisms that play a key role in aquatic ecosystems and approximately 40% of global photosynthesis is due to these microorganisms.2 They have different morphological, physiological, and genetic traits that confer the ability to produce different biologically active metabolites.3 Markouk *et al.*4reported that microalgae can produce different chemical compounds with different biological activities, such as carotenoids, phycobilins, polyunsaturated fatty acids, proteins, polysaccharides, vitamins, and sterols among other chemicals. The chemical composition is variable and mainly due to environmental factors such us, water temperature, salinity, lights, and nutrients.

 Foods rich in antioxidants should play an important role to avoid numerous pathology such as, cancer and neurodegenerative diseases as well as cell inflammatory complications generated by cutaneous agents.5 Fight to cancer drugs and their reaction are of interest.6 Epidemiological studies and experimental analyses have oriented the scientific agreement of native ingredients able of favorably regulating carcinogenesis. There has been focused in the therapeutic effect of foods as antioxidants in decreasing such free radical–activated tissue injury.7Commercial *Nannochloropsis oculata* (*N. oculata*) and *Isochrysis galbana* (*I. galbana*) are well applicated in aquaculture industry. They are an important food source and feed additive in the commercial rearing of many aquatic animals, and widely used especially live foods organisms and human alimentation.8*I. galbana* is a golden brown microalga has good nutritive characteristics since it’s rich in polyunsatured fatty acids and used commonly as feed in bivalve hatcherie.9Tsung-Yuan*et al.*10reported that green water produced with *N. oculata* was shown to inhibit the blooming of vibrio an opportunistic pathogen of fish. Cárdenas*et al.*11showedan inhibition of shrimp’s vibrio pathogens by *I. galbana* in batch cultures. Both marine microalgae,*N. oculata* and *I. galbana,* are rich sources of functional compounds and known to have high soluble and insoluble polysaccharides and proteins contents as well as significant percentages of polyunsatured fatty acids.12 Over the last two decades, many studies have focused on natural polysaccharides isolated from: plants, fungi, bacteria, animal sources, and algae and can improved disease resistance of fish13. Polysaccharides are giant polymers of monosaccharides, all connected by glycosidic linkages. Polysaccharides from algae have several biological activities such as anticoagulant activities, antithrombotic, antimetastatic, antitumoral, antiviral activities, and antioxidant activities.14,15,3

The present study is an attempt to valorize the prevention from human disease of both microalgae *N. oculata* and *I. galbana*focused on the (**1**) extraction of polysaccharides, (**2**) evaluation of their cytotoxic and antimicrobial activities against Gram positive bacteria, Gram negative bacteria and Candida strains, and (**3**) finally, the study of the antioxidant and anticholinesterase activities.

EXPERIMENTAL

*Culture conditions and samples*

Microalgae are obtained from NCMA-CCMP ([https://ncma.bigelow.org](https://ncma.bigelow.org/)) cultures are produced semi-continuous during two months (Partial harvests and dilutions to 24 or 48 hours). Microalgae were grown in cylindric photobioreactor that were airlift. The temperature and salinity were 22 ± 2 °C and 28 ± 3 ‰, respectively. Irradiance is 140 ± 20 µEinstein (µmolm-2.sec-1) from the roll surface. The photoperiod is always light (24 hours light and 0 hours dark). Culture medium (f/2 without silicate) sterilized (UV + ultrafiltration).

*Extraction of water soluble polysaccharides*

Microalgae pastes, *I. galbana* (clone T-iso) and *N. oculata* CCMP-1325were obtained from NutrOcean (Rimouski, Canada). Each lyophilized microalgae cells (20 g each) were extracted three times with 200 mL of methanol, the first time during 48 h and the two lasts extractions for 24h. Each methanolic extracts were evaporated and the yields were determined. Then, each microalgae paste treated with methanol, were extracted twice with 200 ml of deionized water for 72h and 24h, respectively. Microalgae aqueous extracts were lyophilized and the yields were determined.16

*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.17 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 where glucose was used as standard. Sulfate content was determined using barium chloride/gelatin method18with some modifications. 0.2 mLof aqueous extract was treated with 3.8 mL of 4% trichloroacetic acid followed by the addition of one 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.

Proteins content were measured from nitrogen percentage obtained by combustion in elemental analysis.The percentage of crude protein (*CP*) in the samples is calculated by multiplying the nitrogen percentage (*N*) by a conversion factor according to19 using the following equation: *CP* = *N* x 6.25

*Hydrolysis and silylation of polysacharidic extracts*

Samples preparation was carried out according20 with some modifications. A total of 10 mg of Lyophilized aqueous extracts were hydrolyzed with 10 mL of aqueous trifluoroacetic acid 2 M at 120 °C during 8 hours. The solution was evaporated to dryness with a nitrogen flow. Samples were reacted with 0.2 mL of *N*,*O*‑bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% chlorotrimethylsilane in 0.2 mL anhydrous pyridine during 3 h at 70 °C. The resulting solution was evaporated with a nitrogen flow. The solid was then extracted with 2 mL *n*-hexane prior to analysis21.

*Monosaccharide analysis*

The methylated-silylated and silylated saccharide samples were analyzed using a Hewlett-Packard 6890 gas chromatograph (GC) equipped with a DB-5 capillary column (30 m x 0.25 mm x 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 oC. The column temperature began at 80 °C during 5 min, then increased at a rate of 4 °C/min 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. Arabinose was used as internal standard. A volume of 1 µL of sample was injected splitless. 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).21

*DPPH Assay*

The 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma) radical scavenging activity was measured by the method of.22Microalgae polysaccharidic extracts was dissolved in 10 mL of distilled water to a final concentration of 100 µg/mL. Two milliliter of 0.2 mM DPPH in ethanol and added to 1 mL of each microalgae 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 a formula: DPPH radical scavenging activity (%) = (1- absorbance of sample/absorbance of control) x 100. Vitamin E (Sigma Aldrich) was used as positive control and all measurements were performed in triplicate.

*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, *Candida glabrata* ATCC 90030, *Candida kreusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019). Bacteria or Candida species were grown in Nutrient Broth23and incubated aerobically without shaking for 24 h at 37 °C and sub-cultured 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 by use of a micro-dilution assay as described by16.PEA and PEB stock solution were prepared by dissolution 10 mg of PEA or PEB in 2 mL of 10% dimetyl sulfoxyde (DMSO, Sigma Aldrich). Overnight broth cultures were adjusted to yield approximately 1 x 106 CFU/mL of bacteria or fungus. A sample from each extract (200 μL) was added to four wells of the first column of each plate and then serially diluted with dimethyl sulfoxide (DMSO) (10%) solution as doubling dilutions up to the well number eight of first column dilution factor (1:1). Each well was then inoculated with 50 µL of bacteria or Candida species and microplates were incubated for 24 h at 37 °C. Controls (wells inoculated with the tested culture without added Polysaccharide extracts) and blanks (wells containing un-inoculated broth with added Polysaccharide extracts) were run on each micro-plate. Imipenem and vancomycin were used as positive control for bacteria strains and fluconazol for Candida species. All Antibiotics (Sigma-Aldrich) were tested at final concentration of about 1 mg/mL. The MIC was the lowest concentration of tested agent giving the complete inhibition of growth (i.e. OD equal to OD of the blank). The micro-plate assay was repeated at least three times for each polysaccharide extracts and the MIC was the average of the three independent repetitions.

*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 % CO2.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-[di](https://en.wikipedia.org/wiki/Di-)[methyl](https://en.wikipedia.org/wiki/Methyl)[thiazol](https://en.wikipedia.org/wiki/Thiazole)-2-yl)-2,5-di[phenyl](https://en.wikipedia.org/wiki/Phenyl)tetrazolium bromide) assay with slight modifications.24 HeLa cells (5x103) were seeded into chosen 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 were 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 (%) = [A (sample)-A (control)/A (control)].

*Anticholinesterase activity*

The anticholinesterase activity was studied by the colorimetric method of25 with some modifications,26using Cholinesterase Kit (Chronolab, Spain). PEA and PEB stock solution were prepared by dissolution 20 mg of PEA or PEB in 2 mL of 10 % dimetyl sulfoxyde (DMSO, Sigma-Aldrich). Human Plasma was provided from the Biochemistry-Toxicology Laboratory, University Hospital “Fattouma Bourguiba” of Monastir, Tunisia and used us a source of Butyrylcholinesterase (BChE). Five hundred µL of PEA or PEB (10 mg/mL) were added to 500 µL of plasma and the mixture was incubated at 37 °C for at 5, 10, 15, 20 and 30 minutes. BChE activity was measured by COBAS INTEGRA® 400 (Roche diagnostics). The control (plasma and distilled water) was treated under the same condition. The anticholinesterase activity was calculated by the following formula: % inhibition = [(Activity control – Activity of sample)/Activity of control)]\*100. All assays were carried out in triplicate.

RESULTS AND DISCUSSION

*Total sugar, proteins, and Sulfate composition*

Fig. 1 shows the composition in sugars, proteins and sulfate content in the microalgae aqueous extracts. Total sugar content in aqueous extract from *I. galbana* and *N. oculata* were about 86.9 ± 0.8 % (22.8 % of total dried matter) and 59 ± 0.1 % (4.1 % of total dried matter), respectively. For sulfate groups represent only 7.9 ± 1.2 % and 6.2 ± 0.1 % of aqueous extract *I. galbana*, *N. oculata,* respectively. The presence of sulfates compounds band were confirmed by Infrared spectroscopy analysis (data not shown). The percentage of proteins in the microalgae aqueous extract were about 5.2 ± 0.17 % and 21.0 ± 0.2 % percent for *I.*

*galbana* and *N. oculata,* respectively. According to literature, carbohydrates represent around 13 % of *I. galbana*’s dry matter.27 Brown 28 showed that *N. oculata* presents 35 % of proteins, and 7.8 % of carbohydrates. However,29 reported that carbohydrates represent 29.4 % at 25 °C (22 °C in our study). Many studies reported that chemical composition of carbohydrates, proteins and lipids in *N. oculata* and *I. galbana* is dependent of environmental growing conditions like salinity, light intensity, nitrogen content, photoperiod, and stage of harvest.30,31,28,32



Fig. 1. Monosaccharide chromatogram of polysaccharidic extracts from *Isochrysis galbana* (PEA) and *Nannochloropsis oculata* (PEB) determined by trimethylsilylation method.

*Monosaccharide analysis*

Polysaccharides from commercial microalgae were hydrolyzed with TFA into individual monosaccharides, which were further trimethylsilylated obtained volatile compounds for GC‑MS analyses. Table I presents monosaccharide composition of polysaccharidic extracts from microalgae. Glucose is the major component in *I. galbana* and *N. oculata* extract with respectively 56.9 % and 68.3 %. Mannitol (38.8 %) and inositol (20.32 %) are the second major compound in PEA and PEB, respectively (Fig. 2).



Fig. 2. Total sugar, proteins and sulfate content in water-soluble polysaccharides extracts from *Isochrysis galbana* and*Nannochloropsis oculata.* All assays were carried out in triplicate.

Mannitol represents a percentage of 5.8 % in *N. oculata* extracts and inositol is absent in *I. galbana* extract. Chu*et al.*33,using trimethylsilylation (TMS) method, demonstrated the presence of manitol percentage in *I. galbana* was about 1.35 %. Xylose, mannose and galactose are minor constituents of the both PEA and PEB microalgae extracts (<2 %). Glucose, galactose, mannose and xylose have been reported in various proportions in extract from *I. galbana*.34 Brown28reported that glucose is the major compound in *N. oculata* and *I. galbana,* with a corresponding percentage of 68.2 % and 70.3 %, respectively. During, exponential and stationary *I. galbana* growth phase, the percentage of glucose reached 60 % and 80 %, respectively.33 In our study, the percentage of sorbitol in *I. galbana* and *N. oculata* were about1.02 % and 3.38 %; respectively. Sadovskaya*et al.*34andBrown28 reported the absence of sorbitol in *I. galbana* and *N. oculata,* respectively,using aditol acetate method. Templeton*et al.*35, showed that it is impossible to distinguish between neutral sugar (glucose) and sugar alcohol (sorbitol) in the original mixture with Aditol acetate-derivatization method.

TABLE I. Monosaccharides composition of *Isochrysis galbana* and *Nannochloropsis oculata* microalgae aqueous extracts determined by trimethylsilylation method.

|  |  |
| --- | --- |
| **Retention time (min)** |  **Monosaccharide composition (%)** |
|  |  | *Isochrysis galbana* | *Nannochloropsis oculata* |
| 28.253 | xylose | 0.43 | 0.90 |
| 29.827 | mannose | 1.26 | 1.03 |
| 31.049 | galactose | 1.73 | 0.96 |
| 31.591 and 34.044 | glucose | 56.88 | 68.23 |
| 33.044 | mannitol | 38.74 | 5.78 |
| 32.392 | D-sorbitol | 1.02 | 3.38 |
| 36.200 | Inositol | - | 20.32 |

*DPPH radical scavenging activity*

DPPH is a free radical compound that has been widely used to evaluate the ability of antioxidant to scavenge radicals.36Fig. 3 shows the scavenging power of DPPH radicals by PEA and PEB. The antioxidant capacities of both PEA and PEB are dose dependent manner. The inhibition percentage of PEB and PEA (at 1 mg/mL) was about 24.79 ±0.05 % and 15.71±0.03 %, respectively. At final concentration of about 10 mg/mL, PEB (59.28±0.04 %) showed higher antioxidant activities than in presence of PEA (41.45±0.03 %). This difference of activity can be explained by presence of proteins and high branched polymers. Vitamin E (0.12 mg/mL) had significant antioxidant capacities of about 90.312 ±0.005 %, compared to both PEA (9.74 ± 0.003 %) and PEB (16.14 ± 0.005 %). Custódio*et al.*37indicated that Organic extracts from *N. oculata* have antioxidant properties with IC 50 (half maximal inhibitory concentration) values between 4.93 % and 7.31 %. Balavigneswaran*et al.*38 reported that ethanol soluble polysaccharides extract from *I. galbana* was active against DPPH (almost 40 %) at 10 mg/mL.

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 hydrogen atom. Reductones are reported to react with certain precursors of peroxide, thus preventing peroxide formation.39Carboxyl groups may play an important role in scavenging radicals, possibly because carboxyl groups donate hydrogen more readily than hydroxyl groups, proteins and sulfate groups.36 The low percentage of sulfates 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,40 branched degree,41 monosaccharide composition, sulfates and configuration.42



Fig. 3.DPPH scavenging power of polysaccharidic extract from *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 or PEB) from microalgae *I. galbana* and *N. oculata*. All bacterial strains tested are sensitive to PEA or PEB. Results showed that Gram negative bacteria (GNB) are more sensitive to PEA than Gram positive bacteria (GPB). MICs of PEA against *Escherichia coli* ATCC 25922 (*E. coli*), *Pseudomonas aeruginosa* ATCC 27950 (*Ps. aeruginosa*),and *Enterococcus faecalis* ATCC 29212 were about 1250, 1870 and 3750 µg/mL, respectively. MICs of PEB against *Ps. aeruginosa* and *E. coli* were about 1870 and 2500 µg/mL, respectively. Sun*et al.*43show that *I. galbana* methanolic extract is active against shrimp’s pathogens as *Vibrio parachaemolyticus* and *Vibrio alginolyticus*. In other hand the same author shows that methanolic extract is not active against multiresistant pathogens GPB (*Staphylococcus aureus*, *Bacillus subtilis*,) and GNB (*Ps. aeruginosa* and *Klebsiella pneumomiae)*.Bruce*et al.*44 reported that *I. galbana*acetonic extract is active against *Staphylococcus aureus* and *Micrococcus sp*. with a corresponding inhibition zone diameter of about 10 and 15 mm, respectively.

For antifungal activity, *Candida Krusei* ATCC 6258 showed a higher sensitivity to PEA (60 µg/mL) and PEB (80 µg/mL) than other Candida species. *Candida parapsilosis* ATCC 22019 was inhibited at PEB and Fluconazole MICs of about 118 and 15.62 µg/mL, respectively. No inhibitory activity of both PEA and PEBwere detectedagainst *Candida albicans* ATCC 90028 (Table II). *Candida glabrata* ATCC 90030 was resistant to Fuconsaol (1 mg/mL), and appeared to be sensitive to PEA or PEB, with corresponding MICs of about 117 and 100 µg/mL, respectively. The mechanisms involved in antimicrobial activity of polysaccharides extracts are worthy further investigation.45 Polysaccharides influence cytoplasm permeability, DNA decomposition after polysaccharide/DNA binding, and protein, essential for bacteria.46 In the other hand, the activity against microorganisms can be related to the GPB composition membrane, resistance capacity of yeasts, structure, degree of ramification and sulphate degree. Goy*et al.*47 reported that polysaccharides inhibit the fungi growth by reacting with enzymes in hyphae.

TABLE II. Antimicrobial activity of polysaccharidic extracts from *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. MIC was the average of the three independent repetitions.

|  |
| --- |
|  **Minimum Inhibitory Concentration (µg/mL)** |
|  | **PEA** | **PEB** | **Imipenem** | **Vancomycin** | **Fluconazole** |
| **Gram positive bacteria (GPB)** |  |
| *Enterococcus faecalis* ATCC 29212 | 3750 | 2500 |  NA\* | 62.5 |  ND\* |
| *Staphylococcus aureus* ATCC 25923 | 3750 | 3750 | NA | 3.9 | ND |
| **Gram negative bacteria (GNB)** |
| *Escherichia coli* ATCC 25922 | 1250 | 2500 | NA | 1.95 | ND |
| *Pseudomonas aeruginosa* ATCC 27950 | 1870 | 1870 | 1.95 | NA | ND |
| **Candida yeasts** |
| *Candida albicans* ATCC 90028 | NA | NA | ND | ND | NA |
| *Candida glabrata* ATCC 90030 | 117 | 100 | ND | ND | NA |
| *Candida parapsilosis* ATCC 22019. | 117 | 118 | ND | ND | 15.62 |
| *Candida Krusei* ATCC 6258 | 60 | 80 | ND | ND | 15.62 |

*Cytotoxic activity*

Fig. 4 shows that cell proliferation decreases with increasing of PEA or PEB content. The proliferation cells reached 42.7 % and 13.8 %, at PEA concentration of about 31.25 and 500 µg/ml, respectively. After PEB treatment (125 and 250 µg/mL), the proliferation cells percentages were about 51.5 % and 38.36 %, respectively. Both PEA and PEB were shown to inhibit HeLa cells proliferation at final concentration of about 1mg/mL. Remarkably, HeLa cells proliferations were more abundant in presence of *N. oculata* aqueous extract (PEB) than *I. galbana* extract (PEA) at different extracts concentrations. The proliferation cell percentages of PEA and PEB (at final concentration of about 62 µg/mL) are respectively 44 and 59 %. Sadovskaya*et al.*34 showed that the polysaccharides extracts from *I. galbana* inhibits U937 human leukemic monocyte lymphoma cells proliferation (30 % at 100 µg/mL) and consequently as potential anti-tumor activity. [Atasever-Arslan](http://www.sciencedirect.com/science/article/pii/S0928098715300750)*et al*.48 reported that essential oils from *N. oculata* extract (at 500 µg/mL) cause K562 cell lines cytotoxicity (human chronic myeloid leukemia cell line) of about 45.64 %.



Fig. 4.Percentage of proliferation cells 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*

Fig. 5show the effect of PEA or PEB (10 mg/mL) on anticholinesterase activity at different incubation times (5 to 30 min). Remarkably, Time-Dependent Inhibition of butyrylcholinesterase (BChE) was observed after PEA or PEB treatment. PEB showed more active than PEA (Fig. 5). For PEA, percentage of BChE inhibition at 5 and 30 min were respectively, 1.25 ± 0.25 % and 7.30 ± 0.48 %. After 30 min PEB treatment, the BChE inhibition reached a maximum of about 11.53 ± 0.12 %. Custódio*et al.*49 evaluated the BChE activity of *N. oculata* organics and water extracts. Maximum inhibition (21 %) was observed after treatment with *N. oculata* water extract at final concentration of about 0.5 mg/mL.



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

CONCLUSION

*I. galbana* and *N. oculata* are used widely in aquaculture for feeding and pathogens prevention. PEA and PEB has important functional activities such as antioxidant, antimicrobial, anticholinesterase, and antiproliferation activity that prove their importance value as commercial microalgae. Both *I. galbana* and *N. oculata* can be tested for the further nutritional and medical human application. The study of polysaccharidic extracts mode action on pathogenic bacteria or fungus constitutes also our next focus.

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