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Antioxidant Properties of Metabolites from New Extremophiles Microalgal Strain (Southern, Tunisia)

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Abstract

With the demand for bioproducts that can provide benefits for biotechnology sectors like pharmaceuticals, nutraceuticals, and cosmeceuticals, the exploration of microalgal products has turned toward extremophiles. This chapter is intended to provide an insight to most important molecules from halotolerant species, the cyanobacteria *Phormidium versicolor* NCC-466 and *Dunaliella* sp. CTM20028 isolated from Sfax Solar Saltern (Sfax) and Chott El-Djerid (Tozeur), Tunisia. These microalgae have been cultured in standard medium with a salinity of 80 PSU. The *in vitro* antioxidant activities demonstrated that extremolyte from *Dunaliella* and *Phormidium* as, phycocaynin, lipids, and polyphenol compound presents an important antioxidant potential.

Keywords: microalgae, halophile, biomolecule, antioxidant properties

1. Introduction

The primary producers of oxygen in aquatic environments are algae, especially planktonic microalgae. They play an important role in carbon dioxide (CO₂) recycling through photosynthesis [1]. Microalgae have been divided into ten groups, which refer to the color of the cell including: Cyanobacteria, blue-green algae; Chlorophyta, green algae; Rhodophyta, red algae; Glaucophyta; Euglenophyta; Haptophyta; Cryptophyta; photosynthetic Stramenopiles; Dinophyta; and Chlorarachniophyta [2]. Cyanobacteria are much closer to bacteria in terms of structure and their cells lack both nucleus and chloroplasts. Cyanobacteria are also known as a source of pigments, chlorophyll (a), phycocyanin, phycoerythrin, xanthophyll, and β -carotene. Microalgae are widely distributed in nature and adapted to different environments from fresh to hypersaline water ecosystems. Salt lakes in arid regions (sabkhas) and solar salterns are an examples of high salty environments inhabited by extremely halophilic microorganisms that include halophilic Archaea (halobacteria), halophilic cyanobacteria, and green algae [3–5]. These microorganisms must have specific adaptive strategies for surviving in high salinity conditions to prevent the loss of cellular water under high osmolarity in

hypersaline conditions [6]. Halophiles generally develop two basic mechanisms: (i) halobacteria and microalgae accumulate KCl (potassium chloride) in their cells to maintain high intracellular salt concentrations, osmotically at least equivalent to the external concentrations (the “salt-in” strategy); (ii) other halophiles produce or accumulate low molecular weight compounds (osmolyte or compatible solute) that have osmotic potential.

Microalgae provide many biotechnology applications in various industrial sectors such as food, cosmetics, pharmaceuticals, energy and environmental industries. Hyperhalophilic microalgae and their bioproducts, has gained a great deal of attention in the last decade. They are well known for their production of high value products such as β -carotene, lipids, and omega 3 fatty acids.

There are high demands for novel lead molecules for new classes of pharmaceutical and research biochemicals, and in combination, these drivers have led to an increased interest in microalgae and cyanobacteria as sources of both bioactive natural products.

Cyanobacteria species contain potential products for medicinal [7] and energy applications [8]. Some of this group has secondary metabolites that can potentially be used as therapeutic agents, such as antivirals, immunomodulators, inhibitors, cytostatics and antioxidants [9]. Several natural compounds such as vitamin C, tocopherol, and numerous plant extracts have been commercialized as natural antioxidants to fight against oxidative stress associated with various chronic diseases including atherosclerosis, diabetes mellitus, neurodegenerative disorders, and certain types of cancer [10]. Antioxidants are a crucial defense against free radical-induced damage [11].

Microalgae are abundant in nature and can be used as a renewable source of natural antioxidants [12]. Free radicals including reactive oxygen species (ROS), such as superoxide ($O_2^{\bullet-}$), hydroxyl ($OH^{\bullet}$) and Hydrogen Peroxide (H_2O_2), and reactive nitrogen species (RNS) are generated during normal cellular metabolism. These free radicals are highly reactive species and play a dual role in humans as both beneficial and toxic compounds depending on their concentration. At low or moderate concentration, these reactive species exert beneficial effects on cellular redox signaling and immune function. At high concentration, however, these radical species produce oxidative stress, a harmful process that can lead to cell death through oxidation of protein, lipid, and DNA [11, 13].

A number of microalgae have been used in the commercial production of pigments with antioxidant properties, for example: astaxanthin from *Haematococcus pluvialis*, β carotene from *Dunaliella salina*, as well as phycobiliproteins from *Arthrospira* and *Porphyridium* [12]. The review here in is about antioxidant capacity of the majors compounds extracted from new strain of hyperhalophilic microalgae (*Dunaliella* sp.) from salt lake Chott El-Djerid and cyanobacteria (*Phormidium versicolor*) from Sfax Solar Saltern (Tunisia).

2. Methods of cultivation and antioxidant assays

2.1 Isolation and principal production of the culture of new highly halophilic microalgae strains

Although most species of green algae (Chlorophyceae) are moderately halophilic, a few of them, including *Dunaliella salina*, are extremely halophilic species [3]. They are responsible for most of the primary production in hypersaline environments [4]. *Dunaliella salina* is the most important species of the genus for

beta-carotene production. Several investigations have demonstrated that *D. salina* produces more than 10% of the dry weight [14]. Lutein, chlorophyll, and other pigments and carotenoids are also produced by the genus of *Dunaliella*, under the same stressful environmental conditions [15]. Lipids for aquaculture, human nutrition, and biodiesel production have also been investigated in *Dunaliella* species [16].

Dunaliella sp. CTM 20028 have been isolated for the first time from Chott El-Djerid (Southern Tunisia) with a mean salinity of 142 PSU [17]. Chott El-Djerid (5.000 km²) consists of salty shallow pools and marshes, and it is covered by a large salt pan during the dry season (June to August). The water emerges into the Chott El-Djerid through a thin clay aquiclude of Quaternary age [18]. This generally allows temporary flooding of the Chott during winter. The climate of the area is arid-saharian with a mean annual rainfall between 80 and 140 mm and mean temperature of 21 °C. The elevation of the Chott surface is controlled by the position of the water table and the associated capillary fringe [19].

After acclimatation and purification, *Dunaliella* sp. was cultured in optimized f/2 Provasoli medium. Culture was carried out in 200 ml flask at 31 °C, 21 rad/s agitation and 54 mmol photon/m²/s continuous illumination intensity supplied by cool-white fluorescence tubes and in a saturated atmosphere to 0.1 v/v/m CO₂.

Cyanobacteria *Phormidium versicolor* NCC466 have been isolated from hypersaline ponds (75 PSU) of Sfax Solar Saltern (Central Tunisia). The solar saltern studied is located in the central-eastern coast of Sfax (Tunisia, 34°39'N and 10°42'E), and consists of a series of shallow interconnected ponds (20–70 cm depth) extending over an area of 1.500 ha. The salinity of water ponds varied from 45 to 450 PSU. The morphometric characteristics of the Saltern were reported elsewhere [20]. This Saltern shows high microalgae diversity, 13 diatoms, 26 Dinoflagellates, 5 cyanobacteria and 2 Chlorophyceae [5]. *Phormidium versicolor* was identified according to its internal transcribed spacer sequence based on the rDNA sequence (GenBank accession number NCC 466). It was grown in 250 mL Erlenmeyer flasks in batch containing 100 mL of a modified BG11 medium. The flasks were placed in a homeothermic incubator at 25 °C under a light intensity of 100 μM photons m⁻² s⁻¹, with a 14/10 h light/dark cycle for 11 days.

2.2 Extraction of metabolite and in vitro antioxidant evaluation

Total lipids were extracted at the end of the exponential phase of growth of *Dunaliella*'s cells according to the method of [21]. The phycocyanin pigment was isolated from *P. versicolor* using the method developed by [22]. However, the phenolic and total flavonoids content were determined in ethanolic extract according to [23, 24], respectively.

2.2.1 In vitro free radical scavenging and antioxidant assays

The antioxidant potential of the lipid extract (LE) of *Dunaliella* from Chott El-Djerid in batch culture was assessed on the basis of the 2,2-Diphenylpicrylhydrazyl (DPPH) and superoxide anion radical-scavenging activities. When DPPH radicals encounter a proton donating substrate, such as an antioxidant, the radicals would be scavenged and the absorbance would be reduced [25]. Antioxidant potential of C-PC was evaluated by Superoxide (O₂•⁻) scavenging, Hydroxyl (OH•) and Nitric oxide (NO) scavenging capacity. Moreover, the ability of C-phycocyanin to inhibit the lipid peroxidation was assessed using the method described by [26].

The free radical scavenging capacity of phenolic and flavonoids compounds extracted from *P. versicolor* was assessed through DPPH, NO and 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) tests. The antioxidant activities of polyphenol were expressed as IC₅₀, defined as the concentration of the these compounds required causing a 50% decrease in initial DPPH, NO and ABTS concentration.

3. Lipid antioxidant properties of *Dunaliella* sp. from Chott El-Djerid

Lipid compounds such as wax, fat, fat-soluble vitamins, oil, triacylglycerols, phospholipids, co-enzymes (ubiquinone), pigments (carotenoids), and more, could be found in plants or animals. Lipids are formed from long-chain hydrocarbons and sometimes contain other functional groups of oxygen, phosphorus, nitrogen, and sulfur. They are insoluble in water, but soluble in organic solvents such as chloroform, hexane, and ether. As invascular plants, microalgae produce both polar and neutral lipids. There is a wide range of bio-based lipid products that can be harvested from microalgal biomass. Microalgae lipids offer great potential in terms of biotechnology applications (e.g. food, food supplements, energy, cosmetics, and pharmaceuticals). In functional food, the use of microalgal lipids has already been established as an industry. The type and quality of the lipid products depend on microalgae species, culture conditions, and recovery methods.

The present study is the first comprehensive *in vitro* study revealing the protective effect of the lipidic extract (LE) of the *Dunaliella* sp. from Chott El-Djerid [17]. The *in vitro* antioxidant activity demonstrated that LE presents an important antioxidant potential. The DPPH radical-scavenging activity was investigated at different concentrations from 0.1 to 3 mg/mL of the LE. LE exhibited an interesting radical scavenging activity that was concentration dependent (**Figure 1A**). The IC₅₀ value obtained was about 0.1 ± 0.02 mg/mL which, is only 1.4 times higher than those of control, ascorbic acid and BHT. The antioxidant effect of *Dunaliella* sp. lipid extract was assessed at a concentration of 1, 2, and 3 mg/mL. The results show that the concentration of 2 and 3 mg/mL of *Dunaliella* sp. Lipid extract indicate a high radical scavenging ability compared with the ascorbic acid and BHT and that of 1 mg/mL of LE presents high activity compared with BHT as positive standard.

The low IC₅₀ indicates the higher free radical-scavenging ability of *Dunaliella* sp.-LE, which contained a high amount of essential fatty acid [17]. In addition, these authors reported that *Dunaliella* sp.-LE exhibited a strong NBT (Nitroblue-terazolium) photoreduction inhibition. Omega-3 EFAs is well documented for the

Figure 1. Antioxidant activities of *Dunaliella salina* lipid extract (LE) determined by two methods: DPPH-scavenging activity (A) and superoxide anion scavenging (B) and compared with synthetic antioxidants: Vitamin C (Vit C) and BHT. Data are presented as mean $\pm$ SD [17].

attenuation of oxidant mediated organ damage induced by various xenobiotics and disease states [27]. Moreover [17], stated that LE of *D. salina* from Chott El-Djerid enhance the antioxidant effect against Ni-induced toxicity by in vitro and in vivo test.

4. Phycocyanin pigments from *Phormidium versicolor* NCC466 from Sfax solar saltern

Phycocyanin (C-PC) isa hetero-oligomer consisting of a grouping of subunits that are organized into complexes called « phycobilisomes » [28]. C-PC possess a number of unique properties that make it useful colorant, including a higher molecular absorbance, fluorescence quantum yields, stable oligomers, and high photosatbility [29]. Phycocyanin has primarily been used as natural dye; however, it is increasingly being used as nutraceuticals or in ither biotechnological applications [29]. However, to the best of our knowledge, the antioxidant capacity of *P. versicolor* phycocyanin fraction (C-PC) has not been proved.

P. versicolor phycocyanin had a strong ability to scavenge free radicals (Figure 2). The ability of C-PC to scavenge the O2• – and OH• radicals were measured and compared with that of the positive control (ascorbic acid and BHT) (Figure 2(a) and (b)). C-PC presented the highest scavenging activity against O2• – and OH• radicals ((87.42 and 88.75% at 1 mg. mL⁻¹), respectively). Phycocyanin fractions isolated from cyanobacteria species were reported to be very efficient free radical

Figure 2. Antioxidant activity of C-PC extract on (a) superoxide radical, (b) hydroxyl radical, (c) nitric oxide radical and (d) inhibition of lipid peroxidation. BHT, ascorbic acid, TROLOX were used as standard. Values are presented as mean $\pm$ SD ($n = 3$).

scavengers and exhibit the highest antioxidant activity [30]. All phycocyanin extracts showed fairly moderate to high scavenging capacity against free radicals. As for nitric oxide radical (NO•), the C-PC showed a strong NO• scavenging activity reaching up to 84.87% (**Figure 2c**).

Several studies showed that phycocyanin isolated from cyanobacteria species exhibited strong antioxidant properties and can be protected cells against oxidative stress [31, 32]. Moreover, *in vitro* studies suggest that phycocyanin of *Spirulina* enhance antioxidant enzyme activity and inhibit lipid peroxidation in cells. The effect of *P. versicolor* phycocyanin (C-PC) on ferrous sulfate induced lipid peroxidation *in vitro* was illustrated in **Figure 2d**. Indeed, the inhibition rats of lipid peroxidation of C-PC varied between 37.65 and 82.31%.

The results here in suggested that administration of C-PC in reaction mixture significantly inhibited lipid peroxidation. The present finding revealed that C-PC had a strong effect and had antagonized action against ferrous sulfate induced lipid peroxidation *in vitro*. In this regards, Thangam et al. [33] showed that phycocyanin isolated from *Oscillatoria tenuis* possesses excellent antioxidant activity against DPPH radical, OH• and nitric oxide. Similarly, Ou et al. [31] indicated that *Spirulina maxima* phycocyanin protects human hepatocyte cell line L02 against H₂O₂ induced lipid damage. C-PC from halophilic *P. versicolor* could be used to produce a natural antioxidant complement or added to healthy food products.

5. Antioxidant properties of polyphenolic compounds from *P. versicolor* NCC466

Polyphenols represent a group of chemical compounds emerging from a common intermediate, phenylalanine, or a close forerunner, shikimic acid [34]. Polyphenols are able to protect cells from oxidative stress by various mechanisms; they can chelate transition metal ions, can inhibit lipid peroxidation by trapping the lipid alkoxyl radical, or can directly scavenge molecular species of active oxygen [34]. Flavonoids are a class of phenolic metabolites that have strong chelating and antioxidant properties [34]. Their tendency to inhibit free radical-mediated events is controlled by their chemical structure. This structure–activity relationship has been well established *in vitro* as previously reported [35, 36]. *P. versicolor* exhibited a high amount of phenolics and flavonoids reaching 408 ± 18.8 mg GAE g⁻¹ FW and 13,67 ± 0.788 mg QEq g⁻¹ FW, respectively (**Table 1**). These amounts are significantly higher than those recorded in *Dunaliella salina* from Sfax Solar Saltern [37]. These later recorded 0.086 ± 0.002 mg GAE g⁻¹ FW and 0.006 ± 0.0001 mg QEq g⁻¹ FW respectively for phenolics and flavonoids. Total antioxidant capacity (TAC) of phenolics and flavonoids extracted from *P. versicolor* are high about 0.94 ± 0.02 mg Eq g-1 FW. The IC₅₀ concentrations DPPH, ABTS and NO scavenging were low (0.007 to 0.031 mg. l⁻¹), suggested a high antioxidant activity of polyphenols and flavonoids extract from *P. versicolor* on the ROS (**Table 1**).

Antioxidant test	Polyphenols and flavonoids extract	Standard
DPPH (mg. l ⁻¹)	0.031 ± 0.08	0.077 ± 0.06 (BHT)
ABTS (mg. l ⁻¹)	0.015 ± 0.01	0.098 ± 0.02 (TROLOX)
NO (mg. l ⁻¹)	0.007 ± 0.03	0.094 ± 0.01 (Vit C)

Table 1. Antioxydant capacity (IC₅₀ concentrations) of phenolics and flavonoids metabolites extracted from *P. versicolor* NCC466. BHT, Trolox and vitamin C represent the standard.

6. Conclusion

News hyperhalophilic microalgae strains, *Dunaliella* sp. and *Phormidium versicolor* NCC466 are rich in lipid and phycoerythrin even secondary metabolite such polyphenolic compounds. Scavenging activity tests indicated that these extremophytes have an excellent capacity as natural antioxidant.

Acknowledgements

This study was supported by the Ministry of Higher Education and Scientific Research of Tunisia. We thank Dr. Mohammad Ali from Institute for Scientific Research (Kuwait) for correcting the English language.

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References

- [1] Chisti Y. Microalgae as sustainable cell factories. *Environmental Engineering and Management Journal*. 2006;5:261-274.
- [2] Graham LE, Graham J, Wilcox LW. *Algae*. Wilbur B. editor. 2nd ed. San Francisco: Pearson Education; 2009. 100p.
- [3] Grant WD, Gemmel RT, McGenity TJ. Halophiles. In: Horikoshi K, Grant, WD, editors. *Extremophiles: Microbial Life in Extreme Environments*. Wiley-Liss; 1998. p. 93-132.
- [4] Oren A. A hundred years of *Dunaliella* research 1905-2005. *Aquatic Biosystems*. 2005 ;1:1-14. DOI: 10.1186/1746-1448-1-2
- [5] Ayadi H, Elloumi J, Guermazi W, Bouain A, hammami M, Giraudoux P, Aleya L. Fatty acids composition in relation to the microorganisms in the Sfax solar saltern, Tunisia. *Acta protozoologica*. 2008; 47:189-203
- [6] Oren A. Bioenergetic aspect of halophilism. *Microbiology and Molecular Biology Reviews*. 1999;63:334-348. DOI: 10.1128/MMBR.63.2.334-348.1999.
- [7] Rastogi RP, Sinha RP. Biotechnological and industrial significance of cyanobacterial secondary metabolites. *Biotechnology Advances*. 2009;27:521-539. DOI:10.1016/j.biotechadv.2009.04.009
- [8] Angermayr SA, Hellingwerf KJ, Lindblad P, de Mattos MJT. Energy biotechnology with cyanobacteria. *Current Opinion in Biotechnology*. 2009;20:257-263. DOI:10.1016/j.copbio.2009.05.011
- [9] Smith JL, Boyer GL, Zimba PV. A review of cyanobacterial odorous and bioactive metabolites: Impacts and management alternatives in aquaculture. *Aquaculture*. 2008;280:5-20. DOI:10.1016/j.aquaculture.2008.05.007.
- [10] Vadlapudi V. Antioxidant activities of marine algae: a review. In: Cappasso A, editor. *Medicinal plants as antioxidant agents: understanding their mechanism of action and therapeutic efficacy*. Research Signpost: Kerala; 2012. p. 189-203.
- [11] Sen S, Chakraborty R. The role of antioxidants in human health. In: Hepel M, Andreescu S, editors. *Oxidative stress: diagnostics, prevention, and therapy*. American Chemical Society: Washington, D.C; 2011. p 1-37.
- [12] Spolaore P, Joannis-Cassan C, Duran E, Isambert A. Commercial applications of microalgae. *Journal of Bioscience and Bioengineering*. 2006;101:87-96. DOI:10.1263/jbb.101.87
- [13] Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science*. 2008;4:89-95.
- [14] Olmos-Soto J, Paniagua-Michel J, Contreras R, Trujillo L. Molecular identification of β -carotene hyperproducing strain of *Dunaliella* from saline environment using species-specific oligonucleotides. *Biotechnology Letters*. 2002; 24:365-369. DOI: 10.1023/A:1014516920887
- [15] Skjanes K, Rebours C, Lindblad P. Potencial for green microalgae to producer hydrogen, pharmaceuticals and other high value products in a combined process. *Critical Reviews in Biotechnology*. 2013;33:172-215. DOI: 10.3109/07388551.2012.681625
- [16] Da Silva CM, Gomez ADA, Couri S. Morphological and chemical aspect of *Chlorella pyrenoidosa*, *Dunaliella*

tertiolecta, *Isochrysis galbana* and *Tetraselmis gracilis* microalgae. Natural Science. 2013;5:783-791.DOI: 10.4236/ns.2013.57094

[17] Dahmen-Ben Moussa I, Bellassoued K, Athmouni K, Naifar M, Chtourou H, Ayadi, H., Makni-Ayadi F, Sayadi S, El Feki A, Dhouib, A. Protective effect of *Dunaliella* sp., lipid extract rich in polyunsaturated fatty acids, on hepatic and renal toxicity induced by nickel in rats. Toxicology Mechanisms and Methods. 2016; 26(3):221-230. DOI:10.3109/15376516.2016.1158340

[18] Roberts CR, Mitchell CW. Spring mounds in southern Tunisia. In: Frostick L, Reid, I editors. Desert Sediments: Ancient and Modern. Geol. Soc. London, Special Publications;1987. p. 321-334.

[19] Swezey CS. The role of climate in the creation and destruction of continental stratigraphic records: an example from the northern margin of the Sahara Desert. In: Climate Controls on Stratigraphy. SEPM Special Publication; 2003. p. 207-225.

[20] Elloumi J, Carrias JF, Ayadi H, Sime-Ngando T, Boukhris M, Bouain A. Composition and distribution of planktonic ciliates from ponds of different salinity in the solar saltwork of Sfax, Tunisia. Estuarine, Coastal and Shelf Science.2006;67:21-29.DOI: 10.1016/j.ecss.2005.10.011

[21] Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology. 1959;37:911-917.

[22] Silveira ST, Burkert JFdM, Costa JAV, Burkert CAV, Kalil SJ. Optimization of phycocyanin extraction from *Spirulina platensis* using factorial design. Bioresource Technology. 2007; 98 (8): 1629-1634. DOI: 10.1016/j.biortech.2006.05.050.

[23] Hajimahmoodi M, Faramarzi MA, Mohammadi N, Soltani N, Oveisi MR, Nafissi-Varcheh N. Evaluation of antioxidant properties and total phenolic contents of some strains of microalgae. Journal of Applied Phycology. 2010; 22:43-50. DOI: 10.1007/s10811-009-9424-y

[24] Kim Dk, Jeong SW, Lee CY. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chemistry. 2003;81:321-326. DOI: 10.1016/S0308-8146(02)00423-5

[25] Fakhfakh N, Ktari N, Haddar A, Hamza Mnif I., Dahmen I, Nasri M. Total solubilisation of the chicken feathers by fermentation with a keratinolytic bacterium, *Bacillus pumilus* A1, and the production of protein hydrolysate with high antioxidative activity. Process Biochemistry. 2011;46:1731-1737.DOI: 10.1016/j.procbio.2011.05.023.

[26] Halliwell B. Free radicals, reactive oxygen species and human disease: a critical evaluation with special reference to atherosclerosis. British Journal of Experimental Pathology. 1989; 70(6):737-757.

[27] Fayez AM, Awad AS, El-Naa MM, Kenawy SA, El-Sayed ME. Beneficial effects of thymoquinone and omega-3 on intestinal ischemia/reperfusion induced renal dysfunction in rats. Bulletin of Faculty of Pharmacy, Cairo University. 2014;52:171-177.

[28] Wiedenmann J. Marine proteins. In: Oceans and Human Health. Risks and Remedies from the Sea. Walsh PJ, Smith SL, Fleming LE, Solo-Gabriele HM, Gerwick WH, editors. Academic Press: St. Louis, MO; 2008. p. 469-495.

[29] Becker W. Microalgae in human and animal nutrition. In: Richmond A, editor. Handbook of Microalgal Culture: Biotechnology and Applied Phycology:

Blackwell Publishing Ltd, Oxford; 2004. p.312-351.

[30] Bermejo P, Piñero E, Villar ÁM. Iron-chelating ability and antioxidant properties of phycocyanin isolated from a protean extract of *Spirulina platensis*. Food Chemistry. 2008;110:436-445. DOI: 10.1016/j.foodchem.2008.02.021

[31] Ou Y, Zheng S, Lin L, Jiang Q, Yang X. Protective effect of C-phycocyanin against carbon tetrachloride-induced hepatocyte damage in vitro and in vivo. Chemico-Biological Interactions. 2010;185 (2):94-100. DOI: 10.1016/j.cbi.2010.03.013.

[32] Niu YJ, Zhou W, Guo J, Nie ZW, Shin KT, Kim NH, Lv WF, Cui XS. C-Phycocyanin protects against mitochondrial dysfunction and oxidative stress in parthenogenetic porcine embryos. Scientific Reports. 2017;7(1):16992. DOI: 10.1038/s41598-017-17287-0.

[33] Thangam R, Suresh V, Princy WA, Rajkumar M, SenthilKumar N, Gunasekaran P, Rengasamy R, Anbazhagan C, Kaveri K, Kannan S. C-Phycocyanin from *Oscillatoria tenuis* exhibited an antioxidant and in vitro antiproliferative activity through induction of apoptosis and G0/G1 cell cycle arrest. Food Chemistry. 2013;140(1-2):262-272.DOI: 10.1016/j.foodchem.2013.02.060

[34] Rodrigo R, Libuy M. Modulation of Plant Endogenous Antioxidant Systems by Polyphenols. In: Watson RR, editor. Polyphenols in Plants Isolation, Purification and Extract Preparation. ISBN: 978-0-12-397934-6

[35] Heim KE, Tagliaferro A, Bobiya D. Flavonoid antioxidants: chemistry, metabolism and structure–activity relationships. Journal of Nutritional Biochemistry. 2002;13:572-584. DOI: 10.1016/s0955-2863(02)00208-5

[36] Amić D, Davidović-Amić D, Beslo D, Rastija V, Lucić B, Trinajstić N. SAR and QSAR of the antioxidant activity of flavonoids. Curr Med Chem. 2007;14:827-845. DOI: 10.2174/092986707780090954.

[37] Belghith T, Athmouni K, Bellassoued K, El Feki A, Ayadi H. Physiological and biochemical response of *Dunaliella salina* to cadmium pollution. Journal of Applied Phycology. 2015;28(2):991-999. DOI: 10.1007/s10811-015-0630-5.