erevistas.saber.ula.ve/avancesenquimica
Avances en Química, 16(3), 71-79 (2021)
Artículo científico

Phytochemical study and *in vitro* biological activities of *Chlorella vulgaris*, *Chlorella pyrenoidosa* and *Chlorella minutissima* extracts

Antonio Menezes-Filho[1*], Matheus Ventura[1], Hellen Batista-Ventura[1], Carlos Castro[2], Celiana Triches[3], Cinthia Porfiro[3],Jacqueline Guimarães[3], Marconi Teixeira[1], Frederico Soares[1], Aparecida Taques[4].

[1)] Department of Agrarian Sciences, Doctorate in Agrarian Sciences, Goiano Federal Institute, Campus Rio Verde, Goiás, Brazil.

[2)] Department of Agrochemistry, Master in Agrochemistry, Goiano Federal Institute, Campus Rio Verde, Goiás, Brazil.

[3)] Department of Pharmacy, UniBRAS College, Campus Rio Verde, Goiás, Brazil.

[4)] Department of Engineering, Mato Grosso Federal Institute, Campus Bela Vista, Mato Grosso, Brazil.

(*) antonio.menezes@estudante.ifgoiano.edu.br; astronomoamadorgoias@gmail.com

Recibido: 30/11/2021 **Revisado**: 24/12/2021 **Aceptado**: 30/12/2021

Resumo

Estudo fitoquímico e atividades biológicas *in vitro* dos extratos de *Chlorella vulgaris, Chlorella pyrenoidosa* e *Chlorella minutíssima*. O estudo teve como objetivo a triagem de compostos fitoquímicos e avaliação das atividades biológicas dos extratos de *Chlorella vulgaris*, *Chlorella pyrenoidosa* e *Chlorella minutissima* (aquoso e hidroetanólico). Foi realizado fitoquímica, varredura em espectrofotometria, fenólicos e flavonoides totais, eliminação do radical DPPH e a atividade antioxidante total, atividade antibacteriana foi realizada sobre *Staphylococcus aureus*, *Escherichia coli*, *Salmonella sorovar* Enteritidis e Thyphymurium e citotóxica sobre *Artemia salina*. Os extratos exibiram a presença de diversos grupos fitoquímicos, alto conteúdo de flavonoides e fenólicos totais, expressivas atividades redutoras para DPPH e %AA. A inibição foi positiva para cepas bacterianas e baixa atividade citotóxica.

Palavras-chave: Atividade antioxidante; Compostos fenólicos, Clorofilas; *Escherichia coli*; *Staphylococcus aureus*.

Abstract

The study aimed to screen phytochemicals and evaluate the biological activities of *Chlorella vulgaris*, *Chlorella pyrenoidosa* and *Chlorella minutissima* extracts (aqueous and hydroethanolic). Phytochemistry, spectrophotometric scanning, total phenolics and flavonoids, DPPH radical scavenging and total antioxidant activity were performed, antibacterial activity was performed on *Staphylococcus aureus*, *Escherichia coli*, *Salmonella serovar* Enteritidis and Thyphymurium and cytotoxic on *Artemia salina*. The extracts showed the presence of several phytochemical groups, high content of flavonoids and total phenolics, expressive reducing activities for DPPH and %AA. Inhibition was positive for bacterial strains and low cytotoxic activity.

Keywords: Antioxidant activity; Chlorophylls; *Escherichia coli*; Phenol compounds; *Staphylococcus aureus*.

Introducción

Chlorella Beijerinck is a genus of eukaryotic green unicellular microalgae that shows spherical shape ≈ 2 to 10 μm (diameter) with high photosynthesis capacity, fast reproduction requiring only sunlight, CO_2, water and a small amount of nutrients[1,2]. According by Huss *et al.*[3] the lack of obvious morphological characters combined with an exclusively asexual reproductive cycle by means of autospores has caused considerable problems in the taxonomic description and identification of *Chlorella* species. Currently, this diverse genus presents remarkable phylogenetic studies that support the morphological characteristics of the species of green microalgae included in *Chlorella*. According by Andrade *et al.*[1] the name *Chlorella* derived from the Greek "*chloros*" and from the Latin "*ella*", which mean green and small. *Chlorella* microalgae have been present on earth since the pre-Cambrian period around 2.5 billion years ago. Japan is currently the world leader in *Chlorella* microalgae consumption.

Chlorella species live in freshwater and marine ecosystems having bioactive compounds such as proteins, vitamins, chlorophyllian pigments, polyunsaturated fatty acids, sterols and especially polyphenolics being represented by phloroglucinol, *p*-coumaric acid, ferulic acid and apigenin[4], which makes this genus very interesting from a health-beneficial point of view, being used as forage, in medicine and as food additives[1,5].

The different species of this genus such as *Chlorella vulgaris*, *Chlorella pyrenoidosa* and *Chlorella minutissima* have a very diversified chemical constitution, although they share in common several polysaccharide molecules, involved in bio-

logical activities such as antioxidants, antifungals, antibacterials, antivirals, antitumor, cytotoxic and anti-radiation agent[6,7]. In addition to the countless biological activities, this group of green microalgae are nutritional sources of proteins, lipids (palmitoleic, oleic, linoleic, α-linoleic, γ-linoleic, and homo γ-linoleic), chlorophylls (*a*, *b* and *c*), β-carotene, soluble vitamins, choline, dietary fiber and mineral salts such as iron, calcium, potassium, magnesium and phosphorous[8,9].

The green microalgae *Chlorella* have experienced a strong surge in their applications last years, but are still not fully exploited as source resource in medical, biological, biotechnological process, and agricultural science. This growing knowledge generates a large number of considerable studies, however, little is known about the numerous and potential biological activities with photo-protective action, antioxidant for numerous free radicals, especially reactive oxygen species such as singlet, antibacterial and cytotoxic oxygen[7,9-11].

The present research work was planned to examine the phytochemical and bioactive compounds of *C. vulgaris*, *C. pyrenoidosa* and *C. minutissima* marine algae extracts.

Material and methods

The reagents used were ethanol (LSchemicals, Brazil), iodine (Synth, Brazil), mercury II sulfate (Synth, Brazil), petroleum ether (Neon, Brazil), acetone (Neon, Brazil), Folin-Ciocalteu reagent (Sigma Aldrich, Singapore), ferric chloride (Neon, India), aluminum chloride (Neon, China), quercetin (Gemini, India), 2,2-diphenyl-1-picrylhydrazyl (Sigma Aldrich, Singapore), linoleic acid (Vetec, Brazil), sodium chloride (Neon, India), count plate agar (Kasvi, U.S.A), chlorophyll a (Sigma Aldrich, China), chlorophyll b (Sigma Aldrich, China), chlorophyll c (Sigma Aldrich, China), xanthophyll (Sigma Aldrich, Singapore), β-carotene (Sigma Aldrich, Singapore), sodium carbonate (Neon, China), gallic acid (Sigma Aldrich, Singapore), sodium nitrate (Neon, China), sodium hydroxide (Neon, Brazil), 3,5-di-tert-4-butylhydroxytoluene (Sigma Aldrich, Singapore), potassium phosphate (Neon, India), azithromycin, cephalexin and tigecycline (CenterLab, Brazil) and Tween 20 (Sigma Aldrich, U.S.A).

The three Chlorella algae species were supplied in the form of lyophilized powder: *C. vulgaris* by Qingdao Fraken International Trading Co. Ltd. (China); *C. pyrenoidosa* by Qingdao Hilda-Jingyi Trading Co. Ltd (China) and *C. minutissima* by Xi'an Tongze Biotech Co. Ltd (China). The identification of Chlorella species was carried out using a dichotomous key for the Chlorella genus, HPLC and UV, and certification rules the ISO, FDA, HACCP and Kosher. Initially, they were dried in an oven 50 ± 2 °C and crushed in a mill (7lab, Mod. Micro910, Brazil). The powder obtained was stored in an amber bottle in a refrigerator at -12 °C. 150 g of powdered samples were separately extracted by reflux using destilled water and 70% ethanol as the solvents for 12 h. The aqueous extract was reduced in a hot bath at 80 ± 5 °C, and the hydroethanolic extract was reduced in a rotary evaporator with reduced pressure. Then, the extracts were frozen and lyophilized until constant mass according by Sembiring *et al.*[12].

Phytochemical tests were carried out on the aqueous and hydroethanolic extracts for qualitative determination according to Sembiring *et al.*[12], Madike *et al.*[13], and Mehdi *et al.*[14]. The three algae materials were tested for groups of alkaloid, flavonoid, tannin, saponin, quinone, terpenoid and steroids, reducing sugars and non-reducing sugars, resins, amino acids, coumarins, glycosides, purines, organic acids, aromatic and aliphatic, phenolics, xanthoproteins, leucoanthocianins, polysaccharides, phlobatannins, carboxylic acids and oxylates.

Analysis by thin-layer chromatography (TLC) was performed on chromatoplates (Xtra SIL G/UV_{254}). Five (5 µL) of the algae extract was added 1 cm from the lower edge of the chromatoplate. Then, the plate was transferred to a vat for chromatography. The chromatographic run was stopped when the mixture reached 1 cm from the upper edge of the chromatoplate. The development system consists of a mixture of ethanol: petroleum ether: acetone in a 1:1:1 volume ratio. After development, the plate is air dried, marked the center of the pigment point, measured the distance traveled by the solvent front and the distance traveled by each pigment. The Retardation factors (R*fs*) are then calculated. Chlorophyll (*a*), chlorophyll (*b*), Chlorophyll (c), xanthophyll and β-carotene standards were used for R*fs* comparison[15].

The total phenolic contents were determined according to colorimetric Folin-Ciocalteu method as described by Labiad *et al.*[16] (modified). Aliquot containing 0.5 mL of sample solution was mixed with 2.5 mL of Folin-Ciocalteu reagent diluted with distilled water (1:9, *v/v*), followed by the addition of 5 mL of sodium carbonate (7.5%, *w/v*). The solution was stored in a dark room for 60 min., and the absorbance (Abs) was measured at 765 nm using a UV-*Vis* spectrophotometer (Bel-Photonics, Mod. M-51, Italy) and a glass cuvette (5 mL). The standard curve of gallic acid is obtained under the same conditions as above using solutions with a range of concentrations between 0-500 $mg.L^{-1}$, which were prepared in 96% ethanol, and $R^2 = 0.9997$. The total phenolic content was measured as gallic acid equivalents (mg GAE g^{-1} dry extract algae).

Flavonoid contents were measured using a modified colorimetric method described by Labiad *et al.*[16]. Aliquot containing 0.25 mL of algae extract solution was added to a test tube containing 1.25 mL of distilled water. Then, 0.075 mL of an aqueous sodium nitrite solution (5%, *w/v*) was added to the mixture and maintained for 5 min. Then, 0.15 mL of an aluminum chloride solution (10%, *w/v*) was added and homogenized for 1 min. After 6 min., 0.5 mL of 1 M sodium hydroxide was finally added. The solution was diluted with 0.275 mL of distilled water, and homogenized for 5 min. The absorbance (Abs) of the final solution was measured at 510 nm; the standard curve of quercetin was obtained under the same conditions as above, using solutions with a range of concentrations between 0-650 mg L^{-1}, prepared in 96% ethanol and $R^2 = 0.9991$. The total flavonoid content is expressed as mg quercetin equivalent (QE g^{-1} of dry extract algae).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging ability assay was used to evaluate the antioxidant activity of each algae extract. Test was conducted in a 96-well plate according to Sembiring *et al*.[12] (modified). 20 µL stock solution for each algae extract was prepared at different concentrations (between 5-2.000 ppm, *v/v*) and 180 µL of DPPH solution 0.147 mmol.mL^{-1} were added to each well. After 60 min incubation at room temperature in dark room, absorbance was read at 517 nm using the micro-plate reader of UV-*Vis* spectrophotometer. Hydroethanol solution was used as blank. The scavenging ability (%) was calculated according to equation (1), and ascorbic acid and 3,5-di-*tert*-4-butylhydroxy-toluene (BHT) was used as positive standards.

$$\% \text{ reduction} = \frac{(\text{Abs standard} - \text{Abs crude extract})}{\text{Abs standard}*100} \quad \text{Eq. 1}$$

All tests were performed in triplicate. Concentrations of algae extract samples resulting in 50% inhibition on DPPH (IC_{50} value, expressed in µg.mL^{-1}) were calculated.

The antioxidant activity (%AA) of *Chlorella* algae extracts was determined according to the thiocyanate method proposed of Mitsuda *et al*.[17] and described by Gulçin *et al*.[18] 10 mg of lyophilized water extracts were dissolved in 10 mL water. 10 mg of each algae hydroethanolic extract were dissolved in 10 mL hydroethanolic solution (between 5-100 µg.mL^{-1}) or standard samples in 2.5 mL of potassium phosphate solution buffer (0.04 M, pH 7.0), was added to 2.5 mL linoleic acid emulsion. The 50 mL linoleic acid emulsion consists of 175 µg Tween 20, 155 µL linoleic acid, and 0.04 M potassium phosphate buffer. 50 mL control contains 25 mL linoleic acid emulsion and 25 mL potassium phosphate buffer. The solution was incubated at 37 ± 2 °C in tubes assay in the dark roon. After, the solution was stirred for 3 min., the peroxide value was determined by reading the Abs at 500 nm in a spectrophotometer UV-*Vis*. Therefore, high Abs indicates high linoleic acid oxidation. Solutions without added extracts or standards were used as control. All data are the average of quadruplicate analyses. The inhibition percentage of lipid peroxidation was calculated by following equation 2:

$$\% \text{ Inhibition} = (A_0 - A_1/A_0)*100 \quad \text{Eq. 2}$$

where: A_0 = Abs of the control reaction; A_1 = Abs in the presence of the samples.

Antibacterial activity was determined according to Tuama and Mohammed[19] (modified). The antibacterial assay was investigated applying the standard agar well diffusion. The assay pathogens *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *S. serovar* Enteritidis (ATCC 13076) and *S. serovar* Thyphymurium (ATCC 14028) were uniformly homogenized on count plate agar (CPA) using sterile Drigalski-spreader, then, five wells of 9 mm diameter were made using sterile well tip. 50 µL of different concentrations were added to each well (25, 50, 75, and 100 µg.mL^{-1}). Then, the plates were incubated at 36 ± 2 °C for 36 h for the bacterial strains; after incubation the zones of inhibition were recorded. A minimum 5 mm antibiosis halo was determined using a digital caliper (Eda, Mod. 8", China).

The photo-protection activity was adopted as the methodology described by Medeiros *et al*.[20] (modified). The critical wavelength scan was obtained scanning from 250 to 400 nm in a UV-*Vis* spectrophotometer, using a 1 cm single-field quartz cuvette. *Artemia salina* cytotoxic assay was conducted in according to Silva *et al*.[21], as proposed by Meyer *et al*.[22] (modified). Each algae extract (20 mg) was dissolved in 2 mL of hydroethanolic solution (45%, *v/v*) and samples of this solution (500, 375, 250, 125, 50 and 25 µL) were transferred, in triplicate, to the 5 mL vials. After total removal of the solvent, 5 mL of a saline solution (NaCl, 0.38 g.L^{-1}), was added in each of the bottles, resulting in final concentrations of 1.000, 750, 500, 250, 100 and 50 µg.mL^{-1}. Larvae of *A. salina* nauplii type (10 per vials) were added and after of 12 h contact, the survivors were counted. As a negative control, saline aqueous solution (0.38 g.L^{-1}) was used. The lethal concentration LC_{50} (expressed in µg.mL^{-1}) was derived from the best fit line obtained by linear regression analysis.

Assays for total phenolics and flavonoids, DPPH free radical reduction and total antioxidant activity (%AA) and antibacterial activity were performed in quadruplicate. When significant differences were observed, they were analyzed using the Duncan's test ($p < 5\%$) using the Statistica software (SPSS).

Results and discussion

In this study, aqueous and hydroethanolic extracts of three algae of the *Chlorella* genus exhibited a rich and varied complexity of positive phytochemical groups that are involved in several biological activities of therapeutic use such as alkaloids, flavonoids, tannins, saponins, reducing sugars, amino acids, glycosides, organic acids, aromatics, phenolics, xanthoproteins and polysaccharides (Table 1). The aqueous extracts of *Chlorella* exhibited the highest number of positive phytochemical groups.

Kannan *et al*.[23] studied the seaweed genera *Gracilaria* (*G. corticata*) and *Spirulina* (*S. platensis*) where also through TLC they found positive results for alkaloids, flavonoids, glycosides, phenols and saponins similar to this study with the genus *Chlorella*. Phytochemical screening of *Caulerpa racemosa* exposed the presence of alkaloids, phenolics, flavonoids and steroids in the study by Srivastav *et al*.[24]. Algae in several families and genera share relatively common phytochemical groups, which are important both for these organisms and for food and medicinal use.

Several phytochemical groups of special metabolism in algae have important biological activities, such as cytotoxic agents (tannins), analgesic and anti inflammatory (terpenoids), anti inflammatory, estrogenic, antimicrobial, antiallergic, antioxidant, vascular and cytotoxic antitumor (flavonoids), congestive heart failure and cardiac arrhythmia (glycosides), and antibacterial and antifungal saponins[25].

Chlorella algae extracts were tested for the presence of chlo-

Table 1. The phytochemical of aqueous and hydroethanolic of *C. vulgaris*, *C. pyrenoidosa* and *C. minutissima* extracts.

Phytochemical	Type extract					
	Aqueous			Hydroethanolic		
	1	2	3	1	2	3
Alkaloids	+	+	+	-	-	-
Flavonoids	+	+	+	+	+	+
Tannins*	+	+	+	-	-	-
Saponins	+	+	+	-	-	-
Quinones	-	-	-	-	-	-
Terpenoids and Steroids	+	+	+	+	+	+
Reducing sugars	+	+	+	-	-	-
Non-reducing sugars	-	+	+	+	+	-
Resins	+	+	+	+	+	-
Amino acids	+	+	+	-	-	-
Coumarins	-	+	-	-	-	-
Glycosides	-	+	+	+	+	+
Purines	-	-	-	-	-	-
Organic acids	+	+	+	+	+	+
Aromatics and Aliphatics**	+	+	+	+	+	+
Phenolics	+	+	+	+	+	+
Xanhoproteins	+	+	+	-	-	-
Leucoanthocyanins	-	-	-	-	-	-
Polysaccharides	+	+	+	-	-	-
Phlobatannins	-	-	-	-	-	-
Carboxylic acids	-	-	-	-	-	-
Oxylates	-	-	-	-	-	-

Notes: *C. vulgaris* (1). *C. pyrenoidosa* (2). *C. minutissima* (3). *Tannins (Green). **Positive result for aliphatic substances. (-) absent. (+) presence. Source: Authors, 2021.

rophyll *a*, chlorophyll *b*, chlorophyll *c* xanthophyll and β-carotene using the TLC technique. Table 2 shows the R*fs* obtained, which indicate high separation efficiency by method used. It is suggested that the mobile phase constituted by ethanol, petroleum ether and acetone played an important role in this step of separation of compounds for *Chlorella*. Similar effects to this study were reported during the separation of pigments and other important groups of molecules performed by Jeffrey[26] for several species of seaweed using classical planar chromatography (paper) and mobile phases acetone, ethyl ether, ethanol, pyridine and carbon disulphide.

Pigment separation occurred exhibiting the following pattern for chlorophyll *a* (blue spots), chlorophyll *b* (greenish yellow), chlorophyll *c* (light green), xanthophyll (yellow spots), chlorophyll degradation products (grey spots) and β-carotene at the highest point, being similar when compared to the standards. According by Kannan *et al.*[23], Mendiola *et al*.[27] and Kannan *et al*.[28], a lot phytomolecules have been recognized in algae extracts equivalent to various carotenoids formerly known in *S. platensis* microalgae along with numerous degradation products.

The pigments observed in the three *Chlorella* samples in this study corroborate the research by Mello *et al*.[29], where researchers discuss the purpose of these pigments such as chlo-

Table 2. R*fs* values for separation on TLC, the monodimensional method of aqueous and hydroethanolic of *C. vulgaris*, *C. pyrenoidosa* and *C. minutissima* extracts.

Identified component	R*fs* values of algae extracts*					
	Aqueous extract			Hydroethanolic extract		
	1	2	3	1	2	3
Chlorophyll *a*	2.01	2.09	2.13	1.96	1.90	1.99
Chlorophyll *b*	1.83	1.86	1.82	1.80	1.78	1.85
Chlorophyll *c*	0.55	0.91	0.77	0.69	0.60	0.72
Xantophile	5.53	5.60	5.49	5.40	5.55	5.60
β-carotene	7.30	7.21	7.34	7.28	7.26	7.31

Notes: *R*fs* values are dimensionless. (1) *C. vulgaris*, (2) *C. pyrenoidosa*, (3) *C. minutissima*. Source: Authors, 2021.

rophylls, xanthophylls and carotenoids that, in addition to being large and complex molecules, also participate in the absorption of electromagnetic radiation, playing an important role in the conversion of solar energy into chemical energy.

The aqueous extracts of *Chlorella* in this study showed similarity although at different band intensities in the UV-*Vis* spectrophotometric analysis (Figure 1-A). In Figure 1-B, a similar behavior can be observed both for the extracts of *C. vulgaris* and *C. pyrenoidosa* and different for *C. minutissima*, which showed similarity with the aqueous extracts. Although extracts showed differences in UV-Vis bands (with both extracting solvents), all of them showed absorption bands related to groups composed of chlorophylls *a* (600-700 ~10 nm), *b* (400-500 ~10 nm) and *c* (450-500 ~10 nm), xanthophylls (400-700 ~10 nm) and β-carotene (300-500 ~50 nm) corroborating the TLC analysis.

It is noteworthy that the index on the quantitative or qualitative rate of chlorophylls varies according to the absorption of light by the alga, and that the bands with higher intensities generally occur between 645 to 663 nm, where still in Figure 1 (A and B) it is possible to see such statement proposed by Tamburic *et al*.[30]. Also in the study by Tamburic *et al*.[30] and Oliveira *et al*.[31], bands with medium to high intensities tell about the health of these organisms, where bands with low and no intensity between this UV-Vis range are highly indicatives of damage to cell culture due to discoloration. Thus, this study demonstrates that the three *Chlorella* species analyzed have a high degree of pigmentation, thus, exhibiting excellent quality in the health of these marine organisms.

Hydroethanolic extracts are the best option for obtaining β-carotene, due to its molecular conformation and the mixture of non-toxic solvents. In Figure 1 (B) UV-Vis spectra show homogeneity and absorption between 300-550 ~50 nm in *C. pyrenoidosa*, *C. minutissima* and *C. vulgaris* (superimposed on *C. pyrenoidosa* as described above). As discussed by Hornero-Méndez and Britton[32], β-carotene is an important source of vitamin A, in addition to presenting high photoprotective activity against harmful damage from energizing radiation emitted in UV wavelengths (A, B and C) both for algae and

for humans using photoprotective emulsions, preventing these energy sources from damaging several biomolecules, including promoting the appearance of skin cancers and premature aging. According Silva *et al*.[21] the carotenoids identified in these microalgae are astaxanthin, zeaxanthin, violaxanthin, and lutein which are already industrially produced synthetically for use in a variety of food products and cosmetics.

This has been also discussed by Rinawati *et al*.[33], who also analyzed by UV-*Vis* spectrophotometry the content of chlorophylls and carotenoids in algae. The chlorophyll content in microalgae in the logarithmic phase was: *C. vulgaris* 200-1.500 mg.L^{-1}, *Nannochloropsis* sp. 100-500 g.L^{-1}, *Porphyridium cruentum* 500-800 g.L^{-1} and *Spirulina platensis* 1.000-3.500 mg.L^{-1} and for stationary phases microalgae *C. vulgaris* 100-1.000 mg.L^{-1}, *Nannochloropsis* sp. 200-500 g.L^{-1}, *P. cruentum* 900-2.000 mg.L^{-1} and *S. platensis* 2.000-6.000 mg. L^{-1}. While the carotenoid content of microalgae in the logarithmic phase of *C. vulgaris* 10-40 g.L^{-1}, *Nannochloropsis* sp.

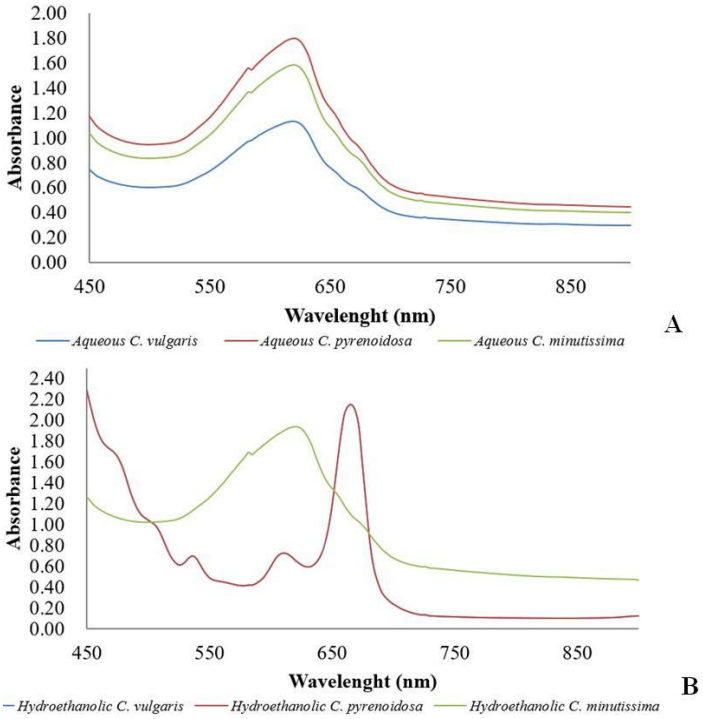

Fig. 1: UV-*Vis* spectra between 450-900 nm of the aqueous (A) and hydroethanolic (B) extracts of *C. vulgaris*, *C. pyrenoidosa* and *C. minutissima*. The *C. vulgaris* scan line is superimposed on the *C. pyrenoidosa* line in (B). Source: Authors, 2021.

10-60 g.L^{-1}, *P. cruentum* 10-60 ug.L^{-1} and *S. platensis* 20-40 ug.L^{-1} and for stationary phases microalgae *C. vulgaris* 10-50 g.L^{-1}, *Nannochloropsis* sp. 10-70 g.L^{-1}, *P. cruentum* 70-130 ug.L^{-1} and *S. platensis* 20-1.100 mg.L^{-1}.

All *Chlorella* extracts in both extracting solvents showed remarkable extraction of total phenolic compounds (Table 3). Among the samples of aqueous extracts of *C. vulgaris* and *C. minutissima* there was no significant difference according to Duncan's test, although they showed higher values compared to the other extracts. Similar extraction results were also observed for total flavonoids, however, the hydroethanolic extract of *C. minutissima* had a higher flavonoid content compared to the other extracts, showing a statistically significant difference. Again, it is observed in this study that water as an extracting solvent proves to be the best option for obtaining these groups of compounds with notable antioxidant activities.

Our values for phenolic compounds were higher than those obtained by Miranda *et al*.[34] evaluating the methanol extract of *C. vulgaris* with a value of 24.95 mg in 100 g^{-1} of dry alga matter, and 0.65 to 3.17 mg GAE 100 g^{-1} by the study in *C. vulgaris* extracts[7]. Results similar to those of this study were obtained by Siddhanta *et al*.[35] investigating the extract of the seaweed *Himanthalia enlongata* with high levels of phenolic compounds of 151.3 mg GAE 100 g^{-1} and flavonoids of 42.5 mg QE 100 g^{-1} of dry extract.

Potential DPPH free radical reducing activity was also verified for all aqueous and hydroethanolic extracts of *Chlorella*. Among the other extracts, the *C. pyrenoidosa* extract showed greater reduction capacity, which could be due to the numerous phytochemical classes (Table 1) verified in the qualitative test. The algae aqueous and hydroethanolic extracts showed high antioxidant activity but some lesser than the antioxidant ascorbic acid and BHT (IC_{50} 1.97 ± 0.06 and 3.14 ± 0.09 µg.mL^{-1}). Similar results were obtained by Miranda *et al*.[34] for the methanolic extract of *C. vulgaris* cultivated at 30 °C, which presented higher antioxidant activity = 85%, quite similar to BHT = 86%. By the Rancimat test (lipid medium) two fractions of methanolic extracts showed much higher antioxidant activity with induction times > 37.50 h at 60 °C and 11.5 h at 100 °C. According to the researchers, salicylic, transcinnamic, synaptic, chlorogenic, and caffeine phenolic compounds found in the methanolic extract of *Chlorella* may be responsible for its greater antioxidant activity.

Table 3. Total phenolic content, flavonoid content, DPPH radical reduction and antioxidant activity found for the studied algae.

Assay	Results					
	Aqueous extract			Hydroethanolic extracts		
	1	2	3	1	2	3
TPC (mg GAE.g^{-1})	187.32±0.63a	133.18±0.21b	182.09±0.33a	117.11±0.39c	100±0.93cd	121±0.15c
TFC (mg QE.g^{-1})	56.66±0.21b	51.72±0.19b	62.27±0.60c	61.15±0.95c	53.09±1.02b	72.04±1.07a
DPPH (IC_{50} µg.mL^{-1})	99.15±0.26c	87.77±0.98b	94.18±0.19c	178.09±0.18e	190.44±1.00f	156.01±0.93d
%AA (%AA)	81.17±1.26d	86.09±1.60c	83.20±2.09d	90.56±1.97b	97.90±1.99a	90.18±2.84b

Notes: TPC = Total phenolic compounds. TFC = Total flavonoid compounds. DPPH = Free radical reduction expressed as 50% Inhibition Concentration. %AA = Percentage of antioxidant activity. (1) *C. vulgaris*. (2) *C. pyrenoidosa*. (3) *C. minutissima*. Equal letters on the same line do not differ significantly by Duncan's test ($p < 5\%$). Source: Authors, 2021.

Among the studies that corroborate our results we can mention the study by Yu *et al*.[6] with *C. vulgaris*, where it was observed a high DPPH reducing activity ranging from 60.01 to 65.1%. Song *et al*.[36] also studied algae extracts of the *Chlorella* sp., attributing the antioxidant potential on polysaccharide compounds with removal of 49.10% of the DPPH radical, 56.60% for the hydroxyl radical and 32.10% for the superoxide radical. Hu *et al*.[37] also found potential DPPH radical reduction activity on *C. pyrenoidosa* extracts with a reduction between 29.67 to 54.16%. These studies corroborate our results, demonstrating the formidable antioxidant activity of the algae *C. vulgaris*, *C. pyrenoidosa* and *C. minutissima* extracts obtained in different organic solvents.

Important antioxidant activities are reported for several seaweed extracts, Kannan *et al*.[23] were also successful for methanolic extracts of the algae *G. corticata* and *S. platensis* with significant reduction using the free radical method by Fenton reagent, with reduction values equal to 50% and 94.4%, respectively. Also by these authors, an important reduction value over the DPPH free radical was observed between 53.8-70.4% and 51.8-64.7% for the methanol extracts of the algae *G. corticata* and *S. platensis*, respectively, resulting in values similar to those obtained in this study for the three *Chlorella* species. It is suggested that numerous phytochemical groups are involved in the antioxidant activity, which is also proposed by Premalatha *et al*.[38] who evaluated the extracts of the algae *Ulva fasciata* and *Chaetomorpha antenniana* as potential reducing agents in the DPPH assay, and the same was reported for the extract of *H. enlongata* by Siddhanta *et al*.[35] with IC_{50} = 0.125 µg.mL^{-1}.

When evaluating extracts in determining their capacities to reduce free radicals, it should always be verified in more than one method, therefore, this study verified this biological activity in the %AA assay where *Chlorella* extracts presented a reduction rate higher than 81%. It was observed through Duncan's test that the hydroethanolic extract of *C. pyrenoidosa* 97% presented the highest antioxidant activity for this model (%AA). Additionally the numerous species of algae capable of reducing free radicals present protective barriers characteristics against oxidative stress by reactive species in the environment and in the cellular production in living organisms. The oxidative stress, which in turn result in oxidative damage of cellular components in the form of lipid peroxidation, protein denaturation or DNA conjugation finally cell death[7]. Furthermore, oxidative stress has been associated with many diseases such as neural degeneration, Parkinson's and Alzheimer disease, AIDS, and aging and cardiovascular diseases, and cancer[7].

Chlorella aqueous and hydroethanolic extracts showed potential capacity to inhibit *S. aureus* and *E. coli* strains at the highest concentrations 75-100 µg.mL^{-1} which proved to be dose-dependent (Table 4). For the hydroethanolic extracts of *C. vulgaris* and *C. pyrenoidosa*, a slight inhibition activity on *S. serovar* Enteritidis was observed, although no statistically significant difference was observed in both extracts and concentrations 75-100 µg.mL^{-1} by Duncan's test. Although *Chlorella* extracts have shown potential values as natural antibacterial agents, the synthetic antibacterial references azithromycin, cephalexin and Tigecycline are still the best options in combating these bacteria of interest to health, according to manufacturers (CenterLab, Brazil).

Uma *et al*.[39] found antibacterial activity superior than this study for *S. aureus* between 6-25 mm, *E. coli* between 9-21.4 mm; high sensitivity was also verified on strains of *Klebsiella pneumoniae*, *Pseudomonas*, *Vibrio cholerae* and *Streptococcus pyogenes*, in extracts of *C. vulgaris* from different extracting solvents. Algae extracts act in different ways, as it is a natural product obtained in different parts of the world. Possibly the difference between the results of antibiosis on the tested bacteria has an influence on colony health, biological activities and environmental variation. Pratt *et al*.[40] attributes high sensitization activity to chlorellin against a large and complex group of potentially pathological microorganisms, especially Gram-positives and Gram-negatives bacteria: *S. aureus*, *S. pyogenes*, *Bacillus subtilis*, *Bacterium coli* and *Pseudomonas pyocyanea* (*P. aeruginosa*).

Marines algae are potential source organisms with important special functions that can symbolize functional clues in the development of new pharmaceutical drugs, as well as potential improvements over their use in the form of herbal medi cines. According Dhargalkar and Verlecar[41] the marine algae

Table 4. Antibacterial activity on *Staphylococcus aureus*, *Escherichia coli*, *Salmonella serovar* Enteritidis and *Salmonella serovar* Thyphymurium by aqueous and hydroethanolic of *C. vulgaris*, *C. pyrenoidosa* and *C. minutissima* extracts.

Microorganisms	**Inhibition zone (mm)**					
	Aqueous extracts 25, 50, 75, and 100 µg.mL^{-1}			**Hydroethanolic extracts 25, 50, 75, and 100 µg.mL^{-1}**		
	1	**1**	**2**	**1**	**2**	**3**
[a]*S. aureus*	0c/0c/0c/10b	0c/0c/8b/11b	0c/0c/9b/11b	0c/0c/8b/11b	0c/0c/9b/11b	0c/0c/8b/10b
[b]*E. coli*	0d/0d/6c/8c	0c/0c/0c/7b	0c/0c/8b/10b	0c/0c/0c/7b	0c/0c/8b/10b	0c/0c/0c/0c
[c]*S. serovar* Enteritidis	0b/0b/0b/0b	0c/0c/0c/6b	0c/0c/7b/9b	0c/0c/0c/6b	0c/0c/7b/9b	0c/0c/0c/0c
[c]*S. serovar* Thyphymurium	0b/0b/0b/0b	0b/0b/0b/0b	0b/0b/0b/0b	0b/0b/0b/0b	0b/0b/0b/0b	0b/0b/0b/0b

Note: (1) *C. vulgaris*. (2) *C. pyrenoidosa*. (3) *C. minutissima*. [a]Azithromycin, [b]Cephalexin and [c]Tigecycline. Antibiotics: *S. aureus* 23a mm, *E. coli* 28a mm, *S. serovar* Thyphymurium 28a mm, *S. serovar* Enteritidis 27a mm. Equal letters on the same line do not differ statistically by Duncan's test (p < 5%). Source: Authors, 2021.

products likes fibers execute a diverse array of functions such as antioxidant agents, anticoagulant, antimutagenic and anti-tumor.

According by Siddhanta *et al*.[35] and Kannan *et al*.[23] many bioactive and pharmacologically important compounds such as alginate, carrageen and agar are obtained from marine algae and treatment used in herbal medicine and pharmacy, and microbiology studies.

According to Bobin *et al*.[42] and Violante *et al*.[43] one of the factors that determine the photoprotection activity and effectiveness of a natural product is directly involved in terms of its chemical composition, consecutively its activity in absorbing high energy waves such as UV, in addition to the molar extinction coefficient and solubility. The UV critical wave assay is an *in vitro* test that in numerous studies has shown to be a pleasant option due to its simplicity and speed when compared to *in vivo* assays. In this essay Mansur *et al*.[44], Ferrari[45] and Violante *et al*.[43] address the correlation of absorption of a certain compound, isolated or not, on the erythematogenous effect caused by UV radiation.

The aqueous extracts exhibited bands of medium intensity in the scan between the critical wavelengths in the UVC, where *C. vulgaris* presented two bands, a broad one at 259 nm and a smaller one at 268 nm, still at 259 nm *C. pyrenoidosa* and *C minutissima* showed similar bands, although at different absorption intensities (Figure 2). Hydroethanolic extracts behaved heterogeneously when compared to aqueous extracts, where *C. minutissima* exhibited a strong and broad band with maximum absorption at 268 nm, followed by hydroethanolic extract of *C. vulgaris* with maximum absorption at 259 nm and *C. pyrenoidosa* at 268 nm.

Synthetic chemical filters show maximum absorption in regions other than UV, for UVC between 100-290 nm, for UVB the range comprises between 290-320 nm, UVA between 320-400 nm[43,46]. Algae have a special absorption capacity in the ultraviolet region due to their constitution on photosynthetic pigments. Furthermore, the photoprotection activity is not exclusively on pigments, an important portion involves groups of phytomolecules such as flavonoids, tannins, anthraquinones, alkaloids and polyphenols[43,47].

The topical photoprotective activity using emulsions conjugated with extracts proven to act as a temporary barrier on the dermis, reduces the incidence of certain types of cancers such as non-melanoma and melanoma[48]. The melanoma type originates from cells responsible for melanin synthesis and the non-melanin type is found in sun-exposed areas of the body, such as the neck, arms, ears and face[49].

Chlorella aqueous extracts exhibited higher median lethal concentration activity (LC_{50}) with values of 931.50; 929.26 and 838.07 µg.mL^{-1} for *C. vulgaris*, *C. pyrenoidosa* and *C. minutissima*, respectively. The hydroethanolic extracts exhi bited a low median lethal concentration (LC_{50}) with values of 1.177; 1.213 and 1.158 µg.mL^{-1}, respectively for *C. vulgaris*, *C. pyrenoidosa* and *C. minutissima*.

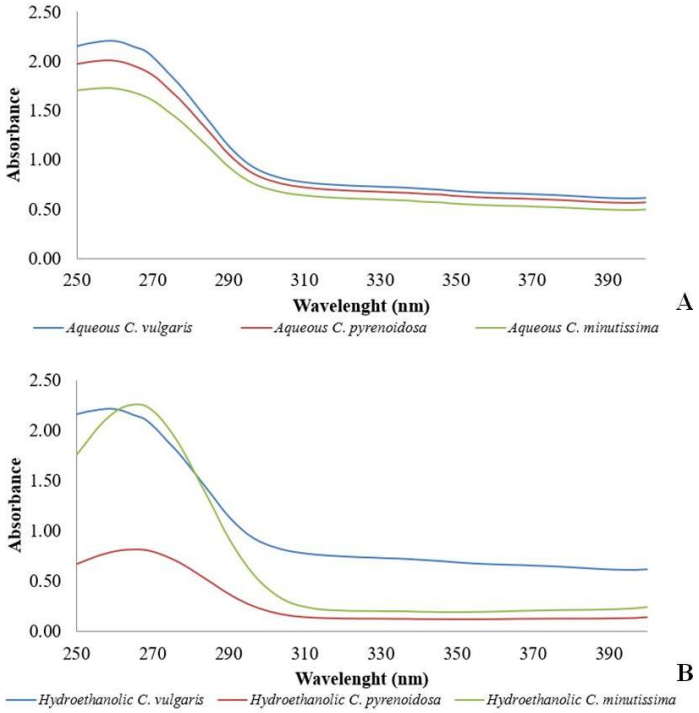

Fig. 2: UV 250 to 400 nm scanning spectrum critical for aqueous (**A**) and hydroethanol (**B**) extracts of *C. vulgaris*, *C. pyrenoidosa* and *C. minutissima*. Source: Authors, 2021.

According to Calazans *et al*.[50] and Meyer *et al*.[22] natural extracts that present LC_{50} values higher than 1.000 µg.mL^{-1} are considered non-toxic, and values lower than 1.000 µg.mL^{-1} are considered potentially toxic. Thus, it is observed that all aqueous extracts exhibited weak, although positive lethal cytotoxicity against *A. salina*. The hydroethanolic extracts, on the other hand, presented values higher than the recommended 1.000 µg.mL^{-1} and were considered non-toxic.

It is worth noting that there is little literature on toxicity of eukaryotic microalgae on cell lines and *A. salina*, while a larger number of papers deal with cyanobacteria. Nicolai *et al*.[51] evaluated several algae extracts, and among them commercial strains of *C. vulgaris* were considered non-toxic and suitable for feeding in studies in the European Union, in addition, of these *in vitro* studies they did not demonstrate toxicity neither for fibroblasts nor for *A. salina*. Although our study showed values below 1.000 µg.mL^{-1}, the extracts are safe for food use due to very low toxicity.

Conclusions

In this study aqueous and hydroethanolic extracts of green microalgae *Chlorella vulgaris*, *Chlorella pyrenoidosa* and *Chlorella minutissima* showed a great variety of special bioactive compounds, especially alkaloids, flavonoids, terpnoids and steroids, organic acids and phenols. Furthermore, these extracts showed variation in their antioxidant potential (DPPH and %AA), especially in hydroethanolic extracts. This antioxidant capacity was significantly correlated with their quantitative of total phenolic and flavonoid contents. Results of the UV-Vis supercritical wave test and the low cytotoxic activity shown in the biological test with *A. salina* indicate that Chlo-

rella extracts have high potential as a photoprotective materials and transmitting confidence for their possible use in various industrial segments such as the food, pharmaceutical and biological. However, future studies are needed to evaluate the feasibility of *C. vulgaris*, *C. pyrenoidosa* and *C. minutissima* extracts for developing potent antioxidant, photoprotective, antibacterial and cytotoxic drugs.

References

1. CJ Andrade, LM Andrade. An overview on the application of genus *Chlorella* in biotechnological processes. **J. Adv. Res. Biotechnol., 2**, 1-9 (2017).
2. FRG Silva, TMS Matias, LIO Souza, TJ Matos-Rocha, AS Fonseca, KC Mousinho, AF Santos. Phytochemical screening and *in vitro* antibacterial, antifungal, antioxidant and antitumor activities of the red propolis Alagoas. **Braz. J. Biol., 79**, 452-459 (2019).
3. VAR Huss, C Frank, EC Hartmann, M Hirmer, A Kloboucek, BM Seidel, E Kessler. Biochemical taxonomy and molecular phylogeny of the genus *Chlorella Sensu Lato* (Chlorophyta). **J. Phycol., 35**, 587-598 (1999).
4. K Goiris, K Muylaert, S Voorspoels, B Noten, DD Paepe. Detection of flavonoids in microalgae from different evolutionary lineages. **J. Phycol., 50**, 485-492 (2014).
5. EV Tretiakova, PG Beliaeva, AI Saralov. Substantiation of using *Chlorella* genus microalgae as a raw material for preparation of chemopreventive substances. **I. J. P., 5**, 774-780 (2018).
6. M Yu, M Chen, J Gui, S Huang, Y Liu, H Shentu, J He, Z Fang, W Wang, Y Zhang. Preparation of *Chlorella vulgaris* polysaccharides and their antioxidant activity *in vitro* and *in vivo*. **In. J. Biol. Macromol., 137**, 139-150 (2019).
7. OH Abdel-Karim, SF Gheda, GA Ismail, AM Abo-Shady. Phytochemical screening and antioxidant activity of **Chlorella vulgaris**. **D. J. S., 41**, 81-91 (2020).
8. MLS Queiroz, MC Rocha, CO Torello, J Souza Queiroz, C Bincoletto, MA Morgano, AK Calgarotto. *Chlorella vulgaris* restores bone marrow cellularity and cytokine production in lead-exposed mice. **Food Chem. Toxicol., 49**, 2934-2941 (2011).
9. J Matos, CL Cardoso, P Falé, CM Afonso, NM Bandarra. Investigation of nutraceutical potential of the microalgae *Chlorella vulgaris* and *Arthrospira platensis*. **I. J. Food Science Technol., 55**, 303-312 (2020).
10. LM Andrade, CJ Andrade, M Dias, CAO Nascimento, MA Mendes. *Chlorella* and *Spirulina* microalgae as sources of functional foods, nutraceuticals, and food supplements; an overview. **MOJ Food Process Technol., 6**, (2018).
11. HJ Hussein, SS Naji, NMS Al-Khafaji. Antibacterial properties of the *Chlorella vulgaris* isolated from polluted water in Iraq. **J. Pharm. Res., 10**, 2457-2460 (2018).
12. EM Sembiring, B Elya, R Sauriasari. Phytochemical screening, total flavonoid and total phenolic content and antioxidant activity of different parts of *Caesalpinia bonduc* (L.) Roxb. **Pharmacogn. J., 10**, 123-127 (2018).
13. LN Madike, S Takaidza, M Pillay. Preliminary Phytochemical screening of crude extracts from the leaves, stems, and roots of *Tulbaghia violácea*. **In. J. Pharmacogn. Phyto. Res., 9**, 1300-1308 (2017).
14. MAH Mehdi, FYS Alarabi, M Farooqui, V Pradhan. Phytochemical screening and antiamebic studies of *Tamarindus indica* of leaves extract. **Asian J. Pharm. Clin. Res., 12**, 507-512 (2019).
15. E Torres-Rodríguez, YA Núnez-Romero, Y Mojena-Guerra, Y Fung-Boix, Y Fonseca-Turruella. Análisis fitoquímico de partes aéreas de *Sida pyramidata* Cav. Yerba de aura. **Rev. Cub Quími., 33**, 401-414 (2021).
16. MH Labiad, H Harhar, A Ghanimi, M Tabyaoui. Phytochemical screening and antioxidant activity of Maroccan *Thymus satureioïdes* extracts. **J. Material Environ. Sc., 8**, 2132-2139 (2017).
17. H Mitsuda, K Yuasumoto, K Iwami. Antioxidation action of indole compounds during the autoxidation of linoleic acid. **Eiyo Shokuryo, 19**, 210-214 (1996).
18. İ Gülçin, I Güngor Sat, Ş Beydemir, M Elmastaş, Ö İrfan Küfrevioğlu, Comparision of antioxidant activity of clove (*Eugenia caryophylata* Thunb) buds and lavender (*Lavandula stoechas* L.). **Food Chemistry, 87**, 393-400 (2004).
19. AA Tuama, AA Mohammed. Phytochemical screening and in vitro antibacterial and anticancer activities of the aqueous extract of *Cucumis sativus*. **Saudi J. Biol. Scien., 26**, 600-604 (2019).
20. MAC Medeiros, B Santos, FMC Marques,MFMS Leite, MM Simões, RM Anjos, L Brito Júnior, GLA Maia, MASG Alves, AP Sousa, AA Oliveira Filho. Avaliação da atividade fotoprotetora do extrato aquoso de *Rhaphiodon echinus* (Nees & Mart.) Schauer. **Sci Plen., 17**, 1-5 (2021).
21. J Silva, C Alves, S Pinteus, J Reboleira, R Pedrosa, S Bernardino. *Chlorella*. **Nonvit. Nonm. Nutri. Suppl.**, 187-193 (2019).
22. BN Meyer, NR Ferrigni, JE Putnam, LB Jacobsen, DE Nichols, JL McLaughlin. Brine shrimp: a convenient general bioassay for active plant constituents. **Planta Med., 45**, 41-34 (1982).
23. M Kannan, A Pushparaj, B Dheeba, K Nageshwari, K Kannan. Phytochemical screening and antioxidant activity of marine algae *Gracilaria corticata* and *Spiruluna platensis*. **J. Chem. Pharm. Res., 6**, 312-318 (2014).
24. N Srivastav, K Saurav, V Mohanasrinivasan, K Kannabiran, M Singh. **British J. Pharmacol. Toxicol., 1**, 72-76 (2010).
25. M Sonam, RP Singh, S Pooja. Phytochemical screening and TLC profiling of various extracts of *Reinwardtia indica*. **Int. J. Pharmacog. Phytochem. Res., 9**, 523-527 (2017).
26. SW Jeffrey. Paper-chromatographic separation of chlorophylls and carotenoids from marine algae. **Biochem. J., 80**, 336-342 (1961).
27. JA Mendiola, S Santoyo, A Cifuentes, G Reglero, E Ibáñez, FJ Señoráns. Antimicrobial activity of sub-supercritical CO_2 extracts of the green alga *Dunaliella salina*. **J. Food Prot., 71**, 2138-2143 (2008).
28. M Kannan, AJA Ranjit Singh, M Narayanan. Phytochemistry and immunopharmacological investigation of *Rubia cordifolia* Liin. (Rubiaceae). **Pharmacologyonline, 3**, 653-662 (2009).
29. AJM Melo, EM Saito, B Schulze, SC Sticca. Expressão de pigmentos em algas pardas (*Canistrocarpus cervicornis* e *Padina*

gymnospora): uma abordagem quimioecológica. Estudos ecológicos da Ilha de Santa Catarina, 2011.

30. B Tamburic, FW Zemichael, GC Maitland, K Hellgardt. Parameters affecting the growth and hydrogen production of the green alga *Chlamydomonas reinhardtii*. **Int. J. Hydrogen Energy, 36**, 7872-7876 (2011).
31. FS Oliveira, MC Soares, RL Costa, FRX Batista. Padronização do procedimento de determinação da clorofila utilizado como parâmetro para a estimativa da viabilidade celular em algas verdes. *In:* XIX Jornada em Engenharia Química, de 3 a 8 de Março de 2014.
32. D Hornero-Méndez, G Britton. Involvement of NADPH in the cyclization reation of carotenoid biosynthesis. **FEBS Letters, 515**, 133-136 (2002).
33. M Rinawati, LA Sari, KT Pursetyo. Chlorophyll and carotnoids analysis spectrophotometer using method on microalgae. IOP Conference Series: Earth and Environmental Science, 441, 2nd **Int. Conf. Fisheries Marine Sc.,** 26 September, 2019, Surabaya, Indonesia, p. 1-21.
34. MS Miranda, S Sato, J Mancini-Filho. Antioxidant activity of the microalga *Chlorella vulgaris* cultered on special conditions. **Bollett. Chimi. Farm., 140**, 165-168 (2001).
35. AK Siddhanta, KH Mody, BK Ramavat, VD Chauhan, HS Garg, AK Goel, MJ Doss, MN Srivastava, GK Patnaik, VP Kamboj. Bioactivity of marine organinsms: Part VIII-Screening of some marine flora of western coast of India. **Indian J. Exp. Biol., 36**, 638-643 (1997).
36. H Song, M He, C Gu, D Wei,Y Liang, J Yan, C Wang. Extration optimization, purification, antioxidant activity, and preliminary structural characterization of crude polysaccharide from an artic *Chlorella* sp. **Polymers, 10**, (2018).
37. Q Hu, B Pan, J Xu, J Sheng, Y Shi. Effects of supercritical carbon dioxide extraction conditions on yields and antioxidant activity of *Chlorella pyrenoidosa* extracts. **J. Food Eng., 80**, 997-1001 (2007).
38. M Premalatha, P Dhasarathan, P Theriappan. Phytochemical characterization and antimicrobial efficiency of seaweed samples, *Ulva fasciata* and *Chaetomorpha antennina*. **Int. J. Pharm Bio Sci., l2**, 2-16 (2011).
39. R Uma, V Sivasubramanian, S Niranjali Devaraj. Preliminary phytochemical analysis and in vitro antibacterial screening of green micro algae, *Desmococcus olivaceous*, *Chlorococcum humicola* and *Chlorella vulgaris*. **J. Algal Bio. Util., 2**, 74-81 (2011).
40. R Pratt, TC Daniels, JJ Eiler, JB Gunnison, WD Kumler, JF Oneto, LA Strait, HA Spoehr, GJ Hardin. Chlorellin, an antibacterial substance from *Chlorella*. **Science, 99**, 351-352 (1944).
41. VK Dhargalkar, XN Verlecar. Southern ocean seaweeds: a resource for exploration in food and drugs. **Aquaculture, 287**, 229-242 (2009).
42. MF Bobin, M Raymond, MC Martini. UVA/UVB absorption properties of natural products. **Cosmet Toiletries, 109**, 63-78 (1994).
43. IMP Violante, IM Souza, CL Venturini, AFS Ramalho, RAN Santos, M Ferrari. Avaliação *in vitro* da atividade fotoprotetora de extratos vegetais do cerrado de Mato Grosso. **Rev. Bras. Farmacog., 19**, 452-457 (2009).
44. JS Mansur, MVR Breder, MCA Mansur, RD Azulay. Correlação entre a determinação do fator de proteção solar em seres humanos e por espectrofotometria. **An. Bras. Dermatol., 61**, 167-172 (1986).
45. MM Costa, APA Farias, CAB Oliveira. Importância dos fotoprotetores na minimização de danos a pele causados pela radicação solar. **Braz. J. Devel., 7**, 101855-101867 (2021).
46. AM Fernandes, SMC Siqueira, ML Furtado, AEQR Campos, AFV Pinheiro. Avaliação das atividades antioxidante e fotoprotetora da espécie *Syzygium cumini* (L.) Skeels. **Braz. J. Devel., 6**, 64719-64725 (2020).
47. ML Furtado, SMC Siqueira, AM Fernandes, RA Montes, AO Alcântra, DP Oliveira, NAP Pinheiro, AFV Amorin. Avaliação *in vitro* da atividade fotoprotetora e antioxidante de extratos vegetais de *citrullus lanatus* (Thunb.) Matsum. & Nakai. **Braz. J. Devel., 7**, 6793-6812 (2021).
48. M Ferrari, MSC Oliveira, AK Nakano, PA Rocha-Filho. Determinação do fator de proteção solar (FPS) in vitro e in vivo de emulsões com óleo de andiroba (*Carapa guianensis*). **Rev. Bras. Farmacogn., 17**, 626- 630 (2002).
49. MCMR Souza, TG Horta, ES Melo, FDB Rocha. Câncer de pele: hábitos de exposição solar e alterações cutâneas entre agentes de saúde em um município de Minas Gerais. **Rev. Enf. Cen. Oes. Mineiro, 6**, 1945-1956 (2016).
50. RSP Calazans, LO Bulian, LO Alves, JO Salvi. Estudo fitoquímico e avaliação da citotoxicidade aguda frente à *Artemia salina* (Leach) de plantas comercializadas em feira-livre. **Rev. Uni. Vale Rio Verde, 17**, 1-10 (2019).
51. A Niccolai, E Bigagli, N Biondi, L Rodolfi, L Cinci, C Luceri, MR Tredici. *In vitro* toxicity of microalgal and cyanobacterial strains of interest as food source. **J. Appl. Phycol., 29**, 199-209 (2017).