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CHEMICAL CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF RAW EXTRACT OF PHOTOSYNTHESIZING MICROORGANISMS FROM THE CHAPADA DAS MESAS NATIONAL PARK BRAZILIAN CERRADO

CARACTERIZAÇÃO QUÍMICA E ATIVIDADE ANTIOXIDANTE DO EXTRATO BRUTO DE MICRORGANISMOS FOTOSSINTETIZANTES DO PARQUE NACIONAL DA CHAPADA DAS MESAS, CERRADO BRASILEIRO

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ABSTRACT

Cyanobacteria and microalgae are photosynthetic microorganisms that produce bioactive compounds such as carotenoids, flavonoids, and fatty acids, which are of importance to the pharmaceutical, cosmetic, food, and biofuels industries. This study aimed to investigate the bioactive potential of extracts from the microalgae *Chlorella* sp. (GBBB06) and the cyanobacteria *Nostoc* sp. (GBBB01) and *Synechococcus* sp. (GBBB07), collected in the Chapada das Mesas National Park, Cerrado Maranhense. Methanolic extracts of the samples were obtained to chemically characterize them using chromatographic techniques, such as thin-layer chromatography, high performance liquid chromatography coupled with a diode arrangement detector, and gas chromatography coupled to mass spectrometry. To evaluate the antioxidant capacity, DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays were performed**.** Were found on liquid chromatography, peaks with characteristic wavelengths of carotenoids in the sample of *Chlorella* sp. and those characteristic of chlorophyll in all samples. Metabolites such as fatty acids, alcohols, and nitrogen compounds were identified via gas chromatography. In the antioxidant assessment, the samples scavenged 12% (*Nostoc* sp.), 20% (*Chlorella* sp.), and 25% (*Synechococcus* sp.) of the radical DPPH$^{\bullet}$ in extract concentrations of 0.675 mg/mL. The results for ABTS$^{\bullet+}$ radical assay showed high antioxidant potential in the samples, with scavenging of 60% (*Chlorella* sp.) and 70% (*Synechococcus* sp. and *Nostoc* sp.). These results demonstrated that *Chlorella* sp., *Nostoc* sp., and *Synechococcus* sp. are rich in bioactive secondary metabolites with antioxidant properties and potential biotechnological applications; these extracts represent an alternative for economic exploitation of the Brazilian Cerrado in a sustainable manner.

Keywords: Bioprospecting; *Chlorella*; *Nostoc*; *Synechococcus*; Secondary metabolites.

RESUMO

Cianobactérias e microalgas são microrganismos fotossintéticos que produzem compostos bioativos como carotenoides, flavonoides e ácidos graxos, importantes para as indústrias farmacêutica, cosmética, alimentícia e de biocombustíveis. Este estudo teve como objetivo investigar o potencial bioativo de extratos da microalga *Chlorella* sp. (GBBB06) e das cianobactérias *Nostoc* sp. (GBBB01) e *Synechococcus* sp. (GBBB07), coletadas no Parque Nacional da Chapada das Mesas, Cerrado Maranhense. Extratos metanólicos das amostras foram obtidos para caracterização química por meio de técnicas cromatográficas, como cromatografia em camada delgada, cromatografia líquida de alta

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eficiência acoplada a detector de arranjo de diodos e cromatografia gasosa acoplada à espectrometria de massas. Para avaliar a capacidade antioxidante, foram realizados os ensaios DPPH• e $ABTS^{\bullet+}$. Foram encontrados, por cromatografia líquida, picos com comprimentos de onda característicos de carotenoides na amostra de *Chlorella* sp. e picos característicos de clorofila em todas as amostras. Metabólitos como ácidos graxos, álcoois e compostos nitrogenados foram identificados por cromatografia gasosa. Na avaliação antioxidante, as amostras sequestraram 12% (*Nostoc* sp.), 20% (*Chlorella* sp.) e 25% (*Synechococcus* sp.) do radical DPPH• em concentrações de extrato de 0,675 mg/mL. Os resultados do ensaio com o radical $ABTS^{\bullet+}$ mostraram alto potencial antioxidante nas amostras, com sequestro de 60% (*Chlorella* sp.) e 70% (*Synechococcus* sp. e *Nostoc* sp.). Esses resultados demonstraram que *Chlorella* sp., *Nostoc* sp. e *Synechococcus* sp. são ricas em metabólitos secundários bioativos com propriedades antioxidantes e potencial para aplicações biotecnológicas. Esses extratos representam uma alternativa para a exploração econômica do Cerrado brasileiro de forma sustentável.

Palavras-chave: Bioprospecção; *Chlorella*; *Nostoc*; *Synechococcus*; Metabólitos secundários.

INTRODUCTION

Cyanobacteria and microalgae are photosynthetic microorganisms that are environmentally adaptable, present morphological versatility and diverse metabolism, and are capable of colonizing terrestrial, marine, and freshwater environments along the entire temperature gradient (Komárek, 2010; H*u et a*l., 2018).

These microorganisms are considered to have high biotechnological potential as they present complex and diversified secondary metabolism, and many metabolites have identified bioactivities including: (i) anticancer; (ii) antiviral; (iii) antibacterial; (iv) antifungal; (v) antiprotozoal; (vi) algicidal; (vii) anti-inflammatory; and (viii) antioxidant activities as well as (ix) protection against ultraviolet rays (Niedermeyer, 2015; Jerez-Marte*l et a*l., 2017; Bajpa*i et a*l., 2018; Dema*y et a*l., 2019; Ribeiro, Michel*e et a*l., 2022; Martin*s et a*l., 2022).

In addition to bioactivity, some strains have considerable nutritional value due to their high protein content (*Spirulina* or *Chlorella*). These strains are routinely sold as food supplements and, in many cases, without any kind of quality control resulting in economic and health issues to the consumer. In addition to their direct use as dietary supplements, their application in the food industry is promising due to their ability to produce nutraceutical

compounds, such as polyunsaturated fatty acids or functional molecules including the carotenoids beta-carotene (in *Dunaliella*) and astaxanthin (in *Haematococcus*) (Raja; Hemaiswarya; Rengasamy, 2007; Priyadarshani; Rath, 2012; Muy*s et a*l., 2019; Hassa*n et a*l., 2022). Many microalgae and cyanobacteria known to produce large amounts of lipids (isoprenoids) and alcohols are being investigated as platforms for the production of biofuels. An example is the American company Algenol, which is focused on the production of ethanol using genetically-modified cyanobacteria (Nozzi; Oliver; Atsumi, 2013; Aboi*m et a*l., 2016; Farrok*h et a*l., 2019). Thus, photosynthetic microorganisms continue to show biotechnological potential making it important to understand the composition of their metabolomes. Chemical separation techniques such as thin-layer chromatography (TLC), liquid chromatography, and gas chromatography can be employed to identify and evaluate these metabolites (Rodrigue*s et a*l., 2014, 2015).

Though crucial, bioprospecting studies based on *in vitro* activity tests are often laborious and depend on specific culture conditions for the expression of the target compounds at appropriate concentrations, which often produces false negative results. Therefore, it is ideal to include chemical characterization of these microorganisms for optimized bioprospecting (Winnikof*f et a*l., 2014; Dittman*n et a*l., 2015).

Despite the economic potential of these microorganisms, little is known regarding the bioactive constituents of the microalgae and cyanobacteria of the Chapada das Mesas National Park (PNCM), a conservation unit of the Brazilian Cerrado. Therefore, it is extremely important that bioprospecting work be developed to add value to this diversity and thus subsidize economically-sustainable preservation strategies. Due to the great bioactive potential of these photosynthesizing microorganisms, this work aims to chemically characterize the extracts obtained from microalgae and cyanobacteria isolated from the PNCM in the Cerrado biome.

MATERIALS AND METHODS

Samples

The unigal (non-axenic) samples from PNCM were obtained from the collection belonging to the Biodiversity, Bioprospection and Biotechnology (GB3) research group of the Laboratory of Genetics and Molecular Biology of the Federal University of Maranhão. The lineages registered in SISGEN (AACEC6D) were the microalgae *Chlorella* sp. (cepa GBBB06) and the cyanobacteria *Nostoc* sp. and *Synechococcus* sp. (strains GBBB01 and GBBB07).

Chemical extraction

For biomas growth and extraction, 1 mL of the samples were inoculated in multiple 5 L Erlenmeyer flasks containing 3 L of Z8 culture medium at an initial inoculum of 1%, and maintained under concentration of 3–15 μmol. $m^{-2}.s^{-1}$ for 4 weeks at 25ºC.

Samples was transferred to 250 mL bottles and centrifuged at 10,000 rpm for 10 minutes (Sorvall LYNX 6000 Superspeed Centrifuge Thermo Fisher Scientific Inc., Waltham, MA, USA). The supernatant was then discarded and the pellets were stored at −80 ºC. The samples were freeze-dried for 72 hours in a ChristBeta 2-8 LSC plus freezer dryer equipped with Christ Lyo Club 4-8 chambers (Martin, Christ, Ostrode am Harz, Germany).

In a 2 mL plastic tube, 100 mg of lyophilized cells were mixed with 625 mg of glass beads (0.5 mm Glass beads, Scientific Industries Inc., part SI-BG05, Bohemia, NY, USA) and 1 mL of methanol was added. The samples were homogenized for 5 minutes at 3000 rpm at room temperature (Multi Mixer KASVI Model k40-10208).

The samples were then centrifuged at 10,000 rpm for 5 minutes, the supernatant was collected in a 1.5 mL Eppendorf tube, sonicated for 2 min at 40 kHz, frozen with liquid nitrogen, and lyophilized. To obtain the mass of the extract, the tubes were weighed before

and after lyophilization. The extracts were solubilized in 500 µL of HPLC-grade methanol for chemical analysis.

Chemical profile of cyanobacterial extracts

Thin layer chromatography (TLC)

To obtain the initial chemical profile of the samples, TLC analyses were performed in normal phase by adsorption using aluminum chromatophores (Macherey Nagel-Alugram Xtra Sil.Gel 254, 20 cm x 20 cm). Hexane and dichloromethane eluents were used as mobile phase. Initially, 10 mL of hexane eluent was used. Each sample with a volume of 50 µL at a concentration of 200 $mg.mL^{-1}$was applied with the aid of a glass capillary (1–5 µL), with a distance of 0.7 cm between the samples.

The plate was imaged in a UV darkroom (Spencer, SP930) with readings at 254 and 365 nm taken for the visualization of the compounds. The samples were then tested using an eluent mixture of hexane and dichloromethane in a 1:1 ratio.

High-performance liquid chromatography coupled to diode array detector (HPLC-DAD)

To confirm the initial chemical profile of the sample observed in TLC, the extracts were analyzed via high-efficiency liquid chromatography (Shimadzu Prominence UFLC LC-20AT) with diode array detector (Shimadzu Prominence Spd-M20a) using reverse phase partition separation (C18) in gradient elution. The chromatographic separation was performed on a C18 silica column (Phenomenex, 150x 4.60 nm). For the elution gradient, methanol and 0.01% formic acid in water were used as the solvents. Samples (10 µL) were injected manually and eluted for 60 min using 75:25 methanol:formic acid. The UV-vis spectra were obtained between 200 nm and 700 nm. Spectra were compared with literature for chemical identification.

Gas chromatography coupled to mass spectrometry (GC-MS)

For the identification of volatile compounds, samples were analyzed in a gas chromatograph (Shimadzu CG-2010) coupled to a mass spectrometer (Shimadzu CG-EM QP2010 Plus), using a ZB-FFAP capillary column (30 m x 0.25 mm x 0.25 µm). Helium gas flow was kept at a linear velocity of 30 cm/sec and column flow was 1.0 mL/min. Samples were initially heated at 90 °C for 4 min then increased 10 °C/min to 190 °C and held at that temperature for 16 min. The injector and ion source temperatures were 250 °C and 200 °C, respectively. The split injection mode ratio was 1:50.

Quantification was performed by standardizing the peak areas, and compound identification was performed through direct comparison of the retention time and MS fragmentation patterns with data provided by the NIST08 Equipment Library.

Assessment of antioxidant activity

Scavenging of DPPH• radical

Antioxidant capacity was evaluated according to the procedure described by Sánchez-Moreno, Larrauri and Saura-Calixto (1998) with minor alterations. The methanolic solution of DPPH• (2.4 $mg.mL^{-1}$) was prepared at the moment of the experiment and kept in amber bottle. The volume of 10 µL of the extracts (final concentration of 0.625 to 5 $mg.mL^{-1}$) were added to a microtiter plate and the DPPH• solution was added to a final volume of 200 µL per well. Quercetin was utilized as the standard (final concentration of 1.56×10^{-2} to 0.5 $mg.mL^{-1}$). The mixture was kept under light and the minimum reaction time was 30 minutes.

Readings were obtained using a BioTek spectrophotometer with a multidetection Synergy H1 hybrid reader at 515 nm using 99.9% methanol as the blank. The percentage of inhibition (PI) was calculated according to the following formula (Eq. 1):

$$PI \quad \frac{a_c}{a_c}$$

Where a_c, absorbance of control sample; a_t, absorbance of test sample.

The IC_{50} values (effective concentration for 50% inhibition) were determined via non-linear regression. The results are expressed as mean ± standard error of the means with experiments performed in triplicate. GraphPad Prism software (GraphPad Software, Inc., San Diego, CA, USA) was used to plot the graphs.

Scavenging of ABTS$^{\bullet+}$ radical

Evaluation of antioxidant capacity against the ABTS$^{\bullet+}$ radical was performed according to Re *et al.* (1999) with minor alterations. The ABTS reagent was dissolved in water to a concentration of 7 mM. This was then mixed potassium persulphate (final concentration 2.45 mM). This mixture was kept in an amber flask in the dark for 16 hours prior to assaying the extracts to allow for complete oxidation of ABTS and generation of the ABTS$^{\bullet+}$ (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic) chromophore, a highly stable radical cation. The ABTS$^{\bullet+}$ solution was diluted in absolute ethanol until absorbance at 734 nm was 0.7 ± 0.02. The volume of 10 µL of the extracts (final concentration of 6.25 x 10^{-2} to 0.5 mg.mL^{-1}) were added to a microtiter plate and the ABTS$^{\bullet+}$ solution was added to a final volume of 200 µL per well. Quercetin was utilized as the standard (final concentration of 0.0156 to 0.5 mg.mL^{-1}). Samples were solubilized in methanol and dilutions were performed with ethanol. All tests were performed in triplicate with a 20 min reaction time.

RESULTS AND DISCUSSION

Initial chemical profile of *Chlorella* sp., *Nostoc* sp., and *Synechococcus* sp.

Using TLC, it was possible to visualize the presence of spots with different retention factors (Rf) in the chromatographs, indicating different classes of secondary metabolites in all samples. The metabolites showed varied hydrophobicity with some being retained on the nonpolar stationary phase, whereas others being eluted in the polar mobile phase; however, polar compounds were predominant. Cyanobacteria and microalgae can produce a variety of bioactive compounds, such as alkaloids, tetraterpenoids (carotenoids), micosporine-like amino acids, and ficobiliproteins (Valent*e et a*l., 2006; Rastogi; Sinha, 2009; Valdug*a et a*l., 2009; Richa; Sinha, 2015). The more polar the solvent, the greater the mobile phase strength. Thus, it was observed that dichloromethane, which is more polar than hexane, was the eluent that allowed a greater separation of compounds, making it possible to observe a larger number of spots on the plate. It was possible to observe green- and yellow-stained spots for the samples of *Chlorella* sp. GBBB06, *Nostoc* sp. GBBB01, and *Synechococcus* sp. GBBB07.

Chlorophyll has maximum absorption in the blue and red visible region and reflects in the green visible region, whereas carotenoids absorb in the blue and violet region and appear as visible red, orange, and yellow (Schoefs, 2002; Ribeiro, Eliana Paula; Seravalli, 2007). Thus, the green and yellow spots observed on the plate indicate the likely presence of chlorophylls and carotenoids.

Chemical profile by HPLC-DAD

HPLC results corroborated those of TLC, where the identified compounds were predominantly polar while additionally confirming through the technique the presence of carotenoids and chlorophylls. The peaks and the retention times of these compounds are shown in Table 1.

Table 1 - Classes of metabolites found in the HPLC profiles for the methanolic extracts of *Chlorella* sp. GBBB06, *Nostoc* sp. GBBB01, and *Synechococcus* sp. GBBB07.

Samples	Peaks	Λmáx (Nm)	Rt (Min)	Bioactive Compounds
***Chlorella* sp.**	1	436, 464	15,75	Carotenoids
	2	441, 466	17,11	
	3	444, 471	30,72	
	4	464, 649	37,39	Chlorophyll
	5	428, 664	42,77	
	6	406, 664	51,23	
***Nostoc* sp.**	1	422, 663	42,24	Chlorophyll
	2	419, 658	43,71	
	3	407, 665	51,41	
***Synechococcus* sp.**	1	451, 640	36,21	Chlorophyll
	2	422, 663	42,01	
	3	420, 658	45,10	
	4	421, 658	46,45	
	5	405, 665	51,23	
	6	405, 658	55,38	

* Retention time (RT); Maximum wavelength λmax (nm).

The UV/vis spectra indicated that *Chlorella* sp. GBBB06 was the only sample to have three characteristic carotenoid bands. According to Hashtroud*i et a*l. (2013), carotenoids absorb between 400-500 nm in the visible region Therefore, this region of the UV/vis spectrum is valuable for identification, characterization and quantification of different types of carotenoids as the λ_{max} and peak shape are characteristic of specific compounds.

Plaza et al. (2012) and Patias et al. (2017), used HPLC-DAD to identify carotenoids in an extract of the microalga Chlorella vulgaris, and Patias et al. (2017), reported a total of 18 different carotenoids, including cis-lutein (441, 467 nm), β-carotene (445, 470 nm), and cis-zeaxanthin (449, 474 nm). These pigments are of commercial importance and microalgae of the genus Chlorella can potentially be utilized for their production. Carotenoids have demonstrated antioxidant, anti-inflammatory, anti-obesity, cardioprotective, neuroprotective, and hepatoprotective effects (Park et al., 2010; Lee et al., 2011; Ryu et al., 2012; Zhang et al.,

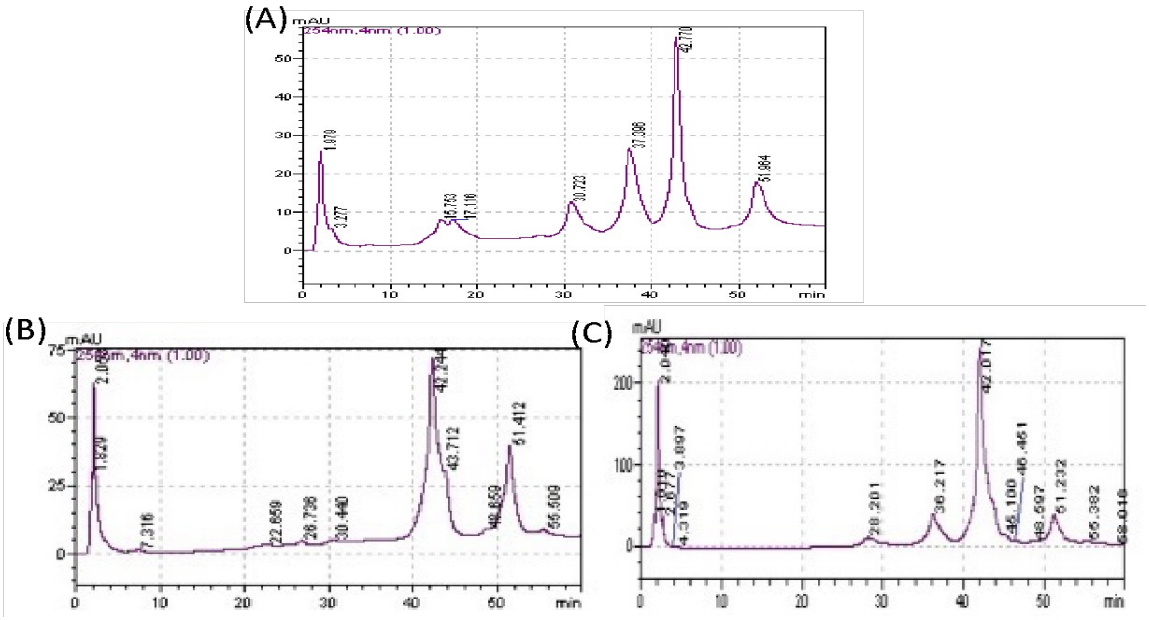
2014; Hu et al., 2018). Carotenoids also play an important role in the treatment of diseases, such as cancer, atherosclerosis, cataracts, macular degeneration, multiple sclerosis, and neurodegenerative and cardiovascular diseases (Obulesu; Dowlathabad; Bramhachari, 2011; Ozawa et al., 2012; Ryu et al., 2012; Zhang et al., 2014; Mezzomo; Ferreira, 2016).

Although TLC indicated all samples with characteristic carotenoid spots, they were only identified in the Chlorella sp. extracts by HPLC-DAD, which is a far more sensitive analytical technique. In addition, the concentration of carotenoids in the other samples may be too low to be detected by HPLC-DAD. Carotenoids are easily susceptible to oxidative degradation and may be oxidized in the presence of light and heat, which may explain why they have not been identified in other samples (Ribeiro, Eliana Paula; Seravalli, 2007).

In addition to the carotenoids, characteristic chlorophyll peaks were identified in Chlorella sp. GBBB06, Nostoc sp. GBBB01, and Synechococcus sp. GBBB07 (Table 1). Chlorophylls exhibit strong absorbance from 460–660 nm in the visible region of the spectrum, similar to what we observed in this study (Kamffer; Bindon; Oberholster, 2010; Rodrigues et al., 2015). When studying the chemical composition and bioactivity in extracts of C. vulgaris, Plaza et al. (2012) identified chlorophyll a and b through HPLC-DAD. According to Romero-Lopez; Lopez-Rodas; Costas, (2012), the genus Chlorella has a high amount of pigments when compared to other genera of chlorophyll containing microalgae.

HPLC-DAD results show that six chlorophyll peaks were present in the extract from the cyanobacterium Synechococcus sp. GBBB07, whereas Nostoc sp. GBBB01 and Chlorella sp. GBBB06 extracts only had three peaks each (see Figure 1). The presence of chlorophyll in all samples was expected, as these microorganisms studied are all photosynthetic. Due to their intense color and physical and chemical properties, chlorophylls are used as food additives in cheese, ice cream, beverages, chocolates and biscuits (Schoefs, 2002; Volp; Renhe; Stringheta, 2009). In addition, these compounds have been reported to have human health benefits, such as antimutagenic effects, antitoxic properties, and antioxidant capacity (Amrani-Allalou et al., 2019).

Figure 1 - Chromatogram obtained via HPLC-DAD. (A) *Chlorella* sp. GBBB06; (B) *Nostoc sp* GBBB01; (C) *Synechococcus* sp. GBBB07.



GC-MS

The chromatograms of eight compounds from the *Chlorella sp*. extract, nine compounds in *Nostoc sp.* extract, and seven compounds in *Synechococcus sp.* The compounds were identified as alcohols, fatty acids, hydrocarbons, and nitrogen compounds. Among these, the most predominant were phytol, glycerol, acetic acid, benzoic acid, and *N*,*N*-dimethylacetamide

All compounds found in *Chlorella* sp. GBBB06, *Nostoc* sp. GBBB01. and *Synechococcus* sp. GBBB07, are show in Tables 2, 3 and 4.

Table 2 - Identified compounds obtained via GC-MS analysis of *Chlorella* sp. GBBB06.

Peak	RT (min)	Compound	Area%
1	5.240	Acetic acid	7.18
2	10.700	9-Eicosene	4.14
3	12.807	2-Pyrrolidinone	2.21
4	14.010	4-ethyoxybenzoic acid ethyl ester	6.91

5	14.429	Hexadecanoic acid methyl ester	2.37
6	15.375	Glycerol	26.98
7	16.972	Benzoic acid	15.07
8	20.282	Phytol	35.14

* Retention time (RT)

Table 3 - Identified compounds obtained via GC-MS analysis of *Nostoc* sp. GBBB01.

Peak	RT (min)	Compound	Area%
1	5.185	Acetic acid	30.35
2	10.961	DL-Alanine, *N* - [(phenylmethoxy) carbonyl]	0.98
3	11.590	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	2.45
4	12.238	Dodecyl acrylate	3.50
5	14.280	1H-imidazole-4-carboxylic acid methyl ester	1.99
6	14.430	Hexadecanoic acid methyl ester	6.75
7	15.026	1,2,3-Propanotriol, monoacetate	3.07
8	15.374	Glycerol	6.44
9	20.280	Phytol	44.47

* Retention time (RT)

Table 4 - Identified compounds via GC-MS analysis of *Synechococcus* sp. GBBB07.

Peak	RT (min)	Compound	Area%
1	5.207	Acetic acid	0.85
2	11.583	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	2.67
3	14.429	Hexadecanoic acid methyl ester	1.93
4	14.674	9-Hexadecenoic acid methyl ester, (*Z*)	1.87
5	15.380	Glycerol	10.00
6	20.287	Phytol	70.26
7	26.781	*N*,*N*-Dimethylacetamide	10.31

* Retention time (RT)

The terpenoid alcohol phytol is present in high percentage in *Chlorella* sp. GBBB06 (35.14%), *Nostoc sp.* GBBB01 (44.47%), and *Synechococcus* sp. GBBB07 (70.26%). This compound stands out for its industrial applications. Phytol is a part of the chlorophyll molecule and is used in the cosmetic industry as an aromatic ingredient in the composition of fragrances. Although it is mainly used as a fragrance constituent, the biological properties of phytol have drawn recent attention for possible application to the pharmaceutical and biotechnology fields (Isla*m et a*l., 2018).

According to Dandekar, Fegade and Vh, (2015), phytol has shown antimicrobial, anti-inflammatory, anticancer, and diuretic properties. Prior studies indicate that it has already been identified via GC-MS in microalgae of the genus *Chlorella* and cyanobacteria of the genera *Nostoc* and *Synechococcus* (Lütke-Brinkhaus; Weiss; Kleinig, 1985; Sale*m et a*l., 2014; Adamakis *et a*l., 2018).

Glycerol was also identified in high concentrations in *Chlorella* sp. GBBB06 (26.98%), *Nostoc* sp. GBBB01 (6.44%), and *Synechococcus* sp. GBBB07 (10.00%). Glycerol is a precursor of triacylglycerols, which can be applied in the production of biofuels (Kobayash*i et a*l., 2013). According to Amaro, Guedes and Malcata, (2011) and Parma*r et a*l. (2011), photosynthetic microorganisms such as cyanobacteria and microalgae have the potential to produce biofuels in an economically efficient and environmentally sustainable manner, as they do not require agricultural soil and because of their rapid growth and high lipid content. Verm*a et a*l. (2010) identified *C. emersonii*, *C. vulgaris*, and *C. minutíssima* as being potentially useful for producing triacylglycerols for biodiesel.

The biotechnological potential of *Chlorella* microalgae is extensive as they produce compounds with many different applications. Khoeyi, Seyfabadi and Ramezanpour (2012) identified highly saturated fatty acids including palmitic acid methyl ester and undecanoic acid methyl ester when studying the strain of *Chlorella sp.* MCCS 040. According to those authors, strains with high amounts of highly saturated fatty acids may be ideal candidates for biodiesel production.

Fatty acids identified from the strains studied here 9-hexadecenoic acid methyl ester in *Synechococcus* sp. GBBB07 (1.87%), 1H-imidazole-4-carboxylic acid methyl ester in *Nostoc* sp. GBBB01 (1.99%), 4-ethoxybenzoic acid ethyl ester in *Chlorella* sp. GBBB06, and the methyl ester of hexadecanoic acid, also known as palmitic acid, in GBBB06 (2.37%), GBBB01 (6.75%), and GBBB07 (1.93%), respectively. Among the fatty acids identified, the methyl ester of hexadecanoic acid, stands out because of its antioxidant, hypocholesterolemic, antiandrogenic, and pesticidal properties, in addition to its application in the food and cosmetics industry (Rajeswari; Murugan; Mohan, 2012; Dandekar; Fegade; Vh, 2015).

This compound was identified along with eicosane, hexadecane, and others by Widiyant*o et a*l. (2018)when studying volatile compounds in *Chlorella* sp. using GC-MS. Ananya and Kamal (2016), also identified hexadecanoic acid in *Nostoc muscorum*, as well as tetradecanoic acid ethyl ester, phthalic acids, and 9-octadecenoic acid ethyl ester, showing that both cyanobacteria and microalgae can produce fatty acids of interest to the biotechnology industry.

Similar to the other genera of microorganisms studied, cyanobacteria of the genus *Synechococcus* also have fatty acid composition. Verm*a et al*. (2019)identified *Synechococcus* sp. PCC7942 as a producer of several fatty acids, long chain linear hydrocarbons, and other high value products, such as fatty alcohols, suggesting that *Synechococcus* sp. PCC 7942 has potential for the production of biofuel such as biodiesel in addition to being a promising natural resource for the food and cosmetic industries.

Assessment of antioxidant capacity

Antioxidant capacity of *Chlorella* sp. GBBB06, *Nostoc* sp. GBBB01, and *Synechococcus* sp. GBBB07 extracts, using the DPPH˙ sequestration assay is presented in Table 5.

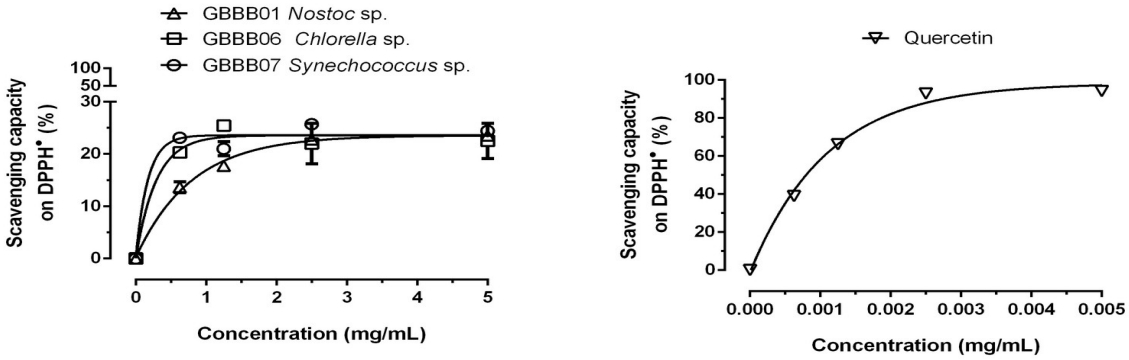
Table 5 - Evaluation of the antioxidant by DPPH• assay from the methanolic extracts of *Chlorella* sp. GBBB06, *Nostoc* sp. GBBB01, and *Synechococcus* sp. GBBB07.

Sample ID	**IC_{50} (mg.mL^{-1})**
GBBB06	8.61 ± 1.31
GBBB01	10.85 ± 1.16
GBBB07	6.65 ± 1.24
Quercetin (standard)	6.5x 10^{-4}± 1.23

* Effective concentration for 50% radical inhibition (IC50**)**

The samples were able to inhibit the DPPH• radical at 12% (*Nostoc* sp.), 20% (*Chlorella* sp.), and 25% (*Synechococcus* sp.) at an extract concentration of 0.675 mg.mL^{-1}, with no increase in antioxidant potential at the highest extract concentrations (Figure 2). Antioxidant activity was identified in extracts of *C. marina*, with radical inhibition of 20.54% in acetone extract, and 23.08% in methanolic extract (Hemalath*a et a*l., 2013). Studies by Jerez-Martel *et a*l. (2017) demonstrated that several cyanobacteria and microalgae possessed effective antioxidant activity. The genus *Nostoc*, for example, demonstrated the ability to scavenger 27.89% of DPPH• and this activity is related to the presence of phenolic compounds in the analyzed extracts. In the cyanobacterium *Synechococcus* sp., isolated from the Cochin estuary in India, 55.83% elimination of DPPH• was observed in methanolic extracts (Lekshmi; A.V., 2018).

Figure 2 - DPPH inhibition for (A) the raw methanolic extracts from *Nostoc* sp. GBBB01, *Chlorella* sp. GBBB06, and *Synechococcus* sp. GBBB07; (B) Positive control (quercetin).



The antioxidant activity of the extracts of *Chlorella* sp., *Nostoc* sp., and *Synechococcus* sp. may be related to the presence of carotenoids and chlorophylls, as identified through HPLC-DAD. Carotenoids interact with free radicals and may have different activities depending on the type of carotenoid present in the sample (Young; Lowe, 2018). Some studies claim that chlorophylls, as well as their derivatives such as copper chlorophyllin, may also exhibit potent antioxidant (Ferruzzi; Blakeslee, 2007; Tumolo; Lanfer-Marquez, 2012; Stinco *et a*l., 2014; Amrani-Allalou *et a*l., 2019).

The results for the antioxidant activity of *Chlorella* sp. GBBB06, *Nostoc* sp. GBBB01, and *Synechococcus* sp. GBBB07 extracts using the $ABTS^{\bullet+}$ assay are presented in Table 6.

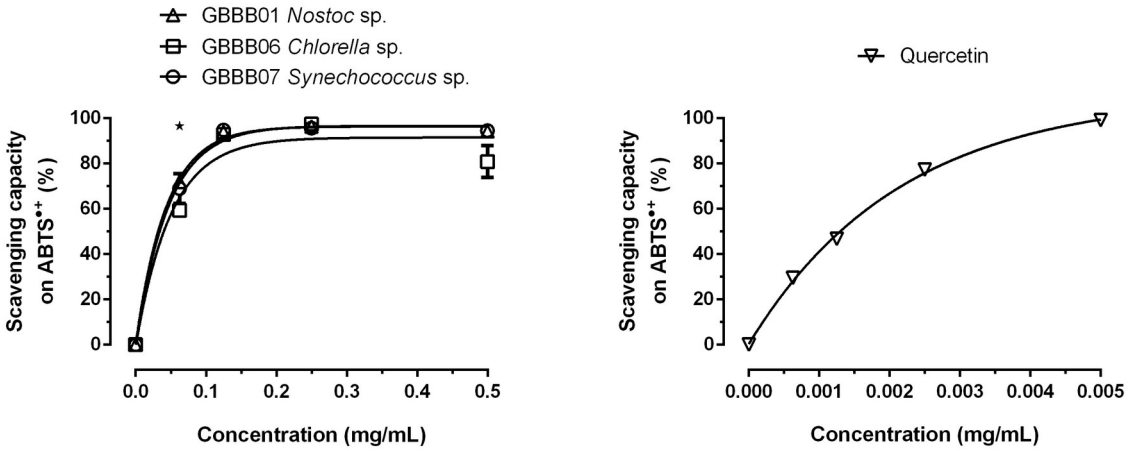
Table 6 - Evaluation of the antioxidant potential via ABTS assay from the methanolic extracts of *Chlorella* sp. GBBB06, *Nostoc* sp. GBBB01, and *Synechococcus* sp. GBBB07.

Sample ID	**IC_{50} (mg.mL^{-1})**
GBBB06	2.64 x10^{-2} ± 2.01
GBBB01	1.63 x 10-2 ± 1.90
GBBB07	1.65 x 10-2 ± 2.05
Quercetin	6.5 x 10-4± 1.23

* Effective concentration for 50% radical inhibition (IC50)

Because it is a more sensitive method, the ABTS•+ assay showed higher antioxidant activity than the DPPH• method. Samples presented consistently lower IC50 values in the ABTS•+ method, resulting in greater antioxidant activity against ABTS•+ radical inhibition of 60% (Chlorella sp.), 70% (Synechococcus sp.), and 70% (Nostoc sp.) at an extract concentration of 0.1 mg.mL-1 (Figure 3). Synechococcus sp. and Nostoc sp. stood out for reaching high antioxidant potential. Similar data were obtained by Shanab et al. (2012). They noted antioxidant activity for aqueous extracts of N. muscorum, with ABTS•+ inhibition of 72.8%. Wu et al. (2005) found inhibition of 40% in 0.2 mg of aqueous extract from C. Vulgaris.

Figure 3 - ABTS radical inhibition for (A) the raw methanolic extracts from *Nostoc* sp. GBBB01, *Chlorella* sp. GBBB06, and *Synechococcus* sp. GBBB07; (B) Positive control (quercetin).



Antioxidants consume reactive oxygen, as well as nitrogen and sulfur species and can protect cell tissues from their deleterious effects, preventing diseases such as cancer (Hemalath*a et a*l., 2013). Thus, microorganisms that produce this class of molecules have the potential to aid in the development of food products and drugs rich in antioxidants with beneficial chemo-preventive effects (Yucharoen; Srisuksomwong; Tragoolpua, 2015).

CONCLUSIONS

The present study demonstrates the potential of photosynthetic microorganisms from the Cerrado Maranhense as sources of bioactive secondary metabolites such as carotenoids and chlorophyll, with applications in the pharmaceutical, nutraceutical, and food industries. The presence of fatty acids also indicates prospective utility in the cosmetic and biofuels industry. Finally, the antioxidant capacity found in these extracts demonstrate the perspective of these microorganisms in antioxidant rich supplements and medicine.

DISCLOSURES AND DECLARATIONS

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Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Authors contribution

Hivana Melo Barbosa Dall'Agnol, Claudia Quintino da Rocha and Leonardo Teixeira Dall'Agnol conceived, obtained funding, and supervised this study. The material preparation, chemical characterization and antioxidant analysis was carried out by Malena Correira Costa, Aldilene da Silva Lima, Cáritas de Jesus Mendonça, Antonio Marcus de Andrade Paes and Vinicyus Teles Chagas. The first draft of the manuscript was written by Malena Correia Costa and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethical Approval

Authorization for the collection and transport of samples was approved by the Instituto Chico Mendes de Conservação da Biodiversidade (SISBIO 55217-1) and this research is registered at the Brazilian National Genetic Heritage and Traditional Knowledge Management System (SISGEN) under the license AACEC6D.

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