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Research Article

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Study of the Secondary Metabolites, Cytotoxic and Antioxidant Activity of the Methanolic *Monoraphidium contortum* Crude Extract

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ABSTRACT

Microalgae are organisms abundant in aquatic environments to be extremely tough. This feature is related to secondary metabolites produced by them, which at present are poorly studied, but may be of great interest to the pharmaceutical industry. In order to characterize chemically the microalgae Monoraphidium contortum, cultivation and the extraction process was performed, in addition, to qualitatively verify its bioactive compounds by analysis of secondary metabolites, to thereby correlate the antioxidant activity and the ability of cytotoxic species. The gathering was held at the fish pond on the property Rosa dos Ventos located in Fazendinha AP-district. The identification and selection were performed by microscopy and subsequently grown in the substrate rich in nitrogen, phosphorus and potassium (NPK) nutrients that allow the growth of these algae, with photoperiod and temperature controlled at 22ºC. The growth was evaluated daily and the extraction of the biomass was performed by maceration, using organic solvents of increasing polarity. The study of secondary metabolites M. contortum indicated the presence of phenols, tannins, and anthraquinones. M. contortum showed a significant antioxidant activity and got a low LC_{50}, which resulted in low toxicity of the extracts. This species proved to be promising; this study emphasizes the importance of knowledge of the composition of bioactive compounds of species of microalgae as a source of the industrial application.

Keywords: Algae; Biomass; *Artemia salina*; Clorococales; Aquatic environment

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INTRODUCTION

Algae are photosynthetic organisms and cover bodies with two kinds of cell structure: eukaryotes (green algae) or prokaryotic (cyanobacteria) have varied color, a characteristic which depends on the presence of pigments and photoautotrophic mechanisms of each species may be filamentous or unicellular forming colonies or not. Have little or no cell differentiation and have a rapid growth as well as survival in various environments such as marine ecosystems, freshwater and land because of its varied structure, it is mainly found in aquatic environments, with large population diversity [1-3].

In recent years, the use of biomass of microalgae in nature or their extracts has shown promising results in different economic sectors, considered a potential source of natural bioactive substances. Since various metabolites such as carbohydrates, proteins, lipids, carotenoids, phycobiliproteins, chlorophylls, sterols, and polyunsaturated fatty acids, have shown diverse biological activity and remarkable health benefits [4-6]. The *Monoraphidium* gender is the most growing enters the clorococales and is distributed in all regions of the world. Generally are more present in fresh water, the species of this genus can be straight or curved, and its reproduction is only by self-sporulation [7,8].

Belonging to Selenastraceae family, *Monoraphidium contortum* presents with elongated cells, condensed spiral, apex gradually tapered with a single chloroplast, and the union occurs only when this in the reproductive process. Its predominant feature is the half-moon shape, its thickness decreasing from the center to the cell ends [7,9].
Moreover, microalgae can produce a range of bioactive molecules with antiviral, anti-inflammatory, antibiotic, the latter activity demonstrated by *M. contortum*, as well as the antifungal capacity of some other species and their enzymatic actions and other pharmacological activities. It also enables the production of biofuels, the biodiesel manufacturing by esterifications reactions of some species of fatty acids (including *M. contortum*) because of its large capacity for energy generation, with a lipid content which can vary from 20 to 80 % of their biomass [3,10-12].
The study of secondary metabolites has been used as a screening system to determine distinct classes of compounds without detailed structural assignment of chemicals. The secondary metabolites play important roles in biochemistry and physiology of plants [12]. Due to lack of more knowledge about bio secondary metabolites synthesized and biological potential of this alga is intended to know the type of more characterized compounds and assess the cytotoxicity of the methanol extract.
The objective of this research was to carry out the analysis of secondary metabolites, cytotoxicity activity front *Artemia salina*, and antioxidant activity of the methanolic crude extract of *Monoraphidium contortum*.

EXPERIMENTAL SECTION

Collection

The study area is located on Highway Salvador Diniz, in Fazendinha district in the city of Macapa – Amapá. Samples were collected in fish tanks that are located on the property known as Rosa dos Ventos.

Cultivation

Cultivation was carried out in 1.0 L Erlenmeyer flask at liquid environment (distilled water) with specific nutrients for the species found, and aeration was performed to supply oxygen dissolved in the culture medium [13]. White fluorescent lamps of 20 w were used with an average luminance of 3200 Lux, and the culture was maintained at a constant temperature of 22°C. The cultivation environment was used BG-11 sterilized by autoclaving for 15 minutes at 121°C [14].

Extraction

In performing, the analysis used the lyophilized and milled biomass the extraction was carried out by thorough maceration method in methanol to yield 3.838g.

Study of secondary metabolites

The analyses were performed at the laboratory of Pharmacognosy/Phytochemistry of UNIFAP, using the Barbosa [15] methodology. The tests for identification of secondary metabolites were phenols, tannins, flavonoids, steroids, triterpenes, and alkaloids. A few milligrams of dry algal extract were used, and followed by the procedures in the internal protocol.

Antioxidant activity

For the antioxidant activity of the extract *Monoraphidium contortum* using free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), it was determined according to the methodology described by Sousa et al. [16] and Lopez-Lutz et al. [17] with modifications. In a test tube containing a solution of 40 mg/mL^{-1} of DPPH previously diluted in methanol. The extracts from the microalgae and cyanobacteria were diluted at concentrations of 5; 1; 0.75, 0.50; and 0.25 mg/mL. In the Later analysis was added to 2.7 mL of the DPPH solution, supplemented with 0.3 mL of diluted extract in methanol, allowed to stand protected from light. After 30 min, the absorbance readings were made at 517 nm on the spectrophotometer. For the analysis was used the control, performed by DPPH calibration curve and the white utilized, was distilled water. The ability to scavenge the radical was expressed as percent inhibitory concentration (CI_{50}), the amount of antioxidant required to decrease the initial concentration of DPPH by 50% [18], the absorption of DPPH solution containing the samples and pattern through radical oxidation inhibition percentage calculated according to equation below

$$(\%AA) = \{[(Abs_{sample} - Abs_{white})] / Abs_{controle}\}$$

Cytotoxicity test

The toxicity bioassay was done according to Meyer et al. [19] with some modifications; the *Artemia salina* cysts to hatch were placed in saline solution under aeration at 25°C after 48 hours the larvae were removed for testing. Serial

dilutions were made of microalgae and cyanobacteria extracts in vials according to the calculation of efficiency, so as to enable to obtain the final concentrations of 1000, 750, 500, 250, 100 e 50 µg/mL. Control tubes were prepared to contain only the solvent dimethyl sulfoxide (DMSO) and saline solution. The test was performed in triplicate, and for each cyanobacterium and microalgae extracts were prepared 3 series of tubes (a number for each concentration, 3 test tubes for each group). To each test tube were transferred 10 larvae. The tube volume was completed to 5 ml with saline solution. After 24 h in contact with the suspension of the extracts was performed by counting the number of surviving larvae. The data of mortality (%) of larvae of *A. salina* in relation to the increased concentration (µL) of *M. contortum* extract was analyzed by SPSS in Probit graph to determine the lethal concentration which causes 50% mortality of the population (LC_{50}).

RESULTS AND DISCUSSION

In the analysis of secondary metabolites for *Monoraphidium contortum*, tests have determined the presence of phenols, tannins, and anthraquinones as shown in Table 1.

Table 1: Study of the secondary metabolites of microalgae *Monoraphidium contortum*

Secondary metabolites	Result
Organicacids	-
Polysaccharides	-
Phenols and Tannins	+
Flavonoids	-
Alkaloids	-
Steroids and triterpenoids	-
Anthraquinone	+
Catechins	-
Saponins espumídicas	-
Reducing sugars	-
Proteins and amino acids	-
Purines	-

Note: Absent (-); Present (+)

The phenols have pharmacological properties such as antiviral or antimicrobial and antioxidant [20]. In a study Scholz; Liebezeit.[12] demonstrated a significant characteristic of bacterial activity in *M. contortum*, which may related to the presence of phenolic compounds. Tannins are phenolic compounds, and therefore are highly chemically reactive, have the ability to form hydrogen bonds, intra- and intermolecular [21], classified into two groups, based on their structural type: tannins hydrolyzable and condensed tannins. It has astringent hemostatic function and its therapeutic applications are related to these properties [22]. The extract also showed reactivity to anthraquinones (using the ammonium hydroxide solution NH_4OH 10%), natural quinone derivatives of anthracene, are formed from the oxidation of phenols they are from the group of quinones, aromatic compounds, their properties are attributed, antifungal, bactericides and repellent to some insects attacks, and especially laxative activity, despite its use limited by adverse effects caused. In the industrial scale, their derivatives are quite used as textile dyes [23,24]. The evaluation of the antioxidant activity of *M.contortum* showed IC_{50} values of 6.3 mg/mL (Figure 1) and correlation coefficient (R2) of 0.9939. The effectiveness of the elimination of DPPH from the through IC_{50}, the lower the value, the higher the antioxidant activity of the sample.

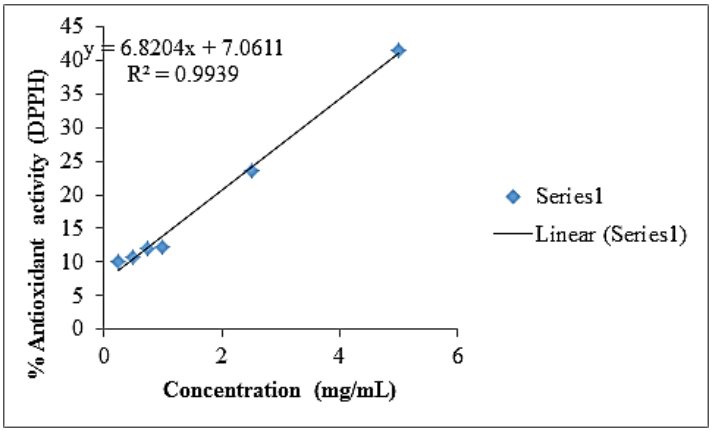
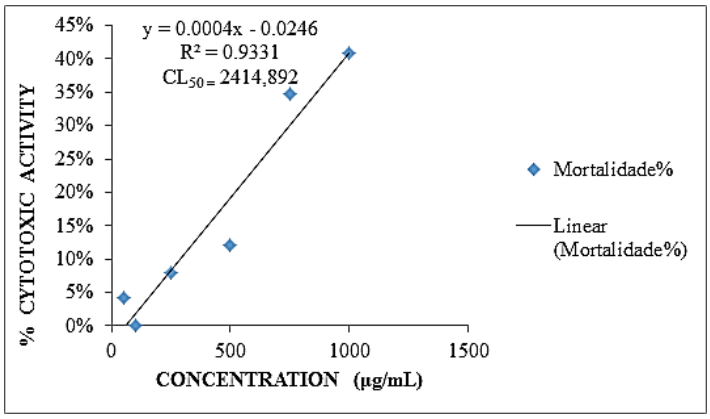


Figure 1: DPPH reduction capacity at the concentrations used in *M. contortum*in30 minutes of time

The highest percentage of inhibitory activity to *M. contortum* concentration was 5 mg/mL with 38% (± 0.688), and 2.5 mg/mL with 21% (± 0.417), and lower concentrations of 0.25, 0.50 and 0.75 mg/mL with 9% (± 1.068) 10% (± 1.548) and 11% (± 0.490) respectively. By comparing with the standard of vitamin C (0.03 mg/mL-1), which has a high antioxidant property, it shows significant inhibition of DPPH values of the microalgae*M. contortum*, which indicates an increase in antioxidant activity to the oxidative degradation process, namely the extract acts as a hydrogen donor to the radical [25]. Chlorophyta species, such as *M. contortum*, showed 10 to 30% inhibition of DPPH, and the upper current result, reaching 38% [26]. As shown by Garcia and Guerrero [27] with ethanol extract of Chlorella vulgaris with a high percentage of the antioxidant activity found.

The substances with antioxidant properties in microalgae can be of various natures, from carotenoids, vitamins, phycobiliproteins and polyphenols, tannins [28]. However, despite few studies, several authors have correlated results for the total phenolic content and antioxidant activity in the presence of algae [29-31]. The phenols are likely to react with free radicals, stabilizing them. This is the ability of the phenolic compounds to counteract the free radical structures due to their chemical structure comprising at least one aromatic ring having hydroxyl groups which are removed by reducing this group to a less reactive form, hydrazine [32,33].

Chaudhuri et al. [34] correlated to the antioxidant activity of microalgae Euglena tuba the tannins and alkaloids and obtained a correlation signification coefficient (R2 = 0.9486), confirming the antioxidant activity of these two compounds. The cytotoxicity assay with *A. saline* determining the lethal concentration of 50% active components and extracts in a saline environment with DMSO and microalgae extract, carried out in a 24-hour period, the larvicidal activity of the species *M. contortum* extract (Figure 2). Where the mortality rate of the values expressed in percentage of mortality (%), where it is observed that the highest mortality rate was found in the concentration of 1000µg/mL with 40.9% demonstrating the extract is non-toxic as stated Nguta et al. [35].



Note: p = 0.282

Figure 2: Cytotoxicity assay results for *M.contortum* relative concentration (µg/ml) x mortality (%)

Relative to *A. salina* test, there was no high LC_{50} toxicity (2414.892), after exposure to 24 hours, it was found a result similar to the study of Scholz and Liebezeit [12], who conducted toxicity tests front of *A.salina* with various kinds of microalgae and cyanobacteria families, including *M.contortum*, collected from salty waters. It showed no toxicity after the first 24 hours, for such results, these authors point out that when the greater the exposure time of the extracts larvae, the greater the sensitivity of the test, thus suggesting a time higher than 48h for more comprehensive results.

However, it is emphasized the importance of the findings for the potential of biotechnological development to products for human use, not to present significant toxicity.

CONCLUSION

Based on these results, it is concluded that *M. contortum* has great potential due to secondary metabolites found and its strong antioxidant activity in addition to, its extract is non-toxic. It is proving to be a promising source of natural resources, used in the pharmaceutical industry.

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