

# Immunomodulatory, antioxidant and anti-tumor capacity of acidic polysaccharides from *Euglena gracilis*.

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## INTRODUCTION:

Microalgae are unicellular organisms and are classified as prokaryotes or eukaryotes, that can grow autotrophic, heterotrophic and in mixotrophic mode. In general, the fixation of O<sub>2</sub> and the use of solar energy to produce biomass, with an efficiency up to four times superior to the plants, are highly efficient. The main characteristic of microalgae is the ability to modulate their metabolism according to environmental conditions, being a great source of bioactive molecules that play a physiological role with themselves and their environment. They are rich in proteins, fatty acids, antioxidant pigments and polysaccharides that show a wide range of biological activities of antimicrobial, immunosuppressive, anticancer and antiviral. It is important to highlight the various physiological activities of sulphated polysaccharides of algae, such as anticoagulants, antivirals, antihyperlipidemics and anticancer. More specifically, *Euglena*, eukaryotic microalgae, has no cell wall, but stores several nutrients such as vitamins and minerals in high concentrations, which can be easily digested, allowing nutrients to be absorbed and used. The ease with which it can be cultivated makes it one of the most studied eukaryotes playing a key role in the development of cell biology and biochemistry. *Euglena gracilis*, in particular, called "Midorimushi" in Japanese, has been investigated as a producer of several important molecules like vitamins A, C and E essential amino acids, polyunsaturated fatty acids, B-carotenes and polysaccharides (paramylon (β-1,3-glucan), being this, one of the most interesting components of *E. gracilis* biomass, with immunomodulatory properties among other).

### Key words:

*Euglena gracilis*, polysaccharides, immunomodulatory, antioxidant capacity, anti-tumor activity cell lines: U-937, HTC116, RAW 264.7 and HGF-1.



Figure 1: Fotografía de *Euglena gracilis*. Disponibles en <http://botany.natur.uni.cuni.cz>

## MATERIAL AND METHODS :

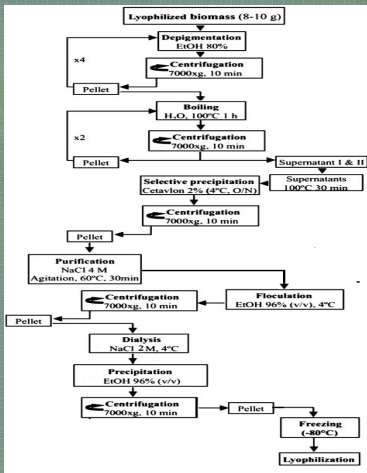


Figure 2: Protocol for the extraction of acid polysaccharides from *Euglena gracilis*.

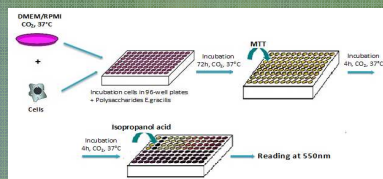


Figure 3: Cell viability assay by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazole bromide (MTT).

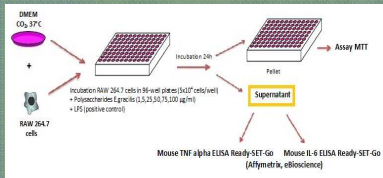


Figure 4: Immunological assay by determination of cytokines.

## RESULTS :

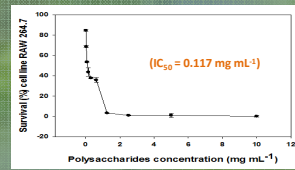


Figure 8: % Survival of RAW 264.7 macrophages exposed to different concentrations of polysaccharides from *E. gracilis*.

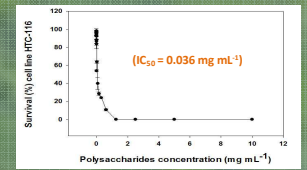


Figure 9: % Survival of cell line HTC-116 (colorectal carcinoma) exposed to different concentrations of polysaccharides from *E. gracilis*.

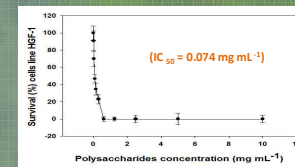


Figure 10: % Survival of cell line HGF-1 (human gingival fibroblasts) very proliferative exposed to different concentrations of polysaccharides from *E. gracilis*.

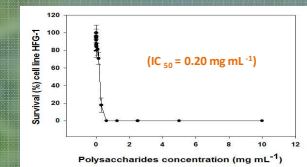


Figure 11: % Survival of cell line HGF-1 (human gingival fibroblasts) low proliferative exposed to different concentrations of polysaccharides from *E. gracilis*.

## RESULTS :

- The amount of S in the acidic polysaccharides of *E. gracilis* is very low compared to the percentage of C, H and N.

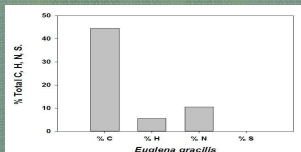


Figure 5: Total Carbon (TC), Hydrogen (TH), Nitrogen (TN) and Sulfur (TS) content in the polysaccharide obtained from *E. gracilis*.

- The data obtained from the MTT cell viability assays for the studied cell lines show different behaviors.

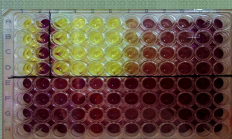


Figure 6: MTT in cell line HTC-116

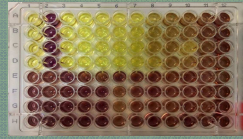


Figure 7: MTT in cell line U-937

## CONCLUSIONS :

- ❖ Determined that IC<sub>50</sub> in polysaccharides isolated from *Euglena gracilis* in the cell line macrophage (RAW 264.7) is 0.117 mg mL<sup>-1</sup>.
- ❖ The polysaccharides have anti-proliferative activity in cancer cells with an IC<sub>50</sub> = 0.036 mg mL<sup>-1</sup> in HTC-116, an IC<sub>50</sub> = 0.027 mg mL<sup>-1</sup> in U-937 and an IC<sub>50</sub> = 0.11 mg mL<sup>-1</sup> in MCF-7.
- ❖ This treatment is more active in proliferative HGF-1 (resemble cancerous conditions) in HGF-1 little proliferative (resemble healthy condition) by an order of magnitude.
- ❖ The antioxidant activity determined in the polysaccharide of *E. gracilis* was of (7.19 μmol TE g<sup>-1</sup>).
- ❖ The polysaccharides isolated from *E. gracilis* have effects on the immune system due to a strong stimulation of macrophages in relation to the synthesis of IL-6 and TNF-α.

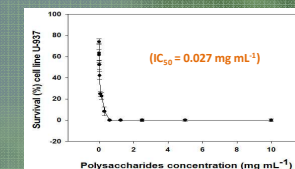


Figure 12: % Survival of cell line U-937 (leukemia human) exposed to different concentrations of polysaccharides from *E. gracilis*.

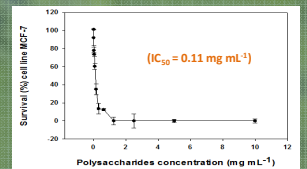


Figure 13: % Survival of cell line MCF-7 (breast adenocarcinoma human) exposed to different concentrations of polysaccharides from *E. gracilis*.

- The polysaccharides isolated from *E. gracilis* show immunostimulatory capacity on the mouse macrophage cell line (RAW 264.7).

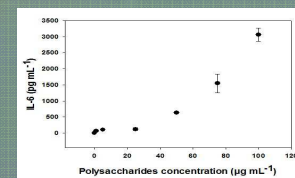


Figure 14: Production of Interleukin-6 by RAW 264.7 macrophages exposed to different concentrations of polysaccharides from *E. gracilis*. Data points represent the average of four samples ± SD.

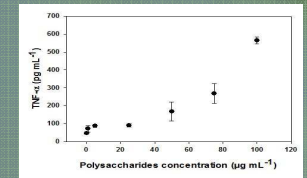


Figure 15: Concentration of Tumor Necrosis Factor-α by RAW 264.7 macrophages exposed to different concentrations of polysaccharides from *E. gracilis*. Data points represent the average of four samples ± SD.

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