SPIRULINA PLATENSIS AS SCAVENGER OF THE REACTIVE OXYGEN SPECIES

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Summary

Spirulina platensis is a microalgae very useful as a nutritional supplement. In this investigation a study was carried out of the antioxidant capacity *in vitro* of different concentrations of hidroalcoholic extract. It was evaluated capturing the superoxide ($O2^{\cdot-}$), the hidrogen peroxide (H_2O_2) scavengers and the hidroxyl radical ($OH^{\cdot}$), as well as chelatting power of iron in *Spirulina*. For the determination the antioxidant capacity the homogenate brain rats was utilized as it is rich in phospholipids and suffer peroxidatives processes that can be measured by the increment of products reactive with acid tiobarbituric. Statistical analysis was carried out using the test Anova and Dunnet. The hidroalcoholic extract the *Spirulina* possesses components such as the ficocianin and the enzyme superoxide dismutase, capable of scavenger the $O2^{\cdot-}$, besides protecting the deoxyribose for its scavenger capacity of $OH^{\cdot}$ and chelate iron, for the presence of compound as carotenes and vitamin E; the capture of H_2O_2 was not demonstrated.With relations to the antioxidant capacity it was demonstrated that this natural substance is capable of to significantly inhibiting the spontaneous peroxidative processes and catalyzed by metals in the concentrations 0.35% and 0.5%. The components: vitamin E, ficocianin, enzyme superoxide dismutase, among other, present in the *Spirulina platensis* confer great antioxidant activity.

Key Words: scavenger, *in vitro*, reactive oxigeno species, *Spirulina platensis*

Introduction

The *Spirulina platensis* is one of the microalgaes most abundant in the Cuban cost, its high protean and vitamin value, as well as its easy obtaining, makes that it is an important natural source. This bioproduct is widely use in the biotechnical industries, pharmaceutical and cosmetic. It is used in the entire world as dietary supplement and for its therapeutic properties, It contains proteins, hydrates carbon, minerals, vitamins, essential fatty acids and aminoacids.(1)

To antioxidant properties reported (1) and the results of the toxicological studies up to this moment demonstrate the relative innocuous this natural substance, we intended to determine the antioxidant capacity the *Spirulina platensis* through *in vitro* test that demonstrated possible mechanism of action.

Methods

For the evaluation the antioxidant capacity *in vitro*, different concentrations were prepared 0.05%, 0.2%, 0.35%, 0.5%, 0.65% of the hidroalcoholic extract of *Spirulina platensis* to determine the capacity the same scavenger the superoxide anion ($O2^{\cdot-}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^{\cdot}$), as well as, its antioxidant capacity.

The scavenger capacity of superoxide it was evaluated through the method inhibition the autoxidation the Pyrogallol(2). The capture of H_2O_2 was measured by means of the colorimetric quantitative determination of reactive oxigen species using the essay based on the oxidation the ferrous iron to ferric iron for H_2O_2 under acid conditions(3). The scavenger capacity of the radical $OH^{\cdot}$ base in the generation $OH^{\cdot}$ for the system H_2O_2 / EDTA/Fe^{3+}/ ascorbic acid and the detection of the damages that it originates by this radical to the sugar deoxyribose. The products degradation of this sugar react with the tiobarbituric acid forming a colored compound that facilitates the detection the damage(4).

The antioxidant capacity was determined using the homogenate brain rat that is rich in phospholipids, these suffer peroxidative processes (catalyzed or not by metals) that can be measured by the increment the levels of tiobarbituric acid reactive substances.(5)

The results were expressed in inhibition percentages of tiobarbituric acid reactive substances (TBARS).

Results

The results obtained of the absorbance (Table1), the different samples assayed in the experiment to measure the scavenger capacity of the radical superoxide anion, describe a decrease in the concentrations 0.05, 0.2, 0.35, 0.5% with regard to the target, and with significant differences of 0.35 and 0.5 %.

In the assay to evaluate the scavenger the hydrogen peroxide, the absorbance measures of the evaluated concentrations are superiors with respect to the target and don't show a decrease with regard to the target, the assay realized it doesn't demonstrate the scavenger capacity the H_2O_2 this substance.

The scavenger effect the hidroalcoholic extract the *Spirulina platensis* in front of the hidroxyl radical show a disminution the absorbance with regard to the target, with significant differences, in the concentrations 0.35% and 0.5% the higher inhibition percentage the formation TBARS for degradation the deoxirybose with 80% and 83.4% respectively.Table 2.

With regard to the chelating iron capacity, the results the experiment they show that the biggest inhibition percentages TBARS of degradation the deoxyribose obtained with the concentrations 0.35 and 0.5% (53.85% and 57.7% respectively), which coincides with the results obtained in the previous assay, but with smaller inhibition percent, that demonstrates that the *Spirulina platensis* presents more scavenger capacity of the $OH^{\cdot}$ radical than chelate capacity iron.

In the capacity antioxidant, it was observed for the enzymatic autoxidation as well as the catalyzed by metals, a decrease in the absorbance statistically significant with more significance for the metalic autoxidation. The inhibition percentage the oxidation are greater for both assays and with similar magnitudes 65.63% -75% for the spontaneous autoxidation and 66.67% -76% for the catalyzed by metals.

Table 1. Concentration of the hidroalcoholic extract of the *Spirulina platensis* tested to measure scavenger of O_2.-.

Sample	Absorbance (A) A ± SD
Target	0.023 ± 0.01**a**
Hidroalcoholic solution	0.02 ± 0.01
0.05%	0.02 ± 0.01
0.02%	0.01 ± 0.01
0.35%	0.006 ± 0.01 **b**
0.5%	0.004 ± 0.01**b**
0.65%	0.03 ± 0.01

A ± SD: Mean value of the 5 repitition and standar desviation
a,b: Statistical signification in comparison to the target $p< 0.05$.

Table 2. Concentration of the hidroalcoholic extract of the *Spirulina platensis* tested to measure scavenger of $OH^{\cdot}$

Sample	Absorbance(A) A ± SD	% de I
Target	0.30 ± 0.010 **a**	-
Hidroalcoholic solution	0.15 ± 0.10 **b**	50
0.05%	0.10 ± 0.01 **b**	66.7
0.2%	0.07 ± 0.02 **b**	76.7
0.35%	0.06 ± 0.01 **b**	80
0.5%	0.05 ± 0.01 **b**	83.4
0.65%	0.02 ± 0.02 **b**	60

A ± SD: Mean value of the 5 repitition and standar desviation
% I: Inhibition Percentage in the production of TBARS by degradation of deoxyribose. a,b: Statistical signification in comparison to the target $p< 0.05$.

Discussion

The decrease of the absorbance with different concentrations of the hidroalcoholic extract the *Spirulina platensis* assayed, when being carried out capture the $O2^{\cdot -}$, indicate that this natural substance possesses compound capable to exhibit scavenger activity of this radical. This result can be verified by the presence of enzyme dismutase superoxide in the *Spirulina sp* which dismutes the superoxide anion to hydrogen peroxide. (6)

The results did not show the capacity of this microalgae to capture the H_2O_2, it seems to be that the compounds present in the extract produce interference with the reagents used in the reaction.

The capacity the *Spirulina* to scavenger $OH^{\cdot}$ radical, it can be justified for the presence in the hidroalcoholic extract of carotenes and ficocianin, elements that are part the defensive system the organism against the free radicals, also it possesses vitamin E, wich has antioxidant activity by its capacity to capture free radicals. Takin into account that the hydroxyl radical is the most potent free radical found in the biological system and that through its reaction with the lipids, protein and nucleic acids is implicated in the genesis or exacerbation of different physiogical processes, we consider as very encouraging this result, for the use of this product as antioxidant agent. (1, 6, 7)

The chelate capacity iron, until the moment is not attributed to any specific component of the *Spirulina platensis*, but exist reports of the treatment with *Spirulina sp* in patients intoxicated with heavy metals (8), if we consider that the heavy metals are eliminated from organism for chelates agents, then we can think that the *Spirulina* presents chelate capacity iron.

With relations to the antioxidant capacity, it is demonstrated this natural substance it is capable of inhibiting the spontaneous peroxidactives processes and catalyzed by metals; this action can be related with the results obtained previously that demonstrate that the hidroalcoholic extract of the *Spirulina platensis* is capable to scavenging superoxide anion, hydroxyl radical and it possesses chelate capacity iron; because it possesses in its composition elements that attribute to its antioxidant capacity. (1, 9)

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