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EFFECT OF AN EXTRACT OF HIBISCUS SABDARIFFA L., ON OXIDATIVE STRESS INDUCED IN SACCHAROMYCES CEREVISIAE

Efecto de un extracto de Hibiscus sabdariffa L., sobre el estrés oxidativo inducido en Saccharomyces cerevisiae

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Resumen

Las infusiones o bebidas a base de *Hibiscus sabdariffa* se caracterizan por presentar un alto contenido de compuestos bioactivos que le proporcionan una capacidad antioxidante de gran interés en el mundo científico. La investigación consistió en evaluar el efecto antioxidante de un extracto acuoso de *H. sabdariffa,* al inducir a *Saccharomyces cerevisiae* a un estrés oxidativo, por la presencia de peróxido de hidrógeno. Se empleó el método de Folin-Ciocalteu para la determinación de fenoles totales del extracto. Para evaluar la capacidad antioxidante se diluyó el extracto, se ajustó una concentración de 4.5 10^6 células/mL de *S. cerevisiae*, para luego añadir alícuotas de extracto puro y diluido. Se

Abstract

Hibiscus sabdariffa-based infusions or drinks are characterized by their high content of bioactive compounds that provide them with an antioxidant capacity that is of great interest in the scientific world. The research consisted in evaluating the antioxidant effect of an aqueous extract of *H. sabdariffa,* by inducing *Saccharomyces cerevisiae* to an oxidative stress due to the presence of hydrogen peroxide. The Folin-Ciocalteu method was used for the determination of total phenols in the extract. To evaluate the antioxidant capacity, the extract was diluted, a concentration of 4.5 10^6 cells/mL of *S. cerevisiae* was adjusted, and then aliquots of pure and diluted extract were added. Oxidative stress

observó inhibición del estrés oxidativo en cada uno de los ensayos con diferencia estadística, en relación con el control positivo. Estos resultados muestran que *H. sabdariffa* tiene una capacidad antioxidante que favorece la actividad biológica de *S. cerevisiae,* contribuyendo a la disminución de los niveles de especies reactivas de oxígeno y minimizando el daño oxidativo.

Palabras clave: cálices; antioxidantes; compuestos fenólicos; flavonoides; enzimas.

inhibition was observed in each of the assays with statistical difference, relative to the positive control. These results show that *H. sabdariffa* has an antioxidant capacity that favors the biological activity of *S. cerevisiae*, contributing to the decrease of the levels of reactive oxygen species and minimizing oxidative damage.

Keywords: calyces; antioxidants; phenolic compounds; flavonoids; enzymes.

Introduction

Hibiscus sabdariffa L. (commonly known as roselle), is an annual or perennial plant with red stems and calyces, belonging to Malvaceae. It is cultivated mainly in the tropical and subtropical areas of both hemispheres (Patel, 2014). In Africa, roselle has two main uses: as a vegetable and for preparation of a beverage (Ghazala & Rajni, 2018). In Guinea-Bissau it is known as ondjo and both leaves and calyces are widely consumed and marketed (Ramírez-Rodrigues *et al*., 2012). The use of this plant comes from ancestral times and is mainly used in the preparation of hot and cold beverages mainly due to its proven antioxidant function in humans, especially against chronic diseases (Kao *et al*., 2016; Moyano *et al*., 2016). These effects have been associated to the presence of some phenolic compounds namely, anthocyanins (cyanidin derivatives), flavonols (quercetin and kaempferol derivatives), phenolic acids (chlorogenic acid) and also due to the presence of a specific organic acid from *H. sabdariffa* (hibiscus acid) (Borras-Linares *et al*., 2015).

A correlation between the total phenolic content of *H. sabdariffa* extracts and their antioxidant properties has been reported (Yang *et al*., 2012; Herranz-López *et al*., 2017). Therefore, it must be presumed that the antioxidant properties of the extracts lie on the polyphenol´s, abilities to scavenge free radicals. This effect has been observed in several oxidative damage models, in both cells and experimental animals. Many scientific investigations have revealed that the calyces of roselle are rich in polyphenols and flavonoids that enhanced the nutritive value of roselle as these compounds are correlated with their antioxidant property (Jabeur *et al*., 2017; Ismail *et al*.; 2018). All organisms have antioxidant defenses, including antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and dietary components (Vitamin E, Vitamin C and beta-carotene) that are used to remove or repair damaged molecules (Halliwell, 1991; Ak & Gulcin, 2008; Stevanovic, *et al*., 2019).

Therefore, the study had the objective to evaluate the antioxidant potential of an abstract of hibiscus *H. sabdariffa* on *S. cerevisiae* in the presence of hydrogen peroxide.

Materials and methods

Origin of vegetal material

The vegetal material was donated by independent farmers in the City of Maracay, Aragua State, Venezuela (Geographical coordinates: 10° 14′ 49″ N, 67° 35′ 45″ W).

Preparation of aqueous extract

The extract was prepared with 2.5 g dry calyxes and 100 mL distilled water. The mixture was boiled for 15 min and the liquid separated from the calyxes by decantation. The extraction was repeated in the same conditions. The extracts were filtered together through Whatman No. 4 filter paper and gauged to 200 mL with distilled water (Reyes-Luengas *et al*., 2015).

Determination of total phenolics

The determination of phenols was carried out by the method colorimetric Folin-Ciocalteu. A volume of 50 µL of extract was mixed with125 µL of the Folin reagent, and 400 µL of sodium carbonate 7.1% (w/v), supplemented with distilled water to 1 mL. This procedure was performed in quintuplicate. Then, 5 concentration standards of 50, 100, 150, 200 and 250 µg/mL were prepared from a standard stock solution of Gallic Acid (phenol) with a concentration of 500 µg/mL. Finally, the reading was performed at 760 nm using the molecular absorption equipment Genesis 20 (Thermo Scintific). The results were expressed as mg of GAE / g of vegetal material (VM) (Singleton & Rossi, 1965).

Oxidative stress test on *Saccharomyces cerevisiae*

This assay seeks to evaluate the ability of the compounds present in the extracts to promote the growth of yeast when subjected to oxidative stress with hydrogen peroxide, for which the following experimental sequence was carried out (Alvarez *et al*., 2012): The positive control was performed with ascorbic acid, the negative without antioxidant and a blank only with yeast and culture medium. Before starting the absorbance measurements, *S. cerevisiae* was inoculated for 24 h under agitation at 37 ° C with 8 mL of YPD culture medium (peptone 2% w / v, glucose 2% w / v), 25 µL of yeast stock (solution of *S. cerevisiae* with a cell concentration of 4.5 x 10^6 cells / mL), 100 µL of ascorbic acid (control positive) and 80 µL of the extract of the samples. In control negative and white were inoculated only 8 mL of YPD and 25 µL of the yeast stock. After this incubation time of the yeast under the different conditions the absorbance was measured at 600 nm, this was approximately 0.240 and corresponded to the starting point of latency period of *S. cerevisiae*. Then 160 were added µL of 1 mM hydrogen peroxide to the positive control, negative and the extract to be evaluated at different concentrations. It was incubated at 37 ° C under stirring for 6 h and readings were taken every 30 min. Before each reading they waved the glass tubes for 10 s in vortex to ensure the homogeneity of the cells in the culture medium, then 100 µL of each test was taken and then taken to plates 96 wells Orbital shaking was performed before each reading 20 s and the absorbance readings were taken at 600 nm.

Statistical analysis

Analyses were done in triplicate, and the results were expressed as means ± standard deviation (SD). Results of antioxidant activity were each subjected to analysis of variance (ANOVA).

Results and Discussion

The concentration of total phenols the aqueous extract of *H. sabdariffa* was of 119 µg/mL (11.8 mg of GAE / g VM). The results show that the pure and diluted extract was able to inhibit the oxidative stress originated, with significant statistical difference in relation to the positive control (figure 1). The observed effect is due to the fact that the phenolic compounds present in the extract can indirectly regulate the expression and activity of enzymes such as CAT, SOD and glutathione (Ray *et al.,*2012) (Figure 2).

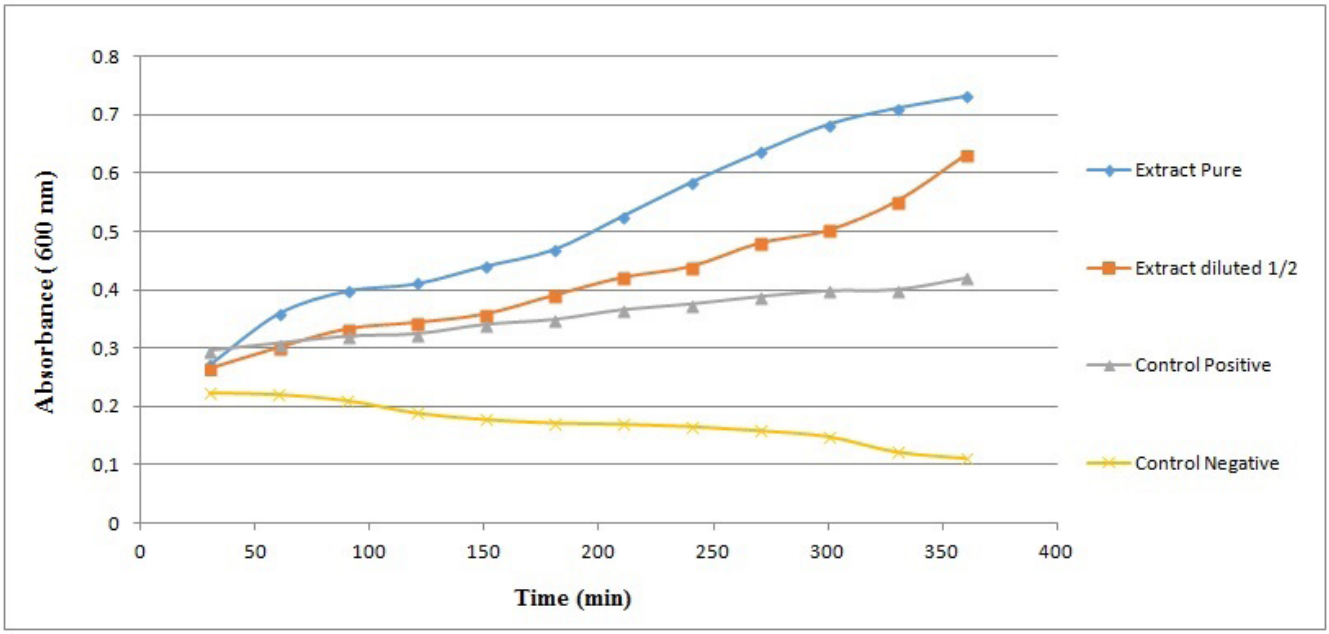

Figure 1. Evaluation of the antioxidant capacity of the pure extract of *H. sabdariffa,* on the oxidative stress induced in *S. cerevisiae.*

Source: own elaboration

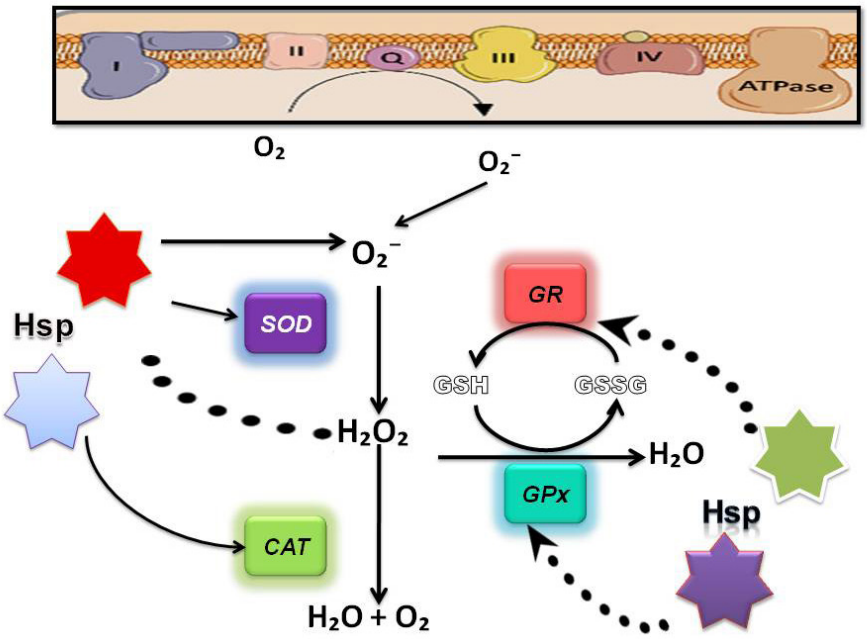

Figure 2. Effect on the enzyme system of polyphenols presentyes in *H. sabdariffa* (Hsp). *The different colored stars represent the polyphenols present in H. sabdariffa.* Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), Glutathione Reductase (GR).

Source: own elaboration

The results coincide with what was found in ethanolic extracts of Hibiscus tiliaceus L, which conferred protection to various strains of S. cerevisiae strains defective in antioxidant defenses against H2O2 and t-BOOH cytotoxicities, showing a clear antioxidant activity. These results suggesting that protection may be due to molecules that act as versatile and wide spectrum no enzymatic antioxidants, such as vitamins or phytosterols (Rosa et al., 2006).

Natural antioxidants are an interesting alternative in view of their variety of structures and chemical interactions, as well as the numerous biological activities they can perform. Intensive research activities are currently being carried out on plant antioxidants to meet this challenge (Kasangana *et al*., 2015).

Conclusions

H. sabdariffa showed a high antioxidant potential even in low concentration. This study is an important issue in the sense of what this polyphenol-rich silver represents, using *S. cerevisiae* as a biological model.

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