# Antioxidant potential of yerba mate (*Ilex paraguariensis* St. Hil.) extracts in *Saccharomyces cerevisae* deficient in oxidant defense genes

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**Abstract**

Yerba-mate (*Ilex paraguariensis St. Hil*) is mainly consumed as "chimarrão", a hot drink highly appreciated in Brazil, Argentina, Paraguay and Uruguay. This study evaluated the antioxidant potential of aqueous extracts of *I. paraguariensis* precipitated with ethanol. The leaves were processed as for tea product (TM) and oxidized (OX). The antioxidant potential was evaluated in cells of *Saccharomyces cerevisiae* deficient in antioxidant defense genes. Three strains evaluated were: a wild (EG) and two mutants (*ctt1*Δ e *ctt1*Δ*sod1*Δ). These strains were pre-treated with the yerba-mate extracts (TM e OX) and submitted to oxidative stress induced by hydrogen peroxide. None of the extracts produced loss of cell viability. The extracts exerted antioxidant activity, protecting the strains (except *sod1*Δ*ctt1*Δ). The TM extract was more effective than OX. *I. paraguariensis* extracts showed a potential to be explored in the development of new products.

*Keywords: I. paraguariensis* St. Hil., mate tea, black tea, *S. cerevisiae*, antioxidant potential.

## Potencial antioxidante de extratos de erva-mate (*Ilex paraguariensis* St. Hil.) em *Saccharomyces cerevisiae* deficientes para genes de defesa oxidante

**Resumo**

A erva-mate (*Ilex paraguariensis* St. Hil) é consumida principalmente como "chimarrão", uma bebida quente muito apreciada no Brasil, Argentina, Paraguai e Uruguai. Este estudo avaliou o potencial antioxidante de extratos aquosos de *I. paraguariensis* precipitado com etanol. Folhas de erva-mate foram processados de maneira semelhante ao processamento do chá-preto (OX) e na forma de mate (TM). O potencial antioxidante foi avaliado sobre células de *Saccharomyces cerevisiae* deficientes para genes de defesa antioxidante. Três linhagens celulares foram estudadas: uma selvagem (EG) e duas mutantes (*ctt1Δ* e *ctt1Δsod1Δ*). As linhagens foram pré-tratadas com os extratos de erva-mate (TM e OX) e submetidos ao estresse oxidativo induzido por peróxido de hidrogênio. Nenhum dos extratos produziu perda de viabilidade celular. Os extratos exerceram atividade antioxidante, protegendo as linhagens (exceto a *sod1Δctt1Δ*). O extrato TM foi mais eficaz em relação ao OX. Extratos de *I. paraguariensis* apresentaram potencial para ser explorado no desenvolvimento de novas formulações.

*Palavras-chave: I. paraguariensis* St. Hil., chá-mate, chá-preto, *S. cerevisiae*, potencial antioxidante.

## 1. Introduction

The genus Ilex, of the family Aquifoliaceae, is distributed in temperate, tropical and subtropical regions. One of the most important species of economic and pharmacological interest in this genus is *I. paraguariensis* St. Hil., popularly known as yerba-mate. This is obtained in the native form and is widely cultivated in northeastern Argentina, eastern Paraguay and southern Brazil (Burris et al., 2012).

The yerba mate is characterized as an important product in the socioeconomic cultural context in their original regions (Sansberro et al., 2001). It is mainly consumed as "chimarrão", a hot beverage prepared by infusion, and used in countries like Brazil, Argentina, Paraguay and Uruguay. There are other products in the market made from the yerba-mate, as blended teas, flavored tea, iced tea, and cosmetics that use *I. paraguariensis* extracts (Mosele, 2002).

Some investigations are expanding the use of *I. paraguariensis* in new products, exploring its probiotic

properties (Preci et al., 2011; Ril, et al., 2011). Studies of physico-chemical characteristics and oxidation of the leaves have been performed (Molin et al., 2011; Dartora et al., 2011), usually by a similar process used in the obtaining of black tea from *Camellia sinensis* leaves. Studies about yerba-mate properties allied to its use as teas is a challenge, considering that the tea is the most consumed beverage in the world, after the water (Namita et al., 2012). As *Camellia sinensis*, yerba-mate also has different biological effects.

Currently, the demand for food containing biologically active substances has increased, considering that consumers are seeking these products for a healthier life (Melo and Guerra, 2002). The yerba-mate fits in this context, due to the numerous benefits to health it provides, such as hypocholesterolemic and hepatoprotective activity (Filip and Ferraro, 2003; Açari et al., 2011); central nervous system stimulation; diuretic action (Castaldelli et al., 2011), inhibition of neoplastic cells proliferation (Mejía et al., 2010); and antioxidant activity, preventing damages caused by free radicals (Bastos et al., 2007). Studies showed that among ten plant species used as teas, the yerba-mate was one that showed the highest antioxidant capacity (Asolini et al., 2006).

Evaluation of antioxidant activity in laboratory animals is generally difficult to perform, requiring a large number of animals to ensure statistically significant results. The tests with microorganisms are easy, fast and can be used a large number of cells with the same genetic characteristics (Soares et al., 2005). Several reasons turn the yeast *S. cerevisiae* one of the best models of unicellular eukaryotic system to study oxidative stress. Its metabolism is similar to that of higher eukaryotes, that have their own mechanisms of metabolic activation, which are absent in bacteria. Furthermore, assays with eukaryotic cells permit evaluation of the antioxidant activity of several compounds in a fast, economic and reproducible way (Henriques et al., 2001; Guarienti et al., 2010).

In this context, the present study aimed to evaluate the antioxidant potential of the *I. paraguariensis* St. Hil extracts, processed as tea and oxidized, in *S. cerevisiae* cells deficient in antioxidant defense genes. Yerba-mate consumption is very common in the South American countries, thus the results can serve as stimulus to the development of new products, as the black tea of *I. paraguariensis*. In socioeconomic context, this species aggregates value in the regions and countries where the cultivation and processing occurs (Canterle, 2005; Heck and Mejia, 2007; Cardozo et al., 2007).

## 2. Material and Methods

### *2.1. Preparation of leave samples*

The leaves used in this study were obtained from a homogeneous culture of production under full sun, located in Barão de Cotegipe, RS, Brazil, (27º 37' 15" S and 52º 22' 47" W), at 765 m altitude.

Mature yerba-mate leaves were collected in the afternoon, packed in cotton bags and taken to the laboratory for processing. The leaves were partitioned into two lots. The lot 1 consisted of the processing type mate (TM), where the leaves were subjected to roasting at 180 °C for 5 minutes at 20 rpm, in a laboratory prototype roaster, as described in literature (Valduga et al., 2003) and drying in an oven with air circulation at 70 °C, for approximately 12 h (moisture less than 3%). The lot 2 consisted of the fraction of leaves that was submitted to the oxidation process (OX). The leaves were placed in an oven at 30 °C for removal of approximately 10% of moisture and submitted to the "rolling" (Heck and Mejia, 2007). Then, the leaves were placed in trays and kept in a climatic chamber at 80% relative humidity and 28 °C for 3 h. Subsequently, the leaves were dried at 70 °C, reaching a moisture content below 3%. Both lots were triturated in a knife mill and the particle size was classified within 16 mesh (2.36 mm). Particles larger than 2.36 mm were discarded.

### *2.2. Preparation of extracts*

About 200 g of each sample (yerba-mate TM and yerba-mate OX) were submitted to aqueous extraction (1.000 mL) in reflux, for 2 hours, with 3 repetitions. After extraction, the material was filtered and the extracts was concentrated in a rotary evaporator and lyophilized. In the next step, 10 g of each extract powder was solubilized in a solution of distilled water and ethanol (3:1, v/v). Each sample was stored at –20 °C for 6 hours, for precipitation of insoluble compounds. The samples were centrifuged (8.500 rpm, 20 min., 4 °C), the supernatant was concentrated and lyophilized (Dartora et al., 2011).

### *2.3. Evaluation of cytotoxic and antioxidant potential of I. paraguariensis St. Hil in vivo*

The *S. cerevisiae* strains used in this study were obtained from Biophysical Department of Federal University of Rio Grande do Sul, and are described in Table 1. The *ctt1*Δ is deficient for the gene that encodes the cytosolic catalase (EC 1.11.1.6), while the double mutant *ctt1*Δ*sod1*Δ is deficient in the genes that encodes catalase and cytosolic superoxide dismutase (EC 1.15.1.1), respectively.

The strains were pre-inoculated from an isolated colony in liquid medium YEL (1% yeast extract, 2% peptone and 2% glucose). The cells ($1 \cdot 10^7 mL^{-1}$) were exposed to concentrations of 150, 300, 600 and 1.200 $\mu g \cdot mL^{-1}$ of each extract (yerba-mate OX and yerba-mate TM). Then, the cells were incubated in phosphate buffer saline PBS (3.2 mM $Na_2HPO_4$, 0.5 mM $KH_2PO_4$, 1.3 mM KCl, 135 mM NaCl, pH 7.4) during 90 minutes, 30 °C and 150 rpm. Aliquots were diluted, plated on solid YEPD (1% yeast extract,

**Table 1.** Strain of *S. cerevisiae* used in this study.

| Strains | Genotype |
|---|---|
| EG103 (Wild) | MATα, *leu 2-3*, *112his3-*Δ*l*, *trp1-289, ura3-52* |
| EG223 (*ctt1*Δ) | Idem EG103, except for ctt1::TRP1 |
| EG213 (*sod1*Δ*ctt1*Δ) | Idem EG103, except for sood1::URA3, ctt1::TRP1 |

2% peptone e 2% glucose and 2% bacteriological agar) and incubated at 30 °C for 3 days before of surviving colonies counting.

The antioxidant activity of *I. paraguariensis* St. Hil. was evaluated by treating the cells ($1 \cdot 10^7 \cdot mL^{-1}$) with PBS for 90 minutes at 30 °C, in a non-cytotoxic concentration of the extracts. Then the cells were washed and treated with $H_2O_2$ (5mM) in PBS (60 minutes, 30 °C, 150 rpm). The cells survival rate was determined by plating the cell suspension in solid YEPD medium, following incubation at 30 °C during 3 days. All the tests were repeated at least three times.

### *2.4. Statistical analysis*

The survival means were analyzed by one-way analysis of variance (ANOVA), following by *Tukey's* test, using the software GraphPad Prism 6.0.

## 3. Results and Discussion

### *3.1. Effect of yerba-mate extracts on the survival of S. cerevisiae cells*

For antioxidant properties evaluation of a plant extract in living organisms, is important that there are not adverse biological effects. In this context, previously to testing the antioxidant effect, the cytotoxic potential yerba-mate extracts (TM and OX) on the survival of *S. cerevisiae* was assessed.

The results showed that yerba-mate TM stimulates the growth in strains EG and *ctt1*Δ in almost all the concentrations tested, except for *ctt1*Δ the lowest concentration (150 $\mu g \cdot mL^{-1}$). In strain *sod1*Δ*ctt1*Δ, the stimulation of growth was observed at 150 $\mu g \cdot mL^{-1}$ and 600 $\mu g \cdot mL^{-1}$ concentrations (Figures 1a, 1b and 1c).

In the case of OX yerba-mate, a significant increase in growth was observed in strains EG and *ctt1*Δ, only at the highest concentration tested (1.200 $\mu g \cdot mL^{-1}$). This yerba-mate processing method did not influence survival rate of strain *sod1*Δ*ctt1*Δ (Figures 1d, 1e and 1f).

The results indicate that there is no loss of cell viability (cytotoxic effect) in any of the cases studied. Both processing methods, in which the yerba-mate was submitted, stimulated the growth of *S. cerevisiae* strains. However, TM yerba-mate showed greater stimulation when compared to OX yerba-mate (Figure 1).

The differences in cell viability described above can be explained by the fact that yerba-mate contains multiple nutrients that can be used by yeast. In both extracts an increase in cell growth was observed, which can be a

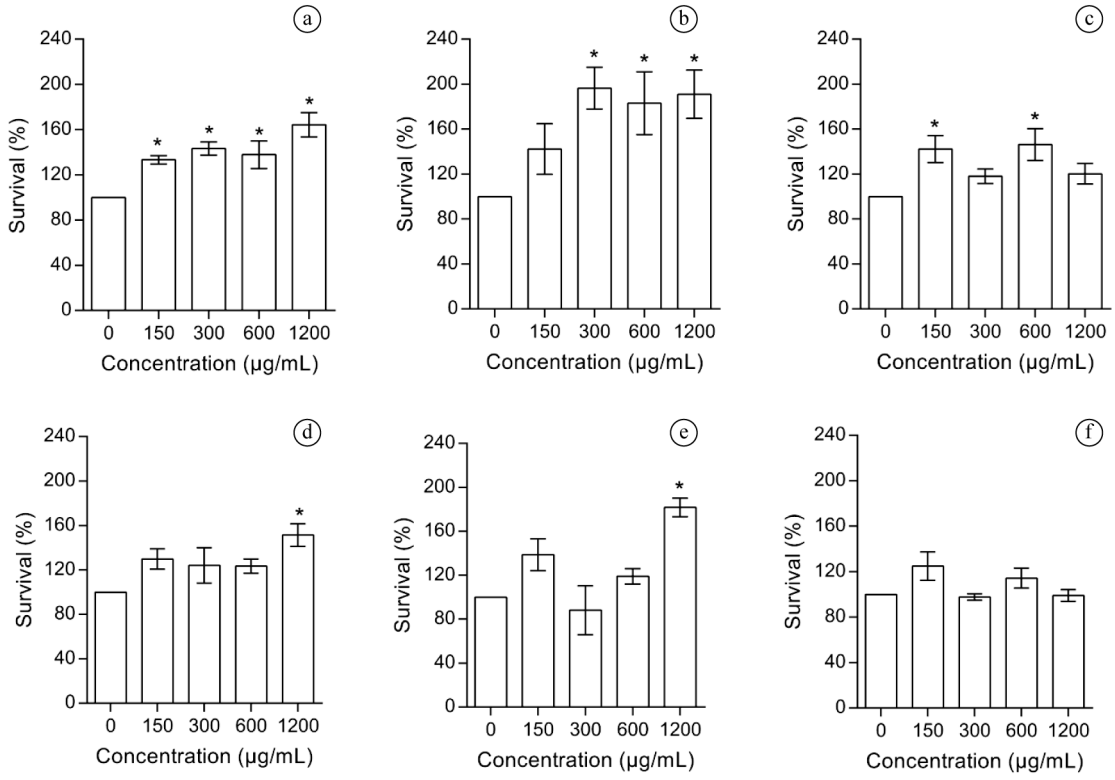


**Figure 1.** Effect of different concentrations of the yerba-mate extract on *S. cerevisae* strains. In (a) TM extract on EG strain, in (b) TM extract on *ctt1*Δ, in (c) TM extract on *sod1*Δ*ctt1*Δ, in (d) OX extract on EG strain, in (e) OX extract on *ctt1*Δ, and in (f) OX extract on *sod1*Δ*ctt1*Δ. The cells ($1 \cdot 10^7 \cdot mL^{-1}$) were treated, with different extract concentrations during 90 minutes. Aliquots were diluted and plated on YEPD médium. The plates were incubated at 30 °C during 3 days to determine the percentage survival. The data are presented with mean ± standard error. *$p<0.05$, comparing the treatments indicated with the respective control without extract.

result carbohydrates presence in the extracts, compared with the control PBS, that does not contain carbohydrates. Different mono and disaccharides were identified in both extracts (Dartora et al., 2011). Polysaccharides isolated from yerba-mate leaves showed activity against murine sepsis (Dartora et al., 2013) and gastric lesion induced by ethanol (Maria-Ferreira et al., 2013). Studies indicate that there are other metabolites present in yerba-mate as methylxanthines, flavonoids, phenolic acids, flavonoids, vitamins, minerals among others (Dartora et al., 2011; Heck and Mejia, 2007).

### *3.2. Antioxidant activity of I. paraguariensis St. Hil in vivo*

For investigating the possible antioxidant effect of the yerba-mate extracts, experiments with *S. cerevisiae* were performed. Wild (EG) and mutants (*ctt1*Δ and *sod1*Δ*ctt1*Δ) strains were tested for antioxidant defenses. The strains were pre-incubated in different concentrations (150 and 600 µg · $mL^{-1}$) of yerba-mate TM and OX extracts, and then exposed to $H_2O_2$ 5 mM.

The results showed that the extract TM has an antioxidant effect significant on strains EG e *ctt1*Δ, at the two concentrations tested (Figures 2a and 2b). For OX extracts, only 150 µg·$mL^{-1}$in *wild* strain and the highest concentration evaluated (600 µg·$mL^{-1}$) in *ctt1*Δ improved the survival rate is cells exposure to $H_2O_2$ (Figures 2d and 2e). However, the mutant *sod1*Δ*ctt1*Δ did not respond to any of the extracts (TM and OX) (Figures 2c and 2f).

Values of $IC_{50}$ very near of reported in this work for *in vivo* antioxidant activity were found for yerba-mate TM and OX antioxidant activity *in vitro*. For yerba-mate TM *in vitro* values ranged 158-254µg·$mL^{-1}$, for yerba-mate OX *in vitro* values ranged from 370-763 µg·$mL^{-1}$ (Dartora et al., 2011), while *in vivo* values reported here ranged from 150-600 µg·$mL^{-1}$. These data shows that yerba-mate TM has a higher antioxidant capacity than yerba-mate OX, *in vitro* and *in vivo*.

The results can be related with differences in the phenolic compounds concentration in each extract. A recent study in fresh yerba-mate leaves, processed as tea (TM), and oxidized (OX) quantified the concentration of these compounds. The OX leaves showed the lower concentration of phenolic compounds, than processed TM and fresh leaves. This decrease on phenolic compounds can reduce antioxidant activity in the oxidized leaves (Dartora et al., 2011). Indeed, the relationship between phenolic content and antioxidant activity is widely described in literature for different plants (Nunes, 2007; Heck and Mejia, 2007; Bastos et al., 2007).

Other studies showed the antioxidant activity of yerba-mate TM. Filip et al., (2000) evaluated the ability of yerba-mate TM extracts in inhibiting lipid oxidation in synthetic

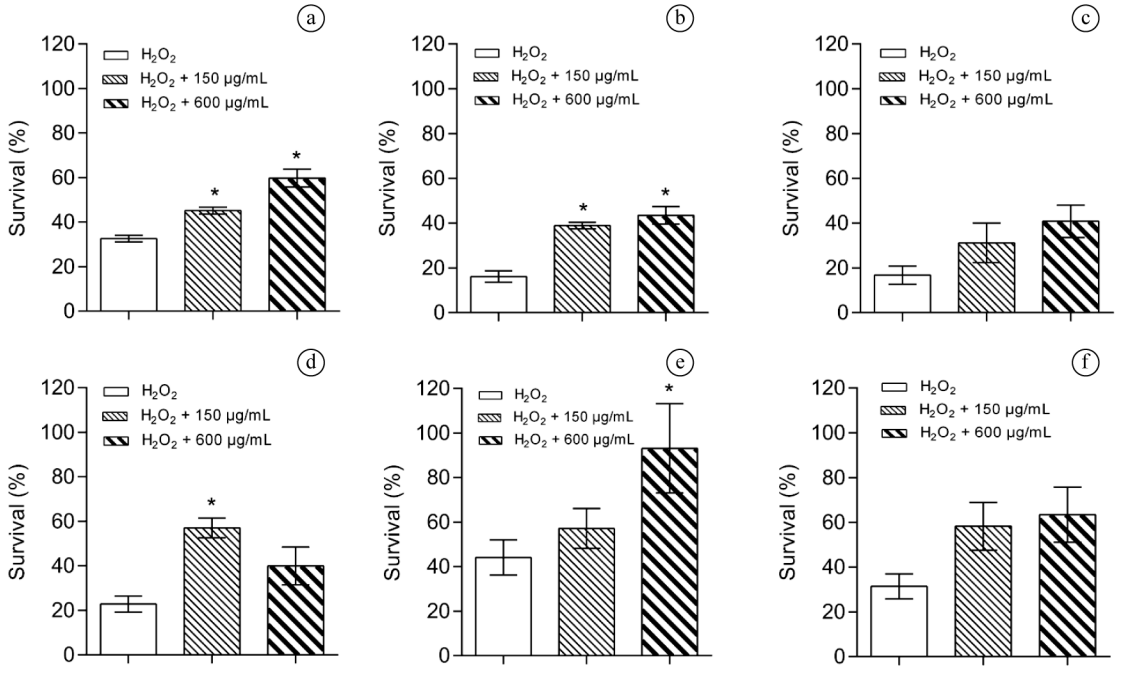


**Figure 2.** Antioxidant activity of TM and OX yerba-mate extract on *S. cerevisiae* strains. In (a) TM extract on EG, in (b) TM extract on *ctt1*Δ,in (c) TM extract on *sod1*Δ*ctt1*Δ, in (d) OX extract on EG, in (e) OX extract on *ctt1* and in (f) OX extract on *sod1*Δ*ctt1*Δ. The cells ($1 \cdot 10^7 \cdot mL^{-1}$) with different concentrations of the extract were pretreated during 90 minutes. After, were washed and incubated with $H_2O_2$ (5mM). Then 60 minutes, the strains were diluted and plated on YEPD médium. The plates were incubated at 30 °C during 3 days for determining the survival. The data are presented with mean ± standard error. *$p$<0.05, comparing the treatments indicated with the respective control without extract.

membranes (liposomes) and verified the occurrence of the protective effect. Schinella, et al., (2000) investigated the antioxidant properties of aqueous extract of *I. paraguariensis* St. Hil TM in rat liver microsomes systems using free radical generators and found that the extract inhibited enzymatic and non-enzymatic, concentration dependent lipid peroxidation, with $IC_{50}$ values of 18 $\mu g \cdot mL^{-1}$ and 28 $\mu g \cdot mL^{-1}$ respectively.

Oral and topic administration of mate tea reduced the damage DNA, lipid peroxidation and protein carbonylation in rats skin tissue induced by radiation UVA and UVB (Barg et al., 2014). Mate tea ingestion produced a decreased lipid peroxidation products in hyperlipidaemic individual, the same research identified increase in serum antioxidant status level and superoxide dismutase activity after supplementation with yerba-mate (Açari et al., 2011). These data corroborate the antioxidant activity of mate TM *in vivo* demonstrated in present work.

The interesting aspect that was observed in this study, is that the *sod1*Δ*ctt1*Δ strain was the only in which the yerba-mate extracts did not have an antioxidant effect (Figure 2). It is probable that these cells have greater availability of $H_2O_2$ (by lack of catalase) and also of the radical $O_2^{\bullet -}$ (by lack of superoxide dismutase). The radical $O_2^{\bullet -}$ can react with $H_2O_2$ as inducer of oxidative stress, generating $OH^{\bullet}$, which is a radical highly harmful to living organisms (Sharmaet al., 2012).

Thus, *sod1*Δ*ctt1*Δ strains may have an increased possibility of generation of $OH^{\bullet}$ relative the capacity of radical scavenging of phenolic compounds present in yerba-mate. Probably, this effect is less pronounced in *ctt1*Δ, for this strain can accumulate $H_2O_2$, it has functional superoxide dismutase, allowing an efficient detoxification of $O_2^{\bullet -}$.

It is important to note, that there are not studies about the antioxidant activity from of oxidized leaves of yerba-mate on life organisms. The use of *S. cerevisiae* strains deficient in genes that encode antioxidant enzymes as a model is an important tool to estimate the protective effects of the extracts against free radicals in living systems.

## 4. Conclusions

None of the evaluated extracts caused loss in viability of *S. cerevisiae* strains. In some cases it was observed a moderate effect of stimulation in cell growth, which may be related with the presence of carbohydrates and other metabolites in TM and OX yerba-mate extracts. Both extracts showed some level of *in vivo* antioxidant activity. However, TM extract was more effective than the OX extract. The yerba-mate antioxidant activity seems to be related with the presence of a "minimum" detoxification system of reactive oxygen species, considering that the protective effect was completely absent in mutant cells *sod1*Δ*ctt1*Δ.

The ingestion of both extracts may probably have a beneficial effect by increasing the organism antioxidant defense system. Even in the case of OX yerba-mate, where the antioxidant effect was lower than the other extract, cytotoxic effects were not observed. This indicates that this extract has a potential to be explored in the development of new products, derived from yerba-mate.

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