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Research Paper

Brazilian propolis protects *Saccharomyces cerevisiae* cells against oxidative stress

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Abstract

Propolis is a natural product widely used for humans. Due to its complex composition, a number of applications (antimicrobial, antiinflammatory, anesthetic, cytostatic and antioxidant) have been attributed to this substance. Using *Saccharomyces cerevisiae* as a eukaryotic model we investigated the mechanisms underlying the antioxidant effect of propolis from Guarapari against oxidative stress. Submitting a wild type (BY4741) and antioxidant deficient strains (*ctt1*Δ, *sod1*Δ, *gsh1*Δ, *gtt1*Δ and *gtt2*Δ) either to 15 mM menadione or to 2 mM hydrogen peroxide during 60 min, we observed that all strains, except the mutant *sod1*Δ, acquired tolerance when previously treated with 25 μg/mL of alcoholic propolis extract. Such a treatment reduced the levels of ROS generation and of lipid peroxidation, after oxidative stress. The increase in Cu/Zn-Sod activity by propolis suggests that the protection might be acting synergistically with Cu/Zn-Sod.

Key words: propolis, antioxidant, oxidative stress, *Saccharomyces cerevisiae*.

Introduction

Aerobic organisms have to deal with the toxic effects of reactive oxygen species (ROS). These reactive species can be formed during stress conditions such as heat shock, dehydration, toxic chemicals, UV and ionizing radiation (Lushchak, 2011). Furthermore, aerobic life style is a potential source of ROS since oxygen can be partially reduced during respiration (Lushchak, 2011; Morano *et al.*, 2011). Indeed, when the generation of ROS overwhelms the cellular antioxidant components a drastic oxidative stress is generated. Oxidative stress promotes several damages to cell structures such as proteins, lipids and nucleic acids. Hence, modifications in such molecules have been strongly related to a number of diseases such as cancer, Alzheimer, Amyotrophic Lateral Sclerosis (ALS) and also to the process of aging (Hwang and Kim, 2007; Valko *et al.*, 2007; Liedhegner *et al.*, 2012) .

Cellular defense mechanisms against ROS-induced oxidative stress involve enzymatic and/or non-enzymatic factors. Enzymatic defense encompasses enzymes such as superoxide dismutases, glutathione transferases, catalase and others involved in removal, repair or detoxification of damaged intracellular components (Scandalios, 2005). On the other hand, non-enzymatic antioxidants such as ascorbic acid (Vitamin C), α-tocopherol (Vitamin E), glutathione (GSH), carotenoids, flavonoids are mainly related to the process of ROS elimination and detoxification of pernicious components damaged by ROS (Scandalios, 2005; Valko *et al.*, 2006).

In the last years, an increasing interest in producing or discovering new antioxidant molecules from "functional foods" is emerging. This term is used for foods that can provide not only basic nutritional or energetic requirements, but also additional components with physiological benefits, such as antioxidants which are involved in protection against ROS (Viuda-Martos *et al.*, 2008). Aiming at reducing diseases and also the process of aging, food industries are developing antioxidant substances and/or enriched foods with antioxidants. In this field, compounds origi-

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nated in the beehive, as honey, propolis and royal jelly have gained prominence (Gómez-Caravaca *et al.*, 2006; Bouayed and Bohn, 2010; Sforcina and Bankovab, 2011).

Propolis is a natural and non-toxic resin produced by honey bees (*Apis mellifera*). It is extensively used in folk medicine presenting several biological applications such as immunomodulatory, antitumor, antiinflammatory, antioxidant, antibacterial, antifungal, antiviral and antiparasite activities (Dobrowolski *et al.*, 1991; Marcucci *et al.*, 2001; Gómez-Caravaca *et al.*, 2006; Souza *et al.*, 2007; Bouayed and Bohn, 2010; Sforcina and Bankovab, 2011). Currently, this product has also been used by food, pharmaceutical and cosmetic industries (Viuda-Martos *et al.*, 2008). The complexity of its chemical composition is mainly due to the site where it is produced by bees. In fact, natural factors such as type of vegetation, zone of temperature and seasonality determine its composition (Gómez-Caravaca *et al.*, 2006; Viuda-Martos *et al.*, 2008; Sforcina and Bankovab, 2011). Although it is possible to find differences in propolis composition, most of the samples share common characteristics in their overall chemistry (Marcucci *et al.*, 2001; Gómez-Caravaca *et al.*, 2006; Sforcina and Bankovab, 2011). Propolis chemistry describes the existence of at least 300 different compounds; it contains 50% resin which is composed mainly by polyphenols (flavonoids, phenolic acids and their esters); 30% wax, 10% essential oils, 5% pollen and 5% other organic compounds (Marcucci *et al.*, 2001). Due to its composition, propolis could act as a promissory antioxidant substance reacting and scavenging ROS, however, the exact protective mechanism displayed by propolis is unknown.

In order to establish new insights regarding the antioxidant properties of propolis, we investigated the protective role of propolis during exposure of the yeast *Saccharomyces cerevisiae* to oxidative stress generated by H_2O_2 and menadione. Due to biochemical and molecular similarities with human cells, the yeast *S. cerevisiae* has been shown to be a powerful eukaryotic model for understanding the cellular response against stress damages (Mager and Winderickx, 2005; Khurana and Lindquist, 2010). In this work, we also report the first evidence for propolis activation of the antioxidant enzyme Cu/Zn-superoxide dismutase.

Material and Methods

Chemicals

All chemicals were purchased from Sigma (St. Louis, MO, USA). Culture media components were purchased from Becton, Dickinson and Company (Franklin Lakes, NJ, USA).

Preparation of propolis extract

Crude propolis from Guarapari was a kind gift by Prof. Monica Freimman de Souza Ramos (Faculty of Pharmacy, Federal University of Rio de Janeiro, Brazil). Guarapari is located in the coastal zone of the Brazilian state of Espirito Santo, with a tropical *Aw* climate possessing reminiscences of the original Atlantic forest. Propolis extract was prepared by static maceration of 6.0 g of the grounded crude propolis with 30 mL of absolute ethanol for one week at 28 °C. After filtration to remove the insoluble residues, the extract was kept in a freezer until use as described by Souza *et al.*, 2007 (Souza *et al.*, 2007).

Yeast strains and growth conditions

Wild type strain of *Saccharomyces cerevisiae* BY4741 (*MATa his3 leu2 met15 ura3*) and its isogenic mutants *ctt1*, *sod1*, *gsh1*, *gtt1* and *gtt2* harboring, respectively, the genes *CTT1*, *SOD1*, *GSH1*, *GTT1* and *GTT2* interrupted by the gene *KanMX4* (Euroscarf, Frankfurt, Germany) were used in this work. Stocks of yeast strains were maintained on solid 2% YPD (1% yeast extract, 2% glucose, 2% peptone, and 2% agar). In the case of mutant strains the medium also contained 0.02% of geneticine. For all experiments, cells were grown in liquid 2% YPD medium using an orbital shaker at 28 °C and 160 rpm with the ratio of flask volume/medium of 5/1.

Oxidative stress conditions

Cells (50 mg) at the first exponential phase growing on 2% YPD were directly stressed (15 mM menadione or 2 mM H_2O_2 during 1 h at 28 °C/160 rpm), or previously treated with propolis (25 μg/mL) during 1 h at 28 °C/160 rpm (Castro, *et al.*, 2007; Fernandes *et al.*, 2007; Dani *et al.*, 2008). Immediately after adaptive treatment, cells were harvested by centrifugation (5,000 rpm/ 5 min/4 °C), washed with distilled water to remove the excess of propolis in the medium and then resuspended in the original growth medium to oxidative stress.

Tolerance determination

Cell viability was analyzed by plating cells (400 μg), after appropriate dilution (1000 x), in triplicate on solidified 2% YPD medium (1% yeast extract, 2% glucose, 2% peptone, and 2% agar) (Castro, *et al.*, 2007; Dani *et al.*, 2008). The plates were incubated at 28 °C/72 h and then colonies counted. Survival, expressed as percentage, was determined before and after oxidative stress condition, using cells treated or not with propolis (25 μg/mL).

Lipid peroxidation assay

Lipid peroxidation was assayed in cells exposed directly or propolis pre-treated to oxidative stress. Cells (50 mg) cooled on ice were harvested by centrifugation (5,000 rpm/ 5 min/ 4 °C), washed twice with distilled water and resuspended with 0.5 mL of 10% trichloracetic acid (TCA) in a test tube containing 1.5 g of glass beads. Cells were disrupted by 6 cycles of 20 s agitation on a vortex mixer followed by 20 s on ice. The extracts were used to de-

tect malondialdehyde (MDA), a final product of lipid oxidation, according to Steels *et al.* (1994) (Steels *et al.*, 1994).

Detection of superoxide dismutase activity

Total protein extract was prepared from exponential cells (50 mg) treated or not with 25 μg/mL propolis according to Mannarino *et al.* (2011) (Mannarino *et al.*, 2011). Protein concentrations were determined by the method of Stickland (1951) (Stickland, 1951). Cu/Zn-superoxide dismutase activity was analyzed loading 30 μg of proteins in native 12% polyacrylamide gel electrophoresis. Electrophoresis was carried out using a Mini-Protean from Bio-Rad (Bio-Rad laboratories Inc, USA) at 25 °C, 100 mV during 2 h. After electrophoresis, the gel was immersed in a solution containing 2.5 mM nitrobluetetrazolium (NBT) and 86 μM riboflavin. The reaction (10 min.) of visible light with riboflavin was sufficient to generate superoxide radicals reducing totally NBT. The activity of Cu/Zn-Sod is responsible to inhibit NBT reduction. Image analysis was performed after capture using UVP BioImaging Systems. The increase of Cu/Zn-Sod activity was determined, as percentage (%), by the ratio of total bands area of propolis (25 μg/mL 1 h) treated and non treated cells.

Determination of intracellular oxidation

The levels of intracellular oxidation were measured using the oxidant sensitive probe, 2', 7'-dichlorofluorescein diacetate. Fluorescence was measured using a Cary Eclipse spectrofluorimeter at an excitation wavelength of 504 nm and an emission wavelength of 524 nm. As a control, fluorescence was recorded over 1 h at 40 °C without any cells and with cells previously killed (data not shown). A fresh 5 mM stock solution of probe dissolved in ethanol was added to the cell culture (to a final concentration of 10 μM) and incubated at 28 °C to allow uptake of the probe. After 15 min, half of the culture was directly exposed to menadione (15 mM) while the other part was treated with propolis (25 μg/mL 1 h) and, thereafter, stressed with menadione. Cell extracts were prepared as previously described by Pereira *et al.* (2001) (Pereira *et al.*, 2001). The results were expressed as a ratio between fluorescence of H_2DCF in stressed and non-stressed cells during oxidative stress.

Data analysis

The results represent the mean ± standard deviation of at least three independent experiments. Statistical differences were tested using t-Test. The latter denotes homogeneity between experimental groups at $p < 0.05$. Different letters mean statistically different results. For lipid peroxidation assay we compared the homogeneity between stressed and non stressed cells of each strain at $p < 0.05$.

Results and Discussion

Propolis protects *S. cerevisiae* cells against superoxide stress

Propolis is recognized as an important pharmacologic substance. Among propolis properties, its antimicrobial action is the most studied and important (Boukraâ and Sulaiman, 2009; Sforcina and Bankovab, 2011). Firstly, we investigated if propolis treatment would kill *S. cerevisiae* cells. Thus, we directly exposed the wild type cells to propolis. Tolerance against propolis was measured after 1 h exposition. According to our results, treatment with propolis in the range of 25-100 μg/mL was not toxic for the wild type strain BY4741. Cells continued to reach 100% tolerance (Figure 1). Since cells were not affected by low doses of propolis we decided to study its protective role against oxidative stress. The antioxidant property of propolis was analyzed exposing *S. cerevisiae* cells, treated or not with propolis (25 μg/mL), to menadione (20 mM) or H_2O_2 (2 mM). Although menadione and H_2O_2 share similarities concerning genetic reprogramming, their mechanism of action and the stress factors involved in primary defense against these agents are quite distinct (Fernandes *et al.*, 2007).

Menadione is a naphtoquinone used as an oxidative stress generator displaying strong ability to produce $O_2^{\bullet-}$ once inside the cells (Mauzeroll and Bard, 2004). In addition, as a mechanism of menadione elimination, a complex with glutathione (GSH) can also be formed through Gtt2 activity (Mauzeroll and Bard, 2004; Castro *et al.*, 2007). Here, as we can see in Figure 2, cells deficient in the glutathione transferase Gtt1 (*gtt1*Δ) showed the same tolerance profile presented by the wild type. On the other hand, the Gtt2 deficient strain (*gtt2*Δ) was drastically affected by menadione stress. In spite of being hypersensitive to a di-

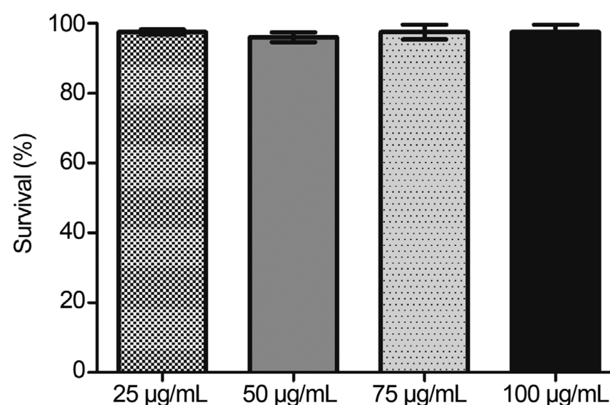

Figure 1 - Survival of *S. cerevisiae* cells exposed to increasing propolis concentrations. Exponential cells of the wild type BY4741 were directly exposed to propolis. After 1 h, cells were plated in triplicate on solidified 2% YPD medium. The plates were incubated at 28 °C/72 h and then colonies counted. The results expressing percentage of survival in relation to non-stressed cells were obtained from the average ± standard deviation of three independent experiments.

rect exposure to menadione stress, this strain acquired tolerance after propolis treatment. In this scenario we are led to suggest that propolis administration is sufficient to overcome Gtt2 deficiency (Figure 2). GSH, γ-L-glutamyl-L-cysteinyl-glycine, is the main and multifunctional antioxidant encountered in all living cells (Hayes *et al.*, 2005; Forman *et al.*, 2009; Pallardó *et al.*, 2009). In order to test whether the antioxidant potential of propolis could replace GSH, we decided to use a mutant of *S. cerevisiae* deficient in GSH synthesis (*gsh1*Δ). This strain presents a disruption in *GSH1*, which encodes the enzyme gamma glutamylcysteine synthetase involved in the first step of GSH synthesis. Despite the fact that cells were very sensitive to menadione stress, propolis treatment strongly increased survival of the *gsh1* mutant (Figure 2). However, protection exhibited by 25 μg/mL propolis was not sufficient for cells to reach 100% survival (Figure 2). Indeed, full protection against menadione in strain *gsh1*Δ, was achieved after 50 μg/mL propolis treatment (data not shown). These results demonstrate that besides being very important for cellular protection against menadione stress, the deficiency in GSH is bypassed by propolis treatment, presumably due to components with antioxidant properties in the propolis extract.

Superoxide dismutases (Sods) are very important metallo-enzymes involved in cellular protection against superoxide ($O_2^{\bullet-}$) toxicity. Among Sods, Cu/Zn-Sod is designed as the first line of defense against $O_2^{\bullet-}$ toxicity and it is found in many of eukaryotic organelles (Bonatto, 2007; Abreu and Cabelli, 2010). Regarding to Mn-Sod function, located in mitochondria, it appears to be restricted to protect cells against $O_2^{\bullet-}$ radicals produced as by-products of respiration and/or other processes inside of mitochondria (Fridovich, 1995). Furthermore as already described yeast strains are highly damaged when *SOD1* mutations are present (Wallace *et al.*, 2005). Thus, in this work using a mutant strain of *S. cerevisiae* defective in Cu/Zn-Sod (*sod1*Δ) biosynthesis, we investigated whether propolis would still be able to protect *sod1*Δ cells after menadione stress. Menadione is a redox cycling agent reacting with cytoplasmic components generating $O_2^{\bullet-}$ radicals. Therefore the Cu/Zn-Sod must be essential in yeast response against this stress. As expected, the *sod1*Δ mutant strain was hypersensitive after menadione stress (Figure 3). Propolis treatment conferred small protection to the *sod1*Δ mutant, not sufficient to recover the hypersensitive phenotype of cells (Figure 3). Different from the data obtained with the *gsh1*Δ mutant, the increase of propolis concentration (50 μg/mL) did not improve *sod1*Δ tolerance (Figure 3). Taken together, our results with *sod1*Δ, suggest that propolis might be acting in synergy with Cu/Zn-Sod or, perhaps, activating this enzyme.

To test our hypothesis, the activity of Cu/Zn-Sod was assessed, as well as, whether propolis had the potential to activate the metal catalyzed reaction of Cu/Zn-Sod. After propolis treatment, a 63% (± 4.2) increase in Cu/Zn-Sod activity was observed in the wild type strain. That non-lethal menadione stress induces Cu/Zn-Sod activity has been previously described (Mannarino *et al.*, 2011). We used the well defined menadione treatment (0.5 mM/60 min) as a reference for the increase in Cu/Zn-Sod activity. The activation of Cu/Zn-Sod, promoted by propolis, was higher than menadione, 63% (± 4.2) vs 50% (± 6.4). We can, therefore, conclude that *sod1*Δ did not acquire tolerance, as observed by the other strains, due to the impossibility of

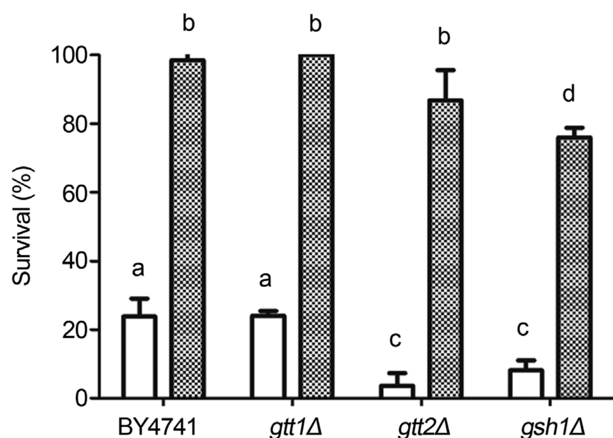

Figure 2 - Effect of propolis treatment on cellular survival against menadione. Wild type (BY4741) and mutants strains *gtt1*Δ, *gtt2*Δ and *gsh1*Δ, harvested in mid exponential phase, were stressed with 15 mM menadione/1 h. Cells were directly stressed (white bars) or previously treated with 25 μg/mL propolis during 1 h before being exposed to menadione stress (hatched bars). The results expressing percentage of survival in relation to non-stressed cells were obtained from the average ± standard deviation of three independent experiments. Different letters mean statistically different results.

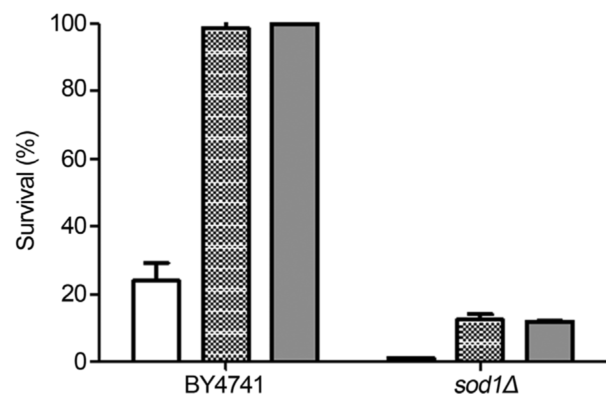

Figure 3 - Dependence of Cu/Zn-Sod for full protection after propolis treatment. Wild type and mutant strains, harvested in mid exponential phase, were stressed with 15 mM menadione/60 min. Cells were directly stressed (white bars) or previously treated with 25 μg/mL (hatched bars) or 50 μg/mL (gray bars) propolis during 1 h before being exposed to menadione. The results expressing percentage of survival in relation to non-stressed cells were obtained from the average ± standard deviation of three independent experiments. Different letters mean statistically different results.

increasing Cu/Zn-Sod activity, which in fact is absent in the *sod1*Δ mutant. Contrasting with current results, Kanbur *et al.* (2008) (Kanbur *et al.*, 2008) obtained in studies of the effect of propolis in drug protection, we did not detect any statistical differences in activity of the antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase when propolis was added to experimental groups. Here, we show the first evidence that propolis triggered the activation of Cu/Zn-Sod, one of the best characterized and most important antioxidant enzymes.

Biomarkers of oxidative stress are extremely useful in evaluating cytotoxicity. Among them, intracellular oxidation is one of the best characterized and explored biomarkers used to detect oxidative stress (Bartosz, 2006). In this work, using the fluorescent probe 2',7'-dichlorofluorescein diacetate (H_2DCF-DA) we determined the levels of intracellular oxidation during menadione stress. H_2DCF-DA is a fluorogenic probe that can permeate the cell membrane by passive diffusion and is deacetylated by cytossolic esterases. H_2DCF is more polar than the parent compound thus being trapped within the cell. Once inside the cell, it becomes susceptible to the attack by ROS, yielding a high fluorescent product (Bartosz, 2006). Recently, propolis from the Slovenian region was described as being able to reduce the levels of intracellular oxidation in cells (wild type) of *S. cerevisiae* (Tanja *et al.*, 2011). The levels of intracellular oxidation were measured only in cells at stationary growth phase without any oxidative treatment. Curiously, although propolis did not increase cell viability, the authors stated that propolis also influences cell energy metabolism and protein patterns. In our approach, we decided to measure the levels of intracellular oxidation in *S. cerevisiae* cells exposed to lethal oxidative stress conditions. Direct exposure to menadione produced an increase of H_2DCF fluorescence in the wild type strain and also in the *sod1* mutant strains (Table 1). However, after propolis treatment, a reduction of H_2DCF oxidation was observed in both strains, indicating a potent antioxidant property of propolis (Table 1). Unexpectedly, although it is easy to correlate the levels of intracellular oxidation with tolerance in the wild type, the reduction in intracellular oxidation in mutant *sod1*Δ, by the propolis treatment was not accompanied by acquisition of tolerance. This result confirms our hypothesis that propolis protected yeast cells by reducing the levels of ROS. However the activation of Cu/Zn-Sod was crucial for cellular adaptation and response to stress condition. Thus, propolis action might be related to components in propolis, which are able to activate the antioxidant enzyme Cu/Zn-Sod and also, presumably, by scavenging ROS during stress.

Table 1 - Effect of propolis treatment in reducing the levels of intracellular oxidation (ROS production) after menadione stress.

	Relative fluorescence	
Strains	Not treated	Treated
Wild type	$1.3^a \pm 0.1$	$0.8^b \pm 0.1$
*sod1*Δ	$2.2^c \pm 0.3$	$1.0^b \pm 0.2$

The Wild type and *sod1*Δ strains were directly stressed with menadione (15 mM) or previously propolis treated (25 μg/mL) during 60 min before being stressed with menadione. The results expressing relative fluorescence were obtained from the average ± standard deviation of three independent experiments. Different letters mean statistically different results.

Cytotoxicity of H_2O_2 is also alleviated by propolis treatment

Hydrogen peroxide (H_2O_2) is the most abundant reactive oxygen species *in vivo*, being continuously produced as a by-product of aerobic metabolism (Kakinuma *et al.*, 1979). Changes in gene expression by H_2O_2 and $O_2^{\bullet-}$ involve similar targets, however, we have previously described that the cellular response to both conditions is quite distinct (Fernandes *et al.*, 2007). In *Saccharomyces cerevisiae*, the response to H_2O_2 seems to be mainly related to the levels of GSH and to the activity of catalase (Ctt1) (Forman *et al.*, 2009). In order to investigate the potential of propolis in protecting yeast cells against H_2O_2 stress we decided to perform experiments using the wild type strain and mutant strains harboring deficiency in either GSH or Ctt1 synthesis. According to Figure 4, cells were drastically affected by direct exposure to H_2O_2. However, after propolis treatment, survival increased almost 3 times. No significant differences were observed between strains, suggesting that propolis compensates deficiencies in both GSH and Ctt1.

Oxidative stress generated by H_2O_2 frequently induces oxidative damages in biomolecules such as lipid, proteins and DNA (Benaroudj *et al.*, 2001; Hwang and Kim, 2007; Nery *et al.*, 2008). However, a reduction in lipid and protein oxidation is observed in cells pre-adapted and

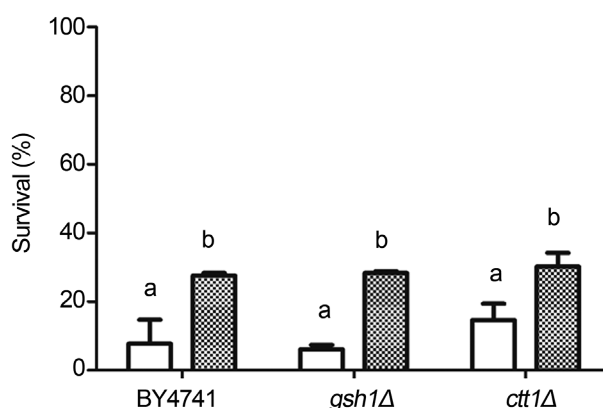

Figure 4 - Effect of propolis treatment on cellular survival after exposure to H_2O_2. Wild type and mutants strains were harvested in the mid exponential phase and stressed with 2 mM H_2O_2 / 1 h. Cells were directly stressed (white bars) or previously treated with propolis (25 μg/mL) during 60 min before being exposed to H_2O_2 stress (hatched bars). The results expressing percentage of survival in relation to non-stressed cells were obtained from the average ± standard deviation of three independent experiments. Different letters mean statistically different results.

subsequently exposed to H_2O_2 (Benaroudj *et al.*, 2001; Fernandes *et al.*, 2007; Nery *et al.*, 2008; Dani *et al.*, 2008). Here, lipid peroxidation was assessed by TBARS (Thiobarbituric Acid Reactive Substances) using exponential cells exposed or not to H_2O_2. Propolis treated cells exposed to H_2O_2 were also examined. As expected, exposure of cells to H_2O_2 increased dramatically the levels of lipid peroxidation (Table 2). In fact, high levels of lipid peroxidation are frequently associated with impairment of growth and survival of yeast cells treated with H_2O_2 (Benaroudj *et al.*, 2001; Fernandes *et al.*, 2007; Nery *et al.*, 2008; Dani *et al.*, 2008). Propolis treatment reduced lipid oxidation in all *S. cerevisiae* strains (Table 2). Despite reducing lipid peroxidation, propolis did not restore basal lipid peroxidation levels, suggesting that H_2O_2 was still exerting its toxic effect on cells (Table 2). This result is in accordance with the observed tolerance of cells which was not fully restored in cells treated with propolis. Protection of carps (Cyprinus carpio) from oxidative damages generated by chromium (VI) by propolis has been recently shown (Yonar *et al.*, 2011). After 28 days of simultaneous administration of propolis and chromium, the levels of lipid peroxidation were decreased together with the increase in activity of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase. Unfortunately, the author did not determine which isoform was involved in that activity.

The Brazilian propolis used in this study is especially rich in phenolic acids contrasting with those originating from European and other temperate regions (Bankova *et al.*, 1995; 2002). It was characterized the presence of caffeic acid, drupanin, *p*-coumaric acid, 3,4-dimethoxycinnamic acid, quercetin, pinobanksin 5-methyl ether, apigenin, kaempferol, pinobanksin, cinnamylideneacetic acid, chrysin, pinocembrin, galangin, pinobanksin 3-acetate, phenethyl caffeate, cinnamyl caffeate, tectochrysin, artepillin C (Marcucci *et al.*, 2001, Souza *et al.*, 2007). Recently, propolis and its components, caffeic and cinamic acid derivatives were shown to prevent oxidative damages in cell membranes and DNA (Benkovic *et al.*, 2008; Prasad *et al.*, 2009). Artepillin C (3,5-diprenyl-4-hydroxycinamic acid), another phenolic substance found in large concentration in Brazilian propolis inhibited lipid peroxidation in different cell models (Shimizu *et al.*, 2004). Souza *et al.* (2007), described that Brazilian propolis from Guarapari is largely composed by phenolic acids such as caffeic acid, drupanin (3-prenyl-4-hydroxycinamic acid), artepillin C and cinamic acid which might be acting as an antioxidant protecting yeast cells against H_2O_2 stress.

Table 2 - Determination of lipid peroxidation in *S. cerevisiae* cells after H_2O_2 stress.

		H_2O_2	
Strains	Non stressed	Stressed	Propolis treated
Wild type	59.6[a] ± 1.6	140.0[b] ± 6.0	111.7[c] ± 1.8
*gsh1*Δ	62.3[a] ± 3.2	183.4[d] ± 4.3	161.4[e] ± 2.7
*ctt1*Δ	46.7[a] ± 2.1	120.5[c] ± 6.5	92.2[f] ± 7.1

Lipid peroxidation was analyzed in exponential cells of the wild type and mutant strains after 2 mM H_2O_2. Non stressed, stressed (2 mM H_2O_2) and propolis treated cells were lysed by TCA 10% and extracts used to determine malondialdehyde (pmoles of MDA/mg of cell dry weight) levels. Lipid peroxidation data was obtained from the average ± standard deviation of three independent experiments. Different letters mean statistically different results.

Conclusions

Based on these results we may conclude that propolis from Guarapari (Brazil) is a promissing antioxidant product due to three main reasons: (i) it contributes to protect membrane lipids from H_2O_2 stress; (ii) in response to an $O_2^{\bullet-}$ stress mediated by menadione, propolis acts maintaining the redox status by scavenging ROS and (iii) it activates Cu/Zn-superoxide dismutase, one of the most important antioxidant enzymes.

Acknowledgments

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