Evaluation and characterization of antioxidant and anti-genotoxic properties of Portuguese propolis

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Propolis is a substance produced by bees (*Apis mellifera* L.) from harvested exudates of plant buds and barks which are subsequently mixed with the salivary enzyme β-glucosidase. Bees use propolis in their combs as protection, to repair damage, to build aseptic locals for the eggs of the queen, and also as a thermal insulator. Propolis composition varies geographically, with the available flora, the time of collection and the race of the bees. Different groups of compounds can be found in propolis such as polyphenols, terpenoids, steroids and amino acids. Some of these compounds have been associated with diverse biological activities: antimicrobial, antioxidant, antigenotoxic, genotoxic and antimutagenic.

Portuguese propolis has deserved little attention by the scientific community. Hence, it is necessary to undertake its chemical and biological characterization, in order to scientifically support the commonly assigned biological properties of this natural product and to add economic value to the resin of national origin. In this work we studied a propolis sample from Beira Alta (Côa), Portugal, to prepare a propolis ethanol extract (PEE). This PEE was used to evaluate polyphenols and flavonoids contents, antioxidant activity *in vitro*, yeast cells viability under oxidative stress, antigenotoxic/genotoxic effect on yeast cells by the comet assay and antioxidant activity *in vivo* by flow cytometry.

Propolis ethanol extract protects yeast cells from oxidative stress

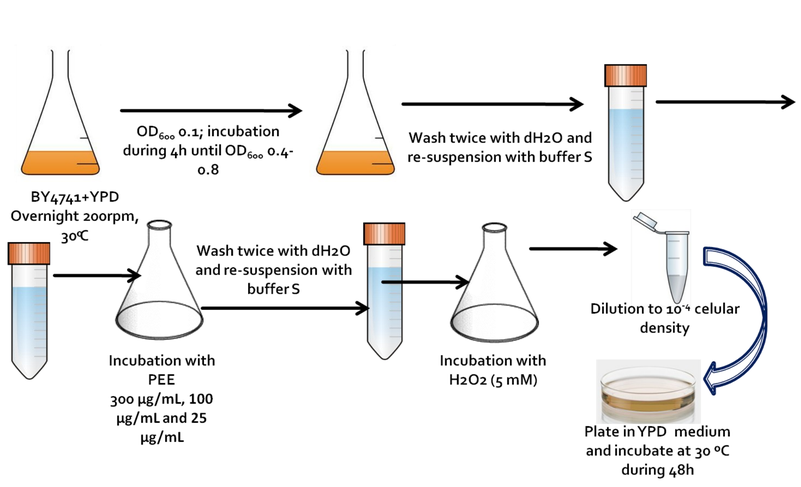

Fig. 1—Viability assay to detect the effect of PEE on the viability of *S. cerevisiae* under oxidative stress.

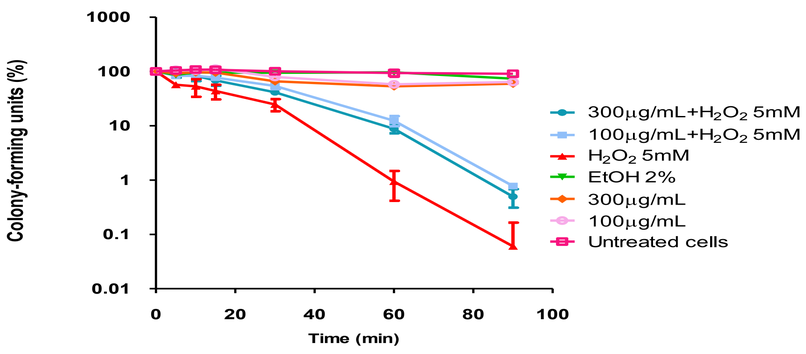

Fig. 2 — Pre-incubation of *S. cerevisiae* with PEE (before H_2O_2 treatment).

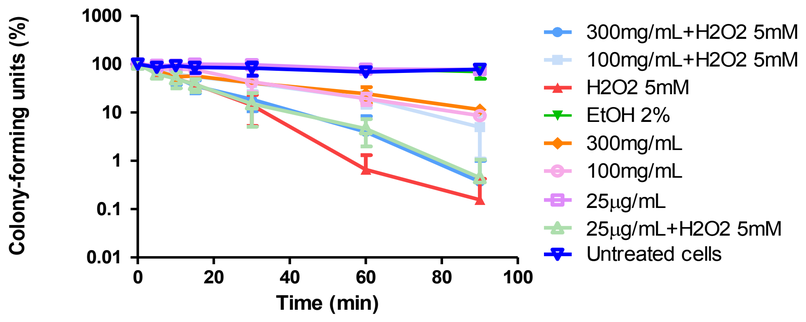

Fig. 3 — Co-incubation of *S. cerevisiae* with PEE and H_2O_2.

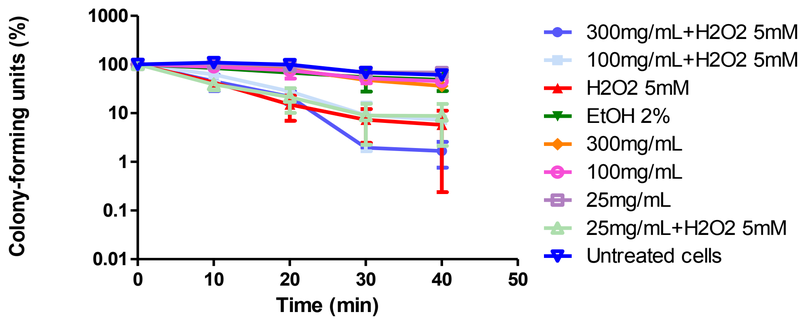

Fig. 4— Post-incubation of *S. cerevisiae* with PEE (after H_2O_2 treatment).

Antigenotoxic activity of Propolis ethanol extract on yeast cells

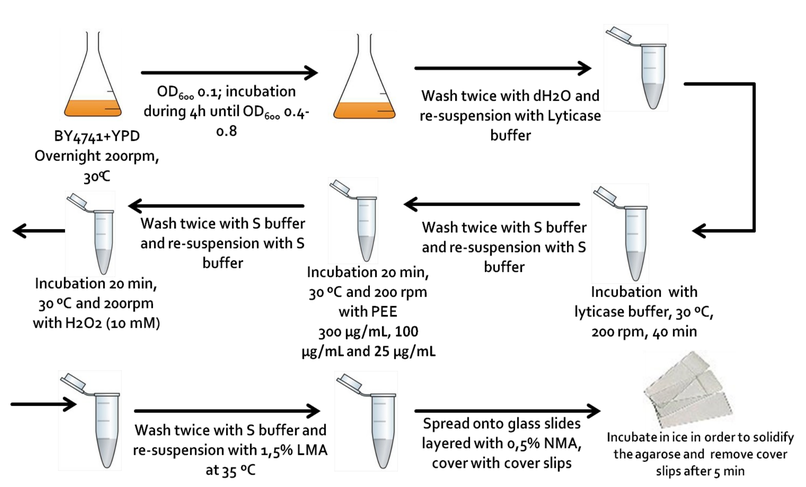

Fig. 5— Comet assay to verify the antigenotoxic effect of PEE on *S. cerevisiae* under oxidative stress. After electrophoresis in micro-gel the comets were visualized by florescence microscopy and the tail length of the comets was measured with the CometScore soft-ware.

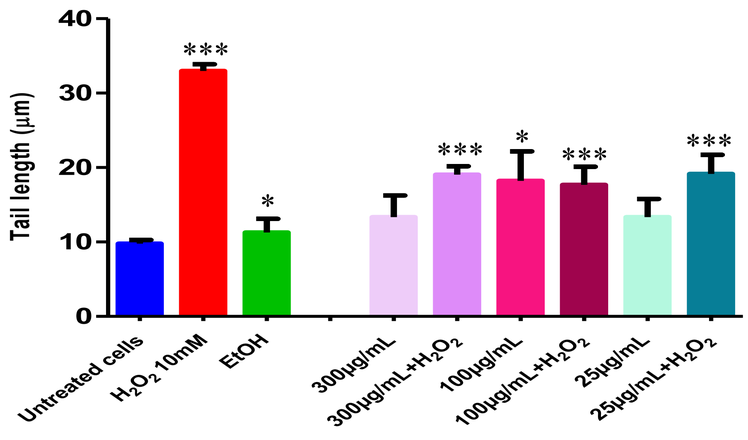

Fig. 6— Pre-incubation of *S. cerevisiae* with PEE (before H_2O_2 treatment).

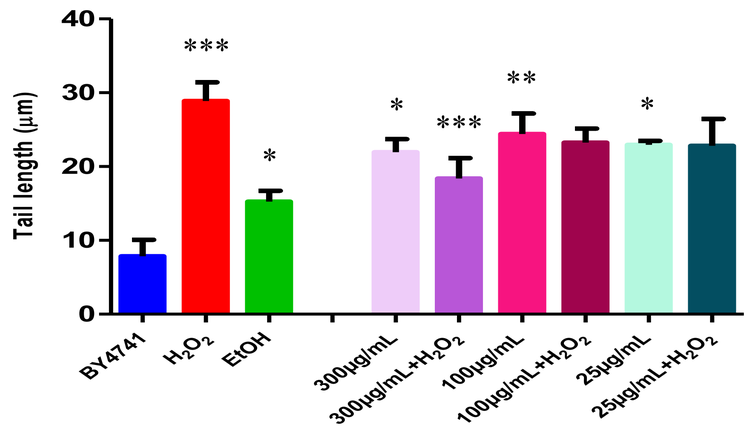

Fig. 7— Co-incubation of *S. cerevisiae* with PEE and H_2O_2.

Antioxidant activity of Propolis ethanol extract on yeast cells

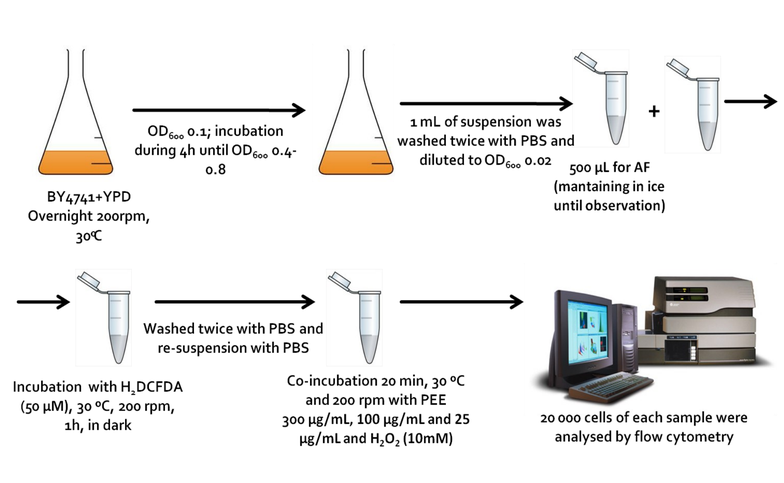

Fig. 8— Flow cytometry to detect the antioxidant activity of PEE *in vivo* using *S. cerevisiae* as biological model.

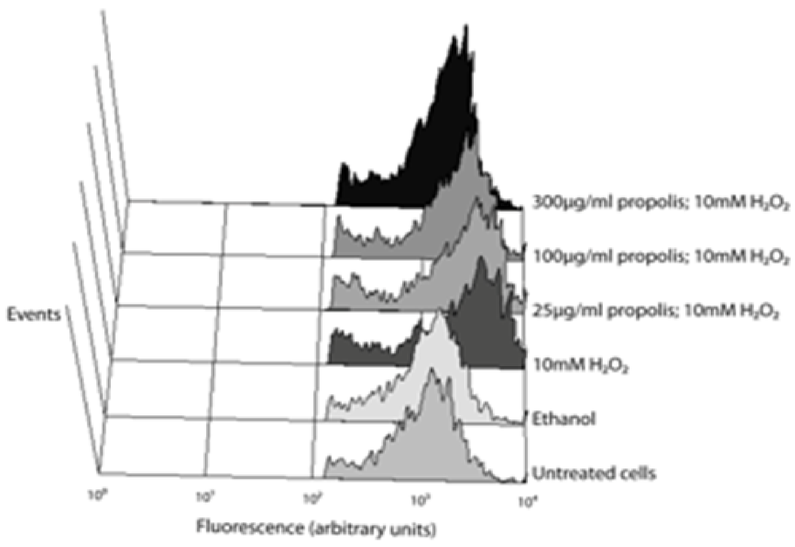

Fig. 9— Co-incubation of *S. cerevisiae* with PEE and H_2O_2, and detection of antioxidant activity of the extract.

Chemical characterization of PEE

Total Polyphenols (mg GAE/g propolis)	Flavonoids (mg QE/g propolis)	DPPH (mg GAE/g propolis)	ABTS (mg GAE/g propolis)
160,40±16,56	30,21±0,52	50,46±3,00	46,29±12,41

Table 1— Plyphenols and flavonoids content in PEE. Antioxidant activity of PEE *in vitro* by DPPH and ABTS assays. GAE—Gallic acid equivalents; QE—Quercetin equivalents

Conclusions

Our results suggest that this sample of Portuguese propolis has an antioxidant activity *in vitro* (Table 1) and *in vivo* (Fig. 9), protects yeast cells from oxidative stress promoted by H_2O_2 (Figs. 2, 3 and 4) and has antigenotoxic and genotoxic effects on yeast cells ("Janus" effect—compound with dual effect) (Figs. 6 and 7). This work can contribute to the economic valorization of the disregarded natural Portuguese propolis, but more studies are required to understand propolis effects on the different cellular pathways.

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