ANTIOXIDANT AND ANTIPROLIFERATIVE ACTIVITIES OF ETHANOLIC EXTRACT OF *ECHINODORUS GRANDIFLORUS* IN THE YEAST *SACCHAROMYCES CEREVISIAE* AND HUMAN CANCER CELL LINES

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Introduction

Echinodorus grandiflorus is popularly known as “chapéu-de-couro”. This plant is an aquatic or semi-aquatic herb with a milky sap. It has been used in the folk medicine as anti-inflammatory and diuretic. To give a scientific basis for traditional usage of this medicinal plant, the leaf extracts were evaluated for their antioxidant and antiproliferative activities.

Methods

We report the in vivo antioxidative properties of the crude ethanolic extract of *E. grandiflorus* studied by using *Saccharomyces cerevisiae* strains proficient and deficient in antioxidant defences. Furthermore, The *E. grandiflorus* cytotoxicity action was investigated on MRC5, MCF-7, HepG2, T-24, PC-3, 22Rv-1, HCT-116, HT-29, and CACO-2 cells by XTT assay, after 24, 48, 72 and 120 h of treatment. The mechanism of cell death as well as DNA damage induction was investigated by flow cytometry and comet assay, respectively. Moreover, to further understand the biological mechanism of the cytotoxic effect of E. grandiflorus, we also investigated its mutagenic effect on *XV185-14c* haploid yeast.

Results

The ethanolic extract of *E. grandiflorus* after 24 h of treatment does not induce DNA damage

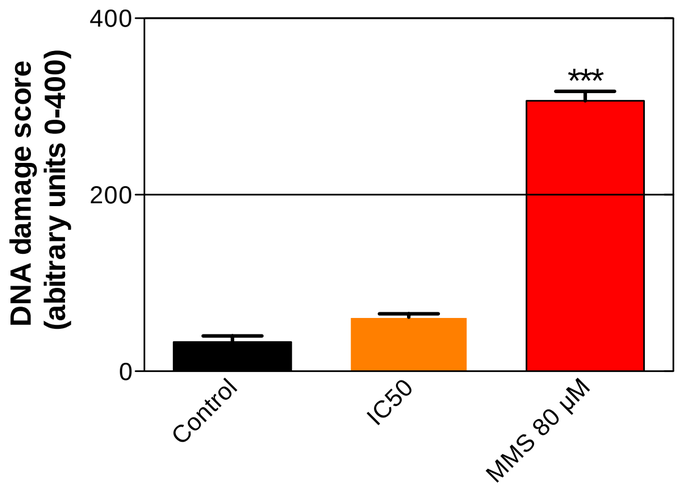

Fig. 1. Effect of ethanolic extract of *E. grandiflorus* after 24 h of treatment on DNA damage index using T24 cells, determined by comet assay. Control (untreated) or treated cells with ethanolic extract of *E. grandiflorus* at concentration IC50 was used. Data are expressed as mean ± SD of three independent experiments.

E. grandiflorus treatment induces apoptosis in T24 cells

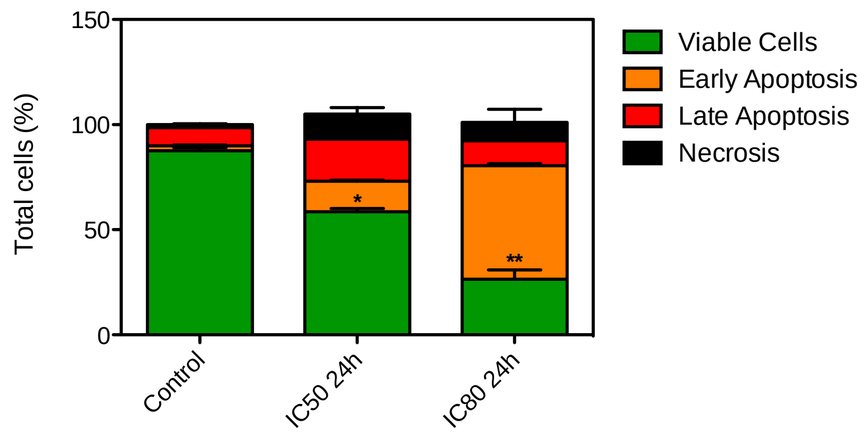

Fig. 2.. Summary of Annexin V assays analyzing in T24 cells treated with IC50 and IC80 at 24 h. The sum of the percentages of Annexin V and 7-AAD-PE-positive cells was calculated. Three independent experiments were pooled and analyzed as a combined data set. Results are expressed as means ± standard deviation (SD). Data were analyzed by one-way analysis of variance (ANOVA), and means were compared using Tukey test, with P ≤ 0.05 considered as statistically significant.

Table 1. Antiproliferative activity of ethanolic extract of *E. grandiflorus* on human cell lines.

Time of Treatment (h)	IC50 (µg/mL)[a] ± SD Selectivity index (SI)[b]/superscript value								
	MRC5	MCF-7	HepG2	T24	HCT116	HT29	CACO-2	PC-3	22Rv-1
24	66,7 ± 0,14	69,68 ± 0.28^{R}	> 100	**12,6 ± 0,05^{5X}**	> 100	> 100	> 100	60,1 ± 1,1^{1X}	55,5 ± 2,22^{1x}
48	37.60 ± 0.44	30.77 ± 0.5^{1X}	> 100	5.20 ± 0.42^{7X}	> 100	> 100	> 100	42,3 ± 2,19^{1X}	39,44 ± 3,55^{1x}
72	21.40 ± 0.20	22.63 ± 0.4^{1X}	> 100	2.99 ± 0.64^{7X}	> 100	> 100	> 100	30,11 ± 0,9^{R}	26,26 ± 2,43^{R}
120	16.84 ± 0.10	10.30 ± 0.8^{2X}	45,3 ± 0,24^{R}	0.39 ± 0.10^{43X}	> 100	> 100	> 100	19,20 ±1,19^{R}	12,22 ± 4,15^{1X}
24/MXTc	2.88 ± 1.44	0.87 ± 1.59	3.5 ± 0.39	2.50 ± 0.62	0.61 ± 0.61	2,4 ± 1,81	2.4 ± 1.81	1,66 ± 4,89	2,77 ± 5,22

[a]Drug concentration required to inhibit the cell growth by 50% after 24 h of incubation.
[b]Selectivity index (in vitro): IC50 in MRC5 cells/IC50 in tumoral cells. Data represent mean ± three separate experiments. [c]Mitoxantrone (MXT) was used as positive control.
R: more resistant in tumoral cells.

Table 2. *Saccharomyces cerevisiae* strains used in this study.

Strain	Genotype	Enzymatic defense lacking	Source
EG103 (SOD-WT)	*MATα: leu2Δ0 his 3-Δ1 trp1-289 ura 3-52*	None	E. Gralla[a]
EG118 (*sod1Δ*)	*Like EG103, except sod1::URA3*	Cu-Zn SOD (cytosolic)	E. Gralla[a]
EG110 (*sod2Δ*)	*Like EG103, except sod2::TRP1*	Mn SOD (Mitochondrial)	E. Gralla[a]
EG133 (*sod1Δsod2Δ*)	*Like EG103, except sod1::URA3 and sod2::TRP1*	Without SOD	E. Gralla[a]
XV185-14c (WT)	*MATα: ade2-2 his1-798 lys1-1 trp5-48 hom3-10 arg4-17*	None	von Borstel *et al.* (1971)[56]

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Table 3. Cytotoxicity and antioxidant effect of ethnolic extract of *E. grandiflorus* in *S. cerevisiae.*

Treatment	Yeast Strains			
	WT	*sod1Δ*	*sod2Δ*	*sod1Δsod2Δ*
NC[a]	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
***E. grandiflorus* 10 µg/mL**	98.30 ± 2.14	89.60 ± 0.95	84.90 ± 3.99	79.20 ± 1.54
***E. grandiflorus* 50 µg/mL**	92.0 ± 2.65	80.93 ± 3.66	72.83 ± 4.34	63.80 ± 2.72
***E. grandiflorus* 100 µg/mL**	83.23 ± 5.01	76.43 ± 10.64	65.0 ± 4.56	55.23 ± 1.84
***E. grandiflorus* 250 µg/mL**	74.63 ± 1.52	67.50 ± 2.12	58.63 ± 2.38	41.23 ± 5.95
PC[b]: H_2O_2 5 mM	63.03 ± 4.12	16.27 ± 4.88	19.10 ± 4.03	19.33 ± 2.10
***E. grandiflorus* 10 µg/mL + H_2O_2**	68.53 ± 1.40	**62.50 ± 7.59*** **	**36.70 ± 7.16** **	**33.20 ± 2.95***
***E. grandiflorus* 50 µg/mL + H_2O_2**	68.33 ± 7.16	**62.17 ± 7.15*** **	**31.20 ± 0.78**	**66.53 ± 5.75*** **
***E. grandiflorus* 100 µg/mL + H_2O_2**	70.80 ± 2.21	**36.87 ± 5.82*** **	**44.07 ± 4.48*** **	**75.15 ± 2.90*** **
***E. grandiflorus* 250 µg/mL + H_2O_2**	69.87 ± 4.75	**36.53 ± 3.75*** **	**27.70 ± 3.11**	**67.95 ± 2.48*** **

[a]NC: negative control (solvent-DMSO). Data significant in relation to negative control group (solvent) at *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$/ one-way ANOVA Tukey’s multiple comparison test.
[b]Positive control (H_2O_2). Data significant in relation to oxidant-treated samples at *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$/ one-way ANOVA Tukey’s multiple comparison test.

Table 4. Induction of reversion of point mutation for his1-7, ochre allele lys1-1, and *frameshift* mutation (hom3-10) in haploid strain XV185-14c of *S. cerevisiae* after treatment of crude ethanolic extract of *E. grandiflorus*.

Agent	Treatment (µg/mL)	Survival (%)	LYS1/10^8survivors[b]	HIS1/10^7survivors[a]	HOM3/10^8survivors[a]
STAT cells treated in PBS					
NC[d]	0	100.00	4.0 ± 2.83[c]	10.50 ± 0.71[c]	4.0 ± 1.42[c]
4-NQO[e]	1.0 µg/mL	39.97***	20.75 ± 4.03**	49.50 ± 9.19***	17.50 ± 1.95*
E. grandiflorus	10 µg/mL	96.70	5.10 ± 1.19	11.84 ± 1.36	3.77 ± 1.73
	50 µg/mL	91.73	6.99 ± 0.16	14.78 ± 1.59	4.91 ± 2.54
	100 µg/mL	88.15	9.39 ± 0.72	17.78 ± 1.25	3.89 ± 0.94
	250 µg/mL	80.33	11.25 ± 0.64	21.25 ± 2.05	6.11 ± 1.41

[a] Locus-specific revertants; [b] Locus non-specific revertants; [c] Mean and standard deviation per three independent experiments; [d] Positive control (solvent); [e] controle positivo;
* Data significant in relation to negative control group (solvent) at * $P<0.05$; ** $P<0.01$; *** $P<0.001$/ One-way ANOVA-Tukey´s Multiple Comparison Test.

Conclusion

Apoptosis is one of the body’s most potent defences against cancer; The apoptotic potency of *E. grandiflorus* suggests that it may be an effective compound in therapy for the treatment of bladder cancer. This work provides a scientific support for the high antioxidant and antiproliferative activity of this plant and thus it may have potential applications in the treatment of the diseases caused by ROS. Further studies are needed to confirm in vivo anti-tumorgenicity and subsequent chemical characterization of its active molecule(s).

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