



TOXICOLOGICAL EVALUATION OF

Pterospartum tridentatum flower water extract

Fernanda M. Ferreira, Susana M. Cardoso, Olívia R. Pereira, M. Rosário M. Domingues, Pedro Azedo, Lia T. Dinis and Francisco P. Peixoto

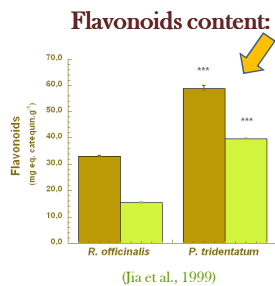
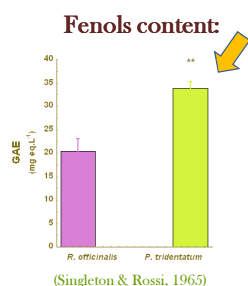
Background:

- *Pterospartum tridentatum* Willk. (prickled broom) is an autochthonous plant, common in Portuguese territory.
- The yellow flowers are widely used in traditional medicine, as a potential cure for all body illnesses, mainly for throat irritation treatment or for diabetes, hypertension and hypercholesterolemia therapy.
- Despite its wide traditional use, no toxicological assessment of this plant has been performed, as far as we know.

Goals

- ☆ chemical characterization of *P. tridentatum* flower extract
- ☆ evaluation of antioxidant activity of *P. tridentatum* extract
- ☆ assessment of potential toxicological effects of *P. tridentatum* flowers water extracts

Chemical characterization:



[M-H] ⁻	Main Fragments ESI ⁻ MS ⁻	Tentative structure	Compound
MS ⁻ [359]: 315, 223, 197, 178, 161; MS ⁻ [197]: 179, 73; MS ⁻ [223]: 205, 179; MS ⁻ [179]: 161, 135; MS ⁻ [161]: 133			Rosmarinic acid
461	MS ⁻ [461]: 446, 299, 285, 284; MS ⁻ [299]: 284		5,6-Dihydroxy-3-methoxy-isoflavanone-7-O-beta-D-glucoside
477	MS ⁻ [477]: 315, 300; MS ⁻ [315]: 299, 300; MS ⁻ [300]: 283, 272, 255, 243, 227, 216, 199		Isorhamnetin-3-O-hexoside
503	MS ⁻ [503]: 461, 443, 399, 285; MS ⁻ [285]: 267, 257, 243, 241, 217, 199, 175, 151; MS ⁻ [443]: 399, 381, 285; MS ⁻ [399]: 355, 327, 285, 263; MS ⁻ [285]: 255; MS ⁻ [255]: 227, 211, 183		Luteolin acetyl glucuronide
641	MS ⁻ [641]: 623, 479, 315/316/317, 301/302		Myricetin-O-glucuronide

Resume of major ions in the ESI-MS spectrum of the water extract of *Pterospartum tridentatum* flowers corresponding to phenolic compounds, with the indication of the main product ions observed in their MS⁻ spectra and the proposed structures. (ClcU: Glucuronide unit; Glc: Glucoside unit; Ac: Acetyl unit) (Cardoso et al., 2011)

Antioxidant activities:

Plant Extract	ABTS (TE, mmol.g ⁻¹)	β-Carotene bleaching test (% AA)	
		30 min of incubation	60 min of incubation
<i>Pterospartum tridentatum</i>	143.0 ± 0.8	101.8 ± 10.7	169.5 ± 17.2 **
<i>Rosmarinus officinalis</i>	144.8 ± 0.4	112.3 ± 12.3	111.6 ± 12.6

A high antioxidant activity of *P. tridentatum* flower water extract was assessed in good agreement with its ESI-MS spectrum that revealed the presence of several important phenolic compounds, such as rosmarinic acid, luteolin-*O*-(*O*-acetyl)-glucuronide and isorhamnetin-*O*-hexoside.

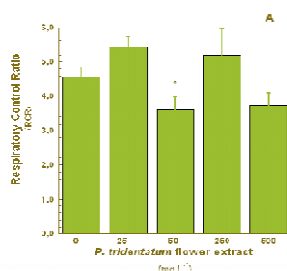
Results are presented as mean ± SD of triplicates of experiments performed with 4 plant water extracts prepared in similar conditions. Statistics: * p < 0.05 as compared to control; ** p < 0.05 as compared to *R. officinalis*. (Miller & Rice-Evans, 1997; Ismail, et al., 2004)

Toxicological evaluation:

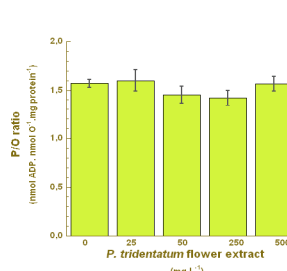
Respiratory rates

Respiratory rate (**)	State 4	State 3	FCCP-stimulated respiration
	(mmol O ₂ .mg protein ⁻¹ .min ⁻¹)		
<i>P. tridentatum</i> flower extract (mg.L ⁻¹ *)			
0	19.2 ± 1.0	73.1 ± 2.0	124.4 ± 4.4
25	11.5 ± 1.5	61.9 ± 5.2	128.0 ± 8.9
50	17.2 ± 2.3	60.6 ± 5.5 *	115.2 ± 8.6
250	12.2 ± 0.8 *	76.0 ± 4.3	142.7 ± 0.6
500	15.8 ± 1.4	60.9 ± 5.1 *	132.0 ± 5.0

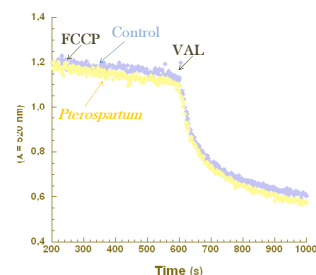
RCR



P/O



Membrane permeability



Values of respiratory rates in state 4, state 3 and FCCP stimulated respiration (respectively V₄, V₃ and FCCP) are expressed as μmol O₂.mg protein⁻¹.min⁻¹. Oxygen consumption of isolated mitochondria was determined polarographically at 30 °C with a Clark oxygen electrode, connected to a suitable recorder in a closed chamber with magnetic stirring (Estabrook, 1967). Respiratory control ratio (RCR) and P/O ratio were determined accordingly to Chance and Williams (1956). State 3 respiration was initiated by the addition of 100 μmol ADP. Values are the means ± SEM of triplicates performed with four different mitochondrial preparations. Results are presented as mean ± SEM of triplicates of experiments performed with 4 mitochondrial preparations. Statistics: * p < 0.05 as compared to control. Mitochondria (1 mg protein) were incubated in 1 mL respiratory standard medium containing succinate (5 mM) and rotenone (1 μM), for 5 min at 30 °C.

Mitochondrial respiratory rates (state 4, state 3 and FCCP-stimulated respiration) and respiratory indexes (respiratory control and P/O ratios) showed no consistent decrease of respiratory and phosphorylative efficiencies for the concentrations tested (up to 500 μg.mL⁻¹), neither affects membrane permeability.

In conclusion, for the concentration range commonly used, *P. tridentatum* flowers usage can be regarded as harmless and trustworthy. Moreover, its great antioxidant properties can be useful to counteract diabetes mellitus associated diseases.

References

- Cardoso, S.M., et al., *Oleuropein ligstrose isomers and their derivatives in Portuguese olive mill wastewaters*. Food Chemistry, 2011. 129 (2): 291-296
- Chance, B. and G.R. Williams, *The respiratory chain and oxidative phosphorylation*. Adv. Enzymol., 1956. 17: p. 65-134.
- Diogo, C.V., et al., *Mitochondrial toxicity of the phytochemicals daphnetoxin and daphnoretin - Relevance for possible anti-cancer application*. Toxicology in Vitro, 2009. 23(5): 772-779.
- Estabrook, R.E., *Mitochondrial respiratory control and the polarographic measurement of ADP:O ratios*. Methods Enzymol., 1967. 10: 41-47.
- Ismail, A., Z.M. Marjan, and C.W. Foong, *Total antioxidant activity and phenolic content in selected vegetables*. Food Chemistry, 2004. 87(4): 581-586.
- Jia, Z., M. Tang, and J. Wu, *The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals*. Food Chemistry, 1999. 64(4): 555-559.
- Miller, N.J. and C. Rice-Evans, *Factors influencing the antioxidant activity determined by the ABTS •• radical cation assay*. Free Radic. Res., 1997. 26: 195-199
- Singleton, V.L., & Rossi, J. A., Jr. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viticul. 16, 144-158.

