Toxicological evaluation of *Pterospartum tridentatum* flower water extract

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Background:

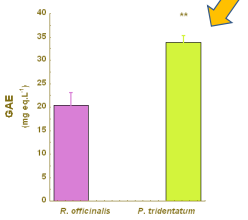
- *Pterospartum tridentatum* Willk. (prickled broom) is an autochthonous plant, common in Portuguese territory.
- The yellow flowers are widely used in traditional medicine, as a potential cure for all body illnesses, mainly for throat irritation treatment or for diabetes, hypertension and hypercholesterolemia 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

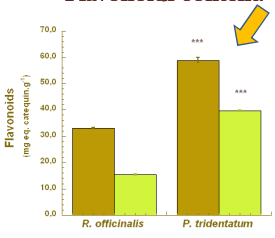
Chemical characterization:

Fenols content:

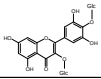


(Singleton & Rossi, 1965)

Flavonoids content:



(Jia et al., 1999)

[M-H]⁻	Main Fragments ESI⁻MSⁿ	Tentative structure	Compound
359	MS²[359]: 315, 223, 197, 178, 161; MS³[197]: 179, 73; MS³[223]: 205, 179; MS³[179]: 161, 135; MS³[161]: 133		Rosmarinic acid
461	MS²[461]: 446, 299, 285, 284; MS³[299]: 284		5,5'-Dihydroxy-3'-methoxy-isoflavone-7-*O*-ß-glucoside,
477	MS²[477]: 315, 300; MS³[315] 299, 300; MS³[300]: 283, 272, 255, 243, 227, 216, 199		Isorhamnetin-3-*O* hexoside
503	MS²[503]: 461, 443, 399, 285; MS³[285]: 267, 257, 243, 241, 217 , 199, 175, 151; MS³[443] 399, 381, 285; MS³[399]: 355, 327, 285, 283; MS³[283]: 255; MS³[255]: 227, 211, 183		Luteolin acetyl glucuronide
641	MS²[641]: 623, 479, 315/316/317, 301/302		Myricetin-*O*-diglucoside

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^n spectra and the proposed structures. (GlcU- Glucuronide unit; Glc- Glucoside unit; Ac- Acetyl unit) (Cardoso et al., 2011)

Antioxidant activities:

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

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)

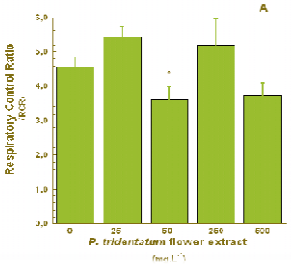
A high antioxidant activity of P. tridentatum flower water extract was assessed in good agreement with its ESI-MS spectrum that revealed the presence of several important phenolic compounds, such as rosmarinic acid, luteolin-*O*-(*O*-acetyl)-glucuronide and isorhamnetin-*O*-hexoside.

Toxicological evaluation:

Respiratory rates

Respiratory rate (➧) / *P. tridentatum* flower extract (mg.L⁻¹ ➧)	State 4 (nmol O_2. mg protein⁻¹.min⁻¹)	State 3 (nmol O_2. mg protein⁻¹.min⁻¹)	FCCP-stimulated respiration (nmol O_2. mg protein⁻¹.min⁻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

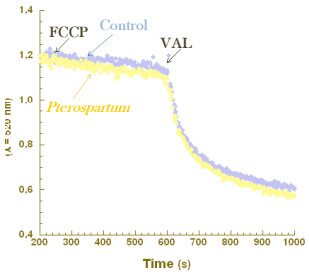
RCR



P/O

Values of respiratory rates in state 3, state 4 and FCCP stimulated respiration (respectively V_3, V_4 and FCCP) are expressed as nmol O_2.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 nmol 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 (1µM), for 5 min at 30 °C.

Membrane permeability



Effect of *P. tridentatum* flower water extract on the permeabilisation to H^+ and K^+ by the inner membrane of rat liver mitochondria, evaluated by passive osmotic swelling of mitochondria suspended in potassium acetate. Where indicated by arrows, 1 µM FCCP, *P. tridentatum* flower extract (250 and 500 mg.L^{-1}) or 1 µg.mL^{-1} valinomycin (VAL) were added (Diogo et al., 2009).

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.

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