

Toxicological evaluation and polyphenols characterization of *Pterospartum tridentatum* leaf extracts

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Background:



• *Pterospartum tridentatum* Willk. (prickled broom) is an autochthonous plant, common in Portuguese territory.



• Leaves are used in cooking, to flavour rice ("arroz de carqueja"), roast meat, game animals or as a condiment in salads.



• Despite its wide traditional use, no toxicological assessment of this plant has been performed previously.

Goals:

• Polyphenols characterization of *P. tridentatum* leaf water extract

• Evaluation of antioxidant activity of *P. tridentatum* extract

• Assessment of potential toxicological effects of *P. tridentatum* leaf water extracts.

Chemical characterization:

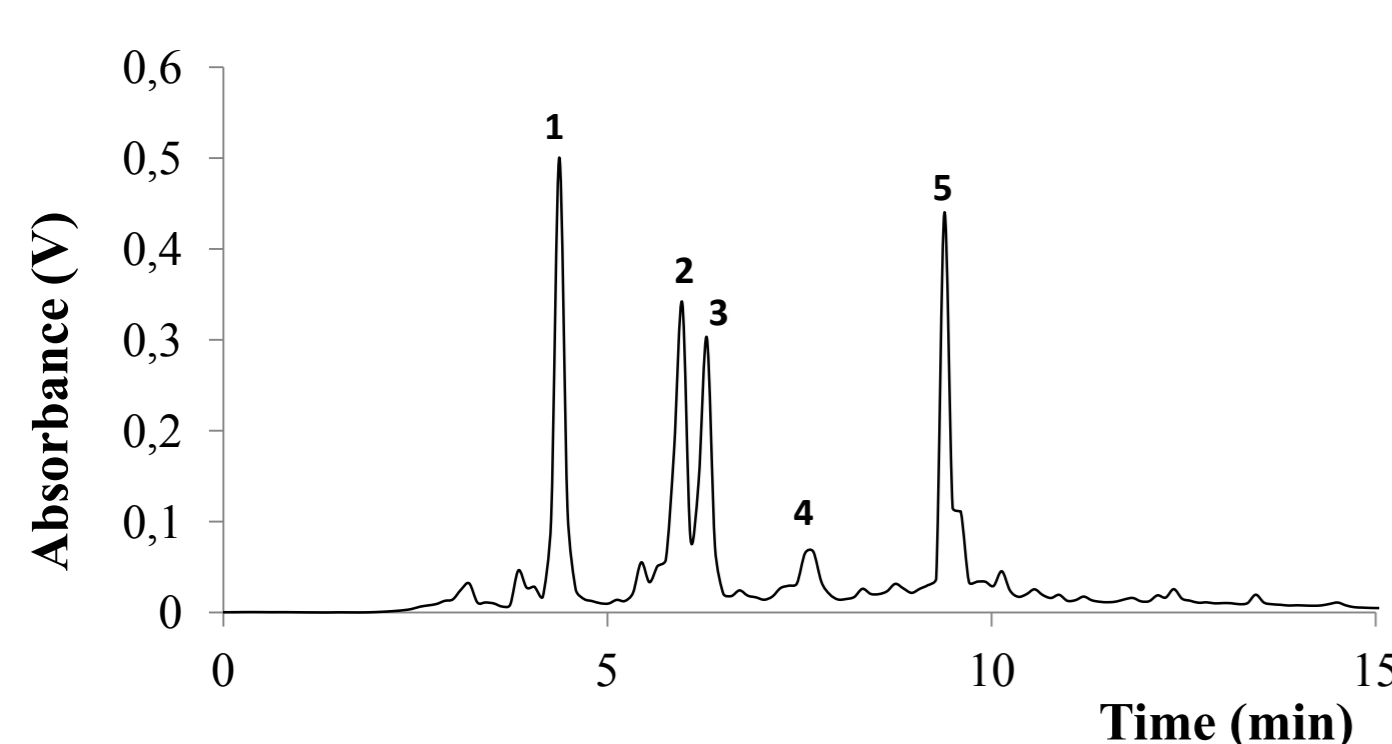


Fig. 1 – Chromatographic profile of aqueous fractions of *P. tridentatum* leaves at 280 nm. The numbers on the figure correspond to the fractions that were collected for by ESI-MS analysis.

Fraction	RT (min)	λ max (nm)	Molecular ion [M-H] ⁻ (m/z)	Main MS ² fragments (m/z) (relative %)	Tentative Identification
1	4.37	289	191	191: 173 (50), 127 (20), 111 (100), 93 (10), 85 (12)	Quinic acid
			465	465: 447 (5), 375 (12), 345 (100), 327 (2), 287 (2), 269 (1)	Dihydroquercetin-6-C-hexoside
2	6.0	292, 340	593	593: 575 (5), 503 (15), 473 (100), 431 (2), 383 (4), 353 (10), 311 (2), 283 (1)	Genistein-di-C-hexoside
			479	479: 389 (5), 359 (100)	Myricetin-6-C-hexoside
3	6.3	285, 340	479	479: 389 (5), 359 (100)	Myricetin-6-C-hexoside
			479	479: 359	Myricetin-C-hexoside isomer
4	7.6	290, 340	431	431: 341 (5), 311 (45), 269 (40), 268 (100)	Genistein-8-C-hexoside
			431	431: 341 (3), 311 (100), 283 (1)	Genistein-8-C-hexoside isomer
5	9.4	261, 332	431	431: 341 (3), 311 (100), 283 (1)	Genistein-8-C-hexoside isomer

Table 1 – Resume of major [M-H]⁻ ions observed in the ESI-MS spectrum of the aqueous extract of *P. tridentatum* leaves corresponding to phenolic compounds, with the indication of the main product ions observed in their MS² spectra.

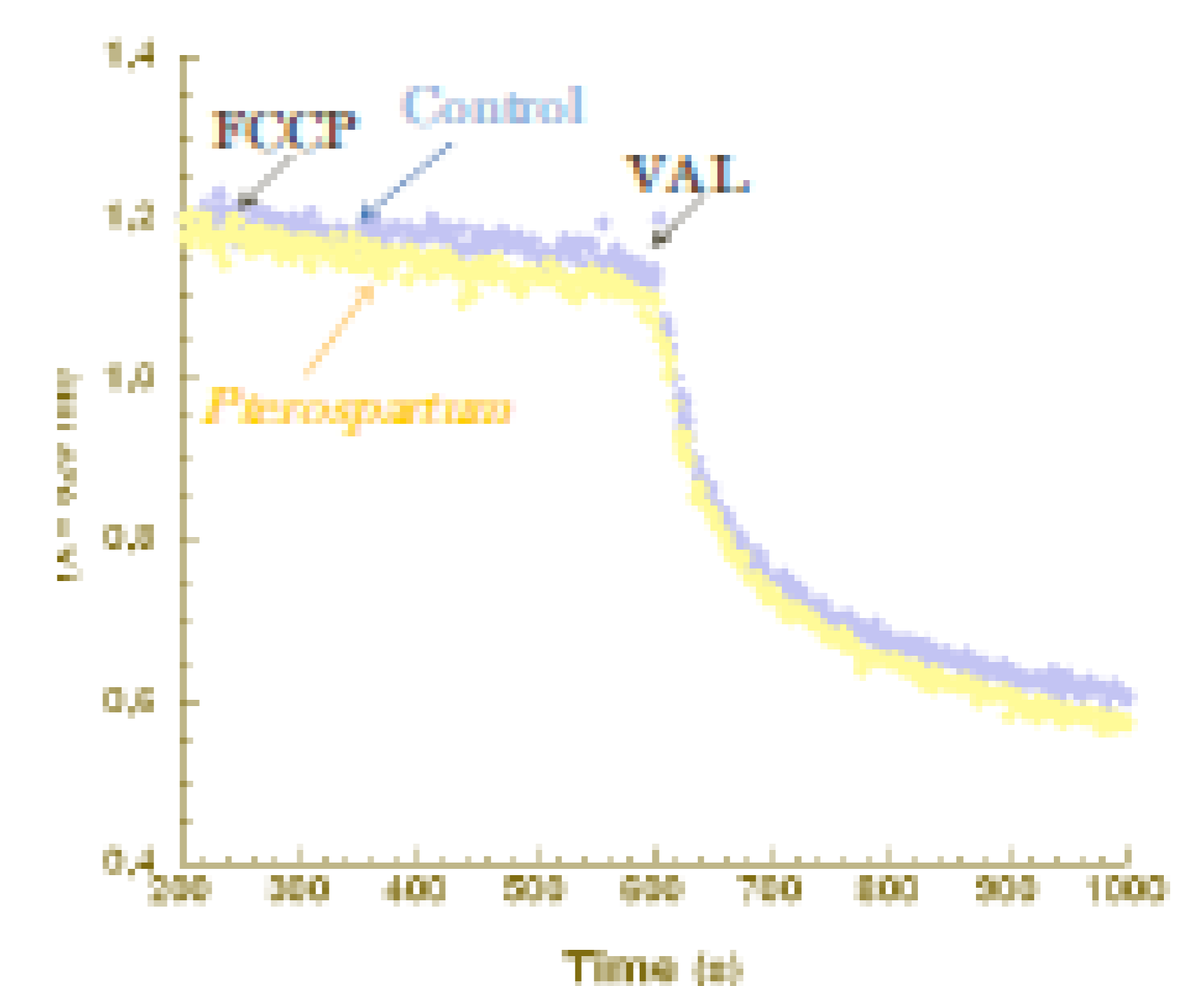
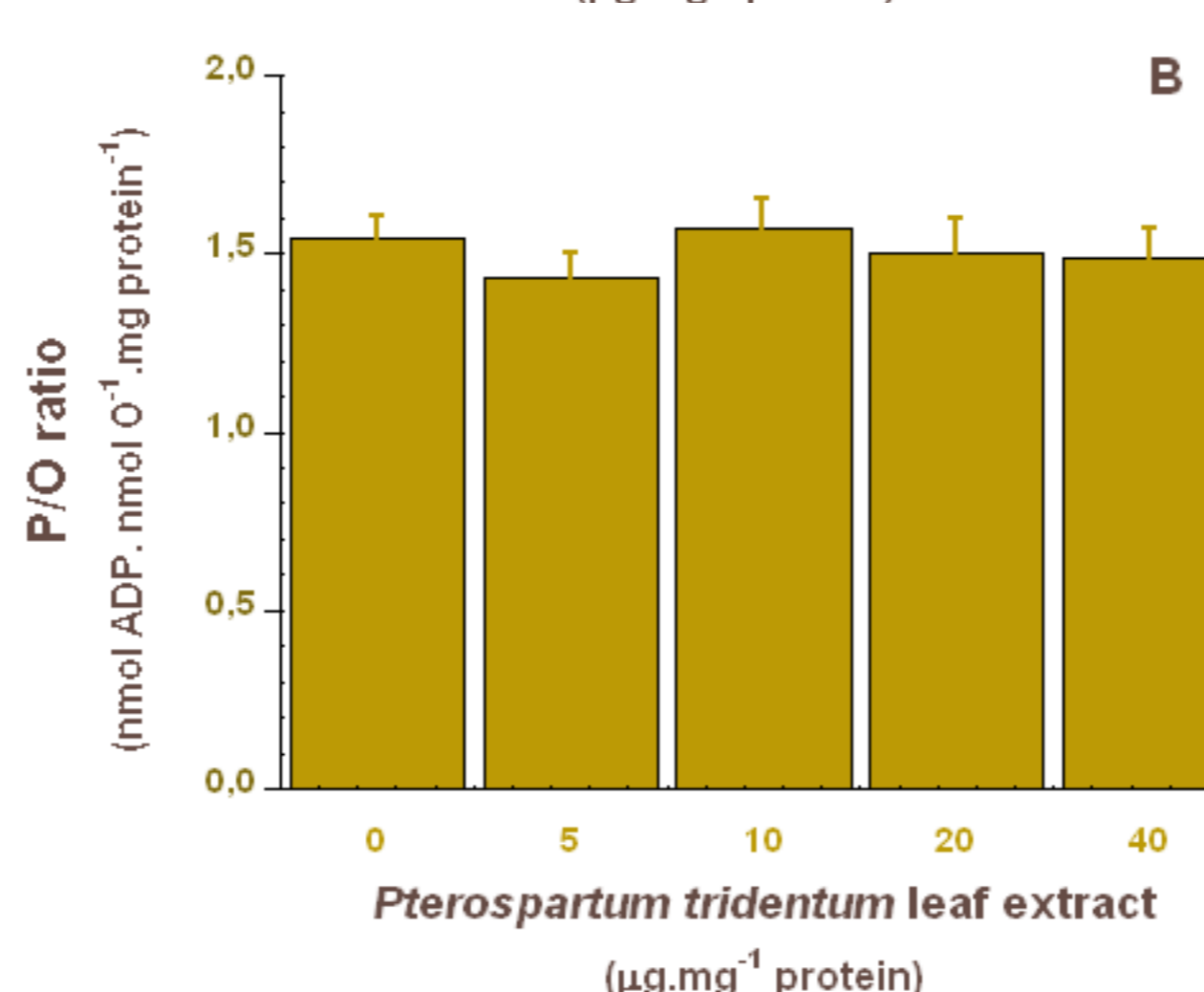
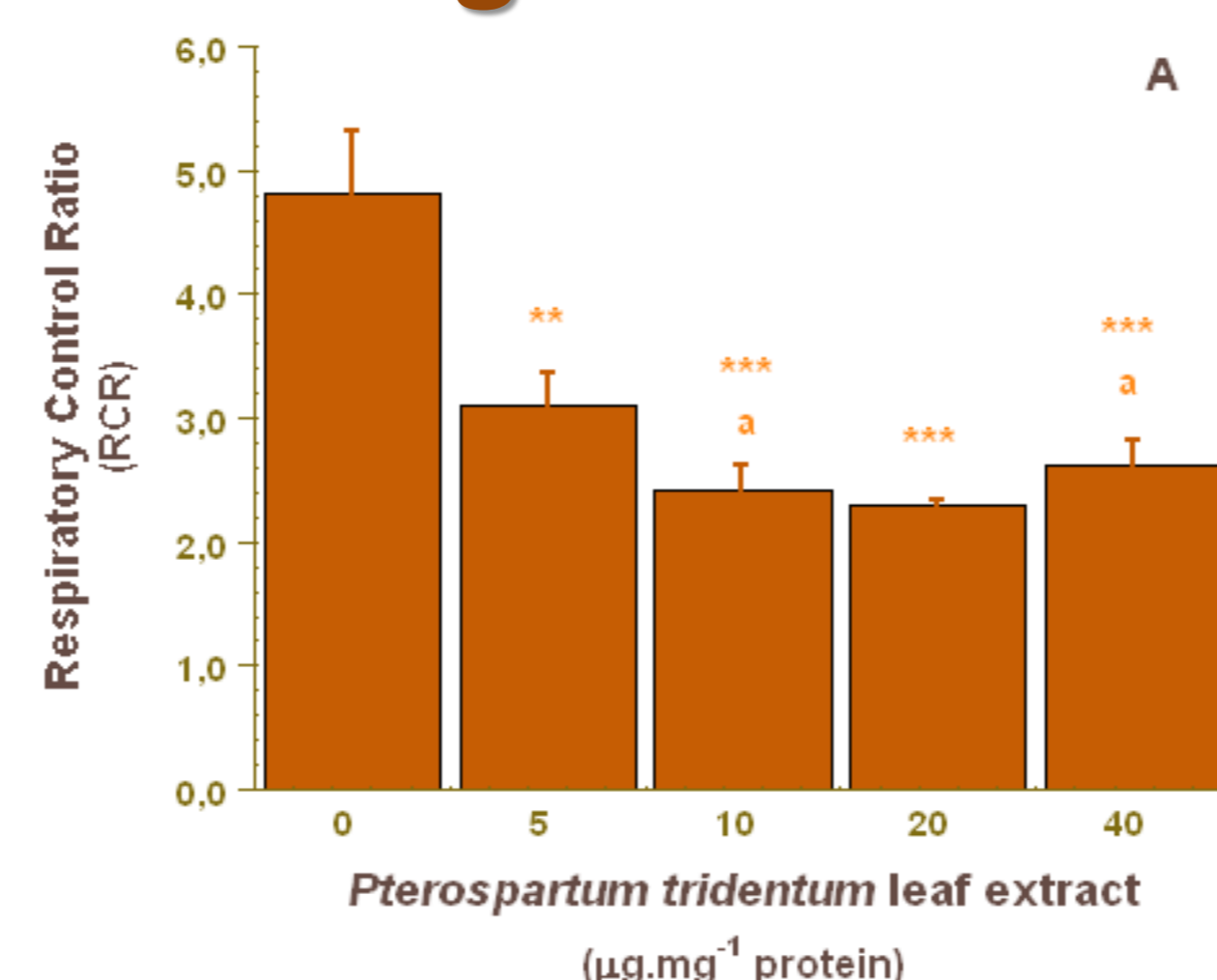
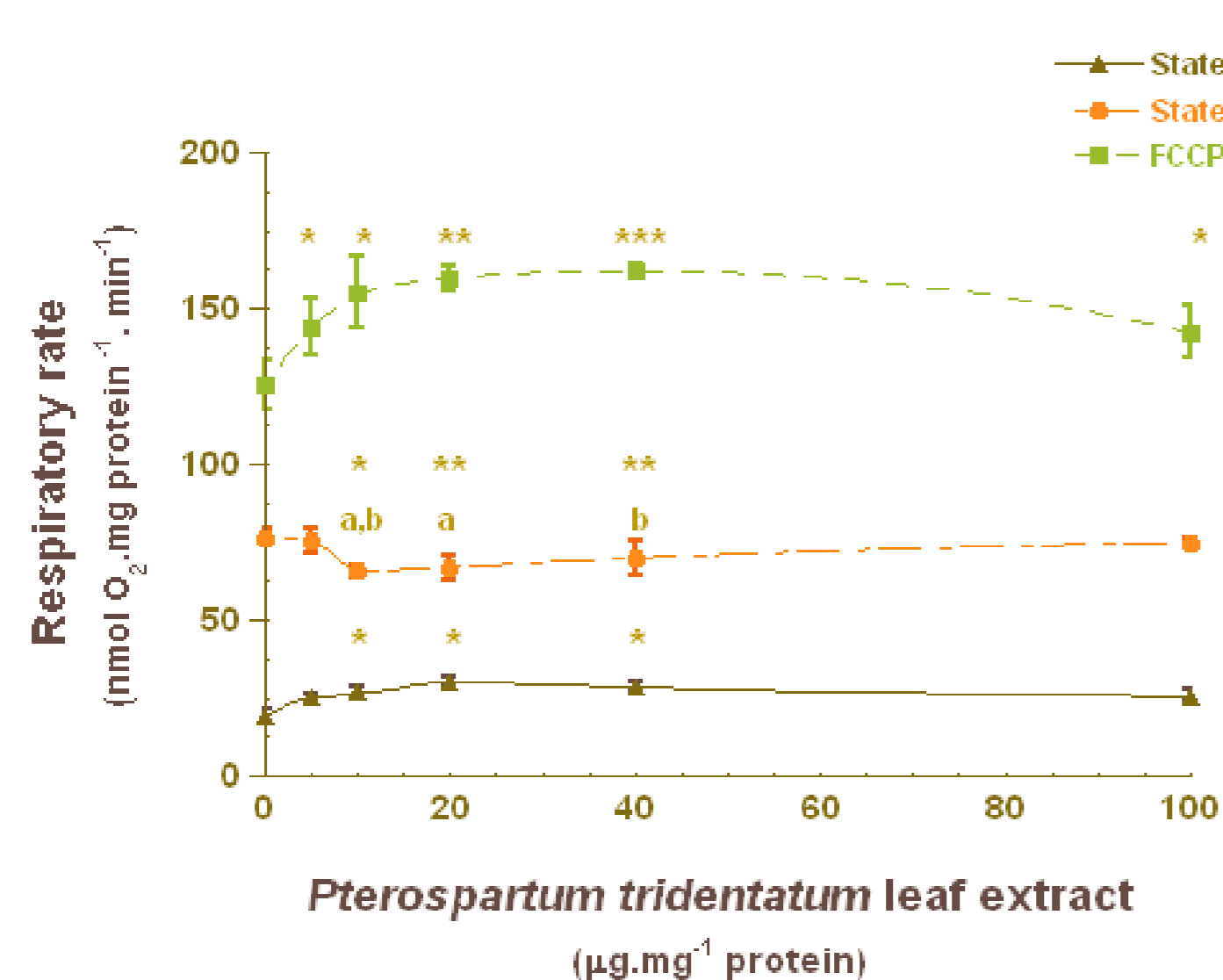
Polyphenols content:

	Flavonoids (catechin equivalents: mg Eq.g ⁻¹)	Phenols (GAE; mg Eq. g ⁻¹)
<i>R. officinalis</i>	33.03 ± 0.20	102.43 ± 0.65
<i>P. tridentatum</i>	62.64 ± 0.42 ***	175.51 ± 2.40 **

• *P. tridentatum* leaf extract showed a higher polyphenols content, than the *R. officinalis* extract (**p<0.001; ***p<0.001).

• *P. tridentatum* leaf extract phenolic compounds are mainly composed of isoflavones (genistein derivatives) and flavonols (dihydroquercetin and myricetin-C-glucosides).

Toxicological evaluation:



• The results suggest that in the range of concentrations used (up to 40 µg · mg protein⁻¹), the mitochondrial phosphorylative system is not directly inhibited by *P. tridentatum* leaves aqueous extract, as P/O ratio is not affected, although a partial dissociation between oxidative and phosphorylative systems must occur.

• A partial energetic uncoupling induced by *P. tridentatum* leaf extract, decreasing membrane potential was observed, suggesting a decrease in ROS production.

• Hence, the mild mitochondrial stress induced by the polyphenols present in *P. tridentatum* extract, act as hormetic stimuli and can account for the antioxidant properties of *P. tridentatum* observed in vitro and contribute also to a higher mitochondrial flexibility.

• Our results show that used as condiment, the daily dosages of *P. tridentatum* (a few grams/day) seem to be not harmful.

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