Polyphenols characterization and toxicological evaluation of *Pterospartum tridentatum* leaf extracts

Fernanda M. Ferreira[a, b] ✉, Francisco P. Peixoto[b,c], Olívia R. Pereira[d, e], Lia T. Dinis[c,f], M. Rosário M. Domingues[g], Carlos M. Palmeira[h] and Susana M. Cardoso[d]

(✉ fmlferreira@gmail.com)

Background:

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

- Leafs are used in cooking, to flavour rice, roast meat or hunting animals or as a condiment in salads.

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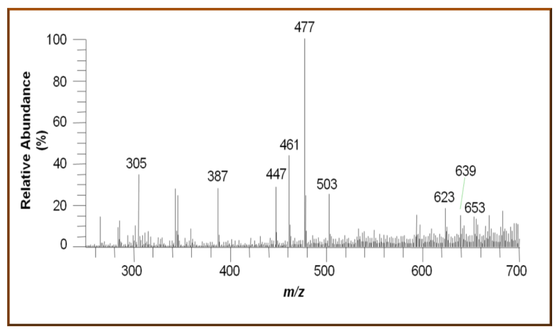
- Despite its wide traditional use, no toxicological assessment of this plant has been performed previously.

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:

Main Fragments ESI⁻MS^n	Compound
MS^2[447]: 285; MS^3[285]: MS^3[285]: 267, 257, 243,	Luteolin-*O*-glucoside
MS^2[461]: 299, 285; MS^3[285]: MS^3[285]: 267, 257,	Luteolin-*O*-glucuronide
MS^2[477]: 315, 300; MS^3[315]: 299, 300, 283, 272,	Isorhamnetin-*O*-glucoside
MS^2[503]: 461, 443, 399, 285; MS^3[285]: 267, 257, 243, 241, 217 , 199, 175, 151; MS^3[443]: 399, 381,	Luteolin-*O*-(*O*-acetyl)-glucuronide
MS^2[593]: 285; MS^3[285]: 267, 257, 243, 241, 217 ,	Luteolin-*O*-rutinoside
MS^2[623]: 447; MS^3[285]: 267, 257, 243, 241, 217,	Luteolin-*O*-(glucuronyl-glucoside)
MS^2[639]: 477, 315, 300; MS^3[315]: 299, 300,	Isorhamnetin-*O*-diglucoside
MS^2[653]: 477; MS^3[477]: 315, 300	Isorhamnetin-*O*-(glucuronyl-glucoside)



Resume of major [M-H]$^-$ ions observed in the ESI-MS spectrum of the aqueous extract of *Pterospartum tridentatum* leaves corresponding to phenolic compounds, with the indication of the main product ions observed in their MS^n spectra.

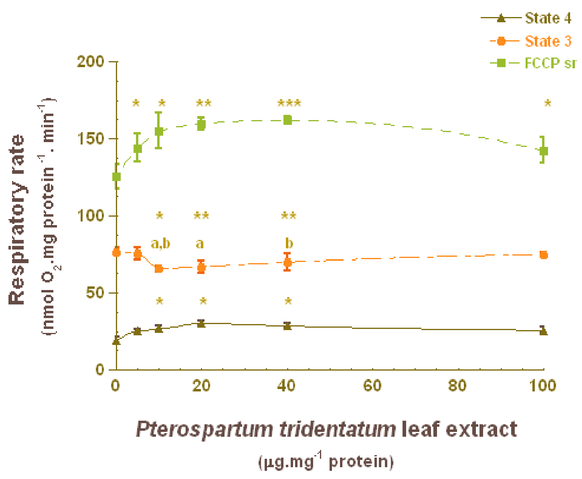
✍ *P. tridentatum* leaf extract phenolic compounds could be grouped in luteolin derivatives and isorhamnetin derivatives. The most abundant [M-H]$^-$ molecular ion in the ESI-MS spectrum was detected at *m/z* 477 and was identified as isorhamnetin-*O*-glucoside .

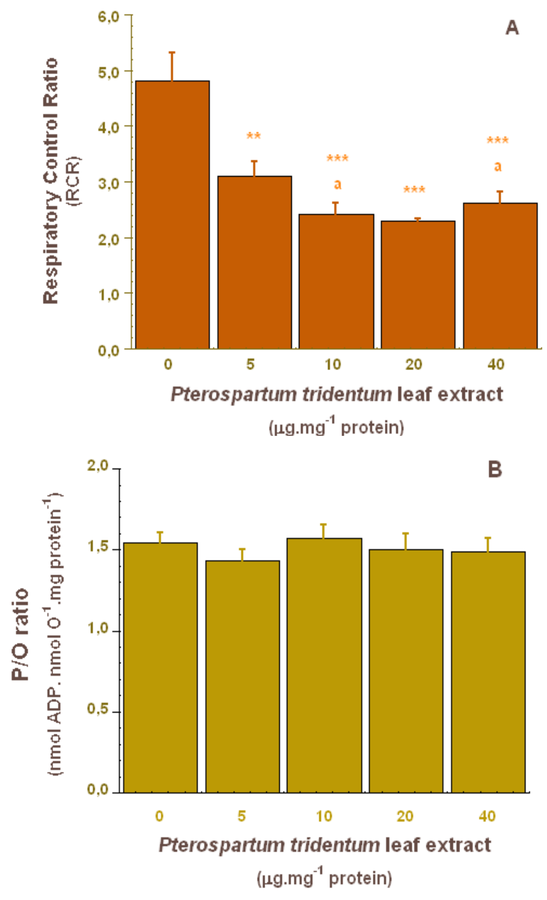
Polyphenols content:

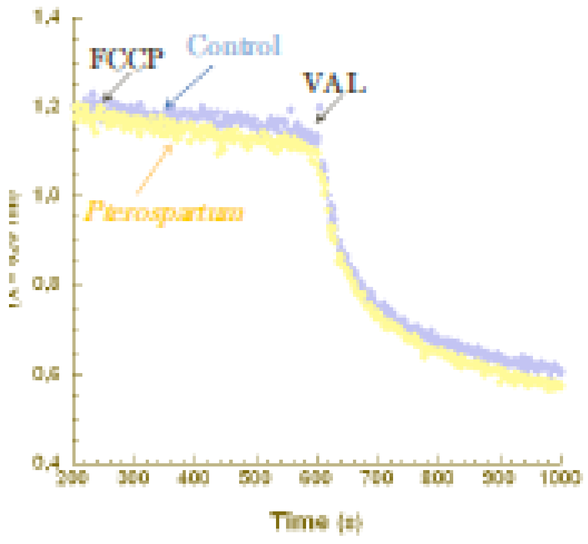
	Flavonoids (catechin equivalents: mg Eq.g^{-1})	Phenols (GAE; mg Eq. g^{-1})
R. officinalis	33.03 ± 0.20	102.43 ± 0.65
P. tridentatum	62.64 ± 0.42 ***	175.51± 2.40 **

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

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.

a. Department of Environment, Agricultural College of Coimbra (ESAC), Polithecnic Institute of Coimbra, Coimbra, Portugal
b. Centre for Investigation and Agro Environmental and Biological Technologies (CITAB) – Vila Real, Portugal
c. Chemistry Department, University of Trás-os-Montes & Alto Douro, Vila Real, Portugal
d. Center for Study of Natural Resources, Environment and Society (CERNAS), Coimbra, Portugal
e. Departament of Diagnostic and Therapeutic Technologies, School of Health Sciences, Polytechnic Institute of Bragança, Bragança, Portugal
f. Department of Biologic and Environmental Engineering (DEBA), University of Trás-os-Montes & Alto Douro, Vila Real, Portugal
g. Department of Chemistry & QOPNA, University of Aveiro, Aveiro, Portugal
h. Department of Life Sciences, Center for Neurosciences and Cell Biology of Coimbra, University of Coimbra, Portugal

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