Polyphenols characterization and toxicological evaluation of *Pterospartum tridentatum* leaf water 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]

Background:

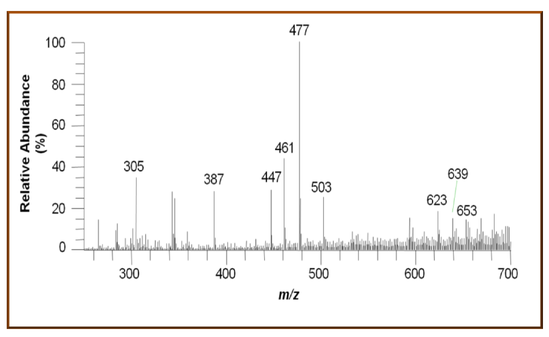
Pterospartum tridentatum Willk. (prickled broom) is an autochthonous plant, common in Portuguese territory.
The leafs and steams are used in used in cooking, to flavour rice, roast meat or hunting animals; leaves are also used as a condiment in salads
Despite its wide traditional use, no toxicological assessment of this plant has been performed, as far as we know.

Goals:

- polyphenols characterization of *P. tridentatum* leaf extract
- evaluation of antioxidant activity of *P. tridentatum* extract
- assessment of potential toxicological effects of *P. tridentatum* flowers 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.

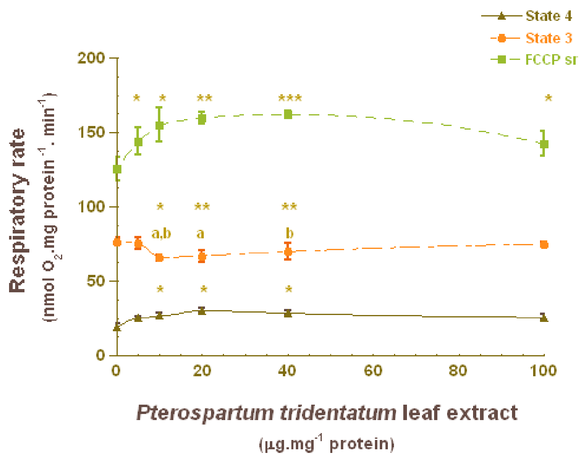
P. tridentatum leaf extract phenolic compounds could be grouped in luteolin derivatives and isorhamnetin derivatives. The most abundant [M-H]⁻ molecular ion in the ESI-MS spectrum was detected at *m/z* 477 and was identified as isorhamnetin-*O*-glucoside .

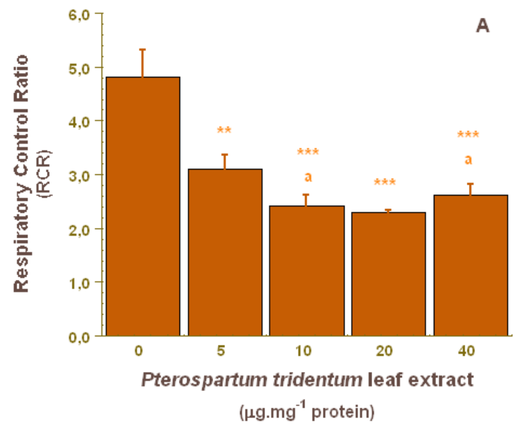
Polyphenols content:

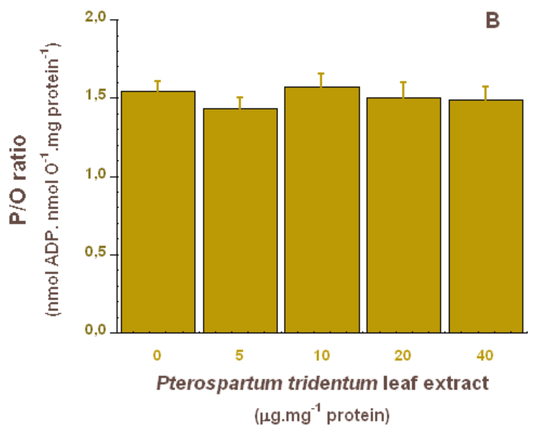
	Flavonoids (catechin equivalents: mg Eq.g^{-1})	Phenols (GAE; mg Eq. g^{-1})
R. officinalis	33.03 ± 0.20	102.43 ± 0.65
P. tridentatum	62.64 ± 0.42 ***	175.51± 2.40 **

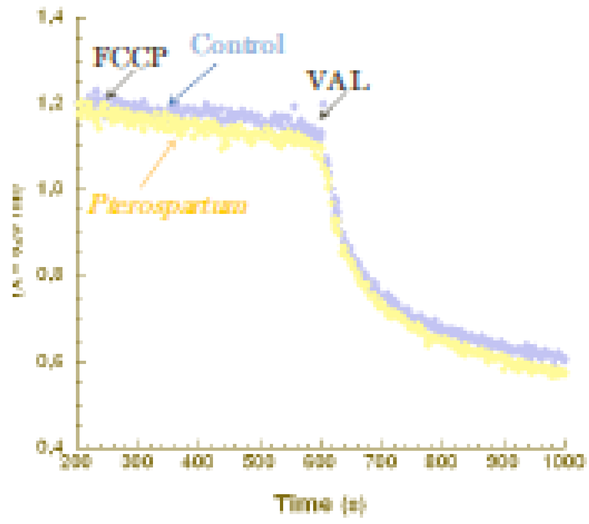
P. tridentatum leaf extract showed a higher polyphenols content, than the *R. officinalis* extract (*** p<0.001).

Toxicological evaluation:









The results suggest that in the range of concentrations used the mitochondrial phosphorylative system is not directly inhibited by *P. tridentatum* leaves aqueous extract, although a partial dissociation between oxidative and phosphorylative systems must occur.

The results account for a partial energetic uncoupling induced by *P. tridentatum* leaf extract, decreasing membrane potential that induce a decrease in ROS production. Hence, the mild mitochondrial stress induced by the polyphenols present in *P. tridentatum* extract, that act as hormetic stimuli, can account for the antioxidant properties of *P. tridentatum* observed in vivo and contribute also to a higher mitochondrial flexibility These hormetic stimuli can also be important in the prevention of other chronic pathologies, related with oxidative stress and the human modern nutritional milieu.

References:
Brand, M. D., and Nicholls, D. G. (2011) Assessing mitochondrial dysfunction in cells, *Biochem. J. 435*, 297-312.
Chance, B., and Williams, G. R. (1956) The respiratory chain and oxidative phosphorylation, *Adv. Enzymol. 17*, 65-134.
Diogo, C. V., Félix, L., Vilela, S., Burgeiro, A., Barbosa, I. A., Carvalho, M. J. M., et al. (2009). Mitochondrial toxicity of the phyotochemicals daphnetoxin and daphnoretin- Relevance for possible anti-cancer application. *Toxicol. in Vitro, 23*, 772-779.
Ferreira, F. M., Dinis, L. T., Azedo, P., Galhano, C. I. C., Simões, A., Cardoso, S. M., Rosário, M., Domingues, M., Pereira, O. R., Palmeira, C. M., and Peixoto, F. P. (2012) Antioxidant capacity and toxicological evaluation of Pterospartum tridentatum flower extracts, *CyTA -J. Food 10*, 92-102.
Jia, Z, Tang, M, Wu, J. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 64:555-559.
Nunn, A., Bell, J., and Guy, G. (2009) Lifestyle-induced metabolic inflexibility and accelerated ageing syndrome: insulin resistance, friend or foe?, *Nutri.Metabol. 6*, 16.
Pereira, O. R., Silva, A. M. S., Domingues, M. R. M., and Cardoso, S. M. (2012) Identification of phenolic constituents of Cytisus multiflorus, *Food Chem. 131*, 652-659.

Adresses:
a Department of Environment, Agricultural College of Coimbra (ESAC), Coimbra, Portugal – fmlfereira@gmai.com
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, Portuga