

***Pterodon emarginatus* Vogel, a Brazilian medicinal plant: antioxidant properties and studies on viability of HT29 cells**

M. Donati¹, F. Benetti¹, A. Mondin², F. M. Miranda³, B.B. do Nascimento Junior⁴, M. Montopoli¹, P. Pastore², L. Caparrotta¹, G. Frolidi¹

¹Dept. of Pharmaceutical and Pharmacological Sciences, University of Padova, 35131 Padova, Italy

²Dept. of Chemical Sciences, University of Padova, 35131 Padova, Italy

³Dept. of Biomedicine and Pharmacy - College Guanambi, Guanambi, Brazil

⁴Dept. of Chemistry and Exact, Southwest Bahia State University, Jequié-BA, Brazil

Pterodon emarginatus Vogel (Fabaceae) is a plant from the *Cerrado* which is commonly known as 'sucupira branca' or 'faveiro'. It is widely used in Brazilian folk medicine for the treatment of rheumatism, sore throats and back problems. Its seeds are commercially available as they are largely used for their properties, mainly as anti-inflammatory remedy (de Moraes et al., 2012). Thus, the aim of this research was the study of *Pterodon emarginatus* seed extracts and essential oil, also to improve their use. For this, we performed different sets of protocols: a) the gas chromatography coupled with mass spectrometry (GC/MS) analysis to determine the sesquiterpenoid content; b) two radical-scavenging assays to determine the antioxidant activity, and 3) the MTT assay on colorectal adenocarcinoma cell line (HT29 cells) to evaluate cellular viability. Thus, the antioxidant activity was evaluated using assays based on two different mechanisms: the Oxygen Radical Absorbance Capacity (ORAC) assay, based on HAT reaction, and DPPH radical scavenging capacity assay, based on SET reaction. The DPPH assay was performed according to the method reported by Brand-Williams et al. (Brand-Williams, 1995) with some modifications (Chen et al., 2013). The ORAC assay was applied according to the method of Gillespie et al. (Gillespie, 2007), appropriately modified (Bertin et al., 2013). We also determined the Total Phenolic Content (TPC) and the Total Flavonoid Content (TFC) for each extract because these compounds are generally related to the antioxidant properties of plants. The *Pterodon emarginatus* seeds were collected from May to August 2013, near the city of Guanambi, Bahia State, Brazil. The ethanolic extract (TME, Traditional Medicinal Extract), the sequential extracts with different solvents (hexane, chloroform, ethyl-acetate and methanol) and the essential oil (EO) were obtained. The GC analysis of *Pterodon emarginatus* extracts showed the presence of β -caryophyllene and farnesol, which were chosen as reference compounds in our research. Relating to the extracts, the TPC was higher for the methanolic extract (ME) with a value of $267.7 \pm 25.6 \text{ mg}_{\text{GAE}}/\text{g}_{\text{extract}}$, following the ethyl-acetate extract (EAE) with $66.4 \pm 19.8 \text{ mg}_{\text{GAE}}/\text{g}_{\text{extract}}$ and TME with $21.6 \pm 10.6 \text{ mg}_{\text{GAE}}/\text{g}_{\text{extract}}$. The values of TFC were of 21.2 ± 3.2 and $10.5 \pm 3.0 \text{ mg}_{\text{QE}}/\text{g}_{\text{extract}}$ for ME and EAE, respectively. With the DPPH assay all extracts exhibited good scavenging activities even if lower than ascorbic acid, with the exception of hexanic extract (HE). The potency order was $\text{EO} > \text{ME} > \text{EAE} > \text{CE} > \text{TME} > \text{HE}$. With ORAC detection, the potency order was $\text{ME} > \text{TME} > \text{EAE} > \text{HE} > \text{CE} > \text{EO}$, whereas ascorbic acid showed lower ORAC activity than all the extracts. Furthermore, the effects of the extracts on HT29 cellular vitality were evaluated. HT29 cells were exposed to each extract and EO in a range of concentration from 0.01 to 50 $\mu\text{g}/\text{ml}$ for 24 hours, without significant inhibition up to 10 $\mu\text{g}/\text{ml}$. These results indicate the absence of cytotoxicity in a concentration range compatible with the traditional use of *Pterodon emarginatus* extracts. In conclusion, the present research shows that *Pterodon emarginatus* essential oil and most of the extracts have antioxidant activities, mainly through HAT mechanism, and are devoid of any significant cytotoxic effects on HT29 cells. All these results support the Brazilian traditional use of *Pterodon emarginatus* in oxidative stress which plays a major role in the development of chronic and degenerative illness such as cardiovascular diseases.

References

- Bertin, R., Chen, Z., Martinez-Vazquez, M., et al., 2014. *Phytomedicine*, 21(5): 586-94.
- Brand-Williams, W., Cuvelier, M. E.; Berset, C., 1995. *Lebensm. Wiss. Technol.*, 28: 25-30.
- Chen, Z., Bertin, R., Frolidi, G., 2013. *Food Chemistry*, 138: 414-420.
- Gillespie, K.M., 2007. *Nature Protocols*, 2: 867-870.
- de Moraes, W.F., Galdino, P.M., Nascimento, M.V., et al., 2012. *Journal of Natural Medicines*, 66: 202-207.