Phytochemical evaluation of *Quercus* species. Antioxidant properties of *Q. suber* leaves in a neurodegeneration cell model

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Abstract. A wide array of plant phenolic substances have been reported to have substantial neuroprotective activity, intervening on multiple biological processes such as iron chelation, radical scavenging, activation of survival genes, cell signaling pathways, regulation of mitochondrial function and possibly the ubiquitin/proteasome system.

Quercus species are potential sources of phytochemical compounds whose bioactivity have not yet been characterized. The aim of this study is to evaluate the phenolic and flavonoid contents, as well as the antioxidant properties of *Quercus* species leaf extracts either *in vitro* and in a neurodegeneration cell model

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A hydroethanolic extraction of leaves from *Q. ilex*, *Q. robur Q. rotundifolia* and *Q. suber* was performed. Total phenolic content in *Q. suber* extracts (89.4 mg GAE.g^{-1} dw) was significantly higher than the values obtained for *Q. robur, Q. rotundifolia* and *Q. ilex* (72.2, 66.4 and 64.9 mg GAE.g^{-1} dw, respectively). *Q. suber* was also the species with the higher flavonoid content (31.4 mg (+) catechin equivalents.g^{-1} dw).

The *in vitro* antioxidant properties of *Q. suber* extracts were then evaluated by the Oxygen Radical Absorbance Assay. The antioxidant capacity detected (885.0 µmol TE.g^{-1} dw) was particularly high when compared with the values reported in the literature for other species.

Toxicity tests of *Q. suber* extracts were performed on a neuroblastoma cell line (SK-N-MC) using CellTiter-Blue® kit. A nontoxic range of concentrations was defined using the SK-N-MC cells. The intracellular radical scavenging activity of the plant extracts in an oxidative stress-induced model of neurodegeneration in SK-N-MC cells is currently being evaluated.

The *in vitro* radical scavenging activity of the *Q. suber* leaf extracts revealed great potential as a source of natural antioxidants. Further studies will confirm their potential use as neuroprotective compounds.

Introduction. Natural antioxidants were found to have great potential in the health sciences. Compounds like polyphenols have been described as possessing valuable properties in human health, with special incidence in diseases where cellular oxidative stress is the main issue of the pathology.

Quercus are important species in the Portuguese native forest and a source of phytochemical compounds whose bioactivity has not yet been characterized.

Materials and Methods. *Extraction of phenolics:* Plant leaves were ground to a fine powder and a hydroethanolic extraction was performed (6 mL.g^{-1} fw). The mixture was shaken for 30 min at room temperature, centrifuged at 12400 *g* for 10 min and the supernatant filtered through 0.20 µm cellulose acetate membrane filter and stored at -80 °C. *Total phenolic compounds*: Determination of total phenolic compounds was performed by the Folin-Ciocalteau method [1]. Gallic acid (GA) was used as standard, and results were expressed as mg GAE.g^{-1} dw. *Flavonoid content*: Determination of flavonoid content was performed by $AlCl_3$ complexation adapted by [2]. Catechin hydrate was used as standard, and the results were expressed as mg CE.g^{-1} dw of plant material. *Peroxyl radical scavenging capacity*: Peroxyl radical scavenging capacity was determined by ORAC (Oxygen Radical Absorbance Capacity) method [3, 4]. Results were expressed as µmol TE.g^{-1} dw. *Cell viability*: Toxicity tests were performed in a neuroblastoma cell line (SK-N-MC)

using CellTiter-Blue® kit. *Antioxidant* in vivo: Neuroblastoma cells were preincubated for 1 hour with *Quercus* phenolic extract and then were treated with H_2O_2 (1 mM) for 1 hour. Cell viability was evaluated as described.

Results and Discussion. Total phenolic content in *Q. suber* hydroethanolic extracts (89.4 mg GAE. g^{-1} dw) was significantly higher than the values obtained for *Q. robur, Q. rotundifolia* and *Q. ilex* (72.2, 66.4 and 64.9 mg GAE.g^{-1} dw, respectively). *Q. suber* was also the species with the higher flavonoid content (31.4 mg (+) catechin equivalents.g^{-1} dw) (Figure 1).

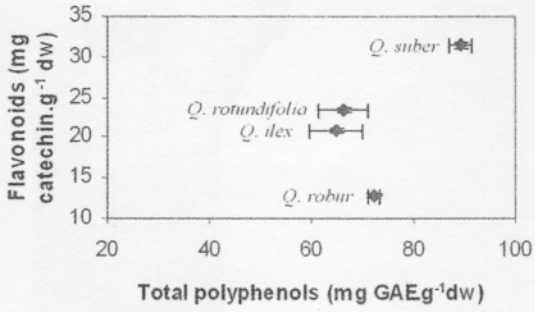

***Figure 1**-Total polyphenols versus flavonoid content for the hydroethanolic extracts from four Quercus species.*

The antioxidant capacity was then evaluated for the *Q. suber* extract. The peroxyl radical scavenging capacity was determined by the Oxygen Radical Absorbance Capacity. The antioxidant capacity detected (885.0 µmol TE.g^{-1} dw) was particularly high when compared with the values reported in the literature for other species.

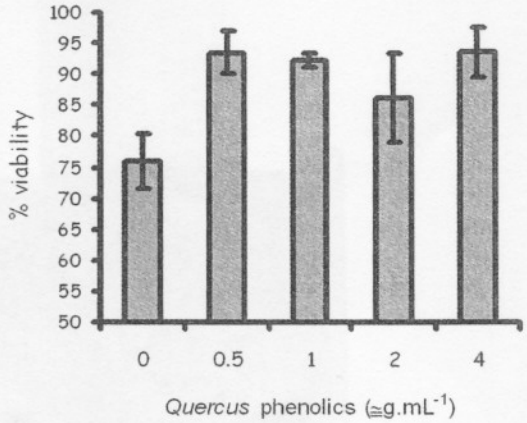

***Figure 2**- Cell viability after oxidative stress with H_2O_2 (1 mM), with and without preincubation for 1 hour with Quercus phenolics extract.*

A non toxic range of concentrations (< 5 µg.mL^{-1}) of *Q. suber* extracts was defined using neuroblastoma cells and the preliminary intracellular antioxidant assay with the non-toxic concentrations of extracts confirmed their antioxidant capacity.

The radical scavenging activity of the *Q. suber* leaf extracts revealed that this species has great potential as a source of natural antioxidants. The intracellular antioxidant capacity must be confirmed by flow cytometry using dichlorodihydrofluoresceindiacetate (H_2DCFDA).

Further studies will confirm the potential of these compounds as active ingredients in the formulation of polyphenols to be used as food supplements, cosmetic or pharmaceutical products.

References

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