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To cite this article: Raffaella Rossi, Carlo Corino, Grazia Pastorelli, Philippe Durand & Michel Prost (2009) Assessment of antioxidant activity of natural extracts, Italian Journal of Animal Science, 8:sup2, 655-657

To link to this article: <http://dx.doi.org/10.4081/ijas.2009.s2.655>



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Published online: 07 Mar 2016.



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Assessment of antioxidant activity of natural extracts

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ABSTRACT - The aim of this study was investigate and compare the antioxidant activity of extracts from Labiatae and Oleaceae cell cultures and a natural Verbenaceae extract on pig whole blood. The antioxidant activity was assessed using the KRL biological test. Extract obtained from *Ajuga reptans* (Labiatae) cell cultures and titrated at 50% of phenylpropanoids expressed as teupolioside (T), extract obtained from *Siringa vulgaris* (Oleaceae) cell cultures and titrated at 50% of phenylpropanoids expressed as verbascoside (V), V and T 50/50 mixture and a natural Verbenaceae extract (NE) titrated at 5% of phenylpropanoids, expressed as verbascoside, were tested for their ability to protect pig red blood cells from 2,2'azobis (2-amidinopropane) dihydrochloride (AAPH) induced hemolysis. The protective effect of the compounds was dose-dependent at concentrations of 0.1 to 1g/L. The active principle in the NE shows an antioxidant activity 2.27 times higher (P<0.01) than the cell culture extract V and 5.11 times higher (P<0.01) than the cell culture extract T. Taking into account the total phenylpropanoids content of cell cultures and natural extract, these results clearly indicate a greater antioxidant activity for the natural Verbenaceae extract.

Key words: Phenylpropanoid glycosides, Antioxidant activity.

Introduction – Dietary antioxidants, including polyphenolic compounds, vitamins E and C, and carotenoids, are believed to be effective nutrients in the prevention of oxidative stress related diseases (Kaur *et al.*, 2001). Epidemiological studies have clearly shown that diets rich in antioxidant protect human against degenerative diseases such as cancer and cardiovascular diseases (Manach *et al.*, 2005). Many studies have been conducted searching for the antioxidant activities of many plant active compounds such as phenylpropanoid glycosides. The antioxidant action of phenylpropanoid glycosides, such as verbascoside and teupolioside has been amply described and is well known in the literature (Aldini *et al.*, 2006). It is of great interest to the health and food science researchers to know the antioxidant capacity of plant constituents, but the biggest problem is the choice of a validated assay that can reliably measure the antioxidant capacity in biological samples. The purpose of the present study was to investigate and compare the antioxidant activity of cell cultures verbascoside and teupolioside and a natural Verbenaceae extract on pig whole blood, using the KRL biological test.

Material and methods – The total antioxidant activity of extract obtained from *Ajuga reptans* (Labiatae) cell cultures and titrated at 50% of phenylpropanoids expressed as teupolioside (T), extract obtained from *Siringa vulgaris* (Oleaceae) cell cultures and titrated at 50% of phenylpropanoids, expressed as verbascoside (V), verbascoside and teupolioside mixture (50% V and 50% T) and a natural Verbenaceae extract (NE) titrated at 5% of phenylpropanoids, expressed as verbascoside, were evaluated using the KRL biological test (Laboratoires Spiral, France). The KRL test (Prost, 1992) allows

Figure 1. Antioxidant activity of cell culture Verbascoside (V), Teupolioside (T), V and T mixture (V+T) and a natural Verbanaceae (NE) extracts in relation to the concentration of the extract in the reaction medium. The four compounds differ for $P < 0.01$.

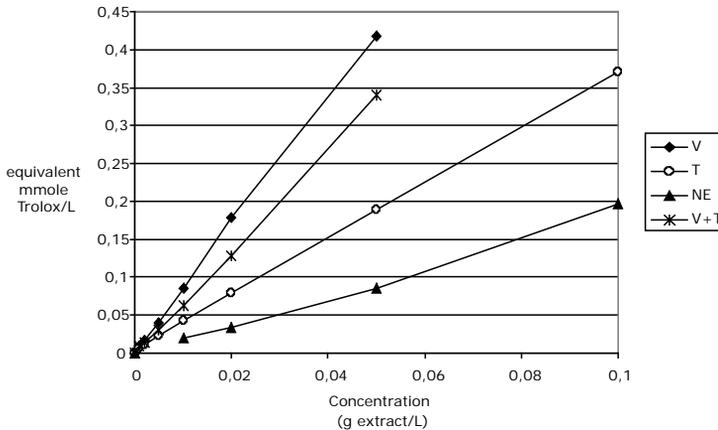
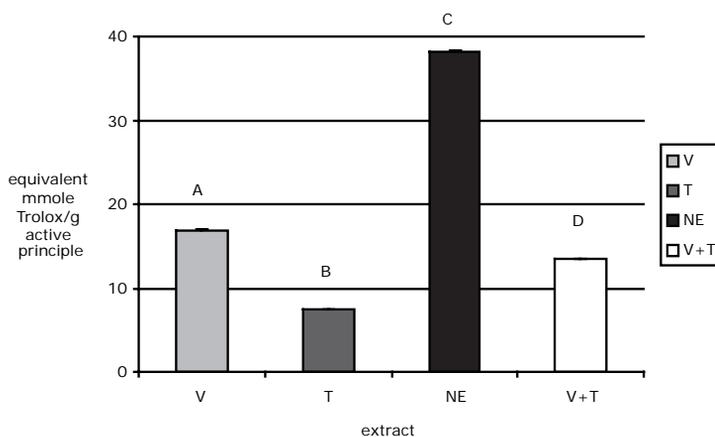


Figure 2. Antioxidant activity of cell culture Verbascoside (V), Teupolioside (T), V and T mixture (V+T) and a natural Verbanacea (NE) extract per gram of active principle. Means without a common letter differ for $P < 0.01$.



the evaluation of red blood cell resistance against the free radicals induced by 2,2'-azobis (2-amidinopropane) hydrochloride (AAPH) that acts by producing peroxy radicals, which induce lipid and protein peroxidation in the cell membrane (Dai *et al.* 2006; Yang *et al.* 2006). Blood samples coming from a healthy pig with a half-hemolysis time near to the median reference value ($\pm 3\%$) found in piglets (Pastorelli *et al.*, 2009). We studied the direct effect of the compounds on the blood without free radical addition and verified that the blank did not present any interference. The blood solutions were diluted to 1:50 in phosphate buffer in isotonic conditions at $pH=7.4$. The phenylpropanoid glycosides were dissolved in aqueous solution at different concentration. Blood solutions were incubated at $37^\circ C$ with different range of concentration (from 0 to 1g by liter of reaction medium) of the three extract and V+T mixture (4 independent experiments, 10 levels and 2 replication for each thesis) for 15 min before being submitted to free radicals produced by a final 50 mM solution of AAPH. Hemolysis was recorded using a 96-well microplate reader by measuring the optical density decay at 450 nm (Laboratoires Spiral, France). Results, expressed

as the time that is required to reach 50% of maximal hemolysis (half-hemolysis time, HT_{50} in minutes) are standardized in Trolox equivalents, a water-soluble analogue of vitamin E. A range from 0 to 1000 $\mu mole/L$ of Trolox® (MW 250.29 g/mole) allowing us to standardize the global antioxidant capacity of the product as compared to vitamin E. One-way ANOVA followed by SNK post hoc test were used to determine significant differences ($P < 0.05$) among the compounds. Data are presented as mean \pm SEM.

Results and conclusions – Our results underline that both cell culture (V, T and V+T) and NE have *in vitro* an important antioxidant capacity, which increases linearly with the dose of the extract until a concentration of 0.1 g/L for T and NE and 0.05g/L for V and V+T mixture (Figure 1). The V, T, NE and V+T mixture show a different ($P<0.01$) antioxidant activity and equivalent to 8.43, 3.73, 1.91 and 6.73 mmoles of Trolox per g of extract.

As reported in figure 2, considering the amount of the active principle in both cell cultures and natural extract, the antioxidant activity of V, T, NE and V+T mixture is different ($P<0.01$) and equivalent to 16.86, 7.47, 38.19 and 13.46 mmoles of Trolox per g of active principle.

In conclusion the active principle in the natural Verbanaceae extract shows an antioxidant activity 2.27 times higher ($P<0.01$) than the cell culture extract verbascoside and 5.11 times higher ($P<0.01$) than the cell culture extract teuopolioside.

In conclusion and according to Aldini et al. (2006), the results of the study clearly indicate that the natural Verbanaceae extract, standardized in polyphenol content, exhibits strong antioxidant activity, due to a cooperative antioxidant interaction between its polyphenol components.

This work has been done as a research project called "Nuove metodologie per la valutazione oggettiva del benessere animale nella specie suina" financed by Regione Lombardia - Agricultural Department, according to the Plan of Research and Development 2007.

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