Antioxidant Activity of Oak (Quercus) Leaves Infusions against Free Radicals and their Cardioprotective Potential

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Abstract: The aim of present study was to evaluate antioxidant capacity and cardioprotective potential of leaves infusions and partially purified fractions of Quercus sideroxyla and Q. eduardii (red oaks) and Q. resina (white oak). Consumption of polyphenol-rich beverages derived from plants, such as oak may represent a beneficial diet in terms of cardiovascular protection. Infusions from Oak leaves were obtained and proved for total phenolics by Folin-Ciocalteu, DPPH and hydroxyl radicals scavenging by DPPH test and Deyco-D-ribose method, the antioxidant capacity was evaluated by FRAP and ORAC tests, inhibition of Low Density Lipoproteins (LDL) oxidation and Angiotensin Converting Enzyme (ACE) activity were measured. HPLC analysis was performed by HPLC-MS. Bioactive polyphenols such as gallic and ellagic acids, catechin, quercetin and derivatives: naringenin and naringin were detected in Quercus infusions. A distinctive HPLC profile was observed among the red and white oak samples. Q. resina infusions have exhibited the highest antioxidant activity in comparison with the other species, although in the inhibition of LDL oxidation no differences were observed. In the inhibition of the ACE, Q. resina was more effective (IC50 18 ppm) than Q. sideroxyla, showing same effect as the control Captopril. From the results it is possible to postulate that not only chelating activity is important in these infusions, especially in Q. resina.

Key words: Angiotensin converting enzyme, antioxidant, infusion, polyphenol, Quercus

INTRODUCTION

Antioxidants prevent the damage to macromolecules and cells by interfering with free radicals implicated in the etiology of several diseases, such atherosclerosis (Srivastava et al., 2007). Tea is a widely consumed beverage all through the world. Beverages that contain bioactive ingredients such as antioxidants should balance nutritional deficits and support health. Currently, consumers tend to favor a healthy lifestyle and natural ingredients are clearly preferred over synthetic ones. New functional beverages such as herbal teas have increased their convenience as healthy drinks. The range of herbal beverages is increasing and usually they are accompanied by claims about benefits including antioxidant, anticancer and cardioprotective activities. Plants, vegetables, herbs and spices used in folk and traditional medicine have gained a wide acceptance as the main source for prophylactic chemicals discovery (Afolayan et al., 2008). For these reasons, information on the overall antioxidant properties of natural products is becoming relevant in the nutrition and nutraceutical fields. Due to the complexity of the oxidation-antioxidation processes, there is not a single method capable of providing a comprehensive picture of the antioxidant profile of a studied sample, for this reason a multimethod approach is necessary in the assessment of antioxidant activity (Singh et al., 2007).

Phenolic compounds are bioactive substances widely distributed in plants and are important constituents of the
human diet because they provide a positive effect due to their antioxidant properties (Di Majo et al., 2008). A possible protective role of flavonoid intake against coronary disease has been reported (Nagao et al., 2007). Several epidemiological studies have shown associations between the regular consumption of flavonoid-rich foods and the decreased risk for cardiovascular diseases. These activities have been identified in the traditional use of oak barks and tea leaves on superficial injuries, gastric ulcers, local inflammation and for some types of cancer (Abou-Karam and Shier, 1999). Humans have evolved with an antioxidant system to protect against free radicals. The use of tea is widespread and plants still present a large source of novel biological compounds with different activities, including anti-inflammatory, anticancer, antiviral, antibacterial and cardioprotective activities (Pietta, 2000; Cespedes et al., 2008).

Peroxidation of LDL constitutes the initial step in a complex process that results in atherosclerosis and its clinical consequences (Berrougui et al., 2007). Several studies have demonstrated that nutrients, antioxidants and/or complementary medicine strengthen LDL oxidation susceptibility and increase the anti-atherosclerotic impact of High Density Lipoproteins (HDL) performing a key role in cardiovascular disease prevention (Jialal and Fuller, 2009). Another risk factor for cardiovascular disease is the hypertension that just in Mexico produces forty thousand deceases every year (Cordova-Villalobos et al., 2008). In this context, the Angiotensin Converting Enzyme (ACE) inhibition is considered to be an important therapeutic approach in the treatment of high blood pressure and the intake of active metabolites as ACE inhibitors would provide positive health effects (Ghazi et al., 2009). The finding that flavonoid rich foods can inhibit ACE activity and reduce blood pressure (Actis-Goretta et al., 2003), open up the possibility that consumption of herbal teas may mimic synthetic ACE inhibitors and provide health benefits without adverse side effects.

Durango, México, has important forestry resources that are not always totally exploited. Thirty-nine species of Quercus are present in Durango’s forests and their major non timber uses are for: (a) food, both fresh and processed; (b) medicinal, related mainly to digestive system diseases; (c) manufacturing handcrafts and toys; (d) fodder, for goats and pigs; (e) tannins for fiber dying and leather tanning (Luna-Jose et al., 2003; Dawra et al., 1988).

Several species of oak including Quercus sideroxyla, Quercus eduardii and Quercus resinosa leaves are used in traditional medicine for treating diverse illnesses. They are used in folk medicine as anticarcinogenic agents (Rocha-Guzman et al., 2007). In a previous study (Rocha-Guzman et al., 2009), it was observed that extracts from Quercus resinosa leaves present high phenolic content, antioxidant activity and anticarcinogenic potential. As part of our ongoing screening on natural products with potential for nutraceutical ingredients, in this work; the antioxidant activity and cardioprotective potential of oak leaves infusions in in vitro assays were investigated, including in vitro assays for inhibition of oxidation of LDLs and inhibition of the ACE.

**MATERIALS AND METHODS**

**Plant materials:** Leaves from Q. sideroxyla (Qs), Q. resinosa (Qr), Q. eduardii (Qe) were collected in March 2007, in Mezquital and El Salto about 85 km South and 95 km west from Durango, México, respectively, leaves were taxonomically identified and voucher specimens deposited at the ISIMA-UIED Herbarium. The leaves were air dried, milled and stored.

**Chemicals:** Folin Ciocalteu reactive (2N), Na₂CO₃, 1,1-diphenyl-2-picrylhydrazyl (DPPH), deoxy-D-ribose, thiobarbituric acid (TBA), triehloroacetic acid (TCA), ethylenediaminetetraacetic acid (EDTA) were obtained from SIGMA (Sigma, St.Louis, MO, USA). For compounds identification from infusions the following standards were used: gallic acid, catechin, quercetin, naringin, naringenin, ellagic acid, HPLC grade solvents: water, methanol and acetonitrile were used; Sodium acetate 2,4,6-tripryridyl-s-triazine, FeCl₃·6H₂O, Fe(II) (FeSO₄·7H₂O), Fluorescein, 6-Hydroxy-2,5,7,8-tetramethylchrom-2-carboxylic acid (Trolox), 2,2'-azobis-(2-amidinopropane dihydrochloride) (AAPH) were from SIGMA-Aldrich (Sigma, St.Louis, MO, USA). n-Butanol and acetic acid were purchased from Fermont (Fermont, Monterrey, Mexico). Hippuryl histidyl leucine (Hip- His- Leu) and ACE were manufactured by SIGMA (Sigma, St.Louis, MO, USA).

**Herb infusions and chromatographic purification:**

Quercus leaves samples (10 g) were added to 250 mL boiling water and extracted under continuous stirring for 10 min. Crude herb infusions (A) were obtained by subsequent filtration and used in the ensuing experiments (Rocha-Guzman et al., 2009). The oak leaves infusions (250 mL) were then purified by permeation gel chromatography using water (B), methanol-water (1:1) (C), methanol (D) and acetone-water (7:3) (E) in a Toyopearl HW 40S column (2.5×50 cm) as shown in Fig. 1.

**Total Phenolic Content (TPC):** Total phenolic content was determined according to the Folin-Ciocalteu (FC) method with minor modifications (Waterman and Mole,
Fig. 1: Chromatographic purification of leaves infusions from three oak species

1994). Standard or sample extracts (0.2 mL) were mixed with 1.0 of FC reagent and 0.8 mL of Na₂CO₃ (7.5%) in a 20 mL vial and allowed to stand for 30 min at room temperature. Absorption was measured at 765 nm with a Varian Cary® 3C spectrophotometer (Varian, Harbor City, CA, USA). Gallic acid was used as standard and the TPC expressed as mg of Gallic Acid Equivalents (GAE) per g of dry extract.

**High Performance Liquid Chromatography (HPLC):** A Thermo Finnigan Surveyor equipped with a quaternary pump (San José, CA, USA), a degasser and auto-sampler was used for the analysis. The separation was carried out in a Phenomenex ODSC₂₅ column (250×4.6 mm, 5 μm). The binary mobile phase consisted of water containing 0.5% acetic acid (solvent A) acetonitrile (solvent B). All solvents and samples were filtered through a 0.45 μm filter prior to use. The flow-rate was kept constant at 1.0 mL min⁻¹ for a total run time of 45 min. The system was run with a gradient program: 0-25 min: 95% A to 70% A; 25-30 min: 70% A to 40% A; 30-35 min: 40% A to 60% A and 35-45 min: 60 to 95% A. The sample injection volume was 10 μL. Peaks of interest were monitored at 280 nm.

**LC-EIS-MS analysis:** LC-MS experiments were carried out using a Finnigan LCQ DECA ion trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA) equipped with electrospray ionization (ESI). The samples were analyzed in under the same chromatographic condition. A negative mode was used for data collection. The sheath gas and auxiliary flow-rates were set at 96 and 7 (arbitrary unit), respectively. The capillary voltage was set at 29 V and its temperature was controlled at 350°C. The inlet lens voltage was fixed at 40 V and the multipole RF amplitude was set at 540 V. The ESI needle voltage was controlled at 4.5 kV. The tube lens offset was 16 V, the multipole lens 1 offset was 8.20 V and the multipole lens 2 offset was 10.5 V. The electron multiplier voltage was set at 980 V for ion detection.

**DPPH radical scavenging activity:** The DPPH assay was carried out according to the method of Brand-Williams *et al.* (1995) with the following modifications. In this study DPPH (2.5 mg/100 mL), all the standards and samples were dissolved in methanol/water (1:1, v/v), which also was used as a blank. Samples were prepared in duplicate at each of the three concentrations used. A standard or sample solution (0.5 mL) was added to 3.5 mL of DPPH solution. The absorbance of the mixture was then determined at 515 nm in the Varian Cary 3C spectrophotometer at 0, 5 and every 10 min until the reaction reached a plateau at room temperature. The antioxidant (antiradical) activity was expressed as the median effective concentration, EC₅₀.

**Hydroxyl radical scavenging activity:** The non-site specific hydroxyl radical-scavenging activity was evaluated according to the method of Dorman *et al.* (2004). The mixture containing FeCl₃ (1.0 mM), ascorbic acid (1.0 mM), EDTA (104 mM), H₂O₂ (1.0 mM), deoxyribose (2.8 mM) and test sample in 500 mL phosphate buffered saline (PBS, 50 mM, pH 7.4) was incubated for 1 h at 37°C. After adding 1.0 mL of trichloroacetic acid TCA (2.8%, w/v) and 1.0 mL of thiobarbituric acid TBA (1.0%, w/v), the reaction mixture was kept boiling for 15 min in a water bath at 100°C for 20 min. The color development was measured at 532 nm and the scavenging activity of tested samples expressed as the inhibition percentage of the deoxyribose degradation to malonaldehyde.

**Ferric Reducing Antioxidant Power (FRAP):** The FRAP was determined using the modified method of Benzie and
Strain (1999) by using a 96-well microplate reader. Standard (FeSO₄·7H₂O) and sample extract (10 μL) were mixed with 300 μL of ferric-TPTZ reagent prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ, in 40 mM HCl and 20 mM FeCl₃ in the ratio of 10:1:1 (v/v) and added to the wells. The plate was incubated at 37°C along the reaction time. The absorbance readings were taken at 593 nm at 4 min using a visible-UV microplate kinetic reader (EL 340, Bio-Tek Instruments Inc., Winoski, Vermont, USA). The FRAP value of samples was expressed as Fe⁷⁺ equivalents (mM L⁻¹).

**Oxygen Radical Absorbance Capacity (ORAC):** The ORAC was determined according to the method of Caldwell (2001) using AAPH as a peroxyl radical generator, trolox as a standard and fluorescein as a fluorescent probe. A diluted sample (25 μL), blank, or Trolox calibration solutions (0-100 μmol) was mixed with 150 μL of fluorescein 8.68×10⁻⁶ M. The plate was incubated at 37°C by at least 30 min in a Synergy HT Multi-Detection Microplate Reader. The reaction was started by the addition of 25 μL of AAPH reagent and kept shaking for 10 s at maximum intensity. Filters were used to select an excitation wavelength of 485 nm and an emission wavelength of 520 nm. The fluorescence was measured every minute during 2 h. All samples were analyzed in duplicate. The final ORAC values were calculated using the area under the decay curves.

**Inhibition of the oxidation of LDL:** A modified lipidic peroxidation method (Loy et al., 2002) was used and results were expressed as percentage of inhibition of LDL. From these results, the median inhibitory concentration (IC₅₀) was determined.

**Angiotensin Converting Enzyme (ACE) inhibition:** ACE inhibition was investigated by using the method of Cushman and Cheung (1971). ACE hydrolyses the angiotensin I analogue hippuryl-L-histidyl-L-Leucine, forming hippuric acid, which level is associated to ACE activity. Captopril was used as a positive control. The samples were analyzed at several concentrations from infusions of Q. resinaea, Q. eduardii and Q. sideroxyla and incubated with the ACE. The absorbance was measured and the median inhibitory concentration (IC₅₀) determined. Briefly, extracted samples were diluted to concentrations of 1, 10, 100 and 1000 μL mL⁻¹, using a 0.1M boric acid buffer containing 0.3 M NaCl, pH 8.3 and charged in amounts of 0.08 mL in test tubes. Then 0.2 mL of hippuryl histidyl leucine (Hip- His- Leu, manufactured by Sigma Chemical Co.), adjusted to 5 mM with a 0.1 M boric acid buffer containing 0.3 M NaCl, pH 8.3 and 0.02 μL of an aqueous enzyme solution (ACE, 0.1 μg mL⁻¹, manufactured by Sigma Chemical Co.) were added in this order as a substrate to the contents of each test tube and the reaction was carried out at 37°C for 30 min. Subsequently 0.25 mL of 1N hydrochloric acid was added to each tube. After termination of reaction, 1.7 mL of ethyl acetate was added and agitation was continued for 20 sec. After centrifugal separation at 3000 rpm for 10 min and recovery of 1.4 mL of the ethyl acetate layer, the solvent was removed. After removing solvent, 1 mL of distilled water was added, the absorption at 228 nm of the extracted hippuric acid was measured and related with the ACE inhibiting activity. The inhibiting ratio was calculated by the following equation:

\[
\text{Inhibiting ratio} = \frac{A-B}{A-C} \times 100\%
\]

where, A is the absorbance at 228 nm of hippuric acid free of sample, B is the absorbance at 228 nm of hippuric acid admixed with a sample and, C is the absorbance at 228 nm of the blank. The ACE inhibitory concentration at 50% (IC₅₀) was then determined.

**Statistical analysis:** All experiments were carried out with two replicates. For each set of obtained data, standard statistical methods were used to determine mean and standard deviations. A two-way analysis of variance with a confidence interval of 95% was performed. Mean differences were analyzed using a Tukey test (p<0.05).

**RESULTS**

The results for the total phenol content assay by FC and antioxidant capacity assays (DPPH and FRAP assays) of infusions and partially purified fractions are presented in Table 1. Highest value of total phenolic content was observed for acetone-water extracts (3:1) of *Quercus sideroxyla*. Best value for DPPH radical scavenging was founded in acetone-water extracts of *Quercus resinaea*. The antioxidant capacities of infusions and partially purified extracts were statistically different from each other (p<0.05). The relative infusions effectiveness for scavenging DPPH radicals was A.Q.r. > A.Q.s. > A.Q.e. Best FRAP value was showed by methanol-water extracts from *Quercus sideroxyla*.

Additional assays used in this screening included the ferric reducing-antioxidant power (FRAP) (Table 1). The relative order found for ferric reducing-antioxidant power of infusion from the three species was A.Q.s. > A.Q.e. > A.Q.r. The highest activity was shown by fractions C.Q.s. (17.78±0.03 EinM Fe⁺) and B.Q.r. (11.73± EinM Fe⁺).
The ORAC protocol is widely used to test natural phenolic antioxidants and teas. Table 1 presents the activity shown by infusions in study that follows the order: A.Q.r. > A.Q.s. > A.Q.e. In red oaks, fraction D (oligomeric polyphenols) showed the highest oxygen radical absorbance capacity expressed in Trolox equivalents.

This study includes the deoxyribose method for assessment of hydroxyl radical scavenging (Halliwell et al., 1987). The tendency found in our infusions was A.Q.s. < A.Q.e. < A.Q.r. (Table 1).

Identified and suggested polyphenol compounds detected in the Quercus leaves infusions are presented in Table 2 and Fig. 2.

The cardioprotective activity of oak leaves infusions was evaluated by two an in vitro assays: the inhibition of oxidation of LDLs and inhibition of Angiotensin Converting Enzyme (ACE). Regarding to the inhibition of LDL oxidation no differences were observed between IC50 from A.Q.s and A.Q.r, but in the test of inhibition of the ACE, A.Q.r was more effective than A.Q.s. Best angiotensin converting enzyme inhibition (Fig. 3) was shown by (A) Quercus resina (IC50, 18 ppm) showing same effect as the hypotensive drug Captopril used as a control. Results obtained (Fig. 2, 3) in this test showed that best IC50 values were obtained by infusions (A) from Quercus sideroxyla and Quercus resina without significant statistical differences.

Table 1: Total phenol content and antioxidant activity of infusions and partially purified fractions from Quercus sideroxyla (A.Q.s.), Q. eduardii (A.Q.e.) and Q. resina (A.Q.r.) leaves

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total phenolics as gallic acid equivalents (mg GAE/g)</th>
<th>DPPH scavenging capacity (mg antioxidant/mg DPPH)</th>
<th>FRAP assay (EmM Fe²⁺)</th>
<th>ORAC (Trolox equivalents, TE)</th>
<th>Inhibition of radical OHLIC50 (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.Q.s.</td>
<td>537.0±0.3</td>
<td>0.62±0.03</td>
<td>11.18±0.05</td>
<td>146±6.82</td>
<td>15±0.1</td>
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<td>A.Q.e.</td>
<td>331.0±0.5</td>
<td>0.72±0.08</td>
<td>3.92±0.10</td>
<td>119±1.61</td>
<td>21±0.1</td>
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<td>A.Q.r.</td>
<td>227.0±0.1</td>
<td>0.22±0.10</td>
<td>1.97±0.00</td>
<td>154±13.25</td>
<td>41±0.1</td>
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<td>B.Q.s.</td>
<td>200.0±0.1</td>
<td>0.70±0.18</td>
<td>1.43±0.01</td>
<td>101±8.26</td>
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<tr>
<td>B.Q.e.</td>
<td>278.0±0.4</td>
<td>2.76±0.00</td>
<td>1.00±0.00</td>
<td>120±6.65</td>
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<td>B.Q.r.</td>
<td>750.0±0.6</td>
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<td>11.73±0.00</td>
<td>188±3.37</td>
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<td>C.Q.s.</td>
<td>300.0±0.2</td>
<td>1.51±0.05</td>
<td>17.78±0.03</td>
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<td>C.Q.e.</td>
<td>603.0±0.3</td>
<td>0.17±0.03</td>
<td>8.25±0.01</td>
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<td>C.Q.r.</td>
<td>565.0±0.3</td>
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<td>11.46±0.01</td>
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<td>D.Q.s.</td>
<td>1614.0±0.9</td>
<td>0.92±0.04</td>
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Table 2: HPLC profile of leaves infusions from three oak species

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<th>Peak</th>
<th>Retention</th>
<th>m/z M-H ions</th>
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<td>2</td>
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<td>3</td>
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<td>301</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>Quercetin*</td>
</tr>
<tr>
<td>22</td>
<td>32.02</td>
<td>151, 271</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>Naringenin*</td>
</tr>
</tbody>
</table>

*Compound identification was confirmed by comparison with authentic standards.
Free radicals are the major cause of the propagation stage in the oxidation process. The high potential for scavenging of free radicals could inhibit the spreading of oxidation (Tachakittirungroj et al., 2007). Flavonoids exhibit typical antioxidant properties, as deduced from a variety of biochemical techniques. It is well known that in vitro antioxidant activity of flavonoids and related metabolites depends upon the arrangement of functional groups around its basic structure. Several attempts have been made to assess the antioxidant activity of beverages and foods. Antioxidant activity assays are useful in obtaining a global picture of relative antioxidant activities in different foods and drinks and how they change after food processing or storage. They may also help detect synergistic interactions of antioxidants (Huang et al., 2005). One of the earliest studies addressing the in vitro antioxidant activity of various teas include the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Ho et al., 1992). Scavenging of this radical is easily followed by loss of its characteristic absorbance. Infusion and partially purified fractions of Q. resina leaves exhibited the highest antioxidant activity in comparison with the other species in study. Thus our results indicate that phytochemicals found in Q. resina have shown a mechanism of action by hydrogen donation and terminated the oxidation process by converting free radicals into more stable products. Similar results were observed in alcoholic extracts of Quercus infectoria galls (Kaur et al., 2008).

FRAP method measures the ability of a compound to reduce Fe³⁺ to Fe²⁺ that depends on the reduction of a ferric tripyridyltriazine (Fe³⁺-TPTZ) complex to a ferrous tripyridyltriazine (Fe²⁺-TPTZ) by a reducing agent at low pH. The generated ferrous ion is a well-known pro-oxidant, due to its reactivity with H₂O₂ to produce OH⁻, the most in vivo harmful free radical. As mentioned, antioxidants are substances that efficiently inhibit pro-oxidant compounds that induce oxidative damage, in this context; the infusion that showed better results was Quercus sideroxyla. A good correlation between total phenolic content and FRAP values was observed for infusions of the three species (Y = 3.0488X-543.82, R² = 0.9823). A significant increase in reducing power was found in infusions from Q. resina leaves when fractioned with several polar solvents. It was demonstrated that these fractions showed a mechanism of action as electron donors and they terminated the oxidation chain reaction by reducing the oxidized intermediates into more stable forms. Similar results were found by Fernandes et al. (2009) for extracts of Q. suber.

The Oxygen Radical Absorbance Capacity (ORAC) assay is a method that takes free radical action to
completion and uses an area-under-curve (AUC) technique for quantification and thus combines both inhibition percentage and the length of inhibition time of free radical action by antioxidants into a single quantity. The ORAC values for individual polyphenols and related phenolics are in reasonable agreement with their structure (Roginski and Lissi, 2005). Values found in the ORAC test agreed with the founded by Dudonne et al. (2009). They report that extracts of *Quercus robur* showed highest antioxidant activity measured by this method in comparison with another plant extracts.

Hydroxyl radicals are generated by a mixture of a ascorbic acid, H₂O₂ and Fe(III)-DTA. The OH⁺ radicals that are not scavenged by other components of the reaction mixture attack the sugar deoxyribose and cause a degradation into a series of fragments, some of which react on heating with thiobarbituric acid (TBA) at low pH to give a chromogen. If an antioxidant is able of scavenging an OH⁺ present in the system (e.g., polyphenols), it will compete with deoxyribose for OH⁺ radicals and will inhibit the deoxyribose degradation (Halliwell et al., 1987).

The mechanisms of action of identified compounds in infusions and in partially purified fractions are of different nature due to a different behavior shown by infusions in the applied tests for the characterization of antioxidant activity. The infusions regarding the partially purified fractions have shown good antioxidant capacity. It was observed a great similarity between the HPLC chemical profile in *Erythrobalamus* species (red oaks), when they are different from *Lencobalamus* species (white oaks), which has shown naringenin and naringin as distinctive constituents (Table 2).

Various chemical properties and biological effects of dietary polyphenols might be involved in protection against cardiovascular risk. It has been proposed that antioxidant properties of polyphenols might protect vascular endothelial function against the deleterious consequences of oxidation of Low Density Lipoproteins (LDLs), as oxidized LDL can impair endothelium-dependent vasorelaxation (Vinson et al., 1995).

An IC₅₀ of 35 μg mL⁻¹ in ACE test was reported for the whole extract from *Pinus maritime* (Packer et al., 1999). It was reported that epicatechin and catechin extracted from Chinese tea showed quite low ACE inhibitory activity but gallatechin showed good ACE inhibitory activity (Liu et al., 2003). It was found that tannin (penta-O-galloyl-D-glucopyranose)-protein (bradykinin) complexes are formed by multiple weak interactions between peptide side chains and galloyl rings (Vergé et al., 2002). Prolin and arginine are good anchoring points and glycine gives certain flexibility in the peptide backbone that allows the polyphenol to approach and to interact with proteins.

However, ACE is a zinc metallopeptidase, in which the zinc ion is essential at the catalytic active site for enzymatic activity. This means that non-specific metal chelators may have apparent ACE inhibition action, thus chelation of Zn⁺ may be, at least in part, responsible for the ACE inhibitory activity of polyphenols (Liu et al., 2003).

The LDL test is a good method to probe two points, a) chelating activity of polyphenols and b) antioxidant capacity. The protocol (Loy et al., 2002) uses Cu⁺ (CuSO₄) to induce LDL oxidation. It was reported (Chirinos et al., 2008) that polyphenols from the flavonoid family as quercetin, catechin or delphinidin-3-glucoside showed higher LDL oxidation inhibition values than phenolic acids. *Q. sideraxyla* showed presence of quercetin and catechin. In vitro tests (Rice-Evans et al., 1996) quercetin and catechin showed the highest efficiency against oxidation of LDL, thus high inhibition of LDL oxidation could be associated to the presence of these compounds. The HPLC profile of *Q. rusciformis* infusions showed presence of catechin and several non identified peaks associated to quercetin plus another flavonoid compounds. Thus, so it is possible to postulate that not only chelating activity of infusions is important in special in *Q. r.*, consequently more experimental work is needed for clarifying this point.

REFERENCES


