http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 13 (11): 537-545, 2010
ISSN 1028-8880
© 2010 Asian Network for Scientific Information

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

[1]M.J. Rivas-Arreola, [1]N.E. Rocha-Guzmán, [1]J.A. Gallegos-Infante, [1]R.F. González-Laredo,
[2]M. Rosales-Castro, [3]J.R. Bacon, [4]Rong (Tsao) Cao, [4]A. Proulx and [5]P. Intriago-Ortega
[1]Instituto Tecnológico de Durango, Departamento de Ings. Química y Bioquímica,
Blvd. Felipe Pescador 1830 Ote., 34080 Durango, Dgo., México
[2]CIIDIR-IPN Unidad Durango. Av. Sigma 119 fraccionamiento 20 de noviembre II,
34220 Durango, Dgo., México
[3]ISIMA-UJED, Carr. a Mazatlán km 5.5, Circuito Universitario, 34120 Durango, Dgo., México
[4]Guelph Food Research Centre. 93 Stone Road West. Guelph, Ontario, N1G 5C9, Canada
[5]Facultad de Medicina, Universidad Juárez del Estado de Durango, Avenida Universidad,
34000, Durango, Dgo., México

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. resinosa* (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 probed for total phenolics by Folin-Ciocalteu, DPPH and hydroxyl radicals scavenging by DPPH test and Deoxy-D-ribose method, the antioxidant capacity was evaluated by FRAP and ORAC tests, inhibitions of Low Density Lipoproteins (LDL) oxidation and Angiotensin Converting Enzyme (ACE) activity were measured. A 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. resinosa* 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. resinosa* was more effective (IC_{50}, 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. resinosa*.

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, costumers 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

Corresponding Author: N.E. Rocha-Guzman, Instituto Tecnológico de Durango, Departamento de Ings, Química y Bioquímica, Blvd. Felipe Pescador 1830 Ote. Col. Nueva Vizcaya, CP. 34080, Durango, Dgo, México Tel/Fax: 52-618-8-18-69-36

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-José *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-Guzmán *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-UJED Herbarium. The leaves were air dried, milled and stored.

Chemicals: Folin Ciocalteu reactive (2N), Na_2CO_3, 1,1-diphenyl-2-picrylhydrazyl (DPPH), deoxy-D-ribose, thiobarbituric acid (TBA), trichloroacetic 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, narigenin, ellagic acid; HPLC grade solvents : water, methanol and acetonitrile were used; Sodium acetate 2,4,6-tripyridyl-*s*-triazine, $FeCl_3{\cdot}6H_2O$, Fe(II) ($FeSO_4.7H_2O$), Fluorescein, 6-Hydroxy- 2,5,7,8- tetramethylchroman- 2-carboxylic acid (Trolox), 2,2azobis-(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,

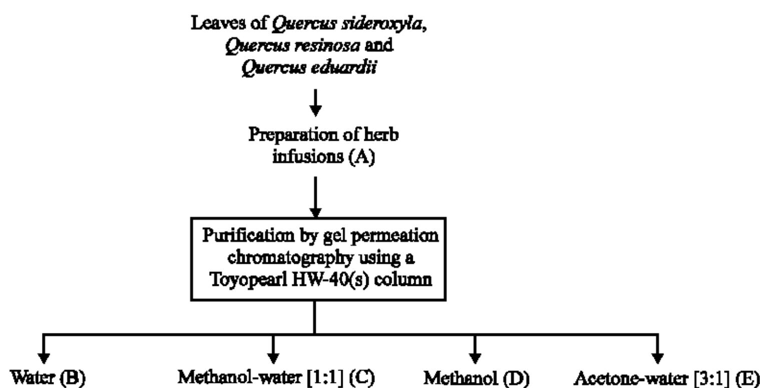

Fig. 1: Chromatographic purification of leaves infusions from three oak species

1994). Standard or sample extracts (0.2 mL) were mixed with 1.0 of FC reagent and 0.8 mL of Na_2CO_3 (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 ODS-C_{18} column (250×4.6 mm, 5 µm). The binary mobile phase consisted of water containing 0.6% 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, EC_{50}.

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 $FeCl_3$ (1.0 mM), ascorbic acid (1.0 mM), EDTA (104 mM), H_2O_2 (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 ($FeSO_4 \cdot 7H_2O$) 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 $FeCl_3$ 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., Winooski, Vermont, USA). The FRAP value of samples was expressed as Fe^{II} 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 mol) were mixed with 150 μL of fluorescein 8.68×10^{-5} mM. 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 (IC_{50}) 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. resinosa*, *Q. eduardii* and *Q. sideroxyla* and incubated with the ACE. The absorbance was measured and the medium inhibitory concentration (CI_{50}) determined. Briefly, extracted samples were adjusted 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 mL 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:

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

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% (IC_{50}) 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 resinosa*. The antioxidant capacities of infusions and partially purified extracts were statistically different from each other ($p<0.05$). The relative infusions effectivity 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 EmM Fe^{II}) and B.*Q.r.* (11.73± EmM Fe^{II}).

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 IC_{50} 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 resinosa* (IC_{50}, 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 IC_{50} values were obtained by infusions (A) from *Quercus sideroxyla* and *Quercus resinosa* 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. resinosa* (A.*Q.r.*) leaves

		Antioxidant capacity			
Samples	Total phenolics as gallic acid equivalents (mg GAE/$g_{d.e.}$)	DPPH scavenging capacity (mg antioxidant/mg DPPH)	FRAP assay (EmM $Fe^{II/I}$)	ORAC (Trolox equivalents, TE)	Inhibition of radical OH IC_{50} (ppm)
A.*Q.s.*	537±0.03	0.62±0.03	11.18±0.05	146±4.82	13±0.1
A.*Q.e.*	331±0.05	0.72±0.08	3.92±0.10	119±1.61	21±0.1
A.*Q.r.*	227±0.01	0.22±0.10	1.97±0.00	154±13.25	41±1.0
B.*Q.s.*	200±0.01	0.70±0.18	1.43±0.01	101±8.26	13±1.0
B.*Q.e.*	278±0.04	2.76±0.00	1.00±0.00	120±6.65	22±2.0
B.*Q.r.*	750±0.06	0.37±0.00	11.73±0.00	188±3.37	39±6.0
C.*Q.s.*	500±0.02	1.51±0.05	17.78±0.03	246±7.32	15±0.1
C.*Q.e.*	603±0.03	0.17±0.03	8.25±0.01	265±11.70	21±1.0
C.*Q.r.*	565±0.03	0.14±0.01	11.46±0.01	178±2.57	116±1.0
D.*Q.s.*	161±0.00	0.92±0.04	0.92±0.00	262±11.11	14±1.0
D.*Q.e.*	420±0.02	0.42±0.01	5.61±0.00	160±3.89	22±3.0
D.*Q.r.*	524±0.04	0.23±0.01	7.91±0.00	129±8.75	82±4.0
E.*Q.s.*	739±0.01	0.47±0.07	4.44±0.02	189±1.42	17±0.1
E.*Q.e.*	---	---	---	---	---
E.*Q.r.*	631±0.03	0.18±0.01	9.60±0.01	151±2.84	95±3.0

Table 2: HPLC profile of leaves infusions from three oak species

Peak label	Retention time (min)	m/z M-H ions	*Quercus sideroxyla*	*Quercus eduardii*	*Quercus resinosa*	Suggested compound
1	6.63	125, 169	√	√	√	Gallic acid*
2	7.25	301, 467, 1084			√	Quercetin polymer
3	7.37	169, 125, 343		√		Gallic acid glycoside
4	8.18	301, 467, 934			√	Quercetin polymer
5	8.60	301, 425			√	Quercetin derivative
6	10.58	153, 315		√		Protocatechuic acid glycoside
7	10.83	301, 425, 469			√	Quercetin derivative
8	14.03	245, 289	√	√	√	Catechin*
9	14.15	375, 435	√	√		Limocitrol
10	15.63	169, 125, 301		√		Quercetin gallate
11	17.33	301, 425			√	Ellagic acid derivative
12	18.97	125, 169, 305, 467, 937		√		Epigalocatechin gallate glycoside
13	19.45	301, 479		√		Ellagic acid glycoside
14	21.53	301	√	√	√	Ellagic acid*
15	21.97	301, 463	√			Quercetin glycoside
16	22.97	227, 255, 285, 593			√	Kaempferol glycoside
17	23.57	301, 477	√			Quercetin glycoside
18	24.58	301, 447		√		Quercetin glycoside
19	25.10	151, 271, 579			√	Naringin*
20	30.13	227, 255, 285, 593			√	Kaempferol glycoside
21	30.70	301	√			Quercetin*
22	32.02	151, 271			√	Naringenin*

*Compound identification was confirmed by comparison with authentic standards

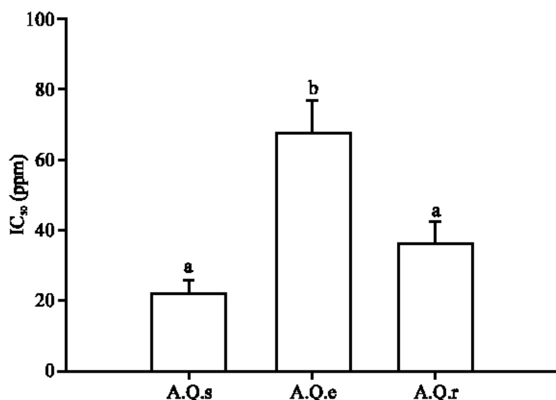

Fig. 2: Median Inhibition Concentration (IC_{50}) for the oxidation of low density lipoproteins (LDLs) by infusions of *Quercus sideroxyla* (A.*Q.s.*), *Q. resinosa* (A.*Q.r.*) and *Q. eduardii* (A.*Q.e.*) leaves

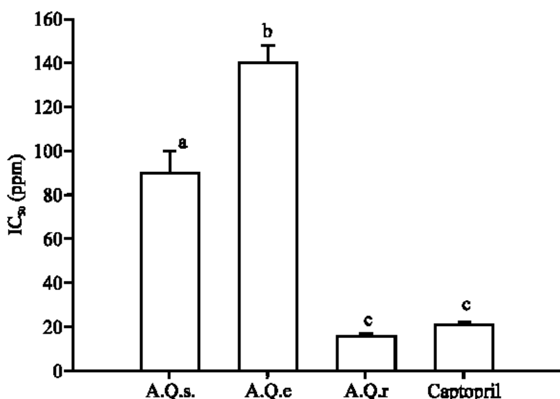

Fig. 3: Median Inhibition Concentration (IC_{50}) of Angiotensin Converting Enzyme (ACE) by infusions of *Quercus sideroxyla* (A.*Q.s.*), *Q. resinosa* (A.*Q.r.*) and *Q. eduardii* (A.*Q.e.*) leaves compared with Captopril, a hypotensive control

DISCUSSION

Total phenolic content in oaks leaves infusions and partially purified fractions differed greatly and were significantly highest in *Q. sideroxyla* infusions (537±0.03 GAE/$g_{d.e}$) and particularly concentrated in its aqueous acetone purified fraction (E.*Q.s.*) (739±0.01 GAE/$g_{d.e.}$). However, results obtained in present study are higher than reported by Rocha-Guzman *et al.* (2009) for *Quercus resinosa* leaves infusions, differences could be attributed to the different age of plant and its growth location different year and location.

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 (Tachakittirungrod *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-picrilhydrazyl (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. resinosa* leaves exhibited the highest antioxidant activity in comparison with the other species in study. Thus our results indicate that phytochemicals found in *Q. resinosa* 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 Fe^{3+} to Fe^{2+} that depends on the reduction of a ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex to a ferrous tripyridyltriazine (Fe^{2+}-TPTZ) by a reducing agent at low pH. The generated ferrous ion is a well-known pro-oxidant, due to its reactivity with H_2O_2 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 *Q. sideroxyla*. A good correlation between total phenolic content and FRAP values was observed for infusions of the three species (Y = 3.0488X-543.82, R^2= 0.9823). A significant increase in reducing power was found in infusions from *Q. resinosa* 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 founded 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 founded 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, H_2O_2 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 *Erythrobalanus* species (red oaks), while they are different from *Leucobalanus* 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 IC_{50} 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 gallocatechin 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). Proline 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^{2+} 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^{2+} ($CuSO_4$) 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. sideroxyla* showed presence of quercetin and catechin. In *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. resinosa* 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 A.*Q.r.*, consequently more experimental work is needed for clarifying this point.

REFERENCES

Abou-Karam, M. and W.T. Shier, 1999. Inhibition of oncogene product enzyme activity as an approach to cancer chemoprevention. Tyrosinase-specific protein kinase inhibition by purpurogallin from *Quercus* sp. Nutgall. Phyto Res., 13: 337-340.

Actis-Goretta, L., J.I. Ottaviani, C.L. Keen and C.G. Fraga, 2003. Inhibition of Angiotensin Converting Enzyme (ACE) activity by flavan-3-ols and procyanidins. FEBS Lett., 555: 597-600.

Afolayan, A.J., O.M. Aboyade and M.O. Sofidiya, 2008. Total phenolic content and free radical scavenging activity of *Malva parviflora* L. (Malvaceae). J. Biol. Sci., 8: 945-949.

Benzie, I.F. and J.J. Strain, 1999. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol., 299: 15-27.

Berrougui, H., M. Cherki, G.A. Koumbadinga, M. Isabelle, J. Douville, C. Spino, 2007. Antiatherogenic activity of extracts of *Argania spinosa* L. pericarp: Beneficial effects on lipid peroxidation and cholesterol homeostasis. Can. J. Physiol. Pharma., 85: 918-927.

Brand-Williams, W., M.E. Cuvelier and C. Berset, 1995. Use of a free radical method to evaluate antioxidant activity. Lebensm. Wiss. Technol., 28: 25-30.

Caldwell, C.R., 2001. Oxygen radical absorbance capacity of the phenolic compounds in plant extracts fractionated by high-performance liquid chromatography. Anal. Biochem., 293: 232-238.

Cespedes, C.L., M. El-Hafidi, N. Pavon and J. Alarcon, 2008. Antioxidant and cardioprotective activities of phenolic extracts from fruits of Chilean blackberry *Aristotelia chilensis* (Elaeocarpaceae), Maqui. Food Chem., 107: 820-829.

Chirinos, R., D. Campos, M. Warmier, R. Pedreschi, J.F. Rees and Y. Larondelle, 2008. Antioxidant properties of mashua (*Tropaeolum tuberosum*) phenolic extracts against Food Chem., 111: 98-105.

Cordova-Villalobos, J.A., J.A. Barriguete-Melendez, A. Lara-Esqueda, S. Barquera and M. Rosas-Peralta *et al*., 2008. Las enfermedades crónicas notransmisibles en Mexico: Sinopsis epidemiológica y prevención integral. Salud Publica de Mexico, 50: 419-427.

Cushman, D.W. and H.S. Cheung, 1971. Spectrophotometric assay and properties of Biochem. Pharmacol., 20: 1637-1648.

Dawra, R.K., H.P.S. Makkar and B. Singh, 1988. Total Phenolic, condensed tanning and protein-precipitable phenolics in young and mature leaves of oak species. J. Agric. Food Chem., 36: 951-953.

Di Majo, D., M. la Guardia, S. Giammanco, L. la Neve and M. Giammanco, 2008. The antioxidant capacity of red wine in relationship with its polyphenolic constituents. Food Chem., 111: 45-49.

Dorman, H.J., O. Bachmayer, M. Kosar and R. Hiltunen, 2004. Antioxidant properties of aqueous extracts from selected Lamiaceae species grown in Turkey. J. Agric. Food Chem., 52: 762-770.

Dudonne, S., X. Vitrac, P. Coutiere, M. Woillez and M. Merillon, 2009. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD and ORAC assays. J. Agric. Food Chem., 57: 1768-1774.

Fernandes, A., I. Fernandes, L. Cruz, N. Mateus, M. Cabral and V. Freita, 2009. Antioxidant and biological properties of bioactive phenolic compounds from *Quercus suber* L. J. Agric. Food Chem., 57: 11154-11160.

Ghazi, F., M. Firoozrai, B. Dabirmanesh and A. Shabani, 2009. Serum angiotensin converting enzyme activity total antioxidants and ascorbic acid in iranian patients with coronary artery disease. J. Biol. Sci., 9: 612-616.

Halliwell, B., J.M.C. Gutteridge and O.I. Aruoma, 1987. The deoxyribose method, a simple test tube assay for determination of rate constants for reactions of hydroxyl radicals. Anal. Biochem., 165: 215-219.

Ho, C.T., Q. Chen, H. Shi, L.Q. Zhang and R.T. Rosen, 1992. Antioxidative effect of polyphenol extract prepared from various Chinese teas. Prev. Med., 21: 520-525.

Huang, D., B. Ou and R.L. Prior, 2005. The Chemistry behind antioxidant capacity assays. J. Agric. Food Chem., 53: 1841-1856.

Jialal, I. and C.J. Fuller, 2009. Oxidized LDL and antioxidants. Clin. Cardiol., 16: 6-9.

Kaur, G., M. Athar and M.S. Alam, 2008. Quercus infectoria galls possess antioxidant activity and abrogates oxidative stress-induced functional alterations in murine macrophages. Chem. Biol. Interact., 171: 272-282.

Liu, J.C., F.L. Hsu, J.C. Tsai, P. Chan and J.H.L. Liu *et al*., 2003. Antihypertensive effects of tannins isolated from traditional Chinese herbs as non-specific inhibitors of angiotensin converting enzyme. Life Sci., 73: 1543-1555.

Loy, S., R. Simon and R. Delgado, 2002. VIMANG, un potencial protector de la peroxidación lipídica en lipoproteínas de baja densidad. Rev. Cub. Inves. Biomed., 21: 167-170.

Luna-José A.D.L., L. Montalvo-Espinosa and B. Rendón-Aguilar, 2003. Los usos no leñosos de los encinos en México. Bol. Soc. Bot. Mexico, 72: 107-117.

Nagao, T., T. Hase and I. Tokimitsu, 2007. A green tea extract high in catechins reduces body fat and cardiovascular risks in humans. Obesity, 15: 1473-1483.

Packer, L., G. Rimbach and F. Virgili, 1999. Antioxidant activity and biologic properties of a procyanidin-rich extract from pine (*Pinus martima*) bark. Pycnogenol. Free rad. Biol. Med., 27: 704-724.

Pietta, P.G., 2000. Flavonoids as antioxidants. J. Nat. Prod., 63: 1035-1042.

Rice-Evans, C.A., N.J. Miller and G. Paganga, 1996. Structure antioxidant activity relationship of flavonoids and phenolic acids. Free Radic. Biol. Med., 20: 933-956.

Rocha-Guzman, N.E., J.A. Gallegos-Infante, R.F. Gonzalez-Laredo, R. Reynoso-Camacho and M. Ramos-Gomez *et al*., 2009. Antioxidant activity and genotoxic effect on HeLa cells of phytophenolic compounds from infusions of *Quercus resinosa* leaves. Food Chem., 115: 1320-1325.

Rocha-Guzman, N.E., R.F. Gonzalez-Laredo, J.A. Gallegos-Infante and I. Lares-Assef, 2007. Evaluacion Biologica Del Efecto de Extractos Polifenolicos de *Quercus resinosa* Sobre Celulas Transformadas. In: Aprovechamiento Biotecnologico de Productos Agropecuarios, Tellez-Luis, S.J., G. Bustos-Vazquez and G.V. de la Cruz (Eds.). Plaza y Valdes-UAT., Mexico, pp: 219-237.

Roginski, V. and E.A. Lissi, 2005. Review of methods to determine chain-breaking antioxidant activity in food. Food Chem., 92: 235-254.

Singh, R., S. Singh, S. Kumar and S. Arora, 2007. Free radical-scavenging activity of acetone extract/fractions of *Acacia auriculiformis* A. Cunn. Food Chem., 103: 1403-1410.

Srivastava, A., L.J. Mohan Rao and T. Shivanandappa, 2007. Isolation of ellagic acid from the aqueous extract of the roots of *Decalepis hamiltonni*: Antioxidant activity and cytoprotective effect. Food Chem., 103: 224-233.

Tachakittirungrod, S., S. Okonogi and S. Chowwanapoonpohn, 2007. Study on antioxidant activity of certain plants in Thailand: Mechanism of antioxidant action of guava leave extract. Food Chem., 103: 381-388.

Vergé, S., T. Richard, S. Moreau, A. Nurich, J.M. Merillon, J. Vercauteren and J.P. Monti, 2002. First observation of solution structures of bradykinin-penta-O-galloyl-D-glucopyranose complexes as determined by NMR and simulated annealing. Biochem. Biophys. Acta, 1571: 89-101.

Vinson, J.A., J. Jang, Y.A. Dabbagh, M.M. Serry and S. Cai, 1995. Plant polyphenols exhibit lipoprotein-bound antioxidant activity using an *in vitro* oxidation model for heart disease. J. Agric. Food Chem., 43: 2798-2799.

Waterman, P.G. and S. Mole, 1994. Analysis of Phenolic Plant Metabolites. Methods in Ecology. Blackwell Scientific publications, London, UK., pp: 238.