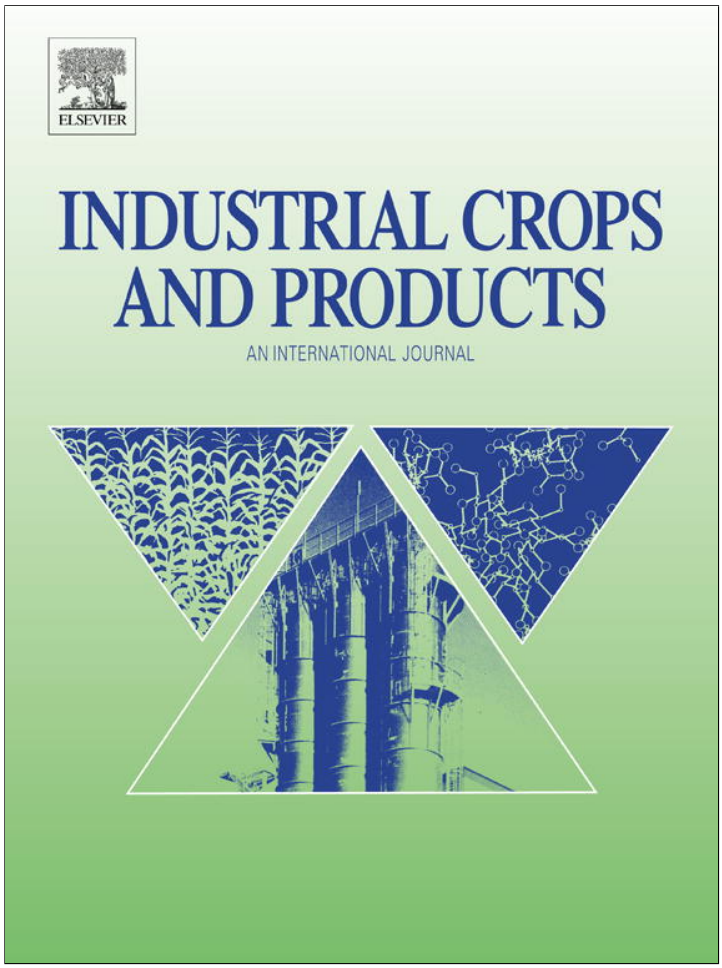

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Mesquite leaves (*Prosopis laevigata*), a natural resource with antioxidant capacity and cardioprotection potential

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ABSTRACT

The antioxidant activity of acetone extracts and purified fractions from *Prosopis laevigata* leaves were evaluated as well its cardioprotection potential *in vitro*. Mezquite leaves were dewaxed with petroleum ether and extracted with aqueous acetone (70%); the polar extract was purified in Sep-Pak® Cartridges and their fractions evaluated. Significant differences among fractions and crude extracts were found in total phenolic content (Folin Ciocalteu), antioxidant capacity by scavenging hydroxyl and DPPH radical assays. Purified fractions showed antihypertensive effects inhibiting angiotensin converting enzyme and cardioprotection inhibiting low density lipoprotein oxidation. The HPLC profile displayed phenolic compounds such as gallic acid, catechin, gallocatechin, epicatechin gallate, rutin, and luteolin that may explain these antioxidant and biological properties. Mesquite leaves can be a source of bioactive phenolics as nutraceutical ingredients.

1. Introduction

Currently, there is a generalized interest for balanced diets rich in fruits and vegetables and their direct relationship with human health and well-being. The contribution of natural phytochemicals present in foods to reduce the risk of chronic diseases is being continuously reported (De La Rosa et al., 2010). Cell damage from unbalance of free radicals that endures as an oxidative stress disorder affects the cellular structure and its components. Then tissues and organs become disturbed as a result of the metabolic syndrome, which is often characterized by oxidative stress that eventually promotes the appearance of degenerative conditions such as cancer, diabetes and cardiovascular diseases (Roberts and Sindhu, 2009).

Plants, herbs and spices have been used as remedies in traditional medicine and attained a wide recognition as sources of bioactive phytochemicals with prophylactic benefits. A distinctive property of these active principles is their antioxidant capacity, which is common in many secondary metabolites from the plant kingdom (Yadav et al., 2012). The major natural antioxidants are polyphenols, although antioxidation is not a property limited only to them. Polyphenols protect macromolecules from the cell structure and its parts from being damaged by free radicals and reactive species, avoiding diseases such as atherosclerosis (Han et al., 2007). Plant phenols, as named in industry, are used as the active principles in many actual formulations (Quideau et al., 2011). Hence, there is interest in searching for bioactive plant antioxidant sources that might prevent from oxidative stress-induced degradation or fight its negative effects in biological systems.

Mesquite (*Prosopis* spp.) is an endemic tree that belongs to the Leguminosae family and Mimosaceae subfamily, and comprises 44 species distributed at arid and semiarid regions over one third of the earth surface (Burkart, 1976). In Mexico there are 11 *Prosopis* species: *P. odorata*, *P. glandulosa*, *P. velutina*, *P. articulata*, *P. tamaulipana*, *P. yaquiana*, *P. vidaliana*, *P. mezcalana*, *P. mayana*, *P. juliflora*, and *P. laevigata* (Cedillo and Mayoral, 1997). Mesquite is an ecologically important plant because it fixes nitrogen in soil, promoting the growing of associated shrub and bushes species, which diminishes soil erosion (Golubov et al., 2001). Its stem and branches are used for wood and charcoal production, and as firewood; from its pods a kind of honey and other edible products are obtained, and the pods along the leaves are also used as forage for cattle and small ruminants (Rodriguez-Franco and Maldonado-Aguirre, 1996). Heartwood from *Prosopis* species has shown resistance against fungal attack, mainly suggesting (−)-mesquitol as the bioactive flavanol compound (Pizzo et al., 2011). However, it cannot be considered as the unique and definite factor to explain the durability of this woody material.

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Leaves from *P. juliflora* are reported containing plant growth inhibitory alkaloids (Nakano et al., 2004), and evaluated for antioxidant and antitumor potential of their phenolic extracts (Sathiya and Muthuchelian, 2010). *P. glandulosa* leaves have shown antiparasitic and antimicrobial properties (Rahman et al., 2011). *P. cineraria* and *P. juliflora* leaf extracts were also tested positively as antibacterial and antifungal agents (Napar et al., 2012). In particular, *P. laevigata* leaves have also been reported with antimicrobial activity (Salinas-Sánchez et al., 2009), with medicinal properties (Monroy and Castillo, 2000), with anthelmintic activity (López-Aroche et al., 2008; De Jesús-Gabino et al., 2010), and as a source for exuded gums (Orozco-Villafuerte et al., 2003). Therefore, the objective of this research was to study the polar extracts from leaves of *P. laevigata* as an unexploited natural resource of bioactive phytochemicals, exploring their antioxidant potential and its cardioprotective effect *in vitro*.

2. Materials and methods

2.1. Plant samples

Leaves from *P. laevigata* were collected at Guadalupe Victoria, Durango, México in October 2008. Botanical samples were positively identified and deposited at the CIIDIR-IPN Unidad Durango Herbarium. Leaves were dried under the shadow, milled (mesh 40), protected from light and kept refrigerated until further use.

2.2. Chemicals

Folin-Ciocalteu reagent, Na_2CO_3, catechin, gallic acid, 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH), trichloroacetic acid (TCA), 2-deoxy-D-ribose, thiobarbituric acid (TBA), ethylenediaminetetraacetic acid (EDTA), *n*-butanol were from SIGMA (Sigma, St Louis, MO, USA). For polyphenol identification the following standards: syringic acid, caffeic acid, gallic acid, coumaric acid, gallocatechin gallate, catechin, epicatechin gallate, gallocatechin, rutin, luteolin, naringenin, morin, and quercetin were used from SIGMA (Sigma, St Louis, MO, USA). HPLC grade solvents water, methanol and acetonitrile; hippuryl-histidyl-leucine (Hip-His-Leu) and angiotensin converting enzyme (ACE) were also from SIGMA (Sigma, St Louis, MO, USA).

2.3. Crude extracts

Sample powder was extensively dewaxed with petroleum ether at room temperature. The air ventilated residue was subjected to extraction, being macerated (18 h) with aqueous acetone (70%) (5×). Common extracts were combined, concentrated by rotoevaporation, and freeze-dried. Extraction yields were calculated as percentage of the dry weight sample, as follows:

$$\text{yield}(\%) = \frac{\text{lyophilized extract(g)}}{\text{dry sample weight(g)}} \times 100$$

2.4. Purified fractions

Acetone crude extract was purified in prepackaged cartridges (Waters Sep-Pak C18) as described in Fig. 1. From the acetone extract three fractions (AE1, AE2, and AE3) were obtained successively. The crude extract and the three purified fractions were concentrated and lyophilized prior to determination of yields, phenolic contents, antioxidant and antihypertensive activities, and chromatographic phenolic profile.

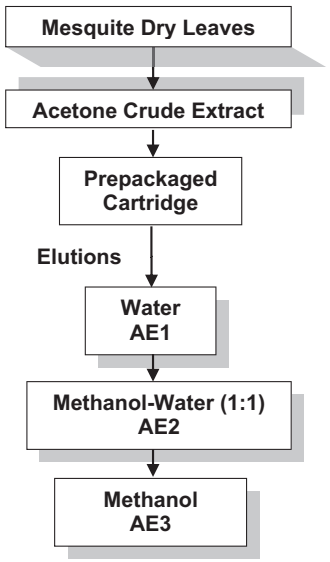

Fig. 1. Purification of acetone crude extracts from mesquite leaves in prepackaged cartridges.

2.5. Total phenolic content (TPC)

The phenolic content of samples was determined using the Folin-Ciocalteu reagent (Waterman and Mole, 1994). A stock solution from every extract was prepared at 1000 μg/mL in distilled water. Sample aliquots of 125 μL were dispensed in tubes containing 500 μL of water and 125 μL of Folin-Ciocalteu reagent (2 N). Tubes were stirred and then left still by 6 min; later 1250 μL of Na_2CO_3 (7%) and 1 mL of water were added and left still in darkness for 90 min; afterwards, the absorbance was measured at 760 nm (Varian Cary® 3C Spectrophotometer, Harbor City, CA, USA). Calibration curves with gallic acid were prepared and results expressed as gallic acid equivalents (GAE) per gram of dry extract (mg GAE/$g_{d.e.}$).

2.6. Chromatographic analysis

Sample analyses were carried out in a High Performance Liquid Chromatography system (HPLC Waters 2487, Autosampler Waters 717, controller with gradient pumps and UV–vis detector). A Supelcosil® LC18 (250 × 4.6 mm, 5 μm) column was used and as mobile phases two eluents were prepared: A, acidified water (acetic acid, pH 2.6) and B, acetonitrile. The sample injection was set at 10 μL, the flow rate kept constant at 1.0 mL/min, and the absorbance reading at 280 nm. The solvent gradient program started at 95% A plus 5% B, then at 70% A plus 30% B (0–25 min), at 40% A plus 60% B (25–30 min), at 40% A plus 60% B (30–35 min), and 95% A plus 5% B (35–40 min).

2.7. Antioxidant capacity

2.7.1. DPPH radical scavenging

This assay was performed according to Brand-Williams et al. (1995). Briefly, DPPH was dissolved in absolute methanol (2.5 mg/100 mL) as well all the samples. A sample aliquot, 100 μL, was mixed with 2900 μL of DPPH solution and the absorbance read at 515 nm (Varian Cary® 3C Spectrometer). The scavenging activity was measured as the median effective concentration (CE_{50}), and as the maximum percentage of inhibition as follows:

$$\text{Inhibition}(\%) = \frac{A_{t=0} - A_t}{A_{t=0}} \times 100$$

where $A_{t=0}$ and A_t are the initial (control) and 30 min absorbance readings, respectively.

2.7.2. Hydroxyl radical scavenging

The •OH radical scavenging activity was determined according to Halliwell et al. (1987) with few modifications. Assay started mixing 100 μL each of deoxy-D-ribose (28 mM), $FeCl_3$ (1 mM), EDTA (1.04 mM), H_2O_2 (1 mM), and ascorbic acid (1 mM) with 500 μL of diluted sample in phosphate-buffered saline (PBS, 50 mM, pH 7.4), and then incubated for 1 h, at 37 °C. Later, 1 mL of TCA (2.8%) and 1 mL of TBA (1.0%) were added, and heated at 95 °C by 20 min. The chromophore formation was monitored at 532 nm and the hydroxyl radicals scavenging activity (AAOH) expressed as the inhibition percentage of the induced degradation of deoxyribose into malonaldehyde as follows:

$$\text{AAOH}(\%) = \frac{\text{absorbance control} - \text{absorbance sample}}{\text{absorbance control}} \times 100$$

2.7.3. Inhibition of low density lipoprotein (LDL) oxidation

The inhibition of LDL oxidation assay was done as described by Loy et al. (2002). Low density lipoproteins were precipitated from human plasma. The reactant ingredients consisted of 700 μL of phosphate-buffered saline (PBS, 50 mM, pH 7.4), 100 μL of $CuSO_4$ (0.5 mM), 100 μL of LDL, 100 μL of samples or standards. For the control 100 μL of PBS was added instead of sample. The mixture was incubated for 3 h at 37 °C, and then, 1 mL of TCA (5%) and 1 mL of TBA (0.37%) were added. The organic phase was separated with *n*-butanol and the absorbance read at 532 nm. Results were expressed as the percentage of inhibition of LDL oxidation and from them as the median inhibitory concentration IC_{50}.

$$\text{Inhibition}(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

2.8. Angiotensin converting enzyme (ACE) inhibition

The ACE inhibition assay was implemented following the spectrometric procedure by Cushman and Cheung (1971). Analysis consisted in mixing 80 μL of sample diluted a several concentrations in boric acid buffer (0.1 M), 200 μL of hippuryl-histidyl-leucine (HHL) diluted in buffer, and 20 μL of the enzyme (0.1 unit/mL). The mixture was incubated for 30 min at 37 °C and then, added with 250 μL of HCl (1 N) to stop the reaction. The product was mixed with 1700 μL of ethyl acetate, stirred for 20 s, and centrifuged at 3000 rpm for 10 min. Then, the organic phase was separated and evaporated, resuspended in 1 mL of distilled water, and stirred for 20 s before reading absorbance at 228 nm to measure hippuric acid formation. Captopril, the first active principles developed for prescribed antihypertensive drugs, was used as standard. The ACE inhibition was measured as follows:

$$\text{Inhibition}(\%) = \frac{A - B}{A - C} \times 100$$

where A is the absorbance reading at 280 nm with no sample, B is the absorbance at 228 nm of hippuric acid plus sample, and C is the reading at 280 nm of the control. The ACE median inhibitory concentration (IC_{50}) was also determined.

2.9. Statistical analysis

All experiments were performed by two replicates and data analyzed by standard statistics methods to determine analysis of variance, averages and standard deviations.

3. Results and discussion

3.1. Extractive yields and phenolic content

An original sample of 500 g of mesquite dry leaves was extracted with aqueous acetone affording 91 g of dry extract (18.2%), which is a significant yield of about one fifth of the raw material (Table 1). An equivalent ethanolic extract from *P. juliflora* produced only 13.75% (Sathiya and Muthuchelian, 2010). The purified fractions from one gram of crude acetone extract gave 60.33 mg of AE1 (6.03%), 58.81 mg of AE2 (5.88%), and 36.25 mg of AE3 (3.62%).

Although many polar organic solvents have been used for phenolic extraction, it seems that aqueous acetone is one of the most polyphenol selective and significant solvents. It has been reported for other natural products, where the greatest amount of total phenolic oligomers and particularly proanthocyanidins was extracted efficiently by 70% acetone (Kallithraka et al., 1995; Karchesy et al., 1989).

The corresponding total phenolic content of crude extracts and purified fractions are shown also in Table 1. The prepackaged cartridge has shown a good performance in retaining the phenolic oligomers from the crude extract. After the Sep-Pak purification, the last fraction (EA3) eluted with methanol has presented the highest phenolic content with 103.6 mg GAE, which is 46% richer than the crude acetone extract. This result is similar to the phenolic content reported for *P. juliflora* leaves (Sathiya and Muthuchelian, 2010). However, these TPC values are relatively lower compared

Table 1
Extract yields, total phenolic content, antioxidant activity by DPPH and deoxyribose assays, and cardio protection analysis by LDL oxidation and ACE inhibitions, in acetone crude extracts and purified fractions from mesquite leaves.

Sample	Yield (%)	TPC[a] (mg GAE/$g_{d.e.}$)	Antioxidant activity		Cardioprotection analysis	
			DPPH scavenging capacity EC_{50} (μg/mL)	Inhibition of radical OH IC_{50} (μg/mL)	Lipid peroxidation IC_{50} (μg/mL)	Angiotensin converting enzyme inhibition (%) IC_{50} (μg/mL)
Acetone extract	18.20	70.84 ± 2.88	2986.29 ± 2.39	1588.34 ± 5.39	368.10 ± 10.87	531.11 ± 22.30
AE1	6.03	47.70 ± 2.28	312.01 ± 4.56	231.91 ± 1.85	208.56 ± 2.39	407.97 ± 3.27
AE2	5.88	49.60 ± 3.56	223.31 ± 2.78	207.81 ± 2.67	151.51 ± 1.91	318.82 ± 5.67
AE3	3.62	103.56 ± 4.74	93.26 ± 3.24	173.04 ± 2.51	76.92 ± 1.16	149.57 ± 0.75

	Antioxidant activity		Cardioprotection analysis	
	DPPH scavenging capacity EC_{50} (μg/mL)	Inhibition of radical OH IC_{50} (μg/mL)	Lipid peroxidation IC_{50} (μg/mL)	Angiotensin converting enzyme inhibition (%) IC_{50} (μg/mL)
Catechin	81.24 ± 2.14	44.60 ± 3.05	13.61 ± 1.66	–
Gallic acid	48.72 ± 2.78	38.48 ± 2.12	11.78 ± 2.76	–
Captopril	–	–	–	17.21 ± 0.44

[a] Milligram of gallic acid equivalents per gram of dried extract.

Table 2
Chromatographic profile of acetone crude extracts and purified fractions from mesquite leaves.

Phenolic compounds (retention time, min)	Concentration (mg/100 g of sample)			
	Crude extract	Purified fractions		
	Acetone	AE1	AE2	AE3
Gallic acid (6.69)	8.0 ± 2.90	–	25.0 ± 5.54	–
Coumaric acid (17.29)	355.0 ± 4.72	635.0 ± 4.32	–	–
Caffeic acid (12.05)	–	–	–	–
Catechin (7.58)	–	–	162.5 ± 2.55	–
Gallocatechin (3.90)	340.0 ± 3.70	668.0 ± 4.88	–	–
Epicatechin gallate (18.33)	10.0 ± 1.23	71.0 ± 1.71	16.0 ± 1.18	12.0 ± 1.23
Quercetin (30.21)	–	–	–	–
Rutin (20.34)	223.8 ± 4.25	256.1 ± 2.19	222.4 ± 1.51	226.9 ± 2.17
Morin (27.62)	236.5 ± 2.65	–	–	–
Naringenin (33.82)	20.0 ± 1.23	–	–	–
Luteolin (30.46)	–	–	–	13.0 ± 2.88

with traditional beverages that are rich in phenolics such as: Cocoa (611 mg GAE), red wine (340 mg GAE), green tea (165 mg GAE), and black tea (124 mg GAE) (Lee et al., 2003).

3.2. Chromatography analysis

The HPLC chromatographic profile of acetone crude extracts and purified fractions from mesquite leaves allowed the positive identification of key polyphenols. The reference compounds in decreasing concentration order were the following: gallocatechin, coumaric acid, morin, rutin, catechin, gallic acid, naringenin, epicatechin gallate, and luteolin. Their concentration and retention times are shown in Table 2. This relative order correlates also with the elution output when purifying the crude extract, starting with water, next using water–methanol mixture, and finishing with methanol. Rutin seems to be abundant and splitting among all purified fractions, while coumaric acid and gallocatechin are more hydrophilic showing up in AE1.

As a reference, polar extracts from *P. juliflora* wood and bark showed important amount of flavanols such as catechin, 4′-O-methyl-gallocatechin and mesquitol (Sirmah et al., 2011). Moreover, aqueous extract from same mesquite leaves contain water-soluble allelochemicals, which could inhibit seed germination and reduce radicle length of germinated wheat (Siddiqui et al., 2009). However, as far our knowledge, there are not specific reports yet on polyphenol composition and antioxidant activity of leaves from *P. laevigata*.

3.3. Antioxidant capacity

3.3.1. DPPH radical scavenging

The EC_{50} for DPPH radical scavenging capacity by acetone crude extracts was about 3000 ppm, which was still two orders of magnitude behind the controls catechin (81 ppm) and gallic acid (49 ppm). However, when analyzing the median effective capacity from the purified fractions, we got a more competitive performance correlated with their phenolic content, finding a graduated enhancement from EA1 (312 ppm) to EA3 (93 ppm) (Table 1, Fig. 2). The acetone purified fractions have shown more capacity to donate hydrogen protons and stabilize the DPPH free radical.

This outcome is better than the partially purified extracts from Mexican pine needles who afforded about 150 ppm in EC_{50} (Saenz-Esqueda et al., 2010), and is equivalent to the methanolic and ethyl acetate extracts from *P. cineraria* leaves, which have shown to have maximum scavenging activity than chloroform and aqueous extracts (Dharani et al., 2011). In another report, *P. cineraria* leaves have also outstood the scavenging activity of *P. juliflora* and *Acacia modesta* methanolic extracts (Napar et al., 2012).

3.3.2. Hydroxyl radical scavenging

When comparing the median inhibition concentration (IC_{50}) of extracts scavenging hydroxyl radicals we got 1588 ppm for the acetone crude extract, while for the standards gallic acid and catechin we have 38.5 and 44.6 ppm (Table 1). However, from the purified fractions of the acetone extract (EA1, EA2, and EA3) we got a substantial enhancement of the •OH scavenging capacity as the IC_{50} ranges from 173 to 232 ppm (Fig. 3 and Table 1). In general, purified fractions from acetone extract were more effective in stabilizing the •OH radical and inhibiting the deoxyribose degradation in a direct relation with their phenolic content. Also, the crude extract and purified fractions showed a straight dose–response relationship up to 10,000 ppm of concentration.

Iron as a transition metal, is vital for the cell homeostasis and it is an essential nutrient of plants. Therefore, it often limits growth, but when is over-accumulated, it might lead to oxidative stress (Walker and Connolly, 2008). In cells, iron is bound to proteins, ferritins, and usually set free by reductive processes (Laulhere and Briat, 1993). As in this assay, reduced iron reacts with H_2O_2 to form hydroxyl radicals (•OH), which have enormous potential to damage organic biomolecules in living cells. In the free radical pathology, the hydroxyl radical is identified as an extremely reactive free radical species formed in biological systems, and if the cell fails to control the levels of ROS, the oxidative damage to DNA is unavoidable (Halliwell, 2006). As we know, hydroxyl radical also have the capacity to cause DNA strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity (Aruoma et al., 1989). In addition, this radical species is considered as one of the rapid indicators of the lipid peroxidation process, removing hydrogen atoms from unsaturated fatty acids (Niki et al., 2005). Thus polar extracts from mesquite leaves may possess antioxidant capacity either to slow down peroxidation processes or to evade cell damaging mechanisms.

3.4. Cardio protection and hypotensive analysis

3.4.1. Inhibition of low density lipoprotein (LDL) oxidation

Cardioprotection potential from mesquite leaves extracts was determined at the first glance by the oxidation inhibition of LDL. Despite of the lipophilic nature of the assay, it was observed a competitive performance from the purified fractions AE2 and AE3, which resulted in the same order of magnitude (76.9 ppm) as the references gallic acid (11.8 ppm) and catechin (13.6 ppm) (Fig. 4 and Table 1).

Purified extracts from mesquite leaves may have the capacity *in vitro* of chelating transition metals such as Cu^{2+} as in this assay or avoiding the generation of peroxide radicals from the oxidized LDLs. Their antioxidant activity may be attributed to the phenolic compounds present such as gallic acid, epicatechin gallate, and

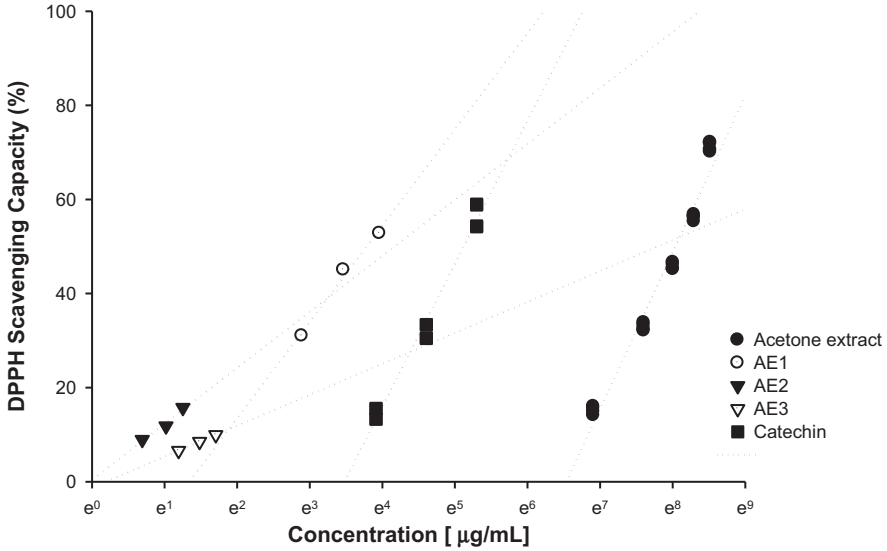

Fig. 2. DPPH scavenging capacity of acetone crude extracts and purified fractions from mesquite leaves compared with catechin.

flavanol glycosides. Epidemiological studies have demonstrated the association between the consumption of catechins or flavonoids (such as from tea) and the reduction of risk reduction of coronary heart disease (Tinahones et al., 2008). Although not all flavonoids are active their hydroxyl group arrangements and related antioxidant capacity could explain their suggested benefit.

A close related species such as *P. cineraria* bark extract has shown hypolipidemic efficacy along with nontoxic effect without interfering the hematology of hypercholesterolemic rabbits (Purohit and Ram, 2012).

3.4.2. Angiotensin converting enzyme (ACE) inhibition

The cardioprotection potential of mesquite leaves extracts was determined also by their evaluation as inhibitors of ACE. Specifically, the antihypertensive activity of acetone extracts and purified fractions from mesquite leaves was estimated by their median inhibitory concentration (IC_{50}) to inhibit ACE. The IC_{50} for the crude acetone extract was 531 ppm, which is relatively distant from the control Captopril (17 ppm), as both are displayed in Fig. 5. From the purified fractions, the more polar one (EA3) showed an IC_{50} of 149.6 ppm, outstanding the previous fractions EA1 and EA2 (Table 1), suggesting also a direct correlation with their phenolic content. In particular, this may be explained from the oligomeric polyphenols present in EA3 fraction that are able to inhibit the angiotensin enzymes I and II in the reaction model with hippuric acid formation.

Takahashi et al. (2007) have reported that the renin inhibitory capacity by legumes can be classified in high and low activities with IC_{50} of 270–420, and 670–1750 µg/mL, respectively. Therefore *P. laevigata* leaves extract can be considered as a highly active

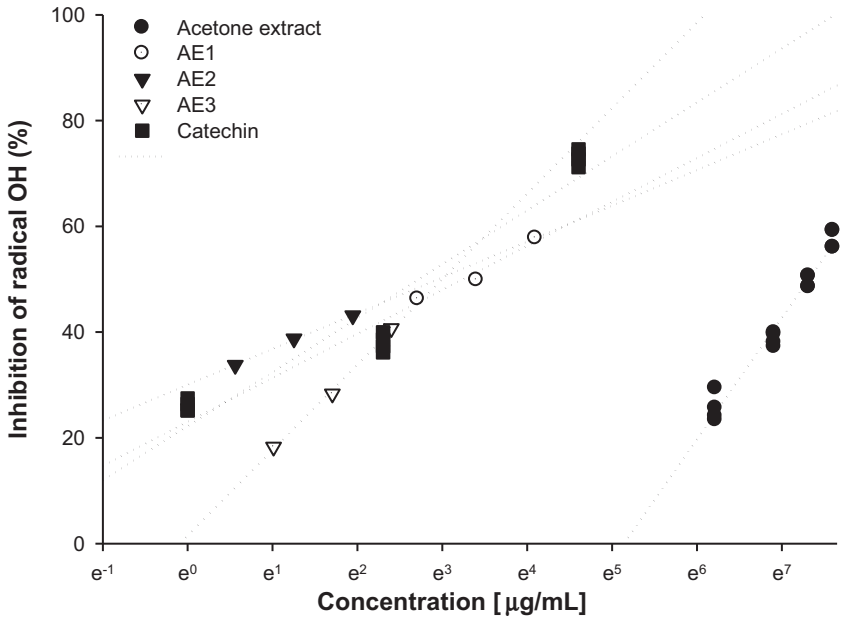

Fig. 3. Hydroxyl radical scavenging activity (%) by the deoxy-D-ribose assay as an indicator of antioxidant capacity of acetone crude extracts and purified fractions from mesquite leaves compared with catechin.

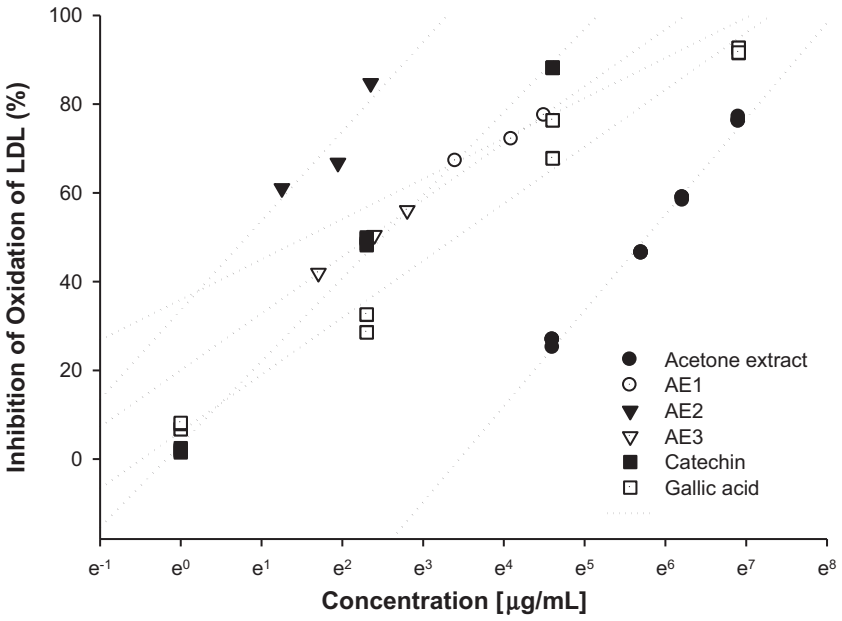

Fig. 4. Inhibition of the low density lipoproteins (LDL) oxidation by acetone crude extracts and purified fractions from mesquite leaves compared with catechin and gallic acid.

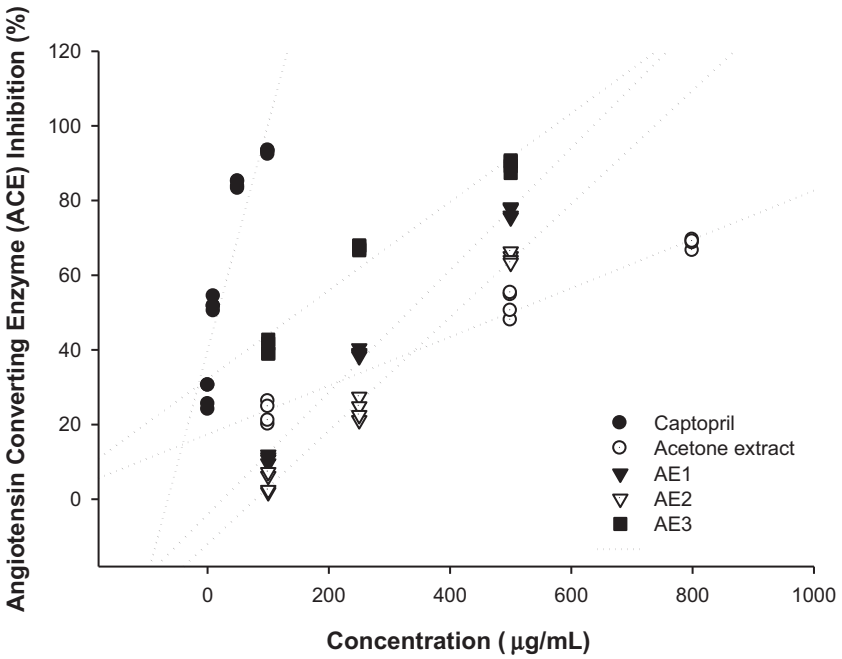

Fig. 5. Angiotensin converting enzyme (ACE) inhibition by crude acetone extracts and purified fractions from mesquite leaves compared with Captopril.

ACE-1 inhibitor. In a parallel study on *P. laevigata* uses, a mesquite pod meal has fallen in the second category (Gallegos-Infante et al., 2012).

Many studies have been reported on single plant species where several classes of ACE inhibitory compounds have been identified and correlated, this is the case of proanthocyanidins, flavonoids, xanthones, peptides, and secoiridoids (Hansen, 1995; Barbosa-Filho et al., 2006). As a reference, an apple skin extract gave an IC_{50} of 49 ppm, consisting mainly of flavonoids and derivatives such as quercetin, quercetin-3-glucoside, quercetin-3-galactoside, quercetin-3-glucuronic, and cyanidin-3-galactoside (Balasuriya and Rupasinghe, 2011). Also, infusions from *Quercus* leaves rich in flavonoid phenolics have shown comparable response than Captopril (Rivas-Arreola et al., 2010). Thus, flavonoids and their glycosides have potential to inhibit ACE *in vitro* and this property varies according to their structure, sugar moiety and its linkage position. Our results suggest that several flavonoids present in mesquite leaves may possess properties for blood pressure regulation; this may be the case of flavanols such as epicatechin gallate and particularly quercetin related flavonols (i.e., luteolin) and their glycosides (i.e., rutin).

4. Conclusions

Polar extracts and their purified fractions from mesquite leaves (*P. laevigata*) have shown antioxidant activity, antihypertensive

response, and cardioprotection effect *in vitro*. This unexploited natural resource might be a source of bioactive phenolics as nutraceutical ingredients. Further work is needed to fully characterize the active principles present in the plant, which are responsible for its biological properties, and elucidate its possible modes of action.

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