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Antioxidant capacity of extracts from hawthorn (*Crataegus mexicana*) skin

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The hawthorn *Crataegus mexicana* is a traditional Mexican fruit with properties that make this fruit useful for the treatment of many ailments, including diseases of the respiratory and urinary tract. This paper reports the antioxidant capacity of the *n*-hexane, dichloromethane, ethyl acetate, acetone, ethanol and methanol extracts of *C. mexicana*. Samples were evaluated for total phenolic and carotenoid contents, 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging, the inhibition of the formation of thiobarbituric acid reactive species (TBARS) and the neutralization of the cation-radical 2,2´-azino-bis(3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt (ABTS). The total phenolic content was 2.65 ± 0.23 mg of gallic acid equivalents per gram, and the carotenoid content was 26.4 ± 0.02 µg/g in dry hawthorn skin. The most active extract in scavenging DPPH radicals and inhibiting TBARS formation was the acetone extract, with activities of 21.9 ± 0.15 and 13.27 ± 0.70%, respectively, at 10 mg/L. The extracts were compared for activity against ascorbic acid, caffeic acid, α- tocopherol and quercetin. The acetone extract was the most active, with an IC_{50} value of 15.2 mg/L in DPPH and 17.7 mg/L in TBARS. A high correlation was observed between the results for TBARS and DPPH. These results demonstrate the potential nutritional and antioxidant value of this Mexican fruit.

Key words: Antioxidant capacity, *Crataegus mexicana,* fruit skin, hawthorn, organic extract.

INTRODUCTION

The worldwide increase in human deaths caused by several chronic degenerative diseases has been well documented (WHO/FAO, 2003). The development of complications from these diseases increases the economic costs of health services. Therefore, it is important to consider how the quality of life in these subjects changes due to a decrease in their working productivity (Biedrzycka and Amarowicz, 2008).

The pathophysiology of many diseases is associated with an increase of free radicals derived from reactive oxygen species (ROS) in the cells (Singh et al., 2008). A higher production of ROS can cause oxidative damage in DNA (Spencer et al., 2012), as well as in proteins and lipids in the cell membranes, and is associated with several inflammatory processes (Dufour et al., 2007). From a physiological perspective, an equilibrium should exist between free radicals and antioxidants in the organism (Blumberg, 2004). Antioxidants play an important role in protecting cells against ROS (Jayakumar and Kanthimathi, 2012). The antioxidant network systems in

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the body include enzymatic defenses, such as the superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH) mechanisms. Other non-enzymatic defense systems utilize ascorbic acid, carotenoid, tocopherol, and phenolic substances (Guimaraes et al., 2010).

Several studies have shown certain clinical benefits associated with the intake of fruits (Crowe et al., 2011). Some fruits have protective properties and help prevent the complications of chronic degenerative diseases, such as atherosclerosis, chronic inflammation, diabetes mellitus, cataracts, coronary diseases and certain types of cancer (Havsteen, 2002).

These characteristics have been attributed to the antioxidant activity of many secondary metabolites in fruits. Many types of fruits also have nutraceutical properties (Andlauer and Fürst, 2002) and a nutritional capacity to prevent chronic degenerative diseases (Willis and Wians, 2003). Studies in fruits from Asia, Europe and America in the *Rosaceas* family and the genus *Crataegus* had shown that contain terpenoids, polyphenols (catechins mainly epicatechin, polymeric and oligomeric proanthocyanidins such as B_2 dimeric procyanidin, cyanidin-3-*O*-galactoside, idaein and chlorogenic acid) and flavonoids (flavonol-*O*-glycoside such as quercetin-3-*O*-galactoside, vitexin-2”-*O*-rhamnoside, acetylvitexin-2”-*O*-rhamnoside, flavone-*C*-glycosides, and hyperóside) (Froehlicher et al., 2009; Shao-Jiang et al., 2011; Calişkan et al., 2012; Edwards et al., 2012). The hawthorn *Crataegus mexicana,* also named “tejocote”, is a traditional fruit in the Mexican diet and in herbal medicine (Cabrera, 1958). The wild type, “criollo”, is found throughout most of Mexico and Northeastern Guatemala. However, there is no scientific evidence that supports the biological and antioxidant properties of *C. mexicana*. In this context, the objective of the present work was to assess the antioxidant activity present in the skin of the hawthorn *C. mexicana*.

MATERIAL AND METHODS

Chemicals and reagents

Polyphenol (quercetin), ascorbic acid, caffeic acid, α-tocopherol, Folin-Ciocalteu reagent, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, trichloroacetic acid, bovine serum albumin (BSA), potassium persulfate, ferrous sulfate, ethylenediaminetetraacetic acid (EDTA), sodium acetate trihydrate, phosphate buffered saline (PBS), 1,1,3,3-tetramethoxypropane, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2´-azino-bis(3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and 2-thiobarbituric acid (TBA) were purchased from Sigma-Aldrich Co (St Louis, MO). Deionized (MilliQ) water was used in the experiments. All of the solvents were of analytical grade.

Plant materials

A total of 4 kg of hawthorn (*C. mexicana)* fruits were harvested 120 days after flowering in the State of Tlaxcala (19°19’20.55”N/98°22’56.15”W; altitude of 2242 m above sea level) in the month of December. Fruits were identified by a biologist according to the guidelines of the botanical garden at the Benemerita Universidad Autonoma de Puebla (voucher 14648). All samples had the same morphological characteristics with respect to the size and color and were free of surface damage. Due to the fact that many fruits contain the highest levels of antioxidants in the skin, only the skin samples of the hawthorn samples were studied. Fruits were hand-washed, and the skin was manually peeled, frozen and lyophilized. A total of 36 g of skin was obtained, which was then processed using a mesh (mesh size 20). Samples were kept frozen at -20°C in a nitrogen atmosphere until use.

Preparation of hawthorn extracts

The hawthorn skin powder (~30 g) was homogenized at room temperature and sequentially extracted for 72 h into 300 mL of the following solvents: *n*-hexane, dichloromethane, ethyl acetate, acetone, ethanol, and methanol. The extracted fractions from each solvent were decanted using a separatory funnel, evaporated, and kept in a sealed amber flask at 5°C.

Quantification of antioxidants

Measurement of total phenolic compounds

The total phenol content of the hawthorn skin was determined using the Folin-Ciocalteu reagent method (Folin and Ciocalteu, 1927) with minor modifications (Singleton et al., 1999; Everette et al., 2010). Briefly, 500 mg of dried skin sample was added to 40 mL of methanol/water (50:50, v/v) for 1 h at 25°C, and the supernatant was then collected and stored (methanol extract). The residual sample was added to 40 mL of acetone/water (70/30, v/v) at 25°C for 1 h (acetone extract). The methanol and acetone extracts were mixed, evaporated and resolubilized in 10 mL of ethanol (ethanol extract). Finally, the residual sample was dissolved in 10 mL of H_2O (aqueous extract). 1 mL of each aqueous and ethanol extract was brought to a volume of 2.5 mL using 0.3% HCl. Subsequently, 50 µL of either this sample or a standard was added to 1 mL of 2% Na_2CO_3, followed by the addition of 50 µL of Folin-Ciocalteu reagent diluted in water (1:1). After a 30 min incubation at 25°C, the absorbance at 750 nm was recorded using a spectrophotometer (Genesys 10 UV, Thermo Electron Co. U.S.A.). The total phenol content was expressed as milligrams of gallic acid equivalent per gram of sample dry weight (GAE/g) using a standard curve (concentration 0.2 to 1.0 mg/mL) for a freshly prepared gallic acid solution (Cai et al., 2004).

Measurement of total carotenoids

Total carotenoids were determined according to Speek et al. (1988). Briefly, 2.0 g of dried skin sample was added into 10 mL of *n*-hexane/acetone/ethanol (50:25:25 v/v). The solution remained at room temperature for 1 h and was then centrifuged at 3000 x *g* for 5 min. The organic phase was dissolved in *n*-hexane to a final volume of 25 mL. A 1.0 mL aliquot was used to measure the absorbance at 450 nm. Concentration values were obtained using the extinction coefficient of β-carotene, ε = 2505, as a standard and were reported as µg/g of dried hawthorn skin according to the following equation:

$$x\ (\mu g) = \frac{A^{*}\ y\ (mL)\ 10^{6}}{A_{1cm}^{1\%}\ 100} \qquad (I)$$

$$x\ (\mu g/g) = \frac{x\ (\mu g}{g\ sample} \quad (II)$$

Where, *x* is the sample concentration of carotenoids, *y* is the sample volume and A* is the extinction coefficient of β-carotene.

Antioxidant activities

Scavenging effect on DPPH

The effects of hawthorn extract antioxidants on free radicals were determined using DPPH (Kim et al., 2005). Briefly, 100 µL of the aforementioned extracts was prepared at a concentration of 20 mg/mL in DMSO. From each sample stock, 1000, 100 and 10 mg/L final dilutions in cool ethanol were prepared. DPPH was dissolved in ethanol (1 mM). A total of 50 µL of sample extract and 150 µL of DPPH were mixed and incubated for 30 min at 37°C in an orbital shaker. The absorbance at 517 nm was recorded using an enzyme linked immunosorbent assay (ELISA) microplate reader (Bio-Tek EL800, U.S.A.). The percent reduced activity of DPPH was calculated and expressed as the IC_{50} (Khattak et al., 2008). Ascorbic acid, caffeic acid, α-tocopherol and quercetin were used as standards.

Lipid peroxidation induced by $FeSO_4$ in rat brain homogenate (TBARS)

The antioxidant activity of the hawthorn skin was estimated using the spectrophotometric method described by Ohkawa et al. (1979). Extract samples at 1000, 100 and 10 mg/L were prepared to inhibit lipid peroxidation induced by Fe^{2+} in rat brain homogenate. Male adult Wistar rats (200 to 250 g) were provided by the Tlaxcala Research Center of Behavior Biology (CTBC) at Universidad Autónoma de Tlaxcala. The study and animal handling were approved by the ethics committee of the Faculty of Health Sciences and the School of Nutrition at the same university. Rats were sacrificed using CO_2 and decapitated as humanely as possible.

The brains were immediately dissected, washed and homogenized in 10 mL of 0.1 M PBS (0.2 g/L KCl, 0.2 g/L KH_2PO_4, 8 g/L NaCl and 2.16 g/L $NaHPO_4.7H_2O$; pH 7.4) at 5°C to produce a 1:10 homogenate (w/v) (Dominguez et al., 2005). The homogenate was centrifuged for 10 min at 3000 x *g*, and the supernatant was adjusted to 2.66 mg of protein/mL in PBS for total protein determination according to the method of Lowry et al. (1951). The analysis of BSA was used to generate the standard curve.

A total of 375 µL (1.0 mg protein content) of brain sample was incubated in an orbital shaker at 37°C for 30 min with 50 µL of 10 µM EDTA and 50 µL of each of the hawthorn extracts to obtain working concentrations of 3 to 50 mg/L. Lipid peroxidation was initiated by the addition of 50 µL of $FeSO_4$ (final concentration = 10 µM) (Ng et al., 2000). After 1 h, the samples were cooled on ice, and 0.5 mL of TBA (1% 2-thiobarbituric acid in 0.05 N NaOH and 30% trichloroacetic acid, 1:1) was added. The samples were centrifuged for 10 min at 3000 x *g*. The final products of the lipid peroxidation from rat brains were assayed by malondialdehyde (MDA) determination using the TBARS assay (Kibanova et al., 2009). The samples were heated at 94°C for 30 min in a water bath. After cooling on ice, 200 µL of each sample and sample blank was placed in an ELISA microplate reader, and the absorbance was recorded at 540 nm. The TBARS results were expressed as the equivalent of MDA relative to 1,1,3,3-tetramethoxypropane as the standard. The percent inhibition is defined as the decrease of TBARS formation due to inhibition caused by the extract (Esterbauer and Cheeseman, 1990).

The inhibition of the lipid peroxidation up to 50% (IC_{50} µg/mL) in each extract was plotted against the *log* of the concentration in each sample of *C. mexicana* and the percent of lipid peroxidation induced by $FeSO_4$ 25 µM (100%).

Neutralization of the ABTS radical cation

The procedure of Delgado et al. (2011) was used to determine the capability of the hawthorn extracts to trap ABTS free radicals. ABTS at 7 mM was mixed with KH_2PO_4 (2.45 mM final concentration) and incubated for 12 to 16 h at 25°C in the dark. The solution was diluted in ethanol to obtain an absorbance of 0.70 at 436 nm. A total of 10 µL of sample extract diluted to 100 mg/L was added to $ABTS^{\bullet+}$ to obtain a final volume of 1.0 mL. The absorbance was measured during 6 min periods to determine the trapping capacity of the extract. The trapping capacity was compared against that of the equivalent of Trolox and was expressed as µM Trolox/g sample extract.

Statistical analysis

Data were expressed as the means ± standard error of mean (SEM) unless otherwise indicated. A one-way analysis of variance (ANOVA) was used to compare the antioxidant capacity and phenolic contents of the hawthorn skin extracts. The Duncan, Tukey, and Pearson correlations were used to compare the extracts. In all cases, significance was accepted at the $p < 0.05$ level. Each experiment was run in triplicate with two control samples. The OrigenLab program (*v*.8) was used for all analyses.

RESULTS AND DISCUSSION

Phenol content of hawthorn skin

A total of 36.0 g of dry hawthorn skin was obtained as previously described. The amount of extract obtained using nonpolar to polar solvents was as follow: *n*-hexane (0.63 g), dichloromethane (0.60 g), ethyl acetate (0.70 g), acetone (0.61 g), ethanol (3.90 g), and methanol (4.10 g). The total phenol content was 2.65 ± 0.23 mg GAE/g of dry sample. These values are similar to the polyphenol contents of other *Crataegus* species such as *C. azarolus* (1.85 mg/g) in Asia and *Crataegus germanica* (3.5 mg/g) in Europe (Edwards et al., 2012), and also in fruits that have an antioxidant activity higher than certain apple varieties (0.46 to 15.1 mg of GAE/g dry sample), as reported in other studies (Cam and Aaby, 2010; Sudha et al., 2007; Garcia et al., 2009). Phenolic compounds can protect against free radicals in aerobic cell metabolic processes. The antiradical activity of the polyphenols is attributed to the interaction of structural parts catalyzing metal chelation, the activation of certain antioxidant enzymes and the inhibition of oxidase enzymes (Ji-Hyun and Kwang-Deog, 2011). Such biological processes could be present in the skin of *C. mexicana.*

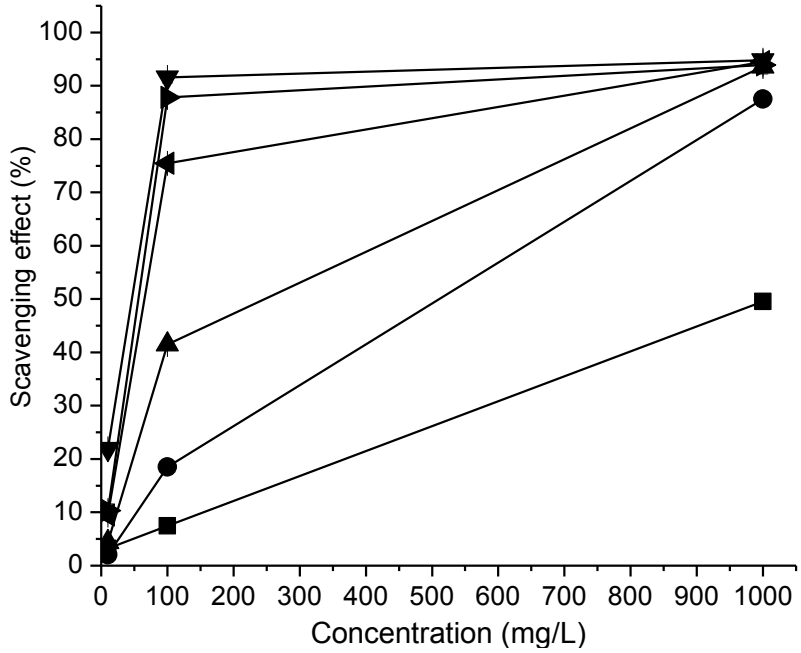

Figure 1. Scavenging of DPPH• radicals in the presence of increasing concentrations of *Crataegus mexicana* extracts at 10, 100 and 1000 mg/L. ■, *n*-Hexane; ●, dichloromethane; ▲, ethyl acetate; ◄, ethanol; ►, methanol; ▼, acetone (*p < 0.05).

Carotenoid content of hawthorn skin

The β-carotenoid content is associated with the intensity of color in fruits and leaves. *C. mexicana* has an intense yellow coloration in both the skin and pulp. This coloration may be associated with the carotenoid content of 26.4 ± 0.02 µg/g measured in dry hawthorn skin. Ying and Oey (2012), reported β-carotenoid contents of cherries, pears and plums of 20, 40 and 30 µg/g, respectively, and Monge-Rojas and Campos (2011) reported a carotenoid content of 27.6 µg/g in apples. A comparison of these results indicates that there is a greater quantity of carotenoids in dry hawthorn skin than in the aforementioned fruits. Therefore, the data demonstrate the large quantity of antioxidant metabolites in this fruit and its nutraceutical properties.

Free radical capture of DPPH

The capacity of *C. mexicana* extract to scavenge DPPH, $O_2^{\bullet -}$, $^{\bullet}OH$ and NO was measured (Figure 1). In this assay, antioxidants react with DPPH, a purple-colored stable free radical, and convert it to a colorless solution. The amount of DPPH reduced can be quantified by measuring the decrease in absorbance at 517 nm (Kaur et al, 2006). The DPPH scavenging ability of the extract may be attributed to its hydrogen donating ability.

The nonpolar extracts (*n*-hexane, dichloromethane and ethyl acetate) were less active in trapping the DPPH radical (7.33 to 41.46%) relative to the polar extracts (acetone, ethanol and methanol) (75.46 – 91.56%) at 100 mg/L. The hawthorn-acetone extract was more active at 10 mg/L, with a DPPH reduction of 21.9 ± 0.15%, compared with the *n*-hexane extract, with a DPPH reduction of 3.2 ± 0.23%. These results show that polar extracts have metabolites with a high capacity to donate hydrogen and electrons, as indicated by the effective neutralization of DPPH (Mishra et al., 2012).

MDA determination by TBARS

Increased lipoperoxide levels have been correlated with cancer, Alzheimer's disease, atherosclerosis and the aging process. The quantitative assessment of conjugated dienes, lipid hydroperoxides, alkanes, aldehydes and isoprostanes has been extensively studied. The effects of free radical damage have received increasing attention (Moore and Roberts, 1998). Therefore, during the last decade, substantial work has been conducted to refine the measurement of lipid peroxidation products.

Rat brain tissue contains both saturated and unsaturated lipids and fatty acids that are susceptible to peroxidation by ROS. Malondialdehyde (MDA) is one of the final products of cell membrane lipoperoxidation. MDA reacts with TBA to form a chromophore that is

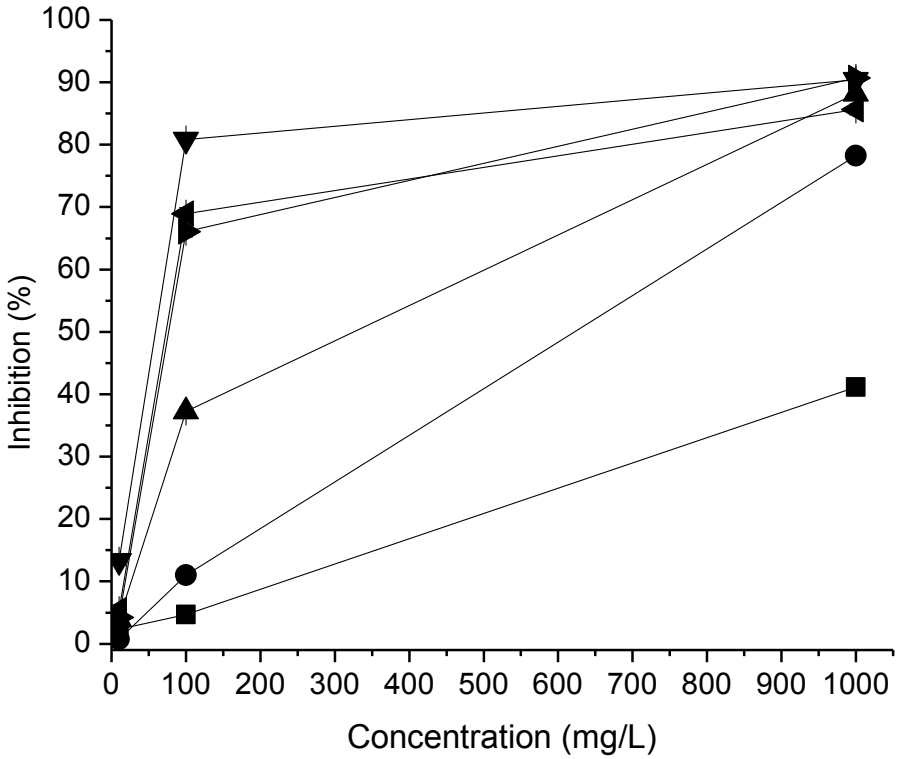

Figure 2. Effects of *Crataegus mexicana* extracts on the production of TBARS in rat brain homogenate. Increasing concentrations of *C. mexicana* extracts at 10, 100 and 1000 mg/L were used. ■, *n*-Hexane; ●, dichloromethane; ▲, ethyl acetate; ◄, ethanol; ►, methanol; ▼, acetone (*$p < 0.05$).

easily measured spectrophotometrically. To determine the antioxidant capacity of hawthorn extracts in rat brain tissue, $FeSO_4$ was used to induce the lipoperoxidation of rat brain cells. The ferrous ion (Fe^{2+}) can form lipid peroxides, which induce the formation of highly reactive substances, such as hydroxyl radicals, perferryl and ferryl species.

To evaluate the ability of the hawthorn extracts to protect the rat brain homogenate from being lipoperoxidated, different concentrations of extracts were used as described above. The polar extracts had a better performance in inhibiting lipid peroxidation, with the most active being the acetone extract (13.27 ± 0.70%) at 10 mg/L and the least active being the *n*-hexane extract (2.35 ± 0.27%) at 10 mg/L (Figure 2).

When compared with the data obtained from the DPPH assay, there was similar trend in the activity of the polar and nonpolar extracts in both experiments.

IC_{50} determination in DPPH and TBARS

To determine the IC_{50} in the DPPH and TBARS experiments, various concentrations of the hawthorn extracts were used according their inhibition response. For the most active extract, concentrations from 3.16 to 56.23 mg/L were used. For the extracts with medium activity, concentrations from 10 to 100 mg/L were used. For the extracts with a low activity, concentrations from 100 to 1000 mg/L were used.

The IC_{50} data for the experiments with DPPH showed that the hawthorn acetone extract was 3-times less active (15.26 ± 0.20 mg/L) than the relative potency of quercetin. The methanol extract ranked second in activity, and the *n*-hexane extract was the least active. Antioxidant activity has been shown to be related to polyphenol content in natural extracts.

The IC_{50} results from the TBARS experiments showed that the acetone extract demonstrated a strong inhibition of lipoperoxidation at a concentration of 16.78 ± 0.33 mg/L, followed by the extracts of methanol, ethanol and *n*-hexane, which was the least active. The acetone extract data were similar to those obtained for the IC_{50} of pure ascorbic acid, caffeic acid, α-tocopherol and quercetin antioxidants at concentrations from 1 to 40 mg/L in TBARS assays. When compared with quercetin (1.00), which had an IC_{50} of 3.56 ± 0.07 mg/L, the acetone extract was five-times less active, with a relative potency of 0.21 for the inhibition of lipoperoxidation (Table 1). In both the TBARS and the DPPH experimental models, the polar extracts were the most active, with a consistent antioxidant activity.

Table 1. [1]Hawthorn (*Crataegus mexicana*) IC_{50} antioxidant capacity compared with synthetic antioxidants determined using DPPH and TBARS assays.

Pure antioxidant	TBARS[1]	Relative potency[2]	DPPH[3]	Relative potency[2]
Ascorbic acid	12.95 ± 0.01	0.27	15.93 ± 0.37	0.33
Caffeic acid	13.55 ± 0.05	0.26	18.65 ± 0.73	0.28
α-tocopherol	33.17 ± 1.13	0.10	23.15 ± 0.67	0.23
Quercetin	3.56 ± 0.07	1.00	5.37 ± 0.14	1.00
Hawthorn extract				
n-Hexane	nd	nd	nd	nd
Dichloromethane	684.90 ± 1.58[a]	5.20×10^{-3}	724.05 ± 1.13[a]	7.41×10^{-3}
Ethyl acetate	550.63 ± 0.72[b]	6.46×10^{-3}	458.49 ± 0.78[b]	0.01
Acetone	16.78 ± 0.33[c]	0.21	15.26 ± 0.20[c]	0.35
Ethanol	54.49 ± 3.56[d]	0.06	54.20 ± 1.93[d]	0.10
Methanol	27.66 ± 0.73[e]	0.12	26.64 ± 0.66[e]	0.20

Means ± SEM of three different experiments. [2]Relative to quercetin as the unitary value. [3]IC_{50} for the neutralization of the radical DPPH in pure antioxidants and hawthorn skin (*C. mexicana*). nd, not determined. Values are expressed as µg/mL (mg/L). Different letters represent a significant difference at $*p < 0.05$ (Duncan analysis).

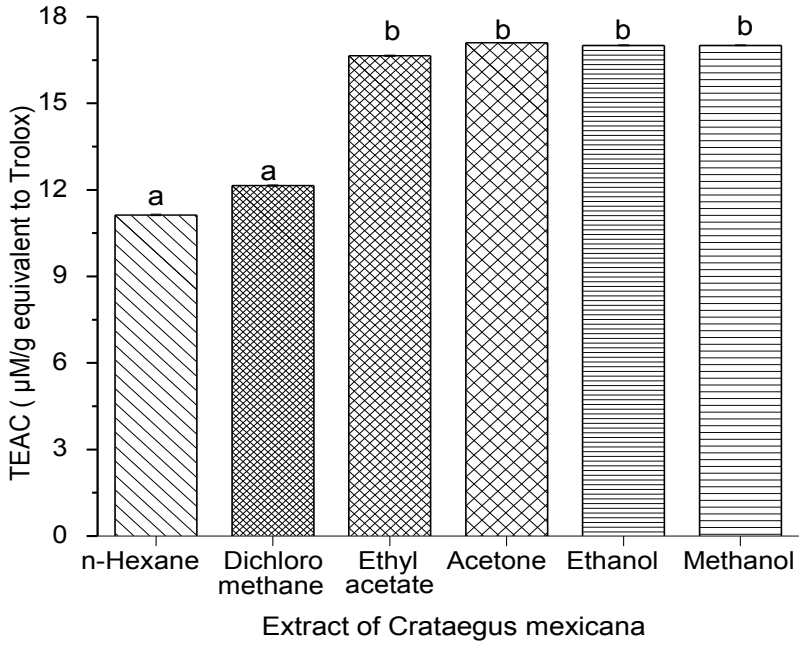

Figure 3. ABTS antioxidant capacity expressed as µM equivalent of Trolox/g of dry skin sample in the six hawthorn extracts of *Crataegus mexicana*. Means ± SEM of three different experiments. Different letters indicate a significant difference at $*p < 0.05$ (Tukey analysis).

ABTS radical scavenging activity

The determination of ABTS radical scavenging activity in hawthorn extracts indicated a lower activity in the nonpolar extracts of 11 and 12 µM of Trolox/g of dry skin sample. In the more polar extracts, the results were between 16 and 17 µM of Trolox/g of dry skin sample (Figure 3). These results are similar to those reported in the skin of dehydrated exotic fruits, with values of 14.6 to 428 Trolox µmol/g (Contreras-Calderón et al., 2011). For comparison, Trolox equivalent antioxidant capacity (TEAC) values of 4.62, 3.87 and 15.18 µM trolox/g have been reported for apple *malus domestica* (red delicious), mango (hainan) and guava (Li et al., 2011), respectively.

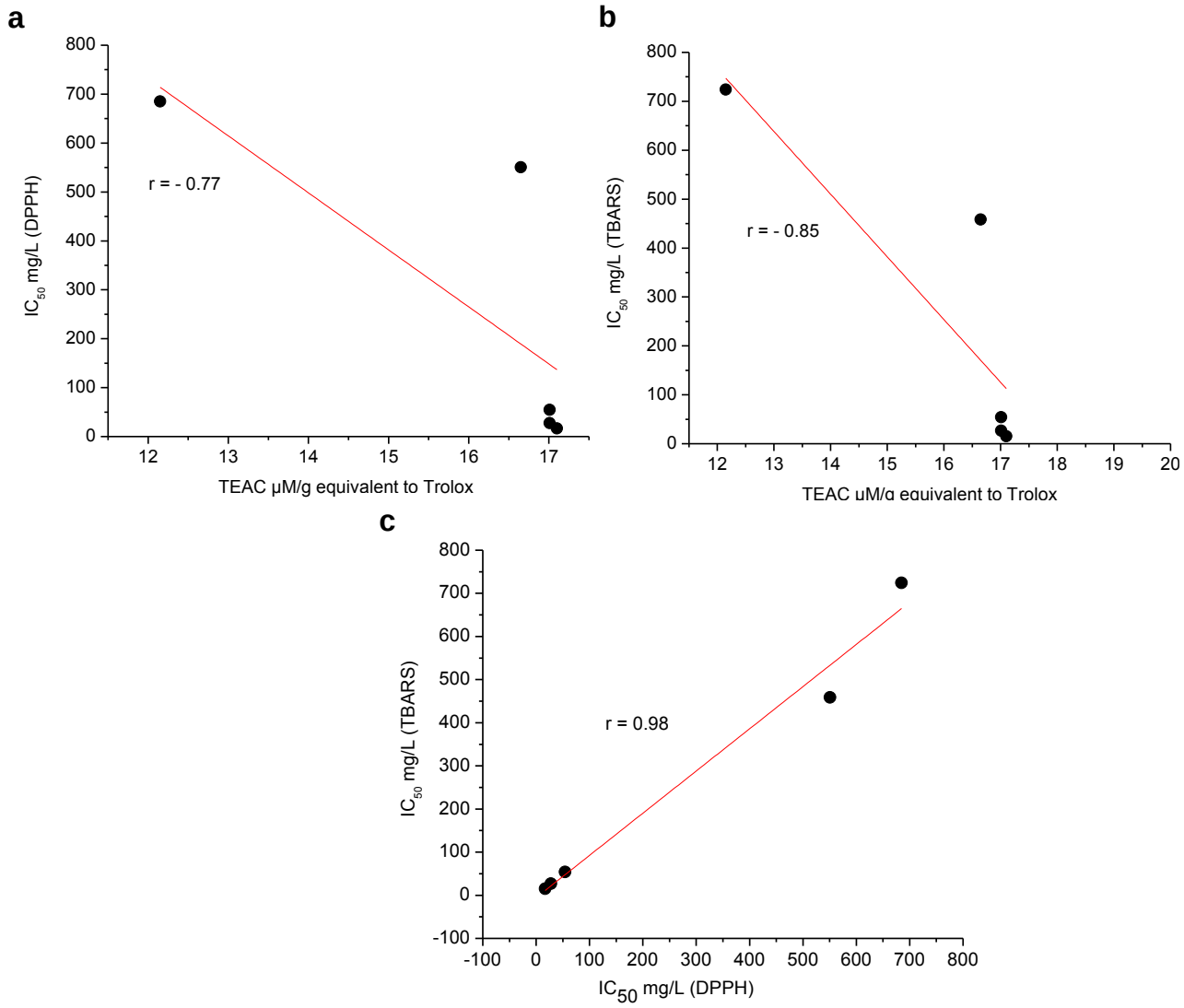

Figure 4. Pearson analyses of the six extracts from hawthorn skin (*Crataegus mexicana*). (a) DPPH *vs.* ABTS, (b) TBARS *vs.* ABTS, **(c)** TBARS *vs.* DPPH.

The data suggests a high polyphenol content in the polar extracts of hawthorn *C. mexicana* (Figure 3).

These results demonstrate that the dry hawthorn skin sample has a greater antioxidant capacity than those of apple, mango and guava. The results of the DPPH and TBARS activity assays using the polar extracts showed a higher correlation with the equivalent of TEAC. The Pearson statistical analysis shows a negative correlation between ABTS and DPPH (R = -0.77). These data show that extracts with low polarity have a low antioxidant capacity (Figure 4).

A lower correlation (R = -0.85) was observed for the TBARS and ABTS results because the methanol extract had a poor response in the TBARS assay and a good response in the ABTS assay. Finally, the correlation between the TBARS and DPPH data (R = 0.98) showed a similar behavior among the different extracts.

Conclusions

Fruits from the *rosaceas* family and the genus *crataegus* contain terpenoids and flavonoids (Shao-Jiang et al., 2011; Calişkan et al., 2012). However, there is insufficient data on the contents of phenolic and terpenic metabolites in these fruits. This work evaluated the antioxidant capacity of *C. mexicana* and revealed the major antioxidant activity of the acetone extract. This activity is likely the result of the action of medium- and high-polarity metabolites.

The total antioxidant capacity of hawthorn (*C. mexicana*) was evaluated *in vitro* using colorimetric techniques. The final data are similar to those reported in other *Crataegus* species in Asia (Calişkan et al., 2012) and Europe (Froehlicher et al., 2009; Zesheng et al., 2001), and also for the empire and golden delicious apple

varieties, strawberry, black thorn, plum and cherry (Lata and Tomala, 2007). These results improve the knowledge of the potential nutritional and healthful effects of the bioactive compounds in this Mexican fruit and support its possible use as a supplement to increase the antioxidant activity of food products. Metabolite analysis of the polar content and protective properties in the red blood cell membranes of these extracts is in progress to identify the chemical structures of the bioactive components. The active components of *C. mexicana* could be used to prevent chronic degenerative diseases and to contribute to the maintenance of human health.

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