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Total phenolic content, *in vitro* antioxidant activity and chemical composition of plant extracts from semiarid Mexican region

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ABSTRACT

Objective: To determine the extraction suitable conditions of total phenolic content (TPC) by heat-reflux system, antioxidant activities and HPLC characterization of the aqueous-ethanolic extracts of *Jatropha dioica* (*J. dioica*) (Dragon's blood), *Flourensia cernua* (*F. cernua*) (Iax bush), *Eucalyptus camaldulensis* (*E. camaldulensis*) (Eucalyptus) and *Turnera diffusa* (*T. diffusa*) (Damiana). **Methods:** TPC was evaluated by the well-known colorimetric assay using Folin-Ciocalteu reagent. The antioxidant activities were assayed by three methods based on scavenging of DPPH, ABTS and by lipid oxidation inhibition. The chemical composition of the extracts obtained was subject to HPLC analysis. **Results:** TPC in the plant extracts ranged from 2.3 to 14.12 mg gallic acid equivalents/g for *J. dioica* and *E. camaldulensis*, respectively. The plant extracts of *F. cernua*, *E. camaldulensis* and *T. diffusa* showed similar strong antioxidant activities on scavenging of DPPH and lipid oxidation inhibition. In contrast, *J. dioica* extracts had lowest potential antioxidant in three assays used. HPLC assay showed the presence of several phenolic compounds in the extracts used. **Conclusions:** The results obtained suggest that *F. cernua*, *E. camaldulensis* and *T. diffusa* are potential sources to obtain bioactive phenolic compounds with high antioxidant properties which can be used in the factories as antioxidant agents or for treatments in diseases.

1. Introduction

Recently synthetic products are being restricted in the industries, because of harmful effects observed such as human toxicity and environmental pollution[1] and besides have been reported to be carcinogenic[2,3]. Additionally, the new demands by the consumers which pressure on the industries particularly for safer products are presented[4].

From this point of view, an increasing tendency towards the use of natural products instead of synthetic products has been observed in a high demand for food, cosmetics

and pharmaceutical manufacturers. For thousand years the plant natural products have been used in the medicine, cosmetics, nutrition and flavoring without or less harmful effects. Thus, plant extracts appear to be a feasible alternative for this problem and the industries have put the attention in the bioactive phytochemicals present in the plants.

Generally, phytochemicals present in the plant extracts are nontoxic, effective at low concentrations, low cost and friendly with the environment. Besides, recent studies have shown that the ingestion of vegetables, fruit and herbs is associated with prevention of some bactericidal, anti-viral, analgesic, anti-inflammatory and anti-carcinogenic disorders, due to their antioxidant activities[5-7]. In the food industry, phytochemicals are interesting due to these compounds retard oxidative degradations of lipids, improve

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the food quality and nutritional value[5] and contributing to prevent microbial deterioration[4,8–10].

Plant extracts consist in a complex mixture of several compounds as alcohols, esters, aldehydes, ketones, carbohydrates, terpenes, polyphenols, etc[11]. In addition, crude extracts, purified fractions and pure compounds have already been used in an antioxidant approaches[12] and several responses have been obtained. Generally, there is not compound or extract that can be used as universal antioxidant. However, nowadays is necessary search new sources and compound of specific antioxidants for determined objectives. Phenolic compounds are commonly reported to have the most antioxidant activity[13].

In this context, Mexico is an attractive country for its large endemic plant variety. In the semiarid regions of Mexico several plants take part of a great source of antioxidative compounds, mainly because their ability to grown under extreme climatic conditions[13,14]. This kind of plants have developed in most cases a pool of chemical compounds produced as secondary metabolites as environmental defense mechanism. Chemical compounds of semiarid region plants has been well studied to develop potential agents against certain pathogenic and phytopathogenic microorganisms present in the food and agricultural industries[4,10,13–16]. In addition, semiarid region endemic plants are commonly used in the Mexican traditional medicine. Among the plants most used are: *Jatropha dioica* (*J. dioica*) (Dragon's blood), *Flourensia cernua* (*F. cernua*) (Tar bush), *Eucalyptus camaldulensis* (*E. camaldulensis*) (Eucalyptus) and *Turnera diffusa* (*T. diffusa*) (Damiana).

J. dioica has been used as analgesic in toothache and *T. diffusa* as aphrodisiac[17]. On the other hand *F. cernua* is widely used to treat diarrhea, rheumatism, venereal disease, sores, bronchitis, chicken pox and common cold[15,18]. Nevertheless, actually there is a lack of knowledge about some plants that have not been extensively studied and neither theirs phytochemical compounds which could be used as antioxidants on control of certain diseases or in the food industry. Therefore, the purpose of this work was to evaluate the extraction conditions, antioxidant potential and chromatographic profiles (HPLC) of extracts from *J. dioica*, *F. cernua*, *T. diffusa* and *E. camaldulensis*.

2. Materials and methods

2.1. Plants collection

Plant materials were proportioned by the company Fitokimica Industrial de Mexico SA de CV (Table 1). The

plants were transported to the Department of Food Research at Universidad Autonoma de Coahuila, in black plastic bags. Immediately the samples were dried for 24–48 h at 60 °C in an oven (LABNET International, Inc.). The dry samples were ground in a mechanical mill and screened at 0.6–0.8 mm size particle. The fine and dried powder was stored in black plastic bags with sealed hermetically and at room temperature under darkness.

Table 1

Source and tissue of the plants used.

Plant material	Area of recollection	Period of recollection	Tissue
<i>J. dioica</i>	Parras de la Fuente, Coahuila	January, 2011	Stem and root
<i>F. cernua</i>	Saltillo, Coahuila	February, 2011	Stem and leaf
<i>E. camaldulensis</i>	Saltillo, Coahuila	May, 2011	Leaf
<i>T. diffusa</i>	Saltillo Coahuila	June, 2011	Stem and leaf

2.2. Preparation of the plant extracts

Phenolic extracts were obtained by heat–reflux extraction system evaluating two independent factors. It was necessary to select the best extraction time to assure the complete TPC extraction. Then, the first factor evaluated was the extraction time of heat–reflux. Mixtures of water and ethanol were used because it is well known the phenolic compounds are soluble in polar solvents as water and hydroalcoholic solutions, in addition, ethanol is a safe solvent (FDA 2012). However pure water is not the best solvent for phenols extraction[19,20]. Each dried powder sample (5 mg) (tissues ratio 1:1, w/w, where was necessary) were placed in an Erlenmeyer flask covered with aluminum foil to avoid light exposure and mixed with aqueous–ethanol (70%, v/v) (solid–liquid ratio 1:4, w/v, except in *J. dioica*, solid–liquid ratio 1:15, w/v was used). The mixture of the material vegetal and solvent was heat–refluxed in a water bath at 60 °C. A kinetic study on the total phenolic content extracted was carried out at different times: 0, 2, 4, 6, and 8 h of extraction.

Once selected the best extraction time the second factor evaluated was the aqueous–ethanol concentration. Three aqueous–ethanol concentrations were tested: 0%, 35% and 70% (v/v), where 0% is water without ethanol. Then, the heat–reflux extraction was performed under the same conditions as in the first experiment.

After extraction, each extract was filtered using a gauze for eliminate the big particles. Briefly, extracts were centrifuged at 3 500 rpm during 10 min and subsequent were filtered through a filter paper (fine pore, 0.45 µm) under vacuum. The filtered extracts were dehydrated in an oven at 60 °C for 24 h and the extraction yields were obtained in dry base (% w/w). The dried extracts were stored at 4 °C in a dark place before their use in the quantification of total phenolic

content. The experiments were performed in triplicates. In all the next determinations the extracts were re-suspended in water (1 mg/mL).

2.3. Determination of total phenols content (TPC)

TPC was quantified according the methodology reported by Makkar^[21] with some modifications^[22]. First, 800 μ L of the sample were mixed with 800 μ L of Folin-Ciocalteu reagent (Sigma-Aldrich), shaken and left for 5 min. Then 800 μ L of Na_2CO_3 (0.01M) were added and shaken and left for 5 min again. Finally, the solution was diluted with 5 mL of distilled water and the absorbance was read at 790 nm. TPC was expressed as gallic acid equivalents per gram of vegetal material (mg GAE/g).

2.4. Antioxidant activity

Antioxidant activity was measured in the extracts that presented the higher amount of total phenolic content in each vegetal material for three methodologies (Table 3).

Table 2

Extraction yield and total phenolic content of the extracts obtained in the kinetic of extraction.

Plant material	Extraction time (h)	Extraction yield (%)	Total phenolic content (mg GAE/g)
<i>J. dioica</i>	2	9.89 \pm 0.15	2.1 \pm 0.30
<i>F. cernua</i>	2	11.03 \pm 0.87	7.9 \pm 0.93
<i>E. camaldulensis</i>	2	14.85 \pm 0.58	12.8 \pm 0.96
<i>T. diffusa</i>	2	9.64 \pm 3.11	2.5 \pm 0.52

Note: All values are expressed as mean \pm SD of three replicates. Results of 4, 6 and 8 h not shown because no difference were observed at $P < 0.05$.

Table 3

Effect solvent concentration on the extraction yield and total polyphenolic content.

Plant material	Aqueous-ethanol concentration	Total phenolic content (mg GAE/g)
<i>J. dioica</i>	0%	1.36 ^b
	35%	1.55 ^a
	70%	2.34 ^{a*}
<i>F. cernua</i>	0%	2.09 ^b
	35%	10.24 ^{a*}
	70%	7.95 ^a
<i>E. camaldulensis</i>	0%	9.56 ^b
	35%	14.12 ^{a*}
	70%	12.88 ^a
<i>T. diffusa</i>	0%	2.59 ^b
	35%	4.70 ^{a*}
	70%	2.54 ^b

Note: Values followed by different lower-case letters are significantly different at $P < 0.05$.

* Extracts selected for the antioxidant activity assays.

2.4.1. 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity

First, the antioxidant activity in the extracts was evaluated as the DPPH free radical-scavenging activity. This assay was following the method reported by Molyneux^[23]: briefly, the solution of free radical DPPH (2.9 mL, 60 μ M, in methanol) was added to the sample (0.1 mL) and the controls (0.1 mL of water) respectively. The reaction was 30 min standing in a dark room at room temperature and after the absorbance was measured at 517 nm on a Cary-50-Bio Varian spectrophotometer. The free radical-scavenging activity of the extracts was expressed as percentage inhibition of DPPH and was calculated according to the formula:

$$\text{Percentage inhibition of DPPH} = \frac{(C_{\text{abs}} - S_{\text{abs}})}{C_{\text{abs}}} \times 100$$

where C_{abs} is the control absorbance and S_{abs} is the sample absorbance.

2.4.2. ABTS (2,2-azino-bis 3 ethyl benzothiazoline-6-sulfonic acid) radical cation scavenging activity

ABTS radical cation was prepared adding potassium persulphate solution (2.45 mM) and an ABTS aqueous solution (7 mM) and stand in the dark at room temperature for 12 h before use. After 12 h, the final solution was diluted with ethanol to an absorbance of 0.70 \pm 0.02 at 734 nm. Briefly, the extract (50 μ L) or the control (50 μ L of water) was mixed with ABTS solution (1 mL) and immediately the time was taken and the absorbance was read after 1 min using a Cary-50-Bio Varian spectrophotometer. The sample absorbance was compared with the control absorbance. The total antioxidant capacity was calculated as percent inhibition of ABTS radical using the following equation:

$$\text{Percentage inhibition of ABTS} = \frac{(C_{\text{abs}} - S_{\text{abs}})}{C_{\text{abs}}} \times 100$$

where C_{abs} is the control absorbance and S_{abs} is the sample absorbance^[24].

2.4.3. Lipid oxidation inhibition assay

The potential antioxidant of the extracts was obtained by lipid oxidation inhibition assay with the finality of measure the antioxidant activity in a medium close to lipids in a biological system^[6]. First, the linoleic acid solution was prepared by placing 0.56 g of linoleic acid and 1.5 g of Tween 20 in 8 mL of ethanol (96%, v/v). Then, the plant extract (50 L) was mixed with linoleic acid solution (100 μ L) and acetate

buffer (1.5 mL, 0.02 M, pH 4.0). Controls contained 50 μ L of distilled water. The samples were homogenized in vortex and incubated at 37 °C for 1 min. Once achieved 1 min, 750 μ L of 50 M FeCl₂ solution (0.0994 g FeCl₂ and 0.168 g EDTA diluted to 1 L with distilled water) were added to induce the lipid oxidation and incubated for 24 h at 37 °C. Two aliquots (250 μ L) were withdrawn during this period, at 1 and 24 h. Each aliquot was processed in the moment as following: the aliquot were added to NaOH solution (1 mL, 0.1 M, in ethanol at 10%, v/v) to stop the oxidation process; after ethanol (2.5 mL, 10%, v/v) was placed to dilute the sample. Then, the absorbance of the samples was measured at 232 nm. Ethanol (10%, v/v) was used as blank. Percent inhibition of linoleic acid oxidation was calculated with the following equation:

$$\text{Lipid oxidation inhibition(\%)} = \frac{(A-B)}{A} \times 100$$

where A is the difference between the absorbance of the control sample (distilled water) after 24 h and 1 h of incubation, and B is the difference between the absorbance of each extract sample after 24 h and 1 h of incubation [25].

2.5. High performance liquid chromatography analysis

The characterization of the phenols compounds present in the selected extracts (Table 3) was carried out using high performance liquid chromatography (HPLC) analysis following the method reported by Ruiz *et al* [18]. A Varian Pro-Star 330 with DAD detector and Photodiode detection at 280 nm was used. Fractionation of the samples was performed on an Optisil ODS column (5 μ m, 4.6 mm \times 250 mm) under following analytical conditions: using a mobile phase consisting of 3% acetic acid and acetonitrile, during 25 min with flow rate of 1 mL/min (sample previously filtered through a 0.22 μ m nylon membrane; with injection volume of 10 μ L). All the standard solutions of the different phenolic compounds used were injected under the same conditions.

2.6. Statistics analysis

The experiments were established under a blocks completely randomized design for which material vegetal independently. The dates were transformed by log X and analyzed using the software SAS V 9.0. where the comparison test of means of multiple range Tukey ($\alpha = 0.5$) was used. In the graphic the dates observed are the true values.

3. Results

3.1. Extraction of total phenols content

The kinetic extraction of polyphenols allowed to establish the time required for recovery the TPC maximum (Table 2). In the four plants was noted that exist a significant difference in the TPC in the control time (0 h) compared to other extraction times (2, 4, 6 and 8 h). However, no significant difference was found in the times 2, 4, 6 and 8 h in four plants evaluated. Thus, with the purpose of select the suitable time for extraction of phenols, the productivity in each vegetal material was determined (Figure 1). It was observed in all cases that the productivity was higher at 2 h of extraction and decreased at rest of the times evaluated. Using this analysis was possible select 2 h of extraction time as the suitable time for the TPC extraction in the four plants and it was used in the evaluation of the second factor, aqueous-ethanol concentration.

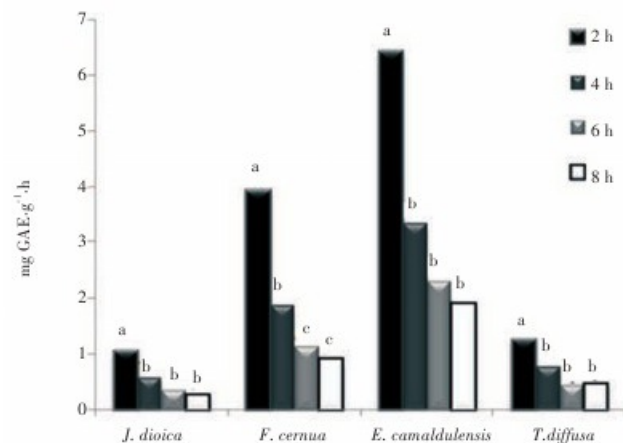


Figure 1. Productivity of the heat-reflux system.

The second factor evaluated was the aqueous-ethanol concentration on the extraction of polyphenols from the four plant used. In this experiment, the extraction of TPC from *E. camaldulensis*, *F. cernua* and *T. diffusa* showed a quadratic effect (Table 3). When aqueous-ethanol concentration was increased from 0% to 35% (v/v) more phenolic compounds were extracted. But lower TPC yield was observed when aqueous-ethanol concentration was increased until 70% (v/v). On the other hand, in *J. dioica* extracts was observed an increase in the TPC extracted which was proportional at the increase of aqueous-ethanol concentration (Table 3). It is possible to mention that under the suitable conditions of extraction time and aqueous-ethanol concentration, *E. camaldulensis* and *F. cernua* were the species with higher amount of TPC while *T. diffusa* and *J. dioica* were the

species with lower TPC yield content (Table 3).

3.2. Antioxidant capacity of the extracts

Four plants of the semiarid Mexican regions were used to assess the antioxidant potential by three methods.

3.2.1. Free radical scavenging activity on DPPH

Figure 2a shows the DPPH scavenging activity of the extracts obtained under the TPC suitable extraction conditions for the four plants. *F. cernua*, *E. camaldulensis* and *T. diffusa* extracts had similar capacity antioxidant ranging between 76.03% to 91.96%. These results were higher (70%) that obtained for *J. dioica* [(15.40±1.88)%]. To our knowledge, there are not concise studies to compare the *J. dioica* potential antioxidant. On the other hand, Salazar et al^[13] evaluated the antioxidant activity using the DPPH assay in *F. cernua* and *T. diffusa* reporting values ranged from 75.3% to 86.8% and from 27.9% to 88.1%, respectively for individual tissues. It is according with our findings since we used a mix of the tissues where antioxidant capacity of each tissue was slight modified.

3.2.2. Free radical scavenging activity on ABTS

Antioxidant activity in the extracts was also investigated using the well-known ABTS method. The results in ABTS assays show that only *E. camaldulensis* extract maintained a high antioxidant activity very similar in both DPPH and ABTS assays with radical inhibition of 88% approximately under the condition used. While, *F. cernua* and *T. diffusa* extracts were more effective in DPPH radical scavenging than ABTS radical scavenging with a loss of 50% in the antioxidant activity decreasing from 80% in DPPH assay to 30% in ABTS assay in both cases (Figure 2). In contrast, *J. dioica* extract had a negligible increasing of 9% inhibition approximately when the ABTS assay was evaluated. In general terms, *E. camaldulensis* extract showed more free radicals scavenging capacity as antioxidant activity.

3.2.3. Lipid oxidation inhibition assay

In the light of the differences among the wide number of test systems available for the antioxidant activity, the results of a single-assay can give only a limited suggestion of the antioxidant properties of plant extracts^[25,26]. In this study, three assays were carried out for have a better interpretation of the antioxidant activities of the obtained extracts. Figure 2c shows the inhibition activity of the extracts against linoleic acid peroxidation caused by FeCl₂ as an oxidant initiator. In this assay the values obtained without antioxidants were taken for 100% lipid peroxidation. As do DPPH and ABTS assays, lower values (13.95%) were obtained with *J. dioica* extract at the concentration used. The rest of the extracts (*F. cernua*, *E. camaldulensis* and *T. diffusa*) presented an average of 65% in the lipid oxidation inhibition. Anew *E. camaldulensis* extract maintained good antioxidant activity compared with the other extracts. These last findings for *E. camaldulensis* extract in the lipid oxidation inhibition are close to results in the DPPH assay.

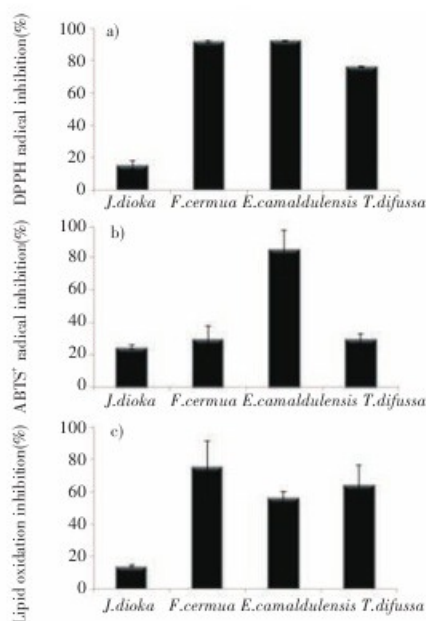


Figure 2. Antioxidant activity of the phenolic extracts selected (1 mg dried extract/mL).

a) DPPH Free radical scavenging activity; b) radical cation ABTS-scavenging activity; c) linoleic acid oxidation inhibition.

Table 4

Phenolic compounds detected by HPLC assay in the different extracts selected.

	STD	PG	GA	RS	CHA	MG	CUA	CAT	HA	EA	QE	CIA
Extracts	<i>J. dioica</i>	-	-	-	-	-	-	-	-	-	-	-
	<i>F. cernua</i>	-	-	-	+	-	+	-	-	-	+	-
	<i>E. camaldulensis</i>	-	-	-	-	-	-	+	-	-	-	-
	<i>T. diffusa</i>	-	-	-	-	-	-	-	-	-	+	-
	RT	6.5	7.46	9.51	11.05	12.99	15.09	15.65	17.76	18.83	20.11	21.05

Retention time (RT), standard (STD), pyrogallol (PG), gallic acid (GA), resorcinol (RS), chlorogenic acid (CHA), methyl gallate (MG), coumaric acid (CUA), catechin (CAT), 2-hydroxycinnamic acid (HA), ellagic acid (EA), quercetin (QE), cinnamic acid (CIA), presence (+) and absence (-).

3.3. HPLC assay

Using the HPLC analytical assay was possible to detect and confirm the presence of some phenolic compounds in *J. dioica*, *F. cernua*, *T. diffusa* and *E. camaldulensis* extracts. Table 4 shows a summary of the analyzed samples. It was noted that only *J. dioica* extract presented none phenolic compound. Possibly because of this is a crude extract and not purified then the phenolic compounds are present in low concentration. Nevertheless, recently Aguilera *et al*[27] confirmed the presence of ellagic acid by HPLC in a methanolic hydrolyzed extract in some arid Mexican plants including *J. dioica*, *F. cernua* and *T. diffusa*. However, these authors reported that the concentration was low (1.8–2.5 mg/g). On the other side, the HPLC assay in the other three extracts, in special *F. cernua*, permitted to detect almost one compound (Table 4). Ruiz *et al*[18] studied the phytochemical profile of the *F. cernua* extracts and fractions, reporting two important phenolic compounds namely 2-hydroxycinnamic and ellagic acids. In our screening these compound was not detected. However, three different polyphenolic compounds were clearly detected as chlorogenic and coumaric acids and quercetin. Quercetin and catechin were identified in *T. diffusa* and *E. camaldulensis* extracts respectively.

4. Discussion

The TPC extracted from each plant was very different among plants despite of the extraction yields were similar in all the samples. Similar reports have been published by other authors[28]. These authors observed that no existed significant differences in the extraction yield evaluating times from 3 to 8 h with heat-reflux system using other plants. Hence, they concluded that highest quantity of compounds extracted is achieved in a lesser time than the times evaluated. Few studies had been developed concerning the polyphenolic content from *F. cernua*[15,29,30]. Interestingly, in the present study was possible to obtain higher TPC. These differences in the results can be attributed at solvent used in their studies as acetone and hexane which extracted certain compounds of low or no polarity. In contrast, in our study, the solvent employed was aqueous-ethanol which is a solvent with higher polarity and it is known that the most phenolic compounds are polar permitting easier the diffusivity in the ethanol and water mixture. Other factor could affect positively the yield extraction was the particle size. In our study was used a small particle size between 0.6–0.8 mm. It is known that a

small size particle increase the contact surface in the vegetal material and therefore, increases the extraction yields of the target compounds[31]. Our findings are also according with previously reports by Castillo *et al*[32], where these authors reported *J. dioica* had lower values of TPC than *T. diffusa* and *F. cernua* before and after fermentation. It is well documented that phenol content in the plants varies depending the specie, the plant tissue and environmental factors as temperatures, water stress, light conditions as well as phenological development[20]. It explains the large differences obtained among the plants used.

It is interesting to note that despite of that *T. diffusa* extract had lower TPC levels respect to *F. cernua* and *E. camaldulensis* extracts, it showed a good potential antioxidant. Several reports have showed that the phenolic compounds are not the unique phytochemicals to possess antioxidant properties[33].

On the other hand Amakura *et al*[34] evaluated the antioxidant activity by DPPH assay of eucalyptus extract and, gallic and ellagic acids isolated from the eucalyptus extract. They observed that the isolated compounds had higher antioxidant activity than BHA and BHT compounds (synthetic antioxidants), isolated terpenes and phloroglucinol. Finally, the authors concluded that the main antioxidant activity in eucalyptus extract can be attributing to gallic and ellagic acids present in the extracts. As expose above, *E. camaldulensis* extract maintained a high antioxidant activity very similar in both DPPH and ABTS assays. These findings shows that one or several antioxidant compounds present in the *E. camaldulensis* extract have the capacity to act in two different mechanisms for the free radical scavenging activity, through a single electron transfer reaction (ABTS assay)[6,35] and a hydrogen transfer reaction (DPPH assay)[23,36]. In this sense, *F. cernua* and *T. diffusa* extracts had more capacity for donate a hydrogen atom instead of transfer an electron. However, it is important to note that antioxidant compounds can respond in a different manner to different radical or oxidant compounds[37]. It could be explained according the mechanism of oxidation proposed by Huang *et al*[6] and Mishra *et al*[36] for DPPH assay and Choe and Ming[33] for lipid oxidation inhibition, both methods are based on the oxidation process could be stopped when another molecule have the capacity of transferring a hydrogen atom to the radical. However, the assay lipid oxidation inhibition is an antioxidant test stricter than DPPH method due to chain reactions are involved[38]. Hence, the antioxidant compounds should be more specific, resistant and higher stoichiometrically for stabilized the several kinds free radical formed; besides exist the possibility that extracts

had a low quantity of fat oxidant agents^[39]. It explains the slight decrease in the lipid oxidation inhibition percentage for *F. cernua*, *E. camaldulensis* and *T. diffusa* extracts.

Antioxidant activity and the phenol content of several extracts had been correlated positively in several studies but, it still is not constant and dependent of the material analyzed^[19,34]. In this context, we can observe that *E. camaldulensis* extract was the best antioxidant extract and it is correlated with the bigger TPC observed during the quantification in the extraction process. In the same understanding *J. dioica* extract was the sample with lower antioxidant capacity and TPC. Finally the antioxidant activity and TPC in the four samples tested were in the order of *E. camaldulensis* > *F. cernua* > *T. diffusa* > *J. dioica*.

The phenolic compounds found in the extracts had been reported to be powerful antioxidants present in vegetables and fruit^[40,41] and known as take part of the mechanism of defense environmental in vegetables, fruit and herbs^[38]. Then, it was presumed that the antioxidant activity in *E. camaldulensis*, *F. cernua* and *T. diffusa* extracts could be due to those typical phenolics.

The better extraction conditions of total polyphenols were clearly associated to the antioxidant capacity in semiarid region plants. The plant *E. camaldulensis* was the better antioxidant source in our study because it maintained the great antioxidant potential in the three antioxidant activity assays with electron transfer and hydrogen atom capacity. However to confirm the close mechanism is necessary subsequent studies as isolation compounds or fractionation of the extract and characterization of the structures. To our knowledge this is the first approach where the phenolic antioxidant activity in these arid Mexican plants is explored. However, it is important to note that is necessary more studies to determine the better conditions of use. Generally, we can suggest that the plants used are a natural source of bioactive compounds of plant widely distributed in Mexico of great value that could be used properly in the food, pharmaceutical and cosmetic industries.

Conflict of interests

The authors disclose no conflicts.

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