Effect of Drying on the Antioxidant Capacity and Concentration of Phenolic Compounds in Different Parts of the *Erythrina americana* Tree

Aurea Bernardino-Nicanor,[a] José Luis Montañéz-Soto,[b] María de los Ángeles Vivar-Vera,[c] José Mayolo Juárez-Goiz,[a] Gerardo Acosta-García,[a] and Leopoldo González-Cruz [a,*]

The *Erythrina americana* tree has been widely studied for its antioxidant and antimicrobial activity, principally of the seeds. Few studies have focused on the other tree parts. This work evaluated the effect of drying on the proximate chemical composition, IC_{50}, and total phenolic content (TPC) of different parts of the plant from *Erythrina americana*. Proximate chemical composition showed significant differences between parts of the plant. Tree bark (TB) exhibited higher protein content, crude fiber, and ash. The IC_{50} value was significantly different in all parts of the plant; the leaves exhibited lower amounts necessary to reach this value. The drying process had a positive influence on the antioxidant activity in all parts of the plant, with mature flowers (MF) and young flowers (YF) samples. These were the samples that required the lowest concentration of all dry samples to reach the IC_{50} value. The TPC values showed significant differences between fresh and dry samples except for MF, according the ANOVA and Tukey test ($P \leq 0.05$). In conclusion, the drying process has potential for retaining the antioxidant activity in YD and MF.

Keywords: Antioxidants; Phenolic compounds; Erythrina; Drying

Contact information: a: Instituto Tecnológico de Celaya, Antonio García Cubas Pte #600 esq. Av. Tecnológico, Celaya, Gto. México, C. P. 3810. Celaya, Gto. A. P. 57; b: Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional del Instituto Politécnico Nacional, Unidad Michoacán, Justo Sierra Nº 28 Jiquilpan, Mich, México, C. P. 59510, México; c: Coordinación de Posgrado e Investigación, Departamento de Ingeniería Química y Bioquímica, Instituto Tecnológico de Tuxtepec, Av. Dr. Victor Bravo Ahuja S/N Col. 5 Mayo, Tuxtepex, Oaxaca, México;
** Corresponding author: leopoldo.gonzalez@itcelaya.edu.mx*

INTRODUCTION

The *Erythrina* tree is a pantropical legume belonging to the sub-family Papilionoideae (Sousa *et al*. 2003), which is divided into 5 sub-genera and 26 sections (Neill 1988; Neill 1993), that comprises 115 species, including trees, shrubs, and some herbs. In Mexico, *Erythrina americana* is found as wild plants in Veracruz, Hidalgo, Morelos, Guerrero, Puebla, and Oaxaca states (Bonilla and Villaseñor 2003; Ruíz Montiel *et al*. 2012). However, some commercial plantations have also been reported in Michoacán, Chiapas, San Luis Potosí, Coahuila, Nuevo León, Tabasco, and Campeche (García-Mateos *et al*. 2001).

The *E. americana* is used mostly as a shade tree (Rivera *et al*. 2008), nitrogen fixer, animal feed, support tree, and ornament. The *E. americana* flowers are frequently used as human food (Sotelo *et al*. 2007) or in the preparation of infusions. Seeds of *E. americana* are used in the medical area as analgesic, tranquilizer, and muscle relaxant

(Garín-Aguilar *et al*. 2000; Sánchez-Herrera *et al*. 2001). In addition aqueous extracts from its leaves have been used as sedatives (Ratnasooriya and Dharmasiri 1999).

The seeds have high alkaloids content, which increases throughout the maturation of the pod (García-Mateos *et al.* 1996, 2004a). In many cases the alkaloids are differentiated according to species (García-Mateos *et al*. 1998; Miguel-Chávez *et al*. 2006). For this reason the alkaloids have been extensively studied, mainly for their antioxidant (Ibarra *et al*. 2011), antimicrobial (Soto-Hernández *et al*. 2011), and pharmacological activities.

However, the alkaloids not only are present in the seeds of *E. americana*; several authors, have reported low quantities of these compounds in flowers, leaves, and tree bark. Alkaloids, α-erythroidine, β-erythridine, 8-oxo-α-erythroidine, erythralin-N-oxid, erysodine, erythraline, erythratine, erythbidin, crystamidine, and β-D- glucoerysopine have been isolated from flowers (Sánchez-Herrera *et al*. 2001; Reimann 2007). Erysoline and erythbidin have been found in leaves, while only erythratidine have been reported in the tree bark (García-Mateos *et al*. 1998). The difference in the type and content of alkaloids in each part of the plant is the principal reason for the diverse applications in the medical area. For example, the tree bark is used as a contraceptive and abortifacient; the seeds are used as central nervous system depressor (Garin-Aguilar *et al*. 2000; Shoaib *et al*. 2000), muscular relaxant, sedative (Sánchez-Herrera *et al*. 2001), toxin against mosquitoes (García-Mateos *et al*. 2004b), and antimycotic (Estrada *et al*. 2009); the flowers are used to treat insomnia. The leaves, seeds, and flowers are used as hypnotics and to treat inflammation of arms, legs, and eyes, as well as to treat insect bites, ulcers, and abscesses, while the whole plant is used to treat malaria, to clean the liver, kidneys, and lungs, and to treat back, teeth and ear pain (Hastings 1990; Gheno-Heredia *et al*. 2011; Araújo-Júnior *et al*. 2012). Some of the beneficial effects found in the *Erythrina* genus are due to the antioxidant activity of the alkaloids and phenolic compounds, which are distributed across all sections of the plant, including fruit, leaves, seeds, roots, and tree bark (Pratt and Hudson 1990).

In addition to the beneficial effects of alkaloids reported to be in the *Erythrina* genus, some authors report that the phenolic compounds could also be responsible for beneficial medical treatments such as analgesic, anti-inflammatory, prevention and treatment of cancer, osteoporosis, diabetes mellitus, cardiovascular disorders and neurodegeneration (Balasundram *et al*. 2006; Sumbul *et al*. 2011). In the food industry, the phenolic compounds are used due to their antioxidant activity, to slow down the oxidative degradation of lipids, and as antimicrobial agents (Cowan 1999; Mathew and Abraham 2006; San Miguel-Chávez *et al*. 2007).

Currently, the main problem in the pharmaceutical, food, and medical industries is the preservation of the raw material with high nutraceutical potential. For this reason, many methods have been used to prolong the shelf life, without losing the nutraceutical properties. One of the methods most widely used to achieve this objective is the drying process (Sagar and Kumar 2010; Hincapié *et al*. 2014). Convection drying is the most popular to the dehydration of fruits and vegetables, since a simultaneous mass and heat transfer process favors the transference of water by means of diffusion, based on moisture content and temperature, respectively, from the interior of the material to the air-food interface, and from there to the convection air current (Márquez and Ciro 2002).

While many studies have been conducted recently on the alkaloids and antioxidant compounds obtained from *E. americana* and their effects on health, few studies have focused on the effect of processing on the chemical composition and the

nutraceutic properties of the different structures of this plant. For this reason, the objective of this study was to determine the effect of drying on the proximate chemical composition, antioxidant activity, and the total phenolic compound content in the different sections of the *E. americana* plant.

EXPERIMENTAL

Physical and Chemical Characterization

Vegetable material

The vegetable material was collected in Tulancingo, Hidalgo, Mexico. Samples were taken from tree bark (TB), leaves (L), and at three stages of flower development: floral bud (FB), young flowers (YF), and mature flowers (MF). Each sample was analyzed before and after the convection drying process.

Proximate chemical analysis

The proximate chemical analysis was conducted according to the methodology established in AOAC (1990). Protein content was calculated by converting the nitrogen content, determined by the micro-Kjeldahl method (method 955.04, factor Nx6.25), crude fiber was quantified with alkaline and acid digestion (method 962.09), crude fat were extracted in a Soxhlet extractor using petroleum ether (method 920.39C), moisture content was determined by drying the samples in an oven (Felisa, Model 243AD, México) at 105 ºC until a constant weight was obtained (method 934.01), and ash content was obtained by calcination in muffle furnace (Thermolyne 1400, model FB1415M, USA) at 550 ºC (method 923.03). Carbohydrate content was determined as the weight difference using protein, lipid, fiber, moisture, and ash content data.

Convection Drying

Drying was undertaken in a forced convection-drying oven (BINDER, model FD115-UL, USA). The samples were placed in the oven for a period of 48 h at 40 °C until the complete elimination of moisture. Tree bark samples of an approximate area of 5 x 5 cm were placed in the dryer, while the leaves and flower samples were placed whole in the dryer.

Determination of Antioxidant Activity (DPPH Method)

The DPPH method was used according to Brand-Williams *et al*. (1995), in which the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, St. Louis, MO, USA) is used. In order to determine the IC_{50} values, the concentration profiles studies were carried out in the range 30 to 700 mg of sample*mL^{-1} of methanol. Each sample was vortex-mixed for 2 min and then centrifuged at 1000 *x g* for 20 min and 0.1 mL of the supernatant was placed in vials. Then 3.9 mL of DPPH in methanol ($6x10^{-5}$ M) was added. The reduction of absorbency was determined at 515 nm from time 0, and then every 10 min until the reaction has been completed using a spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Inhibition (% I) of DPPH radical was calculated using the equation,

$$I(\%) = \frac{A_0 - A_s}{A_0} \times 100\% \quad (1)$$

where A_0 is the absorbance of the control, and A_s is the absorbance of the test sample.

The IC_{50} values were calculated from the graphs by the percentage of inhibition of the DPPH *versus* samples concentrations. IC_{50} denotes the concentration of the compound required to inhibit the initial DPPH value of 50% to an absorbency of 515 nm.

Total Phenolic Content (TPC)

The TPC of each sample was quantified using the Folin-Ciocalteu methodology (Singleton *et al*. 1999). The extraction of phenolic compounds was carried out using 0.5 mg of each sample (fresh or dry), to which was added 2 mL of methanol (at 80%) containing 1% HCl. The prepared dissolution was placed in an orbital shaker (Eberbach Corporation, Ann Arbor, MI, USA) (200 rpm) for 2 h at room temperature. The mixture was centrifuged at 1000*xg* for 15 min in a centrifuge (Hermle Z200A, Germany), and the supernatant was placed in vials. The precipitate was used to carry out a second extraction under the same conditions. The resulting supernatants for both extractions were mixed and used to carry out the quantification of PCC. An aliquot of the extract (100 μL) was mixed with 0.75 mL of the Folin-Ciocalteu reagent (Hycel of México SA de CV, México) (previously diluted in distilled water) and was left to stand at room temperature for 5 min, after which 0.75 mL of sodium bicarbonate was added, for a concentration of 6%. The absorbency was then read at 725 nm after 90 min of rest at room temperature.

The results were expressed as the gallic acid equivalents (GAE) in milligrams per gram of sample, using a gallic acid (Sigma-Aldrich, St. Louis, MO, USA) standard curve.

Statistical Analysis

All the analyses were undertaken in triplicate and the results expressed as median ± standard deviation, while the analysis of variance (ANOVA) was carried out by means of a Tukey test. The software program Statistical Analysis System (SAS Institute Inc., Cary, NC, USA) v. 8.0 was used for the statistical analysis of the data.

RESULTS AND DISCUSSION

Physical and Chemical Characterization

Vegetable material

The measurements for the samples used in this study were as followed: Leaves (L), between 10 and 14 cm in length and 9 and 12 cm in width; floral bud (FB), between 0.7 and 1.5 cm; young flowers (YF), between 2 and 2.4 cm; and mature flowers (MF), between 5.5 and 6.0 cm in length.

Proximate chemical analysis

The proximate chemical composition of *Erythrina americana* showed that exist statistically significant differences ($P \leq 0.05$) among most parts of the plant (Table 1).

The moisture content for all samples was found to be between 68% and 87%, a range comprising samples from the various stages of flower development. The flowers and leaves were the samples with highest moisture levels, which could be attributed to the fact that water is transported through the xylem to the leaves, seeds, flowers, and fruit (Raven *et al*. 1992). In contrast, the tree bark (external cortical layer), comprised principally of cellulose and lignin, and is the structure that accumulates the lowest amount of water (Nutsch 1996).

In particular, the moisture levels in the *Erythrina americana* flowers analyzed were similar to those reported for the same species by Sotelo *et al*. (2007), and lower than those reported by García-Mateos *et al*. (1996). Some authors attributed these variations to the development stage, since there are reports that indicate an inverse relationship between the development stage and moisture levels of the plant (García-Mateos *et al*. 1996, 2005).

Table 1. Proximate Chemical Composition of *Erythrina americana* (g*100 g^{-1} Sample)

	Moisture	Dry Basis				
		Proteins	Crude fat	Crude fiber	Carbohydrates	Ash
L	75.09±0.48^{d}	20.28±0.92^{b}	9.00±0.43^{a}	11.24±0.16^{b}	55.27±0.58^{c}	4.21±0.19^{b}
FB	84.47±0.15^{b}	25.73±0.29^{a}	5.87±0.71^{b}	9.01±0.40^{c}	55.63±0.45^{c}	3.76±0.10^{b}
YF	87.25±0.20^{a}	24.70±0.47^{a}	5.19±0.41^{b}	9.81±0.10^{c}	57.50±0.30^{b}	2.80±0.34^{c}
MF	83.48±0.24^{c}	20.97±0.55^{b}	4.23±0.87^{c}	8.61±0.47^{d}	63.66±0.44^{a}	2.53±0.38^{c}
TB	68.12±0.10^{e}	26.12±0.38^{a}	7.50±0.40^{a}	12.41±0.23^{a}	48.12±0.20^{d}	5.85±0.35^{a}

Different letters in each column indicate significant statistical differences ($P \leq 0.05$). The values are means±SD of triplicate measurements.

On the other hand, the leaves of *E. americana* presented lower moisture levels than the leaves of *E. crista* (Maisuthisakul *et al*. 2008). However, in comparison with the other parts of the tree, the moisture content is lower than the flowers at the several developmental stages, due principally to the dynamic water interchange in the leaves that regulate the evaporative demand of water (Lambrecht and Dawson 2007). The moisture content in the leaves is higher and more stable than in the tree bark. This is because in the tree bark, the water and solutes may buffer the daily or seasonal fluctuations in water availability in the tree tissues (Rosell *et al*. 2014).

The leaves in comparison with the flower at different developmental stage, showed lower protein and carbohydrates content, which confirmed the reports by other authors, who described how the products of the photosynthesis undertaken by the leaves are translocated mainly to the flowers, fruits, and seeds (Maisuthisakul *et al*. 2008).

The protein content in all parts of the plant analyzed was higher than 20%; the leaves (L) and mature flowers (MF) showed the lowest protein content. Higher protein content was observed in the FB and YF, which probably were due to the fact that during these developmental stages high protein levels are required for the elongation of its tissues. On the other hand, it has been demonstrated that during plant development, there is a translocation of nitrogenous compounds, such as proteins, alkaloids, amino acids, and amides from the leaves to the flowers, fruit, and seeds (García-Mateos *et al*. 2004a; Verduzco *et al*. 2008).

Comparing the results of protein content, obtained for *E. americana* with those obtained by other authors, it was observed that the mature flower of the *E. americana* presented a protein content similar to that reported for *E. edulis* (Barrera 1994) but 6.2% lower than the protein content reported for *E. americana* in other studies (Sotelo *et al*. 2007). These results showed that protein content not only varies among species, but also within the same species as a consequence of the geographic zone of development. In

comparison with other legumes, the protein content of the *E. americana* is within of the level reported for the flowers of commonly consumed legumes (Sotelo 1996; Sotelo *et al*. 2007) and higher than the protein content reported for the alfalfa flower (Dien *et al*. 2006; Mullen and Boateng 2008).

The protein content in leaves of *E. americana*, in comparison with other species leaves, such as *E. crista*, is lower around 3.8% (Maisuthisakul *et al*. 2008). This is similar to the protein content of *E. edulis* leaves (Barrera 1994) and higher than edible leaves of other genera (Odhav *et al*. 2007; Maisuthisakul *et al*. 2008).

The carbohydrates content in all parts of the plant were found in the range of 48% to 63%; the mature flowers exhibited the highest content of this component since this structure receives the products of the photosynthesis generated in the leaves and uses them to maintain the progeny of the plant (Maisuthisakul *et al*. 2008). In comparison with other species, the leaves of *E. americana* presented a higher carbohydrate content than the leaves of *E. crista* (Maisuthisakul *et al*. 2008); this variation could be derived, according to other authors, to the species, age, and harvest time (Sánchez-Herrera *et al*. 2001).

The crude fat content obtained from all parts of the plant was found to be in the range between 4% and 9%. The leaves had the highest content of this component due to the fact that these parts of the plant require a higher proportion of lipophilic compounds to protect their surfaces and thus control moisture loss (Muñoz 1987).

While the *E. americana* flowers showed lower fiber and ash contents than those reported by Sotelo *et al*. (2007) and Dien *et al*. (2006) for floral structures from other genera, *E. americana* flowers showed higher contents compared to those reported for *E. crista* (Maisuthisakul *et al*. 2008).

The crude fiber and carbohydrate values in the leaves were found to be below those reported for other *Erythrina* species (Barrera 1994). It is important to consider that the differences in the content of all compounds analyzed according to several authors could be due, principally to the region in which they were collected, the harvest season, the age, and the species of the analyzed plant (Barrera 1994; Sánchez-Herrera *et al*. 2001). However, it has been reported that the products of photosynthesis occurring in the leaves are translocated to the fruit, flowers, and seeds, and for this reason, these parts of the plant have the higher content of protein and carbohydrate.

Antioxidant Activity

In both fresh and dry samples, according to the one-way ANOVA, statistically significant differences exist between all parts of the plant for the IC_{50} value ($P \leq 0.05$). For this reason the IC_{50} value of *E. americana* samples was dependent on the plant part analyzed (Table 2). Leaves exhibited the lower amount necessary to reach the IC_{50}.

According to the classification proposed by Kuete and Efferth (2010), the values of antioxidant activity obtained for *E. americana* in all parts of the plant analyzed, are considered as samples with low antioxidant activity, considering that all parts of the plant showed a value of $IC_{50} > 0.1$ mg*mL^{-1}.

The results showed that the drying process positively influenced the antioxidant activity in all parts of the plant. The drying of samples (MF and YF) diminished the required sample in comparison with the fresh sample to reach the IC_{50} value (Table 2), which could have been due to the fact that melanoidins derived from the Maillard reaction could have been generated and accumulated during the process (Vega-Gálvez *et al*. 2009).

The sample amount necessary of the different parts of the plant were found in the range between 38.5 and 650 mg*mL^{-1}. To reach the IC_{50} value, many authors have indicated that the antioxidant activity of *Erythrina* species is due principally to the high bioproduction of alkaloids in seeds, flowers, and pods of *Erythrina* species (Juma and Majinda 2004; Ibarra *et al*. 2011).

Although the results obtained for TPC showed a decrease after the drying process (Table 3), the antioxidant activity increased in two parts of the plant (MF and YF), due to the high alkaloid content in the flowers (García-Mateos *et al*. 1998). However, in the other parts of the plant (FB, L, and TB), apparently the low content of alkaloids is not sufficient to avoid the oxidation process of other compounds and maintain the antioxidant activity. In addition, these results are important because currently no report has been published about the antioxidant activity and the amount of sample required to reach the IC_{50} value, from different parts of the plant and the importance of the alkaloids in the protecting effect on the antioxidant activity.

Table 2. Amount of Sample Required to Reach the IC_{50} Value in Different Parts of *Erythrina americana* Miller

Structure	IC_{50} (mg*mL^{-1})		
	Fresh		Dry
	mg in fresh basis	mg in dry basis	
MF	650±32.5^{a}	107.3±1.56^{a}	48.5±1.69^{c}
YF	560±14^{b}	71.4±1.12^{b}	38.5±1.54^{d}
FB	400±8^{c}	62.12±0.6^{c}	72.0±3.6^{b}
L	62.6±1.2^{e}	15.59±0.3^{e}	42.0±1.72^{c,d}
TB	166.7±5.4^{d}	52.92±0.17^{d}	90.0±3.15^{a}

IC_{50} (mg*mL^{-1}) is the quantity required for generating 50% inhibition of the DPPH after 30 min. Different letters in each column indicate significant statistical differences ($P \leq 0.05$). The values are means ± SD of triplicate measurements.

The results showed differences in the antioxidant activity in leaves and flowers, which was apparently due to different accessory compounds, principally pigments. For this reason the leaves required the lowest quantity of sample to reach the IC_{50} value. Thus, this part of the plant has a higher antioxidant activity. Many reports have suggested that in the leaves, the stress level is high; for this reason more pigments quantity are generated, and this protects the leaf tissues from photochemical damage (del Baño *et al*. 2004; Chew *et al*. 2009).

Nevertheless, the amount of sample necessary of *E. americana* leaves to reach the IC_{50} value is higher than the following: *E. crista* leaves (Maisuthisakul *et al*. 2008); extracts from legume leaves used in the medical industry (Chew *et al.* 2011); extracts of *B. monandra* leaves (Argolo *et al*. 2004); *Cinnamomum verum* leaves (Mathew and Abraham 2006); edible leaves of other genera (Tachakittirungrod *et al*. 2007; Maisuthisakul *et al*. 2008); as well as the essential oils of the leaves of *Ocimum basilicum* (Hussain *et al*. 2008) and *Biden pilosa* Linn var. *Radiata* (Deba *et al*. 2008). This was the case because the extraction processes used for these samples involve the use of solvents

of different polarities, which facilitates the isolation of various antioxidant compounds, thus increasing their antioxidant activity. However, in this research raw vegetal and dry powder was used, therefore multiple interferences could be generated between different compounds.

On the other hand, the results obtained in this study are within the range of the levels reported for the leaves of edible plants of different genera (Odhav *et al*. 2007). However, in comparison with plants used for medical purposes, the necessary concentration of all *E. americana* parts is high to reach the IC_{50} value (Bruni *et al*. 2004; Deba *et al*. 2008; Maisuthisakul *et al*. 2008; Lu *et al*. 2009; Chew *et al*. 2011).

Total Phenolic Content (TPC)

A similar comportment in the TPC was observed in both fresh and dry samples. All parts of the plant showed significant differences ($P \leq 0.05$), with the higher TPC value obtained for the leaves and the lower TPC value for the tree bark. Statistical calculation for ANOVA and Tukey test showed significant differences ($P \leq 0.05$), between fresh and dry samples, except for the mature flowers.

Phenolic compounds are synthesized as secondary metabolites during the development of plants as a response to stress conditions such as UV radiation, hydric deficit, mechanical damage, and so on. It has been demonstrated that the distribution of phenolic compounds in the plants, both in the structures and at a cellular and sub-cellular level, is not uniform. The insoluble phenolic compounds, for example, are found in the cell walls, while the soluble phenols are present within the vacuoles of the vegetable cells (Randhir and Shetty 2005). The aforementioned fact explains the difference in the concentration of the TPC in each part of the *Erythrina americana* plant analyzed (Table 3).

According to the results obtained, the use of fresh samples to preserve the high content of TPC in all parts of the plant is more effective. All the fresh samples showed a higher TPC compared with the dry samples. The drying process affected the TPC content, apparently due to the fact that during the processing, some phenolic compounds might be suffering thermal or oxidative degradation, combined with the decreased activity of the first committed enzyme in the phenylpropanoids metabolism (Dimberg *et al*. 1996; Loaiza-Velarde *et al*. 1997; Ismail *et al*. 2004; Xu *et al*. 2007; Wojdylo *et al*. 2009). Moreover, based on the moisture content, the quantity of fresh sample expressed in dry basis, used for the TPC determination, was in the range of 0.1275 g to 0.3186 g (YF and TB, respectively), 7.8- and 3.1-fold lower that the amounts of drying samples (1 g).

It has been demonstrated that the processing of food could accelerate the release of phenolic compounds due to the breaking down of the cellular constituents. It must be taken into account that, as this is happening, hydrolytic and oxidative enzymes are being released (Wojdylo *et al*. 2009). Furthermore, it should be noted that the drying temperature used in this research was not sufficiently high to deactivate this set of enzymes. For this reason, it was not possible prevent the loss of phenolic compounds, generating a decrease of TPC in dry samples compared to the fresh samples (Table 3).

On the other hand, the leaf samples presented a higher TPC than the flower samples, which could be explained by the fact that the chlorophylls present in the leaves underwent a process of photosensitization (process of transferring the light energy absorbed). As this process is able to trigger the production of highly reactive chemical species at a cellular level, the leaf tissues are responsible for the generation of highly

effective antioxidants that are capable of eliminating free radicals and minimizing oxidative damage (del Baño *et al*. 2004; Chew *et al*. 2009).

The TPC obtained for *E. americana* leaves and flowers was lower than that reported for the extracts of legume flowers and leaves used in medicine (*Bauhinia kockiana*, *Bauhinia purpurea*, *Caesalpinia pulcherrima*, *Calliandra tergemina*, *Cassia surattensis*, *Leucaena leucocephala*, *Peltophorum pterocarpum*, *Samanea sama*, and *Acacia auriculiformis*); this was due to the specific extraction of phenolic compounds in these reports (Rakic *et al*. 2007; Chew *et al*. 2011), while in the case of the samples, both fresh and dry, analyzed in this study, the entire parts of the plant was analyzed and not an extract, giving rise to the possibility of some interference from other compounds present.

Table 3. Total Phenolic Content in different parts of *Erythrina americana* Miller

Structure	**TPC** (mg*g^{-1} sample)	
	Fresh	**Dry**
MF	417.44 ± 12^{b}	351.78 ± 17^{b}
YF	393.15 ±12^{c}	312.62 ± 11^{c}
FB	362.61 ± 8^{d}	166.98± 3^{d}
L	599.05 ± 3^{a}	402.12 ± 4^{a}
TB	76.42 ± 5^{e}	39.41 ± 3^{e}

*TPC is expressed as gallic acid equivalents (GAE) in milligrams per gram of sample. Different letters in each column indicate significant statistical differences ($P \leq 0.05$). The values are means±SD of triplicate measurements.

On the other hand, the leaves and the flowers in different stages of development presented a higher level of TPC content compared to those reported for other plants used in human food (Ismail *et al*. 2004).

The TPC of the tree bark was lower compared to the other parts of the plant. However, although this value was low, some phenolic compounds of interest for both the medical and food industries have been reported both in the tree bark and the root of *Erythrina sigmoides*, *Erythrina eriotricha*, *Erythrina burttii*, and *Erythrina indica*, among which are the following: sigmoidine L; ferulic acid; isoferulic acid; erytrinasinates C, and D; 5,7-dihydroxy-4´-methoxy-3´,5-di-(3-methylbut-2-enyl) flavonone; 5,7-dihydroxy-4´-methoxy-3´-(3-hydroxy-3-methylbut-1-enyl)-5´-(3-methylbut-2-enyl) flavonone; 5,4´-di-*O*-methylalpinumisoflavone; cajanin; 3-phenylcoumarin (indicanine B, and C); and indicanines D, and E (Nkengfack *et al*. 1997; Yenesew *et al*. 1998; Waffo *et al*. 2000; Nkengfack *et al*. 2001).

Lastly, a positive significant linear relationship between antioxidant activity and total phenolic content has been reported (Cai *et al*. 2004). However, this correlation was not observed in this study. This could be due to the conditions of the drying process, which may influence the antioxidant activity and TPC. Other studies have reported that the drying method, temperature and time of the treatment, type of extraction, and interactions among the different antioxidant reactions could be responsible for the difference in responses of the phenolic compounds to the Folin-Ciocalteu method (Balasundram *et al*. 2006; Que *et al*. 2008; Vega-Gávez *et al*. 2009).

CONCLUSIONS

1. The protein content in FB, YF, and TB was higher than 20%, while that for L and MF were around 20%.
2. The concentration of sample required to reach the IC_{50} value range was between 38 to 107 mg in dry basis.
3. The concentration of sample from *Erythrina americana* required to reach the IC_{50} value was high in comparison with other plants used in the medical area.
4. The TPC levels for L, YF, FB, and MF were higher than those reported for plants used for common consumption.
5. The drying process caused a decrease in TPC content.
6. The drying process resulted in an increase in the antioxidant activity of YF and MF.

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REFERENCES CITED

AOAC. (1990). *Official Methods of Analysis* (15th Ed.), Association of Official Analytical Chemists, Washington, DC.

Araújo-Júnior, J. X., Sant'Ana, A. E., Alexandre-Moreira, M. S., de Oliveira, M. S., and Aquino, P. G. (2012). *A Phytochemical and Ethnopharmacological Review of the Genus Erythrina*, INTECH Open Access Publisher.

Argolo, A. C. C., Sant'Ana, A. E. G., Pletsch, M., and Coelho, L. C. B. B. (2004). "Antioxidant activity of leaf extracts from *Bauhinia monandra*," *Bioresource Technology* 95(2), 229-233. DOI: 10.1016/j.biortech.2003.12.014

Balasundram, N., Sundram, K., and Samman, S. (2006). "Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses," *Food Chemistry* 99(1), 191-203. DOI: 1016/j.foodchem.2005.07.042

Barrera, N. (1994). "*Erythrina edulis*: Multipurpose tree for the tropical highlands," in: *Nitrogen Fixing Tree Association Highlights*, Hawaii, USA, pp. 1-2.

Bonilla, B. J. R., and Villaseñor, R. J. L. (2003). *Catálogo de la Flora del Estado de Morelos*, Centro de Investigaciones Biológicas, Universidad Autónoma del Estado de Morelos, México.

Brand-Williams, W., Cuvelier, M. E., and Berset, C. (1995). "Use of free radical method to evaluate antioxidant activity," *Lebensmittel Wissenschaft and Technologie* 28(1), 25-30. DOI:10.1016/S0023-6438(95)80008-5

Bruni, R., Medici, A., Andreotti, E., Fantin, C., Muzzoli, M., Dehesa, M., and Sacchetti, G. (2004). "Chemical composition and biological activities of Ishpingo essential oil, a

traditional Ecuadorian spice from *Ocotea quixos* (Lam.) Kosterm. (Lauraceae) flower calices," *Food Chemistry* 85(3), 415-421. DOI: 10.1016/j.foodchem.2003.07.019

Cai, Y., Luo, Q., Sun, M., and Corke, H. (2004). "Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer," *Life sciences*, 74(17), 2157-2184. DOI: 10.1016/j.lfs.2003.09.047

Chew, Y. L., Goh, J. K., and Lim, Y. Y. (2009). "Assessment of *in vitro* antioxidant capacity and polyphenolic composition of selected medicinal herbs from *Leguminosae* family in Peninsular Malaysia," *Food Chemistry* 116(1), 13-18. DOI: 10.1016/j.foodchem.2009.01.091

Chew, Y. L., Chan, E. W. L., Tan, P. L., Lim, Y. Y., Stanslas, J., and Goh, J. K. (2011). "Assessment of phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of *Leguminosae* medicinal plants in Peninsular Malaysia," *BMC Complementary and Alternative Medicine* 11(1), 12. DOI: 10.1186/1472-6882-11-12

Cowan, M. M. (1999). "Plant products as antimicrobial agents," *Clinical Microbiology Reviews* 12(4), 564-582.

Deba, F., Xuan, T. D., Yasuda, M., and Tawata, S. (2008). "Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from *Bidens pilosa* Linn. var. *Radiata*," *Food Control* 19(4), 346-352. DOI: 10.1016/jfoodcont.2007.04.011

del Baño, M. J., Lorente, J., Castillo, J., Benavente-García, O., Marín, M. P., Del Río, J. A., and Ibarra, I. (2004). "Flavonoid distribution during the development of leaves, flowers, stems, and roots of *Rosmarinus officinalis*. Postulation of a biosynthetic pathway," *Journal of Agricultural and Food Chemistry* 52(16), 4987-4992. DOI: 10.1021/jf040078p

Dien, B. S., Jung, H. J. G., Vogel, K. P., Casler, M. D., Lamb, J. F., Iten, L., Mitchell, B. R., and Sarath, G. (2006). "Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfalfa, reed canarygrass, and switchgrass," *Biomass and Bioenergy* 30(10), 880-891. DOI: 10.1016/j.biombioe.2006.02.004

Dimberg, L. H., Molteberg, E. L., Solheim, R., and Frølich, W. (1996). "Variation in oat groats due to variety, storage and heat treatment. I: Phenolic compounds," *Journal of Cereal Science* 24(3), 263-272. DOI: 10.1016/jcrs.1996.0058

Estrada, E. I., Morales, R. T., Soto-Hernández, M., Vázquez, M. M., García-Mateos, R., and Miguel-Chávez, R. S. (2009). "Actividad antimicótica *in vitro* de erisovina," *Revista Fitotecnia Mexicana* 32(4), 327.

García-Mateos, R., Lucas, B., Zendejas, M., Soto-Hernández, M., Martínez, M., and Sotelo, A. (1996). "Variation of total nitrogen, non-protein nitrogen content, and types of alkaloids at different stages of development in *Erythrina americana* seeds," *Journal of Agricultural and Food Chemistry* 44(10), 2987-2991. DOI: 10.1021/jf9600768

García-Mateos, R., Soto-Hernández, M., and Kelly, D. (1998). "Alkaloids from six *Erythrina* species endemic to Mexico," *Biochemical Systematics and Ecology* 26(5), 545-551. DOI: 10.1016/S0305-1978(97)00113-0

García-Mateos, R., Soto-Hernández, M., and Vibrans, H. (2001). "*Erythrina americana* Miller ("Colorín"; *Fabaceae*), a versatile resource from Mexico: A review," *Economic Botany* 55(3), 391-400. DOI: 10.1007/BF02866562

García-Mateos, R., Soto-Hernández, M., and Martínez, M. (2004a). "Variation in alkaloid type and content during *Erythrina americana* seed development. Recent advances of research in antinutritional factors in legume seeds and oil seeds," *Publication of the European Association for Animal Production* (Toledo, Spain) 110, 57-62.

García-Mateos, R., Pérez-Pacheco, R., Rodríguez-Hernández, C., and Soto-Hernández, M. (2004b). "Toxicidad de alcaloides de *Erythrina americana* en larvas de mosquito *Culex quinquefasciatus*," *Revista Fitotecnia Mexicana* 27(4), 297-303.

García-Mateos, M. R., Soto-Hernández, R. M., Gutiérrez, R. J. M., and Villegas-Monter, A. (2005). "Alkaloids from several subcultures of *Erythrina americana* Miller calluses," *Revista Chapingo Serie Horticultura* 11(1), 21-26.

Garín-Aguilar, M. E., Luna, J. E. R., Soto-Hernández, M., del Toro, G. V., and Vázquez, M. M. (2000). "Effect of crude extracts of *Erythrina americana* Mill. on aggressive behavior in rats," *Journal of Ethnopharmacology* 69(2), 189-196. DOI: 10.1016/S0378-8741(99)00121-X

Gheno-Heredia, Y. A., Nava-Bernal, G., and Martínez-Campos, Á. (2011). "Las plantas medicinales de la organización de parteras y médicos indígenas tradicionales de Ixhuatlancillo, Veracruz, México y su significancia cultural Polibotánica," *Polibotánica* (31), 199-251.

Hastings, R. B. (1990). "Medicinal legumes of Mexico: *Fabaceae, Papilionoideae,* Part one," *Economic Botany* 44(3), 336-348. DOI: 10.1007/BF03183915

Hussain, A. I., Anwar, F., Sherazi, S. T. H., and Przybylski, R. (2008). "Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations," *Food Chemistry* 108(3), 986-995. DOI: 10.1016/j.foodchem.2007.12.010

Hincapié, Ll., Adolfo, G., Vásquez O. D. C., Galicia M. V. S., and Hincapié, Ll. C. A. (2014). "Technological and functional properties of dietary fiber from mango peel var. Hilacha (*Mangifera indica* L.): Effect of convection drying," *Biotecnología en el Sector Agropecuario y Agroindustrial* 12(1), 153-160.

Ibarra Estrada, E., Pacheco Sánchez, M., García Mateos, R., San Miguel Chávez, R., Ramírez Valverde, G., and Soto Hernández, R. M. (2011). "Actividad antioxidante de alcaloides de *Erythrina americana* Miller," *Revista Fitotecnia Mexicana* 34(4), 241-246.

Ismail, A., Marjan, Z. M., and Foong, C. W. (2004). "Total antioxidant activity and phenolic content in selected vegetables," *Food Chemistry* 87(4), 581-586. DOI: 10.1016/j.foodchem.2004.01.010.

Juma, B. F., and Majinda, R. R. (2004). "Erythrinaline alkaloids from the flowers and pods of *Erythrina lysistemon* and their DPPH radical scavenging properties", *Phytochemistry*, 65(10), 1397-1404. DOI:10.1016/j.phytochem.2004.04.029.

Kuete, V., and Efferth, T. (2010). "Cameroonian medicinal plants: Pharmacology and derived natural products," *Frontiers in Pharmacology* 1, 1-19. DOI: 10.3389/fphar.2010.00123

Lambrecht, S. C., and Dawson, T. E. (2007). "Correlated variation of floral and leaf traits along a moisture availability gradient," *Oecologia* 157, 574-583. DOI: 10.1007/s00442-006-0617-7

Loaiza-Velarde, J. G., Tomás-Barberá, F. A., and Saltveit, M. E. (1997). "Effect of intensity and duration of heat-shock treatments on wound-induced phenolic

metabolism in iceberg lettuce," *Journal of the American Society for Horticultural Science* 122(6), 873-877.

Lu, Y., Zhong, C. X., Wang, L., Lu, C., Li, X. L., and Wang, P. J. (2009). "Anti-inflammation activity and chemical composition of flower essential oil from *Hedychium coronarium*," *African Journal of Biotechnology* 8(20), 5373-5377.

Maisuthisakul, P., Pasuk, S., and Ritthiruangdej, P. (2008). "Relationship between antioxidant properties and chemical composition of some Thai plants," *Journal of Food Composition and Analysis* 21(3), 229-240. DOI: 10.1016/j.jfca.2007.11.005

Márquez, C. J., and Ciro, H. J. (2002). "Deshidratación de mora de castilla (*rubus glaucus*) bajo régimen convectivo con aire forzado," *Revista Facultad Nacional de Agronomía* 55(2), 1587-1600.

Mathew, S., and Abraham, T. E. (2006). "*In vitro* antioxidant activity and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodologies," *Food and Chemical Toxicology* 44(2), 198-206. DOI: 10.1016/jfct.2005.06.013

Miguel-Chávez, S., Soto-Hernández, M., Terrazas, T., and Kite, G. (2006). "Morphology and alkaloidal profile of the seedlings of *Erythrina americana* MILL. and *E. coralloides* A. DC," *Feddes Repertorium* 117(3-4), 232-239. DOI: 10.1002/fedr.200511099

Mullen, C. A., and Boateng, A. A. (2008). "Chemical composition of bio-oils produced by fast pyrolysis of two energy crops," *Energy and Fuels* 22(3), 2104-2109. DOI: 10.1021/ef700776w

Muñoz, F. (1987). *Plantas Medicinales y Aromáticas: Estudio, Cultivo y Procesado*, Mundi-Prensa Libros. México.

Neill, D. A. (1988). "Experimental studies on species relationships in *Erythrina* (*Leguminosae: Papilionoideae*)," *Annals of Missouri Botanical Garden* 75(3), 886-969. DOI: 10.2307/2399377

Neill, D. A. (1993). "The genus *Erythrina*: Taxonomy, distribution, and ecological differentiation," in: *Erythrina in the New and Old Worlds*, S. B. Westley and M. H. Powell (eds.), Nitrogen Fixing Tree Association, Hawaii, USA, pp 15-27.

Nkengfack, A. E., Vouffo, T. W., Vardamides, J. C., Kouam, J., Fomum, Z. T., Meyer, M., and Sterner, O. (1997). "Phenolic metabolites from *Erythrina* species," *Phytochemistry* 46(3), 573-578. DOI: 10.1016/S0031-9422(97)00291-4

Nkengfack, A. E., Azebaze, A. G., Waffo, A. K., Fomum, Z. T., Meyer, M., and van Heerden, F. R. (2001). "Cytotoxic isoflavones from *Erythrina indica*," *Phytochemistry* 58(7), 1113-1120. DOI: 10.1016/S0031-9422(01)00368-5

Nutsch, W. (1996). "Tecnología de la madera y del mueble," *Reverté , S.A*, Barcelona-Bogotá-Buenos Aires-Caracas-México, pp. 26-27.

Odhav, B., Beekrum, S., Akula, U. S., and Baijnath, H. (2007). "Preliminary assessment of nutritional value of traditional leafy vegetables in KwaZulu-Natal, South Africa," *Journal of Food Composition and Analysis* 20(5), 430-435. DOI: 10.1016/j.jfca.2006.04.015

Pratt, D. E., and Hudson, B. J. F. (1990). "Natural antioxidants not exploited commercially," in: *Food Antioxidants*, B. J. F. Hudson (ed.), Elsevier, Amsterdam, pp 171-192.

Que, F., Mao, L., Fang, X., and Wu, T. (2008). "Comparison of hot air-drying and freeze-drying on the physicochemical properties and antioxidant activities of pumpkin (*Cucurbita moschata* Duch.) flours," *International Journal of Food Science and Technology*, 43(7), 1195–1201. doi: 10.1111/j.1365-2621.2007.01590x

Rakić, S., Petrović, S., Kukić, J., Jadranin, M., Tešević, V., Povrenović, D., and Šiler-Marinković, S. (2007). "Influence of thermal treatment on phenolic compounds and antioxidant properties of oak acorns from Serbia," *Food Chemistry* 104(2), 830-834. DOI: 10.1016/j.foodchem.2007.01.025

Randhir, R., and Shetty, K., (2005). "Developmental stimulation of total phenolics and related antioxidant activity in light- and dark germinated corn by natural elicitors," *Process Biochemistry* 40(5), 1721-1732. DOI: 10.1016/j.procbio.2004.06.064

Ratnasooriya, W. D., and Dharmasiri, M. G. (1999). "Aqueous extract of Sri Lankan *Erythrina indica* leaves has sedative but not analgesic activity," *Fitoterapia* 70(3), 311-313. DOI: 10.1016/S0367-326X(99)00027-1

Raven, P. H., Evert, R. F., and Eichhorn, S. E. (1992). *Biología de las Plantas* (Vol. 2), Reverté, S.A, Barcelona, España, pp 541-545.

Reimann, E. (2007). "Synthesis pathways to *Erythrina* alkaloids and *Erythrina* type compounds," in: *Progress in the Chemistry of Organic Natural Products*, W. Herz, H. Falk, and G. W. Kirby (eds), Springer, Vienna, pp. 1-62. DOI: 10.1007/978-3-211-49389-2_1

Rivera, J. A. T., Flores, R. C., and Cano, D. G. (2008). "Cercas de uso pecuario en la cuenca del río La Antigua, México: Inventario florístico y costo de construcción," *Zootecnica Tropical*. 26(3), 279-283.

Rosell, J. A., Gleason, S., Méndez-Alonzo, R., Chang, Y., and Westoby, M. (2014). "Bark functional ecology: Evidence for tradeoffs, functional coordination, and environment producing bark diversity," *New Phytologist*. 201(2), 486-497. DOI: 10.1111/nph.12541

Ruiz Montiel, C., Martínez Hernández, M. D. J., Romero Nápoles, J., and Ríos Reyes, A. V. (2012). "Primer reporte de *Specularius impressithorax* (Pic)(Coleoptera: Bruchidae) alimentándose de semillas de *Erythrina americana* miller en los Estados de Veracruz y Morelos, México," *Acta Zoológica Mexicana* 28(3), 635-639.

Sagar, V. R., and Kumar, P. S. (2010). "Recent advances in drying and dehydration of fruits and vegetables: A review," *Journal of Food Science and Technology* 47(1), 15-26. DOI: 10.1007/s13197-010-0010-8

San Miguel-Chavez, R., Soto-Hernandez, M., Ramos-Valdivia, A. C., and Kite, G. (2007). "Alkaloid production in elicited cell suspension cultures of *Erythrina americana* Miller," *Phytochemistry Reviews* 6(1), 167-173. DOI: 10.1007/s11101-006-9045-x

Sánchez-Herrera, S., Soto-Hernández, R. M., Kite, G., and García-Mateos, M. R. (2001). "Identificación de alcaloides en las inflorescencias de *Erythrina americana* Miller," *Revista Chapingo Serie Horticultura* 7(1), 37-48.

Shoaib, M., Zubaran, C., and Stolerman, I. P. (2000). "Antagonism of stimulus properties of nicotine by dihydro-β-erythroidine (DHβE) in rats," *Psychopharmacology* 149(2), 140-146. DOI: 10.1007/s002139900348

Soto-Hernández, R. M., García-Mateos, R., San Miguel-Chávez, R., Kite, G., Martínez-Vázquez, M., and Ramos-Valdivia, A. C. (2011). "Erythrina, a potential source of chemicals from the neotropics," *Bioactive Compounds in Phytomedicine*, Prof. Iraj Rasooli (ed), InTech, 163-184. DOI: 10.5772/26188

Sotelo, A. (1996). "The nutritive value of wild Mexican legumes with a potential for nutritional use," in: *Food and Feed from Legumes and Oilseeds*, E. Nwokolo, and J. Smartt (eds), Springer, New York, NY, pp 33-48. DOI: 10.1007/978-1-4613-0433-3_3

Sotelo, A., López-García, S., and Basurto-Peña, F. (2007). "Content of nutrient and antinutrient in edible flowers of wild plants in Mexico," *Plant Foods for Human Nutrition* 62(3), 133-138. DOI: 10.1007/s11130-007-0053-9

Sousa, S. M., Ricker, M., and Hernández, H. M. (2003). "An index for the tree species of the family *Leguminosae* in Mexico," *Harvard Papers in Botany* 7(2), 381-398. Retrieved from http://www.jstor.org/stable/41761665

Singleton, V., Orthofer, R., Lamuela-Raventos, R. (1999). "Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent," *Methods in Enzymology* 299(1), 152-178.

Sumbul, S., Ahmad, M. A., Mohd, A., and Mohd, A. (2011). "Role of phenolic compounds in peptic ulcer: An overview," *Journal of Pharmacy and Bioallied Sciences* 3(3), 361-367. DOI: 10.4103/0975-7406.84437

Tachakittirungrod, S., Okonogi, S., and Chowwanapoonpohn, S. (2007). "Study on antioxidant activity of certain plants in Thailand: Mechanism of antioxidant action of guava leaf extract," *Food Chemistry* 103(2), 381-388. DOI: 10.1016/j.foodchem.2006.07.034

Vega-Gálvez, A., Di Scala, K., Rodríguez, K., Lemus-Mondaca, R., Miranda, M., López, J., and Perez-Won, M. (2009). "Effect of air-drying temperature on physico-chemical properties, antioxidant capacity, colour and total phenolic content of red pepper (*Capsicum annuum*, L. var. Hungarian)," *Food Chemistry* 117(4), 647-653. DOI: 10.1016/j.foodchem.2009.04.066

Verduzco, J., Predo, C., and Mercado, R. (2008). "Caracterización e identificación taxonómica del Maguey. Producción y aprovechamiento de plantas en el noroeste de México," RESPYN (Revista Salud Pública y Nutrición). Edición Esepecial No. 2-2009, 4(2), 233-240.

Waffo, A. K., Azebaze, G. A., Nkengfack, A. E., Fomum, Z. T., Meyer, M., Bodo, B., and van Heerden, F. R. (2000). "Indicanines B and C, two isoflavonoid derivatives from the root bark of *Erythrina indica*," *Phytochemistry* 53(8), 981-985. DOI: 10.1016/S0031-9422(99)00615-9

Wojdyło, A., Figiel, A., and Oszmiański, J. (2009). "Effect of drying methods with the application of vacuum microwaves on the bioactive compounds, color, and antioxidant activity of strawberry fruits," *Journal of Agricultural and Food Chemistry* 57(4), 1337-1343. DOI: 10.1021/jf802507j

Yenesew, A., Midiwo, J. O., Miessner, M., Heydenreich, M., and Peter, M. G. (1998). "Two prenylated flavanones from stem bark of *Erythrina burttii*," *Phytochemistry* 48(8), 1439-1443. DOI: 10.1016/S0031-9422(97)00945-X

Xu, G., Ye, X., Chen, J., and Liu, D. (2007). "Effect of heat treatment on the phenolic compounds and antioxidant capacity of citrus peel extract," *Journal of Agricultural and Food Chemistry* 55(2), 330-335. DOI: 10.1021/jf062517I

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