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RESEARCH PAPER

Effect of climate conditions on total phenolic content and antioxidant activity of *Jatropha dioica* Cerv. var. *dioica*

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Abstract

J. Gutiérrez-Tlahque, C.L. Aguirre-Mancilla, J.C. Raya-Pérez, J.G. Ramírez-Pimentel, R. Jiménez-Alvarado, and A.D. Hernández-Fuentes. 2018. Effect of climate conditions on total phenolic content and antioxidant activity of *Jatropha dioica* Cerv. var. *dioica*. *Cien. Inv. Agr.* 45(1): 70-81. The aim of this work was to determine the antioxidant activity, total phenolic content (TPC) and total flavonoid content (TFC) in the rhizomes and stems of *Jatropha dioica* and their relation to collection season, collection location, extraction solvent and their interactions to understand the effect of climate conditions on the synthesis of the antioxidant compounds in *J. dioica*. Plants were collected during different seasons at two locations in Mexico. A 3-factorial experimental design was used for the stems and rhizomes, using the extraction solvent, collection location and season as sources of variation. A Tukey's test with a $P \leq 0.05$ significance level was used to perform the comparison of the means. Significant differences were found when comparing the three sources of variation separately for TPC and antioxidant activity in the stems and rhizomes. In terms of the interactions for the stems, there was a significant difference between the Morelos $\times$ 70% ethanol $\times$ dry interaction and Tetepango $\times$ water $\times$ rain interaction for the TPC and the antioxidant activity; however, no significant difference was found for the TFC. In terms of the rhizomes, a significant difference was found among the 4 variables analyzed. These results indicate that collection location, collection season and extraction solvent affect the TPC and antioxidant activity. In addition, the rhizomes presented higher antioxidant activity and TPC than that of the stems.

Keywords: Antioxidants, drought stress, flavonoids, rhizomes, Sangre de drago, stems.

Introduction

Jatropha dioica, commonly known as “Sangre de drago”, is a plant endemic to Mexico. This plant grows in the wild in arid and semi-arid regions

(Silva-Belmares *et al.*, 2014). It is well known that several factors can affect the concentration of secondary metabolites in plants. An accumulation of metabolites often occurs in plants subjected to stresses including various elicitors or signal molecules. Depending on the biotic and abiotic stresses suffered during growth and development,

plants can produce several compounds that act as chemical defenses. These chemicals have biological activities that can be used to prevent or alleviate several diseases. *J. dioica* rhizomes and stems have been used in Mexican traditional medicine. The sap of stems is applied directly on the eyes to treat some eye diseases such as pterygium or irritation (Fresnedo-Ramírez and Orozco-Ramírez, 2013), and the aqueous dilution of the sap is used as a beverage to prevent different types of cancer (López-Gutiérrez *et al.*, 2014). Similarly, the stems are eaten fresh to treat diarrhea and hemorrhoids (Silva-Belmares *et al.*, 2014), and its decoction is applied topically for the control of alopecia (Razo-Rodríguez and Alvarado-Bárceñas, 2015). In addition, the aqueous extract of the stems has demonstrated antifungal activity (Oliveira-Simone *et al.*, 2013). To the best of our knowledge, the only compound identified in stems of *J. dioica* has been ellagic acid (Aguilera-Carbo *et al.*, 2008). This phenolic compound has been attributed to different medicinal properties such as being antiatherosclerotic (Aviram *et al.*, 2000), anticancer (Carraway *et al.*, 2004), antibacterial (Machado *et al.*, 2002), and antiparasitic (Elkhateeb *et al.*, 2005). On the other hand, the rhizomes of *J. dioica* are chewed raw to alleviate toothaches (Villarreal *et al.*, 1988), and its decoction has been demonstrated as having anti-genotoxic effects in kidneys, liver, and bone marrow (Martínez *et al.*, 2014). The ethanolic extract of the rhizomes of *J. dioica* has been demonstrated as having antifungal and antibacterial properties, while the methanolic extract has been shown as having anti-viral properties (Silva-Mares *et al.*, 2013), and the hexanic extract of the rhizomes of *J. dioica* has been demonstrated as having antibacterial properties (Silva-Belmares *et al.*, 2014). In addition, studies have been conducted on the antioxidant activity, total flavonoid content (TFC), and total phenolic content (TPC) in *J. dioica* by mixing the two parts of the plant, i.e., without separating stems and roots (Wong-Paz *et al.*, 2014). Additionally, several studies have been conducted related to their antioxidant properties, TFC, and TPC, but without considering the collection season or the

collection location, which can vary the concentration of antioxidant compounds in plants. Our hypothesis is that climate conditions affect the synthesis of antioxidant compounds in *J. dioica* due to drought stress and to UV-B radiation in the dry seasons and in locations where rainfall decreases, but it is not clear to what extent the antioxidant compounds are affected. Thus, the aim of this work was to evaluate the antioxidant activity, the TPC, and the TFC in the rhizomes and stems of *J. dioica*, and their relationship with the season of collection, the location of collection, the solvent for extraction and their interactions to understand the effect that climate conditions have on the synthesis of antioxidant compounds in *J. dioica*.

Materials and methods

Plant material

Specimens of *Jatropha dioica* Cerv. var. *dioica* were collected during two different seasons (the rainy season in September 2014, and the dry season in February 2015) from two locations in Mexico: the town of Tetepango located in the state of Hidalgo (20°06'38" N, 99°09'11" W, 2100 MASL) that has a cumulative rainfall of 543 mm and a mean annual temperature of 16.3 °C, and the town of Morelos, located in the state of Zacatecas (22°53'12" N, 102°36'45" W, 2300 MASL) that has a cumulative rainfall of 415 mm and a mean annual temperature of 15.2 °C. The climate at both locations is classified as BSk according to Köppen (García, 2004). The specimens were identified and preserved in the herbarium at the Autonomous University of Hidalgo (voucher number AD Hernandez Fuentes 01). The rhizomes and stems were washed with distilled water to eliminate the residues of soil and dust and were separated using hand shears (Felco F2, Fleisch Holding S.A., Switzerland), and then, the samples were stored in an ultra-low temperature freezer (Thermo Scientific FORMA 703) at -71 °C. Subsequently, the samples were

lyophilized in a freeze drier system (Labconco FREEZONE 6). The lyophilized samples were separated into eight batches: rhizomes from Morelos collected during the rainy season, stems from Morelos collected during the rainy season, rhizomes from Tetepango collected during the rainy season, stems from Tetepango collected during the rainy season, rhizomes from Morelos collected during the dry season, stems from Morelos collected during the dry season, rhizomes from Tetepango collected during the dry season and stems from Tetepango collected during the dry season, and each batch was stored in a resealable bag (Ziploc, SC Johnson, USA) and kept in darkness at 4 °C in a refrigerator (Shel Lab HC30R, Sheldon Manufacturing, Inc., USA).

Extraction method

Maceration was carried out using distilled water or ethanol (70% v/v; Macron, USA) at 25 °C in darkness. The solvent to sample ratio was 15/1 mL g⁻¹ based on Wong-Paz *et al.* (2014). The extraction time was standardized using two h for all the samples. The aqueous and ethanolic extracts were codified adding W or E, respectively, as a subscript for each of the batches previously described. These extracts were filtered through Whatman filter paper number 5. The aqueous extracts were lyophilized in a freeze dryer system (Labconco FREEZONE 6). The ethanol in the extracts was evaporated under reduced pressure at 50 mbar and 60 °C using a rotary evaporator until a dry extract was obtained. A standardized time of 2 h was used to evaporate the ethanol. All dried extracts were stored in Eppendorf tubes in the darkness at 4 °C until they were needed for the study. All extractions were done in triplicate.

Antioxidant activity

The ABTS^{•+} and DPPH assays were performed following the procedures described by Re *et al.* (1999) and Brand-Williams *et al.* (1995), respec-

tively. These two assays were included because *J. dioica* extracts do not absorb in the range of the monitored wavelengths (734 nm for ABTS and 515 nm for DPPH). The results were expressed as percentage inhibition of ABTS^{•+} and percentage inhibition of DPPH according to equations 1 and 2, respectively.

$$\text{Percentage inhibition of DPPH} = [(C_{abs} - S_{abs}) / (C_{abs})] \times 100 \quad (1)$$

$$\text{Percentage inhibition of ABTS}^{•+} = [(C_{abs} - S_{abs}) / (C_{abs})] \times 100 \quad (2)$$

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

Total phenolic content (TPC)

The Folin-Ciocalteu method was employed to estimate the TPC (Waterman and Mole, 1994). A sample of 1 mL was mixed with 5 mL of an aqueous solution of a 1:10 Folin-Ciocalteu reactant (Sigma-Aldrich, USA) in water. This mixture was left for 7 min at room temperature, and subsequently, 4 mL of an aqueous solution of sodium carbonate (7.5% w/w; Sigma-Aldrich) was added and reacted during 2 h at room temperature in darkness. The absorbance was measured at 760 nm using a spectrophotometer (Jenway 6715, Bibby Scientific Ltd. U.K.). A calibration curve was prepared using concentrations of gallic acid (Sigma-Aldrich) that ranged between 0 and 100 mg L⁻¹. The results were expressed as milligrams of gallic acid equivalents (mg GAE) per gram of dry weight (DB).

Total flavonoid content (TFC)

TFC was determined according to Chang *et al.* (2002). An extract of 1 g was mixed with 10 mL of methanol and vortexed for 10 min. Then, 0.5 mL of extract was mixed with 0.15 mL of NaNO₂ (5% w/w; Reasol, Mexico). This mixture was left for 5

min in darkness. Then, 0.15 mL of a methanolic solution of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (2% w/w) and 1 mL of NaOH (Reasol) were added, and the mixture was left for 15 min. The absorbance was measured using a spectrophotometer (Jenway 6715) at a wavelength of 415 nm. A calibration curve was prepared using quercetin (Sigma-Aldrich) as a standard. The results were expressed as milligrams of quercetin equivalents (mg QE) per gram of DB.

Statistical methods

A 3-factorial experimental design was used to determine the effect of season, location, and solvent on the antioxidant activity, TPC, and TFC in the rhizomes and stems. The data were expressed as the mean of the three independent experiments $\pm$ standard deviation. The statistical comparisons of the results were subjected to two-way ANOVA using SAS 9.0 software. The significant differences ($P < 0.05$) were analyzed by the Tukey test.

Results

Antioxidant activity, total phenolic and flavonoid content

Figure 1 A) and Figure 2 A) show the effect of the collection location on the TPC and the TFC of the stems and rhizomes of *J. dioica* and on the free radical scavenging activity by the ABTS^{•+} and

DPPH assays. This effect was significant on the TPC and the ABTS^{•+} and DPPH radical scavenging activity and not significant on the TFC. Morelos displayed the highest values of these variables. This behavior was displayed by both parts of the plant (rhizomes and stems).

The effect of the collection season (Figure 1 B and Figure 2 B) was significant on the four variables, with significantly higher values ($P < 0.05$) obtained during the dry season. The same behavior was displayed by both parts of the plant.

The effect of the extraction solvent on the four variables is shown in Figure 1 C and Figure 2 C. For the stems, the solvent used for the extraction had a significant effect on the antioxidant activity (ABTS^{•+} and DPPH) and on the TPC, with higher values obtained when 70% ethanol was used. Nevertheless, the differences in the TFC were not significantly different between the extraction solvents used. Meanwhile, for the rhizomes, a significant effect was identified on the four variables, with higher values ($P < 0.05$) obtained when 70% ethanol was used as the extraction solvent.

The collection location $\times$ collection season interaction is shown in Figure 3 A and Figure 4 A. These results highlight the effect of the dry season on the DPPH radical scavenging activity and on the TPC of the stems, displaying statistically significant differences among all the possible interactions. With respect to the TFC of the

Figure 1. Effect of A) collection location, B) collection season and C) extraction solvent on total flavonoid content (TFC) and on total phenolic content (TPC) of *J. dioica* stems (S) and rhizomes (R). Different letters between each pair of bars means significant differences between the treatments (Tukey, $P < 0.05$).

Figure 2. Effect of A) collection location; B) collection season and C) extraction solvent on the ABTS and DPPH radical scavenging activity of *J. dioica* stems (S) and rhizomes (R). Different letters between each pair of bars means significant differences between the treatments (Tukey, $P \leq 0.05$).

stems, the Morelos $\times$ dry interaction displayed a statistically significant difference only when compared with the Tetepango $\times$ raining interaction. In the case of the rhizomes, the results showed statistically significant differences among all the interactions on the radical scavenging activity, either by ABTS⁺⁺ or DPPH. Statistically significant differences were also found for the TPC values, and the Morelos $\times$ dry interaction displayed significantly higher values ($P < 0.05$). In relation to the TFC of the rhizomes, the Morelos $\times$ dry interaction displayed statistically significant differences when compared with the Morelos $\times$ raining interaction and with the Tetepango $\times$ raining interaction. A statistically significant difference was also found in the rhizomes when the Tetepango $\times$ dry interaction was compared with the Tetepango $\times$ raining interaction.

Figure 3 B and Figure 4 B show the effect of the collection location $\times$ extraction solvent interaction on the four response variables. For the stems, no statistically significant differences in the TFC among the interactions were identified, nevertheless, significant differences were found in the TPC and radical scavenging activity by DPPH and ABTS⁺⁺ when the Morelos $\times$ 70% ethanol interaction was compared with the Morelos $\times$ water interaction. Similar results were obtained when the Tetepango $\times$ 70% ethanol interaction was compared with the Tetepango $\times$ water interaction. Meanwhile, for the rhizomes, statistically significant differences were found in the TPC values for all the interactions. Like the results for the stems, the results for the rhizomes showed statistically significant differences in the TPC and DPPH and ABTS⁺⁺ activity when the

Figure 3. Effect of the interactions of the pairs of variables: A) collection location $\times$ collection season; B) collection location $\times$ solvent and C) collection season $\times$ solvent on total flavonoid content (TFC) and on total phenolic content (TPC) of *J. dioica* stems (S) and rhizomes (R). Collection locations are represented as M (Morelos) and T (Tetepango). Collection seasons are represented as D (dry) and F (raining). Solvents are represented as W (water) and 70E (70% ethanol). Different lower-case letters between each group of four bars means significant differences between the interactions for the stems (Tukey, $P \leq 0.05$). Different capital letters between each group of four bars means significant differences between the interactions for the rhizomes (Tukey, $P \leq 0.05$).

Morelos $\times$ 70% ethanol interaction was compared with the Morelos $\times$ water interaction and when the Tetepango $\times$ 70% ethanol interaction was compared with the Tetepango $\times$ water interaction.

Considering the Collection season $\times$ extraction solvent interaction, significant differences were found in the TPC (Figure 3 C and Figure 4 C). Meanwhile, no statistically significant differences were found in the TFC for the stems. In terms of the ABTS^{•+} and DPPH radical scavenging activity, statistically significant differences were found for the stems when the dry $\times$ 70% ethanol interaction was compared with the fry $\times$ water interaction. Significant differences were also found for the stems when the dry $\times$ 70% ethanol interaction was compared with the raining $\times$ water interaction and when the raining $\times$ 70% ethanol interaction was compared with the raining $\times$ water interaction. Nevertheless, no significant difference was found in the stems for the ABTS^{•+} and DPPH methods when the dry $\times$ water interaction was compared with the raining $\times$ 70% ethanol. On the other hand, the rhizomes displayed statistically significant differences in the TPC and ABTS^{•+} values among the interactions, with the dry $\times$ 70% ethanol interaction displaying significantly higher values. Statistically significant differences were found in the DPPH values when the dry $\times$ 70% ethanol interaction was compared to the dry $\times$ water interaction, and when the dry $\times$

70% ethanol interaction was compared with the raining $\times$ water interaction. Nevertheless, no statistically significant difference in the DPPH values was found in the rhizomes when the dry $\times$ water interaction was compared to the raining $\times$ 70% ethanol interaction. Meanwhile, the TFC values in the rhizomes showed statistically significant differences only when the dry $\times$ 70% ethanol interaction was compared to the raining $\times$ water interaction.

Figure 5 and Figure 6 show the effect of the collection location $\times$ extraction solvent $\times$ collection season interaction on the four response variables. In terms of the TPC and the DPPH radical scavenging activity of the stems (Figure 5 A and Figure 6 A), the Morelos $\times$ 70% ethanol $\times$ dry interaction showed statistically significant differences when compared with the Tetepango $\times$ 70% ethanol $\times$ dry interaction. A similar behavior was found in stems when the Morelos $\times$ water $\times$ dry interaction was compared with the Tetepango $\times$ water $\times$ dry interaction. Meanwhile, significant differences were found in the DPPH and ABTS^{•+} radical scavenging activity and in the TPC of the stems when the Morelos $\times$ 70% ethanol $\times$ raining interaction was compared with the Tetepango $\times$ 70% ethanol $\times$ raining interaction and when the Morelos $\times$ water $\times$ raining interaction was compared with the Tetepango $\times$ water $\times$ raining interaction. In the case of the rhizomes,

Figure 4. Effect of the interactions of pairs of variables: A) collection location $\times$ collection season; B) collection location $\times$ solvent and C) collection season $\times$ solvent on the ABTS and DPPH radical scavenging activity of *J. dioica* stems (S) and rhizomes (R). Collection locations are represented as M (Morelos) and T (Tetepango). Collection seasons are represented as D (dry) and F (raining). Solvents are represented as W (water) and 70E (70% ethanol). Different lower-case letters between each group of four bars means significant differences between the interactions for the stems (Tukey, $P \leq 0.05$). Different capital letters between each group of four bars means significant differences between the interactions for the rhizomes (Tukey, $P \leq 0.05$).

statistically significant differences were found in the TPC values among all the interactions (Fig. 5 B), except when the Tetepango $\times$ 70% ethanol $\times$ raining interaction was compared with the Morelos $\times$ water $\times$ raining interaction. Statistically significant differences were also found when the Morelos $\times$ 70% ethanol $\times$ dry interaction was compared with the Tetepango $\times$ 70% ethanol $\times$ raining interaction, the Morelos $\times$ water $\times$ raining interaction and the Tetepango $\times$ water $\times$ raining interaction. Statistically significant differences were

also found in the rhizomes when the Tetepango $\times$ 70% ethanol $\times$ dry interaction was compared with the Tetepango $\times$ water $\times$ raining interaction. Meanwhile, for the DPPH and ABTS^{•+} values (Fig. 6 B), statistically significant differences were found when the Morelos $\times$ 70% ethanol $\times$ dry and the Tetepango $\times$ 70% ethanol $\times$ dry interactions were compared with the Tetepango $\times$ water $\times$ dry, Tetepango $\times$ 70% ethanol $\times$ raining, Morelos $\times$ water $\times$ raining and Tetepango $\times$ water $\times$ raining interactions.

Figure 5. Effect of the collection location $\times$ solvent $\times$ collection season interaction on the total flavonoid content (TFC) and on total phenolic content (TPC) of *J. dioica* stems (A) and rhizomes (B). Collection locations are represented as M (Morelos) and T (Tetepango). Collection seasons are represented as D (dry) and F (raining). Solvents are represented as W (water) and 70E (70% ethanol). Different letters between each group of eight bars means significant differences between the interactions (Tukey, $P \leq 0.05$).

Figure 6. Effect of the collection location $\times$ solvent $\times$ collection season interaction on the ABTS and DPPH radical scavenging activity of *J. dioica* stems (A) and rhizomes (B). Collection locations are represented as M (Morelos) and T (Tetepango). Collection seasons are represented as D (dry) and F (raining). Solvents are represented as W (water) and 70E (70% ethanol). Different letters between each group of eight bars means significant differences between the interactions (Tukey, $P \leq 0.05$).

Discussion

Although the climate classifications of both collection locations are equal, a lower amount of precipitation occurred in Morelos during the year. This factor could have influenced the results of the antioxidant activity and TPC, which were found to be significantly higher in Morelos (Figure 1A and Figure 2A) for both parts of the plant. Moreover, when the collection seasons were compared, the dry season displayed significantly higher values for the four variables studied (Figure 1B and Figure 2B). The collection season was the unique factor influencing the TFC among the three factors assessed in this study for both parts of the plant. These results can be explained based on the accumulation of secondary metabolites, including phenolic compounds, and flavonoids, which can be used as a strategy for tolerance towards ultraviolet-B (UV-B) radiation and drought stress (Wolf *et al.*, 2010; Varela *et al.*, 2016), since this plant grows wild in unshaded habitats that are exposed to high levels of light and UV-B radiation intensities. It is well known that ultraviolet radiation is one of the main free radical generators, and plants synthesize secondary metabolites that act as UV-B absorbing compounds and accumulate in the vacuoles of epidermal cells. These compounds are mostly phenolic compounds, including flavonoids (Wolf *et al.*, 2010); therefore, it is also probable that the higher production of antioxidant compounds found in stems is related to UV-B radiation. Furthermore, as the level of drought increases, the level of oxidative stress in the cells and tissues of plants also increased, which implies the initiation of lipid peroxidation. Therefore, the increase in antioxidant levels is attributed to its free radical scavenging capacity, which avoids lipid peroxidation, thus maintaining a cell membrane free of injury (Jaafar *et al.*, 2012). As can be observed in Figures 1 and 2, the results of the TPC, TFC and radical scavenging activity were higher in the rhizomes than in the stems. This result is because in plants the roots are the first tissue to sense a water-deficit condition and induce stress signals (Selote and Khanna-Chopra, 2006).

With respect to the effect of the extraction solvent on the antioxidant activity and TPC, 70% ethanol had higher results (Figure 1C and Figure 2C) for both parts of the plant. This result can be explained based on the polarity of the extraction solvent. Other studies have used 70% ethanol in water and demonstrated good extraction yields, since ethanol facilitates the diffusivity of phenolic compounds when mixed with water (Wong-Paz *et al.*, 2015a). Moreover, it has been demonstrated by other studies that water is not the best solvent for extracting phenolic compounds (Upadrashta *et al.*, 2011). On the other hand, it has been reported that phenolic compounds are more potent DPPH scavengers than flavonoids (Ali *et al.*, 2010), which explains why the extracts obtained with 70% ethanol displayed the highest antioxidant activity, even when TFC was not significantly different between the extraction solvents. The results of the TPC and antioxidant activity in this study were higher than those reported for the mixture of roots and stems of *J. dioica* by Wong-Paz *et al.* (2015b). These differences in the literature can be explained by the gentle treatment of the plant parts used in this work, since the literature reports a convective drying at 60 °C for 48 h of the plant and a heat-reflux extraction, while lyophilization and maceration at 25 °C were used in this work.

The comparisons between collection location × collection season interactions highlighted the effect of drought stress and UV-B radiation (Figures 3A and 4A) for both parts of the plant. The TFC (which did not show changes as a function of location or season, separately) displayed statistically significant differences when the location with the lowest accumulated rain value (Morelos) during the dry season was compared with the location that had the highest accumulated rain value (Tetepango) during the raining season. According to these results, Morelos × dry was the best interaction, presenting the highest values for all the response variables studied. A similar trend between UV-B radiation or drought and high levels of TPC and antioxidant activity has been reported in other studies (Stracke *et al.*,

2010; Gharibi *et al.*, 2016). Varela *et al.* (2016) showed that the accumulation of polyphenols is an important feature in the mechanisms employed by xerophytic species to avoid oxidative damage. Moreover, the effect of climate variations on TPC and antioxidant activity has been reported by Sivaci and Sökmen (2004).

Figures 3 B and 4 B, are useful for identifying the best collection location $\times$ solvent interaction for the TPC and antioxidant activity. As discussed above, Morelos was the location that produced the highest concentration of phenolics in both parts of the plant due to the UV-B radiation and drought stress, since this was the location with lower levels of precipitation and therefore less clouds in the sky. When the plants collected in this location were combined with the use of 70% ethanol, which was the best extraction solvent, the best collect location $\times$ solvent interaction was found. Figures 3 C and 4 C demonstrate that the best collection season $\times$ solvent interaction was obtained when 70% ethanol was used as for the solvent extraction in the samples collected during the dry season. These results confirm our understanding about the effect of the climate conditions ultraviolet radiation and drought stress on the increase in TPC and antioxidant activity.

Finally, the best interaction among the three factors tested in this study (collection location, extraction solvent, and collection season) is shown in Figures 5 and 6. The Morelos $\times$ 70% ethanol $\times$ dry interaction displayed the highest TPC and antioxidant activity in both parts of the plant, and this result was expected, since this interaction was formed by the factors that promote the synthesis of phenolic compounds in the plant (Morelos and dry season) and the factor that allows the best extraction of phenolic compounds (70%

ethanol). Moreover, the increase in TPC under drought stress is attributed to the accumulation of soluble carbohydrates in the plant cells due to the diminishing transport of soluble sugars (Ibrahim and Jaafar, 2011). Thus, phenolic compounds are biosynthesized by the shikimate pathway, which is the responsible for converting simple sugars into aromatic amino acids (Ghasemzadeh *et al.*, 2010). The results of the DPPH radical scavenging activity are consistent with those reported by Wong-Paz *et al.* (2014) for a mixture of roots and stems. The results of the ABTS^{•+} radical scavenging activity are higher than those reported by Wong-Paz *et al.* (2015b) for a mixture of roots and stems, where an inhibition of 24.40% was found.

In conclusion, this study confirmed that abiotic factors such as UV-B radiation due to sunlight and drought stress have a significant effect on the synthesis of antioxidant compounds in *Jatropha dioica*. Ultraviolet-B radiation contributes to the production of phenolics mainly by its incidence on the stems, and drought stress promotes the production of these types of compounds that mainly affect the rhizomes of the plant. On the other hand, ethanol (70% v/v) demonstrated that it could extract more of these antioxidant compounds from the plant in comparison to water. Further studies are required to isolate, quantify and identify the antioxidant compounds produced by *J. dioica* and to assess other bioactivities different from antioxidant activity.

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Resumen

J. Gutiérrez-Tlahque, C.L. Aguirre-Mancilla, J.C. Raya-Pérez, J.G. Ramírez-Pimentel, R. Jiménez-Alvarado, y A.D. Hernández-Fuentes. 2018. Efecto de las condiciones climáticas sobre el contenido de fenoles totales y la actividad antioxidante de *Jatropha dioica* Cerv. var. *dioica*. Cien. Inv. Agr. 45(1): 70-81. El objetivo de este trabajo fue determinar la actividad antioxidante, el contenido de fenoles totales (TPC) y el contenido de flavonoides totales (TFC) en rizomas y tallos de *Jatropha dioica*, y su relación con la época de recolección, la localidad de la recolección, el solvente de extracción y sus interacciones, con el fin conocer el efecto de las condiciones climáticas sobre la síntesis de compuestos antioxidantes en *J. dioica*. Las plantas fueron recolectadas durante diferentes épocas en dos lugares de México. Se utilizó un diseño experimental 3 factorial en tallos y rizomas, usando como fuentes de variación el solvente de extracción, el lugar de recolección y la época de recolección. Se utilizó una prueba de Tukey con un $P \leq 0.05$ para realizar las comparaciones de medias. Se encontraron diferencias significativas cuando se compararon por separado las tres fuentes de variación para TPC y para la actividad antioxidante en tallos y rizomas. Respecto a las interacciones de los tallos, hubo una diferencia significativa entre la interacción Morelos $\times$ 70% de etanol $\times$ seco y la interacción Tetepango $\times$ agua $\times$ lluvia para TPC y para la actividad antioxidante; sin embargo, no se encontraron diferencias significativas para TFC. Respecto a los rizomas, se encontró una diferencia significativa entre las 4 variables analizadas. Esto indica que hay un efecto de la localidad de recolección, la época de recolección y el solvente de extracción sobre el TPC, y en la actividad antioxidante. Además, los rizomas presentaron una mayor actividad antioxidante y TPC que los tallos.

Palabras clave: Antioxidantes, estrés hídrico, flavonoides, rizomas, Sangre de drago, tallos.

References

- Aguilera-Carbo, A.F., C. Augur, L.A. Prado-Barragan, C.N. Aguilar, and E. Favela-Torres. 2008. Extraction and analysis of ellagic acid from novel complex sources. *Chemical Papers* 62:440-444.
- Ali, Q., M. Ashraf, and F. Anwar. 2010. Seed composition and seed oil antioxidant activity of maize under water stress. *J Am Oil Chem Soc* 87:1179–1187.
- Aviram, M., L. Dornfeld, M. Rosenblat, N. Volkova, M. Kaplan, R. Coleman, T. Hayek, D. Presser, and B. Fuhrman. 2000. Pomegranate juice consumption reduce oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in human and in atherosclerotic apolipoprotein E-deficient mice. *Am. J. Clin. Nutr.* 71:1062–1076.
- Brand-Williams, W., M.E. Cuvelier, and C. Berset. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci Technol* 28:25–30.
- Carraway, R.E., S. Hassan, and D.E. Cochrane. 2004. Polyphenolic antioxidants mimic the effects of 1,4-dihydropyridines on neurotensin receptor function in PC3 cells. *J. Pharmacol. Exp. Ther.* 309:92–101.
- Chang, C.C., M.H. Yang, H.M. Wen, and J.C. Chern. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal* 10:178–182.
- Elkhateeb, A., K. Takahashi, H. Matsuura, M. Yam-saki, O. Yamato, Y. Maede, K. Katakura, T. Yoshihara, and K. Nabeta. 2005. Anti-babesial ellagic acid rhamnosides from the bark of *Elaeagnus parvifolius*. *Phytochemistry* 66: 2577-2580.
- Fresnedo-Ramírez, J., and Q. Orozco-Ramírez. 2013. Diversity and distribution of genus *Jatropha* in Mexico. *Genet. Resour. Crop Evol.* 60:1087–1104.

- García, E. 2004. Modificaciones al Sistema de Clasificación Climática de Köppen. 5ª Ed. Instituto de Geografía UNAM, Mexico. 257 pp.
- Gharibi, S., B.E. Tabatabaei, G. Saeidi, and S.A. Goli. 2016. Effect of drought stress on total phenolic, lipid peroxidation, and antioxidant activity of *Achillea* species. *Appl. Biochem. Biotechnol.* 178:796–809.
- Ghasemzadeh, A., H.Z.E. Jaafar, and A. Rahmat. 2010. Elevated carbon dioxide increases contents of flavonoids and phenolic compounds, and antioxidant activities in Malaysian young ginger (*Zingiber officinale* Roscoe.) varieties. *Molecules* 15:7907–7922.
- Ibrahim, M.H., and H.Z.E. Jaafar. 2011. Photosynthetic capacity, photochemical efficiency and chlorophyll content of three varieties of *Labisia pumila* Benth exposed to open field and greenhouse growing conditions. *Acta Physiol. Plant.* 33:2179–2185.
- Jaafar, H.Z., M.H. Ibrahim, and N.F.M. Fakri. 2012. Impact of soil field water capacity on secondary metabolites, phenylalanine ammonia-lyase (PAL), malondialdehyde (MDA) and photosynthetic responses of Malaysian kacipfatimah (*Labisia pumila* Benth). *Molecules* 17:7305–7322.
- López-Gutiérrez, B.N., B.E. Pérez-Escandón, and M.A. Villavicencio-Nieto. 2014. Aprovechamiento sostenible y conservación de las plantas medicinales en Cantarranas, Huehuetla, Hidalgo, México, como un medio para mejorar la calidad de vida en la comunidad. *Bot Sci* 92:389–404.
- Machado, T.B., I.C.R. Leal, I.C. Amaral, K.R. Dos Santos, M.G. Da Silva and R.M. Kuster. 2002. Antimicrobial ellagitannin of *Punica granatum* fruits. *J Braz Chem Soc* 13:606–610.
- Martínez, N., G. Almaguer, P. Vásquez, A. Figueroa, C. Zúñiga and A. Hernández. 2014. Análisis fitoquímico de *Jatropha dioica* y determinación de su efecto antioxidante y quimioprotector sobre el potencial genotóxico de ciclofosfamida, daunorubicina y metilmetanosulfonato evaluado mediante el ensayo cometa. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas* 13:437–457.
- Oliveira-Simone, G.D., P.S. Nascente, R.V. Carvalho, E. Piva, and R.G. Lund. 2013. Evaluation of anti-*Candida* activity and cytotoxicity of *Jatropha dioica* Cerv. extracts. *Afr J Microbiol Res* 7:3903–3907.
- Razo-Rodríguez, C., and E. Alvarado-Bárcenas. 2015. Captosán tónico capilar de sangre gado (*Jatropha dioica*). *Jovenes en la Ciencia* 1:493–497.
- Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice-Evans. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* 26:1231–1237.
- Selote, D.S., and R. Khanna-Chopra. 2006. Drought-acclimation confers oxidative stress tolerance by inducing coordinated antioxidant defense at cellular and subcellular level in leaves of wheat seedlings. *Physiol Plant* 127:494–506.
- Silva-Belmares, Y., C. Rivas-Morales, E. Viveros-Valdez, M.G. De la Cruz-Galicia, and P. Carranza-Rosales. 2014. Antimicrobial and cytotoxic activities from *Jatropha dioica* roots. *Pakistan Journal of Biological Sciences* 17:748–750.
- Silva-Mares, D., E. Torres-Lopez, A.M. Rivas-Estilla, P. Cordero-Perez, N. Waksman-Minsky, and V.M. Rivas-Galindo. 2013. Plants from northeast Mexico with anti-HSV activity. *Nat Prod Commun* 8:297–298.
- Sivaci, A., and M. Sokmen. 2004. Seasonal changes in antioxidant activity, total phenolic and anthocyanin constituent of the stems of two *Morus* species (*Morus alba* L. and *Morus nigra* L.). *Plant Growth Regul.* 44: 251–256.
- Stracke, R., J.J. Favory, H. Gruber, L. Bartelniewoehner, S. Bartels, M. Binkert, M. Funk, B. Weisshaar, and R. Ulm. 2010. The Arabidopsis bZIP transcription factor HY5 regulates expression of the PFG1/MYB12 gene in response to light and ultraviolet-B radiation. *Plant Cell Environ.* 33(1):88–103.
- Upadrasta, L., M. Mukhopadhyay, and R. Banerjee. 2011. Tannins: Chemistry, biological properties and biodegradation. In: Sabu, A., Roussos, S., and Aguilar C.N., eds. *Chemistry and biotechnology of polyphenols*. 1st ed. Cibet Publishing House, India.

- Varela, M.C., I. Arslan, M.A. Reginato, A.M. Cenzano, and M.V. Luna. 2016. Phenolic compounds as indicators of drought resistance in shrubs from *Patagonian shrublands* (Argentina). *Plant Physiol. Biochem.* 104:81–91.
- Villarreal, A.M., X.A. Dominguez, H.J. Williams, A.I. Scott, and J. Reibenspies. 1988. Citlaltirione, a new diterpene from *Jatropha dioica* var. *sessiliflora*. *J. Nat. Prod.* 51:749–753.
- Waterman, P.G., and S. Mole. 1994. Analysis of phenolic plant metabolites. 1st ed. Blackwell Scientific Publications, Oxford. 248 pp.
- Wolf, L., L. Rizzini, R. Stracke, R. Ulm, and S.A. Rensing. 2010. The molecular and physiological responses of *Physcomitrella patens* to ultraviolet-B radiation. *Plant Physiol.* 153:1123–1134.
- Wong-Paz, J.E., D.B. Muñoz-Márquez, P. Aguilar-Zárate, R. Rodríguez-Herrera, and C.N. Aguilar. 2014. Microplate quantification of total phenolic content from plant extracts obtained by conventional and ultrasound methods. *Phytochem Anal* 25:439–444.
- Wong-Paz, J.E., D.B. Muñoz-Márquez, G. Martínez-Ávila, R. Belmares-Cerda, and C.N. Aguilar. 2015a. Ultrasound-assisted extraction of polyphenols from native plants in the Mexican desert. *Ultrason Sonochem* 22:474–481.
- Wong-Paz, J.E., J.C. Contreras-Esquivel, R. Rodríguez-Herrera, M.L. Carrillo-Inungaray, L.I. López, G.V. Nevárez-Moorillón, and C.N. Aguilar. 2015b. Total phenolic content, in vitro antioxidant activity and chemical composition of plant extracts from semiarid Mexican region. *Asian Pac J Trop Med* 8:104–111.