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RESEARCH ARTICLE

# Comparative study of proximate composition, antioxidant and FT-IR profiles of *Flourensia cernua* DC., *Jatropha dioica Sessé* and *Lippia graveolens* Kunth from Mexican semi-arid regions

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## Abstract

*Flourensia cernua* DC ('Hojasén'), *Jatropha dioica* Sessé ('Sangre de Drago') and *Lippia graveolens* Kunth ('Orégano') are three of the main medicinal plants native to the semi-arid regions of northern Mexico. Adaptation to these environments influences their compositional profile and biological activities; however such aspects remain underexplored. Hence this study aimed to compare the proximate composition, antioxidant activity and FT-IR fingerprints of these species collected from different semi-arid regions. The results revealed significant differences ($p < 0.05$) among collection sites. Overall, samples from San Jerónimo (SJ) and Estanque de León (EL) locations showed higher dry matter, crude protein and nitrogen-free extract contents. Conversely, samples from La Tortuga (T) location exhibited higher moisture, ash, crude fat and crude fibre contents. Regarding antioxidant activity, samples from SJ and EL recorded lower $IC_{50}$ values in both $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ assays along with higher FRAP values. Finally, all locations exhibited comparable FT-IR spectra, confirming the presence of several functional groups such as C=O, C-H, C-O, C-N and N-H. Although variability was site-dependent, we highlight the nutritional and functional potential of *F. cernua*, *J. dioica* and *L. graveolens* for future applications.

**Keywords:** native plants; composition; phenolics; bioactivity; FT-IR

## Introduction

Arid and semi-arid regions worldwide comprise unique ecosystems characterized by adverse environmental conditions such as drought, salinity, high temperature and intense solar radiation which often hinder plant growth (1). However certain plant species have successfully adapted to these environments. Evidence has shown that plants evolved intricate genetic, biochemical and physiological mechanisms to cope with stress. These include the synthesis of specialized metabolites, enhanced water retention, improved photosynthetic efficiency and altered leaf structure (2). Notably, such adaptations not only allow plants to survive but also contribute to their nutritional quality and functional properties (1).

In this context, the semi-arid regions of northern Mexico harbor several native plants that have traditionally been used for both food and medicinal purposes (3). Among the most representative species, *Flourensia cernua* DC, *Jatropha dioica* Sessé, *Lippia graveolens* Kunth are widely distributed in the area, easily accessible and collected by rural communities as an economic activity. Indeed, owing to their wide ethnobotanical use, these species have attracted research interest for their complex chemical composition, promising biological activities and high added value (4). For instance, *F. cernua* (Asteraceae; "Hojasén") is a shrub used as an infusion to alleviate various respiratory and gastrointestinal disorders (*e.g.*, asthma, bronchitis, diarrhea and indigestion) (5). Previous studies on crude extracts and fractions revealed the presence of phenolic acids, flavonoids, sesquiterpenes, fatty acids and benzofurans which are associated with antioxidant, anti-inflammatory, anti-obesity and antidiabetic properties (6, 7).

On the other hand, *J. dioica* (Euphorbiaceae; "Sangre de Drago") is a shrub widely recognized for its use as a macerate for the treatment of gastrointestinal and dental disorders, anemia, diabetes, rheumatism, ophthalmia, alopecia and wounds among others (8). Research into the composition of crude extracts has led to the identification of lignans, coumarins, alkaloids, flavonoids, terpenes, cyclic peptides, phytosterols, steroids, phenolic acids and esters. These compounds have been correlated with antioxidant,

antimicrobial, antifungal, antiviral, antitumor, anti-inflammatory, immunomodulatory, anti-obesity and nephroprotective effects (8-10).

Finally, *L. graveolens* (Verbenaceae; "Oregano") is not only a valued food condiment but also a shrub utilized in the form of decoctions or infusions to treat respiratory, gastrointestinal and urinary disorders as well as menstrual and musculoskeletal conditions, allergies, bacterial infections, migraine and diabetes (11). Recent studies have demonstrated that essential oils, crude extracts and fractions contain a range of compounds including flavonoids, terpenes, quinones, steroids, saponins, tannins, coumarins, alkaloids and lactones. The antimicrobial, antifungal, antiviral, antimutagenic, antioxidant, anticancer, anti-inflammatory and photochemopreventive activities of these compounds have also been reported (12, 13).

Although current knowledge supports the use of these species, studies on their compositional profiles and biological activities under different environmental contexts remain limited. Indeed, increasing research has focused on exploring how geographic and environmental variations influence the nutritional and functional properties of plants from arid and semi-arid regions (14-16). Therefore, understanding this variability is essential not only for conservation efforts but also for the exploitation of *F. cernua*, *J. dioica* and *L. graveolens* within the food, nutraceutical and pharmaceutical fields. On this basis, the study aimed to compare the proximate composition, antioxidant activity and FT-IR fingerprints of *F. cernua*, *J. dioica* and *L. graveolens* collected from different semi-arid regions of Mexico.

## Materials and Methods

### Plant materials

Fresh leaves of *F. cernua*, *J. dioica* and *L. graveolens* were collected from three different locations in two semi-arid regions of Mexico (Fig. 1): San Jerónimo (SJ) in Zacatecas (latitude: 22°39'11.95", longitude: 102°29'24.32", elevation: 2275 m a.s.l.), Estanque de León (EL) in Coahuila (latitude: 25°42'35.55", longitude: 103°18'02.27", elevation: 1115 m a.s.l.) and La Tortuga (T) in Coahuila (latitude: 25°51'39.77", longitude: 101°16'30.53", elevation: 984 m a.s.l.). Plant species were authenticated by the herbarium of the Universidad Autónoma Agraria Antonio Narro (https://plants.jstor.org/partner/ANSM). All samples were washed with distilled water, dried at 50 °C for 24 h (Oven HS35-AIA; Novatech, Jalisco, Mexico), ground into powder (Electric grinder HC-500; Homend, Istanbul, Turkey) and sieved (0.6 mm particle size). The resulting powders were collected and stored in air-tight bags at room temperature until use.

### Proximate composition analysis

Proximate composition of leaf samples was determined according to Association of Official Analytical Chemists (AOAC) standard methods (17). Briefly, dry matter and ash contents were assessed using the drying (AOAC 930.04) and incineration (AOAC 930.05) methods, respectively. Crude protein content was determined by the Kjeldahl method (N × 6.25) (AOAC 984.13) while crude fat content was measured using the Soxhlet extraction method (AOAC 920.39). Crude fiber was analyzed by the acid-alkali digestion method (AOAC 962.09). Nitrogen-free extract was calculated by difference.

### Phenolic extraction

Phenolic extraction was performed as reported by Ascacio-Valdés *et al*. (18) with slight modifications. Firstly, 20 g of leaf sample was mixed with 100 mL of distilled water (solid: liquid ratio of 1:5 w/v) and incubated at 60 °C for 30 min. The crude extract was then filtered through filter paper (Whatman N° 41) to remove solids. The resulting filtrate was partially purified by column chromatography using Amberlite™ XAD-16N as the stationary phase. Distilled water was used to remove water-soluble compounds followed by anhydrous ethanol to recover phenolic compounds. The solvent in the phenolic fraction was evaporated at 50 °C for 48 h. Finally, the dried extract was collected and stored in the dark at room temperature until use.

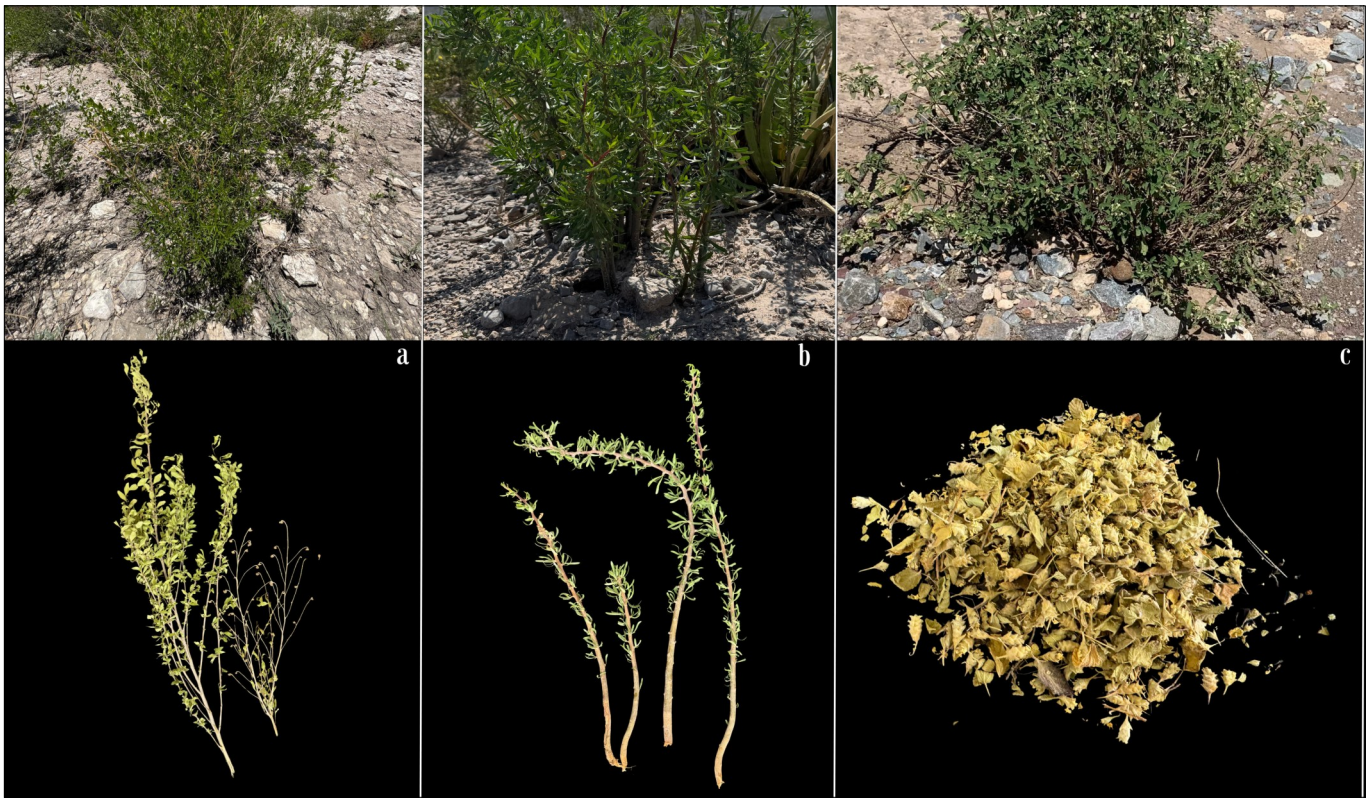


**Fig. 1.** Collected plant material: a) *F. cernua*, b) *J. dioica* and c) *L. graveolens*.

## Antioxidant activity analysis

### DPPH• radical scavenging

DPPH• (2,2-diphenyl-1-picrylhydracyl) radical scavenging activity was assessed according to Brand-Williams *et al*. (19) with slight modifications. Briefly, 50µL of phenolic extract (at concentrations of 250, 500, 750 and 1000µgmL$^{-1}$) was mixed with 2950µL of a DPPH• methanolic solution (60µM) and incubated at room temperature for 30 min. The absorbance of the reaction mixture was measured at 517nm (Spectrophotometer 6320D; Jenway, Essex, UK). The radical scavenging capacity was calculated using Eqn. 1 and results were expressed as $IC_{50}$ values (µgmL$^{-1}$), indicating the concentration of phenolic extract required to inhibit 50 % of DPPH• radical.

$$\text{Inhibition (\%)} = (A_0\text{-}A_1)/A_0 * 100 \qquad \text{(Eqn. 1)}$$

where $A_0$ and $A_1$ are the absorbances of the control and sample, respectively.

### ABTS•+ radical scavenging

ABTS•+ (2,2'-Azino-bis [3-ethylbenzothiazoline-6-sulfonic acid]) radical scavenging activity was evaluated as suggested by van den Berg *et al*. (20) with slight modifications. Firstly, an ABTS aqueous solution (7 mM) was mixed with potassium persulfate (2.45 mM) (liquid: liquid ratio of 2:1 v/v) and incubated at room temperature for 12 h to generate the ABTS•+ radical. The resulting solution was then diluted with anhydrous ethanol until an absorbance of 0.700 ± 0.002 at 734 nm was obtained. Subsequently, 50µL of phenolic extract (at concentrations of 250, 500, 750 and 1000µgmL$^{-1}$) was mixed with 950µL of the ABTS•+ solution and after 1 min of reaction, absorbance was measured at 734nm. The radical scavenging capacity was calculated using Eqn. 2 and results were expressed as $IC_{50}$ values (µgmL$^{-1}$) indicating the concentration of phenolic extract required to inhibit 50 % of ABTS•+ radical.

$$\text{Inhibition (\%)} = (A_0\text{-}A_1)/A_0 * 100 \qquad \text{(Eqn. 2)}$$

where $A_0$ and $A_1$ are the absorbances of the control and sample, respectively.

### Ferric reducing antioxidant power

Ferric reducing antioxidant power (FRAP) was determined according to Çelik *et al*. (21) with slight modifications. Briefly, 50µL of phenolic extract (at concentrations of 250 and 500µgmL$^{-1}$) was mixed with 120µL of phosphate-buffered saline solution (1M, pH 7). Subsequently, 220 µL of potassium ferrocyanide (1 % w/v) was added and the mixture was homogenized and incubated at 50 °C for 20 min. After incubation, 120µL of trichloroacetic acid (10 % w/v), 450µL of distilled water and 100µL of ferric chloride (0.1 % w/v) were added sequentially. The absorbance of the reaction mixture was measured at 734nm. A standard curve was prepared using gallic acid at concentrations ranging from 0 to 150 µg mL$^{-1}$. Results were expressed as µg of gallic acid equivalents per milliliter (µg GAE mL$^{-1}$) of phenolic extract.

## Fourier transform infrared (FT-IR) spectroscopy analysis

Fourier transform infrared (FT-IR) spectroscopy was conducted to identify the functional groups present in the phenolic extract. Briefly, the sample was placed on the plate reader (Spectrometer Cary 630 FTIR; Agilent Technologies, Santa Clara, CA, USA) and the spectrum was recorded over the range of 4000 to 600cm$^{-1}$ by co-adding 32 scans at a resolution of 2cm$^{-1}$. Data analysis was performed using MicroLab Expert software (version 1.1.0.1).

## Statistical analysis

Experimental data are presented as the mean ± standard deviation (SD) of three independent replicates. One-way analysis of variance (ANOVA) followed by Tukey-Kramer's post hoc test was used to determine statistically significant differences among mean values ($p < 0.05$). Statistical analysis was performed using InfoStat software (version 2020e; freely available at http://www.infostat.com.ar).

# Results and Discussion

## Proximate composition

Variations in the proximate composition of *F. cernua*, *J. dioica* and *L. graveolens* leaves are presented in Table 1. As noticed, dry matter and moisture contents ranged from 38.81 to 98.50 % and 1.50 to 61.19 %, respectively. Except for *J. dioica*, significant differences ($p < 0.05$) were found in *F. cernua* and *L. graveolens* collected from different locations. The highest dry matter content was recorded in *F. cernua* from SJ (98.50 %) followed by *L. graveolens* from EL (88.39 %). Inversely proportional, these samples also exhibited the lowest moisture content with values of 1.50 and 11.61 %, respectively. According to Ntshambiwa *et al*. (22), variations in dry matter may be attributed to precipitation and soil moisture conditions at the growing site. Plants exposed to water deficit have been shown to adapt by modifying root water absorption and leaf transpiration which leads to increased dry matter accumulation (23).

Regarding ash content, values ranged from 7.35 to 14.53 %. Significant differences ($p < 0.05$) were detected among samples from different locations. Notably, *F. cernua*, *L. graveolens*and *J. dioica* collected from T exhibited the highest and lowest ash content with values of 14.53, 10.16 and 7.35 %, respectively. Yang *et al*. (24) reported that ash content may vary according to climatic conditions (*e.g*., radiation, temperature and precipitation) as plants modulate their metabolic activity in response to climate-

**Table 1.** Proximate composition parameters of *F. cernua*, *J. dioica* and *L. graveolens* leaves collected from different locations.

| Plant species | Location | Dry matter (%) | Moisture (%) | Ash (%) | Crude protein (%) | Crude fat (%) | Crude fiber (%) | Nitrogen-free extract (%) |
|---|---|---|---|---|---|---|---|---|
| *F. cernua* | SJ | 98.50 ±0.20$^{a}$ | 1.50 ±0.20$^{b}$ | 13.00 ±0.17$^{b}$ | 1.98 ±0.04$^{b}$ | 6.40 ±2.61$^{a}$ | 11.23 ±0.62$^{b}$ | 67.38 ±2.88$^{a}$ |
| | EL | 97.10 ±0.36$^{b}$ | 2.90 ±0.36$^{a}$ | 14.13 ±0.11$^{a}$ | 2.11 ±0.04$^{ab}$ | 8.60 ±0.47$^{a}$ | 11.00 ±0.35$^{b}$ | 64.08 ±0.55$^{b}$ |
| | T | 96.23 ±0.30$^{b}$ | 3.77 ±0.30$^{a}$ | 14.53 ±0.15$^{a}$ | 2.12 ±0.02$^{a}$ | 8.66 ±0.72$^{a}$ | 13.33 ±0.66$^{a}$ | 51.41 ±16.78$^{b}$ |
| *J. dioica* | SJ | 38.81 ±2.04$^{a}$ | 61.19 ±2.04$^{a}$ | 9.26 ±0.07$^{a}$ | 12.70 ±0.22$^{a}$ | 1.99 ±0.17$^{b}$ | 19.20 ±2.27$^{b}$ | 56.85 ±1.92$^{a}$ |
| | EL | 40.12 ±1.68$^{a}$ | 59.88 ±1.68$^{a}$ | 8.87 ±0.30$^{a}$ | 8.04 ±0.08$^{b}$ | 2.04 ±0.16$^{b}$ | 28.25 ±0.30$^{a}$ | 52.90 ±0.56$^{b}$ |
| | T | 39.38 ±1.38$^{a}$ | 60.62 ±1.38$^{a}$ | 7.35 ±0.04$^{b}$ | 9.81 ±0.65$^{b}$ | 3.64 ±0.28$^{a}$ | 26.08 ±0.22$^{a}$ | 53.10 ±0.90$^{b}$ |
| *L. graveolens* | SJ | 74.81 ±0.63$^{c}$ | 25.19 ±0.63$^{a}$ | 9.64 ±0.12$^{b}$ | 9.26 ±0.29$^{c}$ | 3.83 ±0.42$^{a}$ | 14.37 ±0.67$^{a}$ | 62.91 ±0.34$^{a}$ |
| | EL | 88.39 ±0.63$^{a}$ | 11.61 ±0.63$^{c}$ | 8.90 ±0.26$^{c}$ | 13.12 ±0.28$^{a}$ | 3.60 ±0.31$^{a}$ | 11.99 ±1.82$^{a}$ | 62.47 ±1.53$^{a}$ |
| | T | 85.41 ±0.80$^{b}$ | 14.59 ±0.80$^{b}$ | 10.16 ±0.03$^{a}$ | 10.27 ±0.22$^{b}$ | 3.49 ±0.38$^{a}$ | 11.64 ±0.86$^{a}$ | 64.44 ±0.92$^{a}$ |

Different superscript letters within the same column indicate significant differences ($p < 0.05$) among locations of plant species. Parameters are expressed as dry basis. SJ, San Jerónimo; EL, Estanque de León; T, La Tortuga.

induced stress. Similarly, soil properties such as texture, pH and organic matter content play a crucial role in regulating the adsorption, solubility and translocation of mineral elements within the plant system (24).

Crude protein content ranged from 1.98 to 13.12 %. Significant differences ($p < 0.05$) were observed among samples from different locations. The highest crude protein content was recorded in *L. graveolens* from EL (13.12 %) followed by *J. dioica* from SJ (12.70 %); while the lowest content was determined in *F. cernua* from SJ (1.98 %). Plant density and nitrogen availability have been shown to influence crude protein as suggested by Leghari *et al*. (25). These factors can affect the metabolic pathways involved in protein biosynthesis and accumulation ultimately shaping the quality and properties of plant-derived protein (26).

About crude fat content, values ranged from 1.99 to 8.66 %. Except for *J. dioica*, no significant differences ($p > 0.05$) were found in *F. cernua* and *L. graveolens* collected from different locations. Despite the non-significance, *F. cernua* from T exhibited the highest crude fat content (8.66 %). *L. graveolens* from SJ and *J. dioica* from T showed comparable crude fat values (3.83 and 3.64 %, respectively) while *J. dioica* from SJ recorded the lowest (1.99 %). It is well-known that the maturity stage determines the accumulation and redistribution of plant macronutrients especially crude fat. As plants mature, they tend to store more lipids which are essential for membrane biogenesis and cell signaling and serve as energy and carbon sources (27).

Crude fiber content ranged from 11.00 to 28.25%. Except for *L. graveolens*, significant differences ($p < 0.05$) were observed in *F. cernua* and *J. dioica* collected from different locations. The highest crude fiber content was recorded in *J. dioica* from EL (28.25%) followed by *L. graveolens* from SJ (14.37 %) and *F. cernua* from T (13.33%). The lowest value was found in *F. cernua* from EL (11.00%). According to Busuttil-Griffin *et al*. (28), high temperatures and water deficit at the growing site may lead to variations in crude fiber. These conditions accelerate plant maturation during which physiological and structural changes cause the leaves to become woody and fibrous. This response is associated with the regulation of metabolic pathways involved in cellulose and hemicellulose biosynthesis (29).

Finally, nitrogen-free extract (NFE), also referred to as soluble carbohydrate content, ranged from 51.41 to 67.38 %, exhibiting the same significant trend as for crude fibre. *F. cernua* from SJ presented the highest NFE content (67.38 %) while *L. graveolens* from all locations showed comparable values (*ca*. 62-64 %). The lowest NFE was recorded in *F. cernua* from T (51.41 %). Plants are known to sense solar radiation and adjust their carbohydrate content accordingly. Changes in radiation intensity and quality may influence the regulation of photosynthetic pigments, stomatal development and carbohydrate metabolism pathways, ultimately affecting the accumulation of soluble sugars in plant tissues (30). Overall, the proximate composition observed in this study is comparable to or even higher than that reported by Estell *et al*. (31), Afolabi *et al*. (32) and Djengue *et al*. (33) for other *Flourensia*, *Jatropha* and *Lippia* species, respectively.

## Antioxidant activity

Table 2 summarizes the variations in antioxidant activity of *F. cernua*, *J. dioica* and *L. graveolens* phenolic extracts. As noticed, for DPPH$^{\cdot}$ radical scavenging activity, $IC_{50}$ values ranged from 37.20 to 668.25 µg mL$^{-1}$. A lower $IC_{50}$ value indicates higher antioxidant activity of the extract and vice versa; thus significant differences ($p < 0.05$) were found among samples from different locations. The lowest $IC_{50}$ value was recorded in *L. graveolens* from SJ (37.20 µg mL$^{-1}$) followed by *J. dioica* from EL (155.02 µg mL$^{-1}$) and *F. cernua* from T (288.81 µg mL$^{-1}$). The highest value was determined in *J. dioica* from T (668.25 µg mL$^{-1}$).

Regarding ABTS$^{\cdot+}$ radical scavenging activity, $IC_{50}$ values ranged from 14.59 to 139.50 µg mL$^{-1}$ exhibiting the same significant trend as for DPPH$^{\cdot}$ activity. Hence, the lowest $IC_{50}$ values were in the ranking order of *L. graveolens* from SJ (14.59 µg mL$^{-1}$) < *J. dioica* from EL (24.57 µg mL$^{-1}$) < *F. cernua* from T (112.48 µg mL$^{-1}$). The highest value was found in *F. cernua* from SJ (139.50 µg mL$^{-1}$). According to Itam *et al*. (34), the antioxidant activity of plant extracts can be classified based on $IC_{50}$ values as follows: very strong ($IC_{50}$ < 50 µg mL$^{-1}$), strong ($IC_{50}$= 50-100 µg mL$^{-1}$), moderate ($IC_{50}$= 100-250 µg mL$^{-1}$), weak ($IC_{50}$= 250-500 µg mL$^{-1}$) and inactive ($IC_{50}$ > 500 µg mL$^{-1}$). Under this classification and regardless of the collection site, *L. graveolens* exerted very strong antioxidant activity while *F. cernua* and *J. dioica* displayed moderate to inactive activity.

Finally, FRAP values ranged from 18.07 to 44.69 µg GAE mL$^{-1}$. A higher FRAP value indicates higher antioxidant activity. Although all extracts showed a concentration-dependent increase, significant differences ($p < 0.05$) were observed among samples from different locations. *L. graveolens* from SJ exhibited the highest FRAP, with values of 31.36 and 43.76 µg GAE mL$^{-1}$ per 250 and 500 µg mL$^{-1}$ extract, respectively. Meanwhile, *F. cernua* and *J. dioica* showed similar FRAP values at both extract concentrations (*ca*. 18-20 and 20-24 µg GAE mL$^{-1}$, respectively) with only slight variations between species and within locations (Table 2). These results were consistent with those of DPPH$^{\cdot}$ and ABTS$^{\cdot+}$ assays.

**Table 2.** Antioxidant activity of *F. cernua*, *J. dioica* and *L. graveolens* phenolic extracts from different locations.

| Plant species | Location | DPPH$^{\cdot}$ ($IC_{50}$, µg mL$^{-1}$) | ABTS$^{\cdot+}$ ($IC_{50}$, µg mL$^{-1}$) | FRAP (µg GAE mL$^{-1}$, per 250 µg mL$^{-1}$ extract) | FRAP (µg GAE mL$^{-1}$, per 500 µg mL$^{-1}$ extract) |
|---|---|---|---|---|---|
| *F. cernua* | SJ | 325.76 ±1.04$^{b}$ | 139.50 ±0.45$^{a}$ | 20.52 ±0.38$^{a}$ | 24.64 ±0.25$^{a}$ |
| | EL | 349.16 ±0.66$^{a}$ | 137.69 ±0.56$^{b}$ | 19.54 ±0.08$^{a}$ | 20.97 ±0.51$^{a}$ |
| | T | 288.81 ±0.85$^{c}$ | 112.48 ±0.42$^{b}$ | 18.07 ±0.69$^{b}$ | 20.48 ±0.25$^{b}$ |
| *J. dioica* | SJ | 244.07 ±1.98$^{b}$ | 37.87 ±1.27$^{b}$ | 19.99 ±0.34$^{a}$ | 21.12 ±0.39$^{b}$ |
| | EL | 155.02 ±2.26$^{c}$ | 24.57 ±1.01$^{c}$ | 20.52 ±1.26$^{a}$ | 24.55 ±0.22$^{a}$ |
| | T | 668.25 ±1.67$^{a}$ | 108.78 ±0.63$^{a}$ | 19.50 ±0.25$^{a}$ | 21.27 ±0.29$^{b}$ |
| *L. graveolens* | SJ | 37.20 ±0.85$^{b}$ | 14.59 ±1.42$^{c}$ | 31.36 ±0.61$^{a}$ | 43.76 ±1.06$^{a}$ |
| | EL | 44.51 ±0.58$^{b}$ | 22.19 ±0.52$^{a}$ | 25.82 ±0.25$^{b}$ | 33.56 ±0.22$^{b}$ |
| | T | 51.93 ±0.42$^{a}$ | 21.13 ±0.46$^{b}$ | 30.08 ±2.19$^{a}$ | 44.69 ±1.18$^{a}$ |

Different superscript letters within the same column indicate significant differences ($p < 0.05$) among locations of plant species. SJ, San Jerónimo; EL, Estanque de León; T, La Tortuga.

Although a plant's phenolic profile is genotype- and environment-influenced, the antioxidant activity of its constituents depends on their specific reaction mechanisms. In the DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays, phenolics can stabilize free radicals through combined hydrogen atom (H$^{\bullet}$) donation (HAT) and single electron ($e^-$) transfer (SET) mechanisms. Meanwhile, in the FRAP assay which measures the reduction of ferric ions ($Fe^{3+}$) to ferrous ions ($Fe^{2+}$), they rely exclusively on the SET mechanism (35). However, their reaction efficiency is determined by the chemical structure. On this basis, the superior activity of *L. graveolens* compared to that of *F. cernua* and *J. dioica* may be attributed to unique structural features.

For instance, Vuolo *et al*. (36) reported that the number and position of hydroxyl (O-H) groups (*e.g*., presence of two O-H groups in the aromatic ring) correlate with an improved effect. Similarly, the presence of a C2=C3 double bond, coupled with a 4-carbonyl group, improves molecular planarity and electron delocalization, facilitating electron transfer reactions. Even though glycosides have reduced activity compared to free aglycones, glycosylation, particularly in the C-glycoside form may retain greater activity than O-glycoside (36, 37). Conversely, aromatic rings susceptible to O-methyl substitution show decreased effect since methylation leads to steric hindrance that interferes with radical interaction sites (36). Altogether, these features may support the variations observed in this study. Interestingly, previous studies have observed comparable antioxidant activity in other *Flourensia* (7), *Jatropha* (38) and *Lippia* (39) species growing under adverse environmental conditions.

## FT-IR fingerprinting

Fig. 2 to 4 depicts the variations in functional groups present in *F. cernua*, *J. dioica* and *L. graveolens* phenolic extracts, respectively. Overall, all locations exhibited comparable FT-IR spectral patterns with only slight differences in peak intensities. The typical absorption peaks for *F. cernua*, *J. dioica* and *L. graveolens* considered as their molecular fingerprint, were clearly in the range of 650-1700 cm$^{-1}$, 670-1580 cm$^{-1}$ and 680-1600 cm$^{-1}$, respectively. As observed, there were between eight and eleven major peaks, depending on the sample.

Notably, several functional groups corresponding to qualitatively different compounds were identified. Regardless of the sample, peak wavenumbers (Fig. 2 to 4) in the range of 600-950 cm$^{-1}$ were assigned to out-of-plane C-H bending vibration of aromatic compounds, alkenes and alkynes; 1020-1275 cm$^{-1}$ were attributed to C-O symmetric/asymmetric stretching vibration (alcohols, esters, ethers and carboxylic acids) and C-N stretching vibration (aliphatic amines); 1300-1450 cm$^{-1}$ were assigned to C-H symmetric/asymmetric bending vibration (alkanes), C-N stretching vibration (aromatic amines), C-O stretching vibration (amide) and N-O symmetric stretching vibration (nitro compounds); 1500-1650 cm$^{-1}$ were attributed to C=C stretching vibration (aromatic rings in phenolic compounds) and N-H bending vibration (amines); 1700-1760 cm$^{-1}$ were assigned to N-H bending vibration (amino acids) and C=O stretching vibration (aldehydes, ketones, esters and fatty acids); 2000-2150 cm$^{-1}$ were attributed to C°C stretching vibration of alkynes; 2800-2930 cm$^{-1}$ were assigned to C-H symmetric/asymmetric stretching vibration of lipids ($CH_2$ and $CH_3$), methoxy derivatives, aldehydes and alkanes and 3000-3350 cm$^{-1}$ were attributed to O-H stretching vibration (water, hydrogen-bonded alcohols, phenols, carbohydrates and peroxide) and N-H stretching vibration (amines) (40).

It is noteworthy that the FT-IR fingerprints obtained in this study agree with those previously informed for other *Flourensia*, *Jatropha* and *Lippia* species. In the case of the *F. cernua* extracts, peak wavenumbers at ~3338 (O-H and N-H stretch), ~2929 (C-H stretch), ~1702 (C=O stretch), ~1652 (C=C stretch) and ~1017 (C-N stretch) cm$^{-1}$ matched with those reported by Aranda-Ledesma *et al*. (6) and are characteristic of compounds commonly found in *Flourensia* crude extracts. On the other hand, the *J. dioica* extracts exhibited peak wave numbers at ~3250 (O-H stretch), ~2924 (C-H stretch), ~1355 (C-H bend), ~1260 (C-O stretch) and ~1040 (C-N stretch) cm$^{-1}$, aligning with those described by Umar *et al*. (41). These peaks are used as authentication markers of *Jatropha* spp. Finally in the *L. graveolens* extracts, peak wavenumbers at ~3269 (O-H stretch), ~2925 (C-H stretch), ~1596 (C=O stretch), ~1025 (C-O stretch) and ~813 (C-H bend) cm$^{-1}$ were consistent with those observed by de Sá-Filho *et al*. (42), indicating the presence of major constituents found in *Lippia* essential oils and crude extracts.

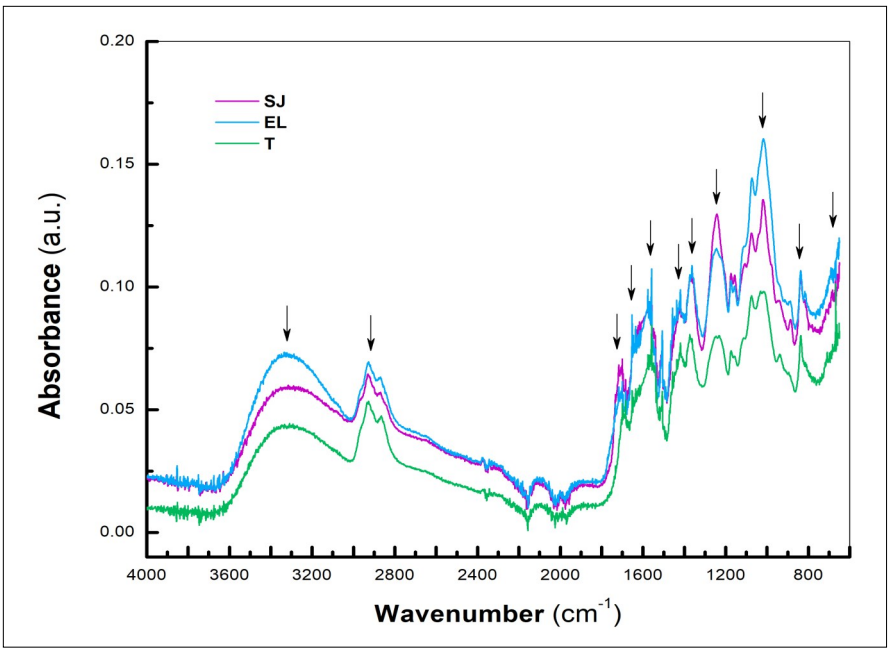


**Fig. 2.** FT-IR fingerprints of *F. cernua* phenolic extracts from (SJ) San Jerónimo, (EL) Estanque de León and (T) La Tortuga locations.

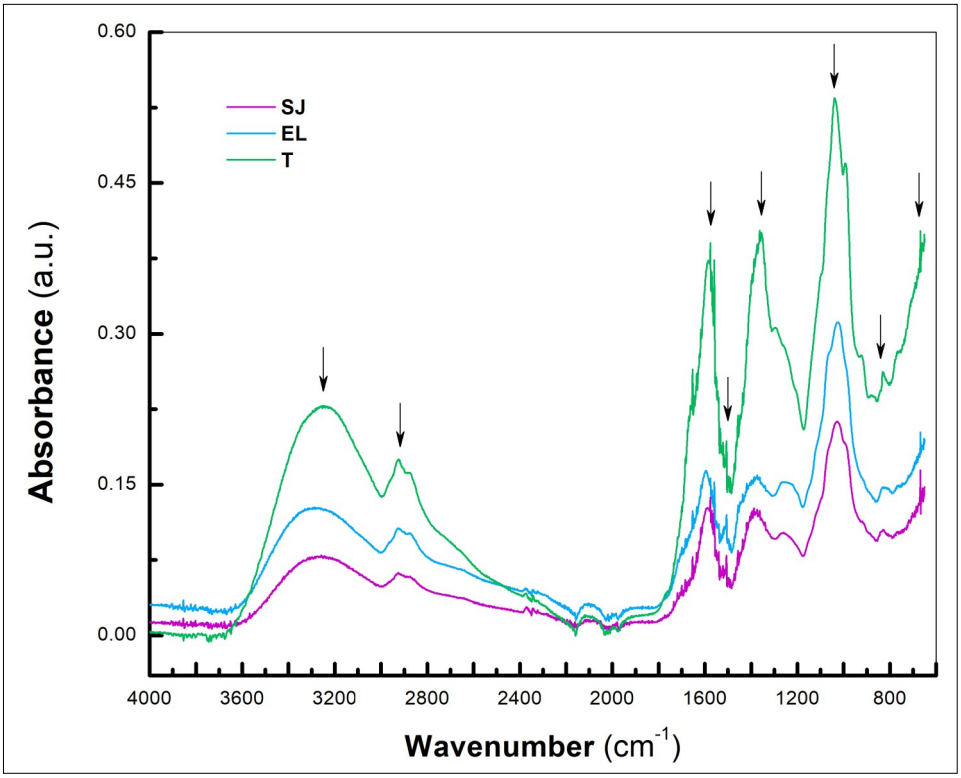


**Fig. 3.** FT-IR fingerprints of *J. dioica* phenolic extracts from (SJ) San Jerónimo, (EL) Estanque de León and (T) La Tortuga locations.

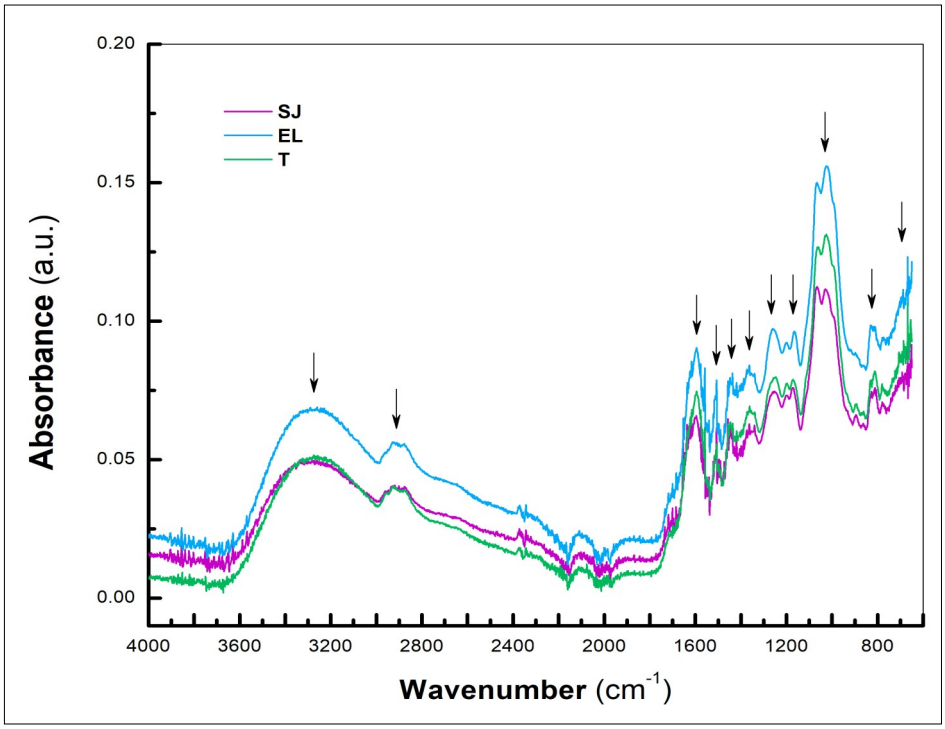


**Fig. 4.** FT-IR fingerprints of *L. graveolens* phenolic extracts from (SJ) San Jerónimo, (EL) Estanque de León and (T) La Tortuga locations.

As mentioned above, plant chemical composition is genetically determined; however, it may be modulated in response to biotic and abiotic stressors at the growing site (43). To counteract stress, plants differentially activate or repress genes that can redirect the metabolic pathways involved in secondary metabolite biosynthesis and accumulation, thereby affecting the type, content and function of compounds produced (44). For instance, solar radiation and temperature stimulate the shikimic acid pathway, leading to the accumulation of different aromatic compounds which play key roles in cell signalling, hormonal regulation and oxidative stress alleviation (44).

According to the FT-IR analysis of phenolic extracts, Sukmawaty *et al*. (43) stated that the peak wavenumber indicates qualitatively the type of compound while the absorption intensity reflects its concentration. Therefore, the FT-IR spectral patterns observed in Fig. 2 to 4 suggest that the types of metabolites present in *F. cernua*, *J. dioica* and *L. graveolens* do not vary among locations, except at the concentration level. However, this statement should be further investigated. Consistent with our findings, studies have reported that *Moringa oleifera* (43), *Laurus nobilis* (45) and *Isotoma longiflora* (46) also maintained similar metabolite profiles despite differing growing sites.

## Conclusion

Based on the findings, *F. cernua*, *J. dioica* and *L. graveolens* could be considered promising sources of macronutrients and bioactive compounds. However significant variations in their compositional profiles and antioxidant activity were demonstrated among collection sites. The variability is mainly attributed to plant physiology, as well as to the soil and environmental conditions prevailing in each location. Overall, this study could help identify suitable cultivation sites for species with enhanced nutritional and functional properties offering prospects for natural product development in the food, pharmaceutical and cosmetic industries (*e.g.*, as additives, preservatives, new drugs and supplements). Despite this, further research should focus on determining the vitamin, mineral and fatty acid composition of *F. cernua*, *J. dioica* and *L. graveolens* leaves. Furthermore, additional studies are needed to evaluate other potential *in vitro* biological activities of phenolic extracts beyond that reported here. Finally, future *in vivo* and clinical studies are required to validate the safety, efficacy and bioavailability of extracts for human use.

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## Authors' contributions

CCL, data collection and analysis, figure preparation and drafting the original manuscript. PSG, sample collection and experimental analysis. GCGMA and RR, methodology supervision and manuscript review. JCTA, conceptualization, methodology supervision, resources and manuscript review. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

**Ethical issues:** None

### Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used Grammarly to check the English grammar. After using this tool/ service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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