



Article

Exploration of Phenolic Content and Antioxidant Potential from Plants Used in Traditional Medicine in Viesca, Mexico

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Abstract: The medicinal plants of the desert can be a potential source of natural bioactive compounds with applications in health and food preservation. This work aimed to investigate the phenolic content and antioxidant potential of twenty-three plants from the arid zone of Viesca, Mexico. The antioxidant potential was determined by three methods (ABTS, FRAP, and DPPH). Two extraction methods and three different concentration levels (1:10 mg/mL, 1:25 mg/mL, and 1:50 mg/mL) were evaluated. According to the results, *Turnera diffusa* (Damiana), *Gutierrezia sarothrae* (Hierba de San Nicolas), and *Leucophyllum frutescens* (Cenizo) present the largest content of phenolic compounds and high antioxidant activity. The highest values of antioxidant activity were achieved using a solid/liquid ratio of 1:50 g/mL for *T. diffusa* and *G. sarothrae* and 1:25 mg/mL for *L. frutescens*. The maceration method was the most suitable for extracting antioxidant compounds from *T. diffusa* and *L. frutescens*, while ultrasound-assisted extraction was more efficient for *G. sarothrae*. The HPLC-MS analysis showed the presence of important phenolic compounds. This study shows for the first time that three plants that naturally grow in Viesca have great potential as a source of natural antioxidant compounds.

Keywords: antioxidant activity; phenolic compounds; medicinal plants; arid zone; natural products; bioeconomy



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1. Introduction

Medicinal plants and their phytochemicals have been used for the treatment of various diseases for a long time [1]. The identification of new plants with medicinal properties is of great importance, as approximately 60% of the new medicines produced since 1981 came from plants [2]. Plant phytochemicals (e.g., phenolic compounds) have attracted attention as bioactive agents exhibiting strong antioxidant potential and are preferably used to treat various diseases to improve human health [3]. Bioactive compounds generated by plants also have applications in the food industry, such as in the manufacture of beverages, flavorings, and food preservatives.

In the human body, antioxidants play a very important role in human health by transferring a hydrogen (H) atom or an electron (e[−]) to inactivate reactive species [4]. The most used antioxidant activity assays are based on these two mechanisms: the H atom transfer (HAT) or the e[−] transfer (ET). HAT-based assays apply a competitive reaction scheme between antioxidant and substrate [5]; meanwhile, ET-based assays measure the capacity of an antioxidant in the reduction of an oxidant agent (which changes color when

reduced) [5]. ET-based assays such as 2,2-Diphenyl-1-picrylhydrazyl (DPPH), the ferric reducing antioxidant power (FRAP), and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic) diammonium acid (ABTS) are the most used to measure the antioxidant capacity of plant extracts [6].

Mexico has an ancestral tradition of using medicinal plants as first-aid remedies. However, research on medicinal plants is scarce in the north of the country [7]. Northern Mexico is a desert rich in endemic flora, accompanied by ample local and traditional knowledge. Natural products of arid-zone plants represent an alternative to the search for new bioactive compounds. The production of phytochemical compounds in desert plants helps them survive in difficult climatic conditions, and these phytochemicals give plants important biological properties such as antioxidant, antimicrobial, and anticancer activities [7].

Studies on plants used in traditional medicine in Coahuila state (northern Mexico) are limited to a few, such as *Larrea tridentata* [8] and *Lippia graveolens* (Mexican oregano) [9,10]. To explore the full potential of the medicinal plants from the semi-desert of Viesca, Coahuila, our work group recently carried out an ethnobotanical study, finding that 77 medicinal plants are used for this purpose in the region [7]. In this study, for the first time, the phenolic content of 23 of these plants will be measured, identifying the antioxidant potential and the compounds of the main plants. The determination of the antioxidant potential of plants used in traditional medicine can contribute to their safe use in local health systems and their valorization under the concept of bioeconomy. Therefore, the objective of this research was to investigate the phenolic content and antioxidant potential of plants used in traditional medicine in the arid zone of Viesca, Mexico.

2. Materials and Methods

2.1. Reagents

Ethanol, Folin–Ciocalteu reagent, Sodium hydroxide, gallic acid, Trolox, 2,2-diphenyl-1-picryl-hydrazyl (DPPH), 2,2'-Azinobis-3-ethyl-benzo-thiazoline-6-sulfonic acid (ABTS), and FRAP reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Plant Material

Twenty-three medicinal plants were collected in Viesca, Coahuila, Mexico (24°45'–25°39' north latitude and 102°28'–103°30' west longitude) and Parras, Coahuila, Mexico (102°11'10" west longitude and 25°26'27" north latitude) (Table 1). The scientific names of the plants were confirmed using <https://www.theplantlist.org/> (accessed on 1 August 2023). These plants were selected based on an ethnobotanical study carried out by Torres et al. [7]. These plants were identified by specialists of the Research Center and Ethnobiological Garden of the Universidad Autonoma de Coahuila (CIJE-UAdeC) and the Botanical Reference Center of the Universidad Autonoma Agraria Antonio Narro (CREB-UAAAN).

Table 1. List of medicinal plants used in this study.

Scientific Name	Common Name	Date of Collection	Part Used
<i>Heterotheca inuloides</i> Cass	Arnica amarilla	9 September 2021	Whole plant
<i>Ruta graveolens</i> L.	Ruda	12 September 2021	Leaves
<i>Lippia graveolens</i> Kunth	Orégano	15 September 2021	Leaves
<i>Ephedra antisiphilitica</i> Berland. ex C.A.Mey	Pito real	17 September 2021	Stem
<i>Larrea tridentata</i> Var.	Gobernadora	18 September 2021	Leaves and stem
<i>Salvia officinalis</i> L.	Salvilla	21 September 2021	Leaves
<i>Flourensia cernua</i> DC	Hojasén	23 September 2021	Leaves and stem
<i>Jatropha dioica</i> Sessé	Sangre de drago	21 September 2021	Stem
<i>Nicotiana glauca</i> Graham	hojas de virginio	22 September 2021	Leaves
<i>Leucophyllum frutescens</i> (Berland.) I.M. Johnst.	Cenizo	23 September 2021	Whole plant

Table 1. Cont.

Scientific Name	Common Name	Date of Collection	Part Used
<i>Bauhinia forficata</i> Link	Pata de res	26 September 2021	Whole plant
<i>Fouquieria splendens</i> Engelm	Ocotillo	30 September 2021	Stem
<i>Parthenium incanum</i> Kunth	Mariola	28 September 2021	Leaves
<i>Parthenium hysterophorus</i> L.	Hierba amargosa	3 October 2021	Leaves and stem
<i>Gutierrezia sarothrae</i> (Pursh) Britton & Rusby	Hierba de San Nicolas	25 September 2021	Leaves and stem
<i>Astragalus nitidiflorus</i> Jimenez & Pau	Garbancillo	26 September 2021	Leaves and stem
<i>Abutilon Coahuilae</i> Kearney	Hierba del buen día	26 September 2021	Leaves and stem
<i>Selaginella moellendorffii</i>	Siempre viva	27 September 2021	Whole plant
<i>Argemone munita</i> Durand & Hilg.	Chicalote	1 October 2021	Whole plant
<i>Prosopis glandulosa</i> Torr.	Mezquite regional	28 September 2021	Leaves
<i>Parkinsonia aculata</i> L.	retama o palo verde	2 October 2021	Leaves
<i>Sphaeralcea angustifolia</i> (Cav.) G.Don	Trompillo	2 October 2021	Leaves and stem
<i>Turnera diffusa</i> Willd. Ex Schult.	Damiana	8 October 2021	Leaves

2.3. Obtaining Plant Extracts

The plant parts used in this study were selected based on a previous ethnobotanical study by our research group [7]. Approximately 500 g of the selected parts of the plants (Table 1) were dried in the absence of light for two days at an average temperature of 30 °C at the Ethnobiological Garden and Research Center located in Viesca, Coahuila. Subsequently, the dried plants were transported to the Phytopharmacology laboratory of the Autonomous University of Coahuila. Immediately, the plants were ground with a laboratory mill (Nixtamatic, NG-02) until a homogeneous powder was obtained (particle size less than 200 µm). Samples were stored in packages for dehydrated products (aluminum bag).

2.4. Evaluation of the Phenolic Content of 23 Plants

The extraction was evaluated in two stages: In the first stage, a solid–liquid extraction of the 23 plants was carried out with 50% ethanol at a ratio of 1:25 g/mL, and the extraction method used was traditional maceration in a shaker (Premiere, TX, USA) at 180 rpm stirring at room temperature (28–30 °C) for 2 h. Subsequently, the samples were centrifuged (ThermoFisher, Waltham, MA, USA) for 4 min at 10,000 rpm. The extracts were stored at 4 °C for subsequent phenolic quantification [11].

2.5. Evaluation of Extraction Methods

In the second stage, the three plants that presented the highest polyphenol content were subjected to extraction, evaluating two methods: ultrasound-assisted extraction (UAE) (Fisher Scientific, Waltham, MA, USA) and maceration (Premiere, TX, USA) with three solid/liquid ratios (1:10, 1:25, and 1:50 g/mL), using the same solvent and centrifugation conditions as in the first stage. The extraction in both methods was carried out for 30 min at 30 °C. The extracts from stage 2 were evaluated for antioxidant activity, phenol content, and the identification of their components by HPLC, as described below.

2.5.1. Quantification of Phenols

The content of phenolic compounds in the extracts was estimated by the Folin–Ciocalteu colorimetric method, according to Blois [12], with the modifications of Magalhães et al. [13] (change sodium carbonate solution for sodium hydroxide to reduce the reaction time). Briefly, 300 µL of 0.2 N Folin’s reagent was added to 300 µL of the plant extracts. Subsequently, 600 µL of 0.35 M sodium hydroxide solution was added. The samples were incubated for 5 min at room temperature, and the absorbance at 760 nm was measured in a spectrophotometer (Velab, Pharr, TX, USA). The concentration of phenolic compounds was calculated according to the calibration curve of gallic acid in Equation (1).

$$y = 0.0147x + 0.0703 \quad (R^2 = 0.9927) \quad (1)$$

The content of phenolic compounds was expressed as milligrams of gallic acid equivalent per gram of dry weight of plant material (mg GAE/g).

2.5.2. Antioxidant Activity Assay

DPPH Assay

The DPPH radical scavenging activity of each extract solution was analyzed using the method reported by Mensor et al. [14]. An amount of 2.5 mL of extract from the plants was mixed with 1 mL of methanolic DPPH• 0.3 mM. The reaction mixtures were incubated for 30 min in the dark at room temperature, and the reduction of radical DPPH was measured at 518 nm (Velab, Pharr, TX, USA). Results were expressed as milligram Trolox equivalent per gram of dry weight of plant material (mg TE/g).

FRAP Assay

Ferric reducing antioxidant power assay was performed as follows. The FRAP reagent was prepared in a 1:1:10 ratio of 10 mM TPTZ, 20 mM FeCl₃, and 0.3 mM acetate buffer with a pH of 3.6, respectively. The mixture was prepared and incubated for 10 min at 37 °C prior to use. An amount of 1425 µL of FRAP reagent was mixed with 75 µL of plant extract, the samples were incubated at 37 °C for 30 min in the dark, and the absorbance was measured in a spectrophotometer (Velab, TX, USA) at 593 nm. Values were expressed as milligram Trolox equivalents per gram of dry weight of plant material (mg TE/g).

ABTS Assay

The ABTS radical cation (ABTS•+) scavenging activity of the sample was measured using the method reported by Re et al. [15]. ABTS•+ was generated by oxidation of 7 mmol/L ABTS with 2.45 mmol/L potassium persulfate after incubation in the dark (12 h). The freshly prepared ABTS•+ solution was diluted with ethanol (1:40) to obtain an absorbance of 0.70 ± 0.02 at 734 nm in a spectrophotometer (Velab, TX, USA). An amount of 10 µL of plant extracts and 1 mL of ABTS•+ were incubated for 1 min in the dark, and the absorbance was then recorded at 734 nm (Velab, TX, USA). The values of the ABTS assay were expressed in a similar way to those in the DPPH assay (mg TE/g).

2.6. RP-HPLC-ESI-MS Analysis

The analyses by RP-HPLC were performed on a Varian HPLC system [16]. An amount of 4 mg of dry extract was diluted in 1 mL of ethanol and filtered (0.2 µm) before injection. A liquid chromatograph ion trap mass spectrometer (Varian 500-MS IT Mass Spectrometer, Palo Alto, CA, USA) equipped with an electro spray ion source was used. Samples were injected into a Denali C18 column (150 mm × 2.1 mm, 3 mm, Grace, USA). The eluents were formic acid (0.2%, v/v; A solvent) and acetonitrile (B solvent) [16]. All MS experiments were carried out in negative mode [M–H]–1. Nitrogen was used as nebulizing gas and helium as damping gas. The ion source parameters were as follows: spray voltage was 5.0 kV, and capillary voltage and temperature were 90.0 V and 350 °C, respectively. Data were collected and processed using MS Workstation software (V6.9). Samples were first analyzed in full scan mode acquired in the *m/z* range 50–2000 [16].

2.7. Statistical Analysis

All studies were performed in triplicate. The influence of the extraction method and solvent-to-solid ratio was investigated using a full factorial design for each medicinal plant (2 extraction processes and 3 solid/liquid ratios). Statistical analysis was performed with the JMP software, trial version 16.2.0. Data were analyzed using a Tukey test ($p < 0.05$) to detect significant differences between treatments. The Student's *t*-test was run to determine significant differences ($p < 0.05$) in the two extraction methods evaluated.

3. Results

3.1. Evaluation of the Phenolic Content of 23 Plants

The phenolic content of a plant is an indicator of its functional potential. Figure 1 shows the polyphenol content of 23 plants used in traditional medicine in Viesca, Coahuila. The plants *Turnera diffusa* (5.09 ± 0.06 mg GAE/g), *Nicotiana glauca* (4.95 ± 0.02 mg GAE/g), *Gutierrezia sarothrae* (4.85 ± 0.06 mg GAE/g), and *Leucophyllum frutescens* (4.81 ± 0.09 mg GAE/g) present the highest content of phenolic compounds (extracts from these plants were selected for the following studies). The *N. glauca* plant was omitted from subsequent analyses due to previous cytotoxicity reports. The values obtained for *T. diffusa* are similar to those previously reported (8 mg GAE/g) [17]. The variation in phenolic content may be due to environmental and agronomic factors. The phenolic content could not be compared with other studies due to the limited information available.

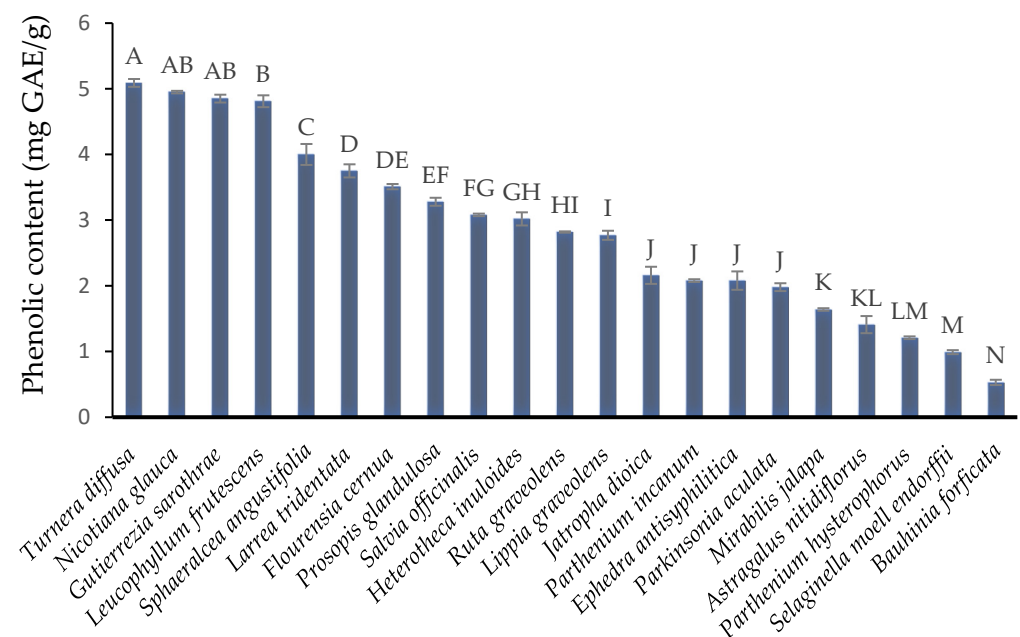


Figure 1. Phenolic content of the 23 plants studied. The different letter indicates significant differences ($p < 0.05$) according to Tukey's multiple range test.

The order of other plants is presented in continuation: *Sphaeralcea angustifolia* (4 ± 0.16 mg GAE/g), *Larrea tridentata* (3.75 ± 0.1 mg GAE/g), *Flourensia cernua* (3.51 ± 0.04 mg GAE/g), *Prosopis glandulosa* (3.28 ± 0.06 mg GAE/g), *Salvia officinalis* (3.0 ± 0.02 mg GAE/g), *Heterotheca inuloides* (3.02 ± 0.1 mg GAE/g), *Ruta graveolens* (2.82 ± 0.01 mg GAE/g), *Lippia graveolens* (2.77 ± 0.07 mg GAE/g), *Jatropha dioica* (2.16 ± 0.13 mg GAE/g), *Parthenium incanum* (2.08 ± 0.02 mg GAE/g), *Ephedra antisiphilitica* (2.08 ± 0.14 mg GAE/g), *Parkinsonia aculata* (1.98 ± 0.06 mg GAE/g), *Mirabilis jalapa* (1.64 ± 0.02 mg GAE/g), *Astragalus nitidiflorus* (1.41 ± 0.13 mg GAE/g), *Parthenium hysterophorus* (1.21 ± 0.02 mg GAE/g), *Selaginella moellendorffi* (0.99 ± 0.03 mg GAE/g), *Bauhinia forficata* (0.53 ± 0.04 mg GAE/g). This investigation classifies plants for the first time by their phenolic content; these studies are very valuable for future studies in ethnopharmacology.

3.2. Evaluation of Extraction Methods

Figure 2 shows the effector of the solid/liquid ratio and the extraction method on the phenolic content of *T. diffusa*, *G. sarothrae*, and *L. frutescens*. At the 1:10 concentration for the three plants studied, there is no significant difference with the UAE or maceration methods. In the solid/liquid ratio of 1:25, the maceration method presented the highest values (4.2 ± 0.04 mg GAE/g) of phenols for the three plants; the values were statistically signifi-

cant. However, in the solid/liquid ratio of 1:50, the opposite effect was present; statistically, UAE was the method that presented the highest values of phenols (4 ± 0.02 mg GAE/g).

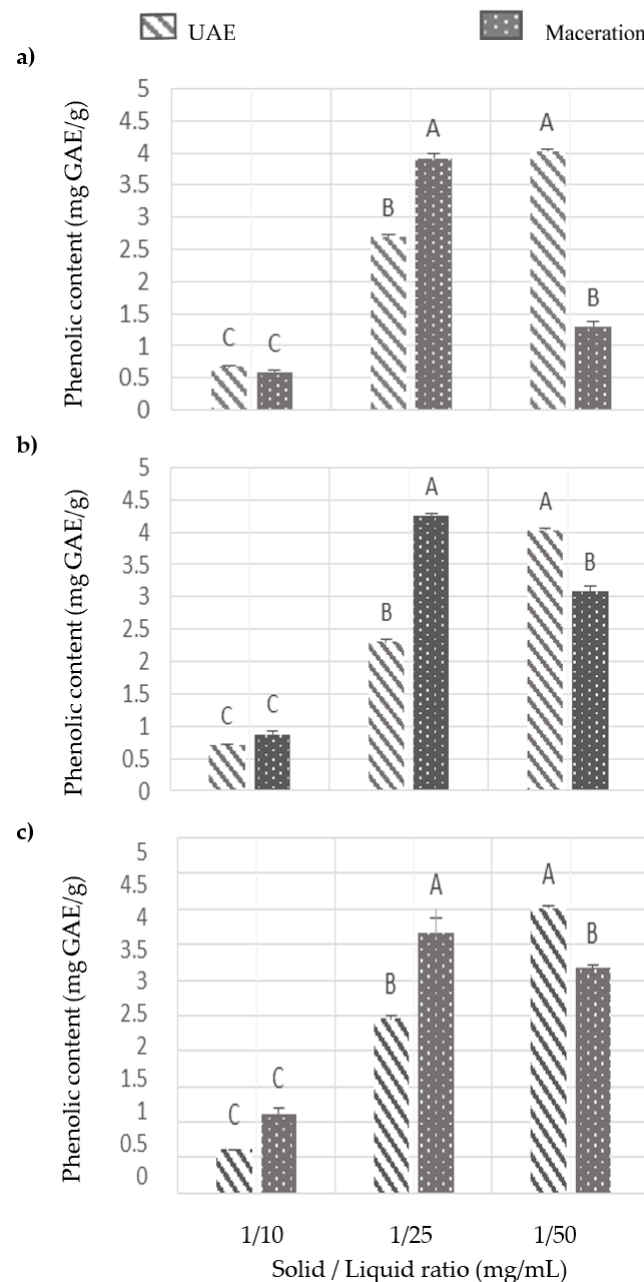


Figure 2. Effect of the extraction method (UAE: ultrasound-assisted extraction and maceration) and the solid/liquid ratio on the phenolic content of (a) *T. diffusa*, (b) *G. sarothrae*, and (c) *L. frutescens*. The different letter indicates significant differences ($p < 0.05$) according to Tukey's multiple range test.

According to the results, when the solute concentration is high, 1:10 or 1:25 mg/mL, the maceration method is more suitable, but when the dilution is higher (1:50 mg/mL), the emerging UAE technology is more appropriate. This behavior is attributed to the principle of mass transfer in a solid–liquid extraction. In the solid/liquid ratio of 1:10, the decrease in phenol content is attributed to a phenomenon of saturation of the solvent; in saturation, the cellular phenomenon of diffusion stops and there is a stabilization rate. According to the principles of mass transfer, the driving force during transfer is the concentration gradient between the solid and the bulk of the liquid. This gradient is greater when a higher solvent-to-solid ratio is used. The interactions of the extracted compounds with the solvent

can modify the activity coefficients and therefore, the solubility of the compounds. Similar results on the effect of the solid/liquid ratio on the extraction of phenolic compounds have been reported [11,18]. The main driving force of UAE is acoustic cavitation, which reduces particle size and facilitates mass transfer in less concentrated solutions. UAE is effective at low concentrations by improving solvent accessibility through the implosion of bubbles on the surface of the plant matrix. According to the results obtained, the UAE generates extracts rich in phenolic compounds with a smaller amount of plant material. This is very important for future studies where the efficiency of extraction methods is evaluated [19]. In summary, the conventional maceration method can be used with high concentrations of material (this is more in line with the method used by local communities to prepare the plant). The UAE method cannot be discarded since it is more efficient with low concentrations of plant material.

3.2.1. Antioxidant Activity Assay

DPPH Assay

In the present study, extracts from *T. diffusa*, *G. sarothrae*, and *L. frutescens* were analyzed for their DPPH• scavenging activity, and the results are shown in Figure 3a for the UAE method and Figure 3b for the maceration method. The effect of three solid/liquid ratios (1:10, 1:25, and 1:50 mg/mL) was also evaluated. According to the results, in the two extraction methods evaluated, *T. diffusa* and *G. sarothrae* present greater antioxidant activity at a solid/liquid ratio of 1:50 mg/mL. At this concentration, there is a statistically significant difference with respect to the other two concentrations ($p < 0.05$).

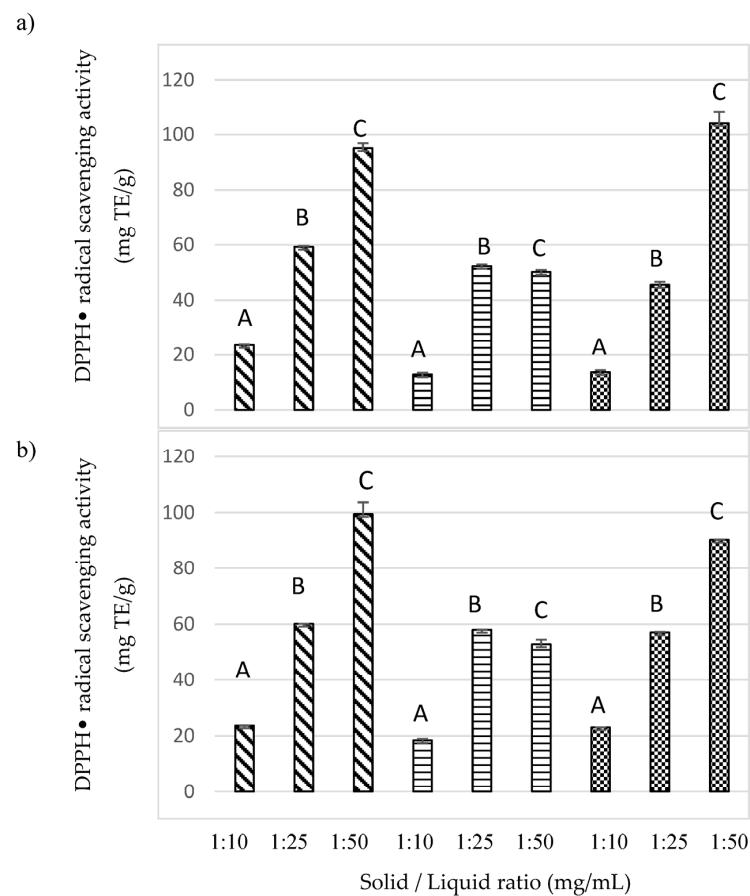


Figure 3. DPPH free radical scavenging activity of *T. diffusa*, *L. frutescens*, and *G. sarothrae* extracts obtained by (a) UAE and (b) maceration. The different letter indicates significant differences ($p < 0.05$) according to Tukey's multiple range test.

G. sarothrae presented the highest antioxidant activity in both methods at a concentration of 1:25 mg/mL ($p < 0.05$). This result is attributed to a modification in the activity of the extracted compounds in *G. sarothrae* during the extraction with the solvent [20].

T. diffusa and *L. frutescens* present the highest values of antioxidant activity with the maceration method at 100 and 57 mg TE/g, respectively. However, the UAE of *G. sarothrae* presented the highest antioxidant activity of the three plants evaluated (103 mg TE/g). The results show the extracts of the three plants present high antioxidant activity. Additionally, such high free radical scavenging properties of the extracts are higher than those reported in hydroalcoholic extracts of medicinal plants, such as *Lavandula stoechas* L. (58 mg TE/g) and *Aesculus indica* (Wall. ex Cambess.) Hook (46 mg TE/g) [3].

FRAP Assay

The results of the ferric reducing antioxidant power assay are presented in Figure 4. The *T. diffusa* and *G. sarothrae* plants presented a similar trend to the results of the DPPH method; the solid/liquid ratio of 1:50 g/mL significantly showed greater antioxidant activity compared to the ratios of 1:10 and 1:25 mg/mL.

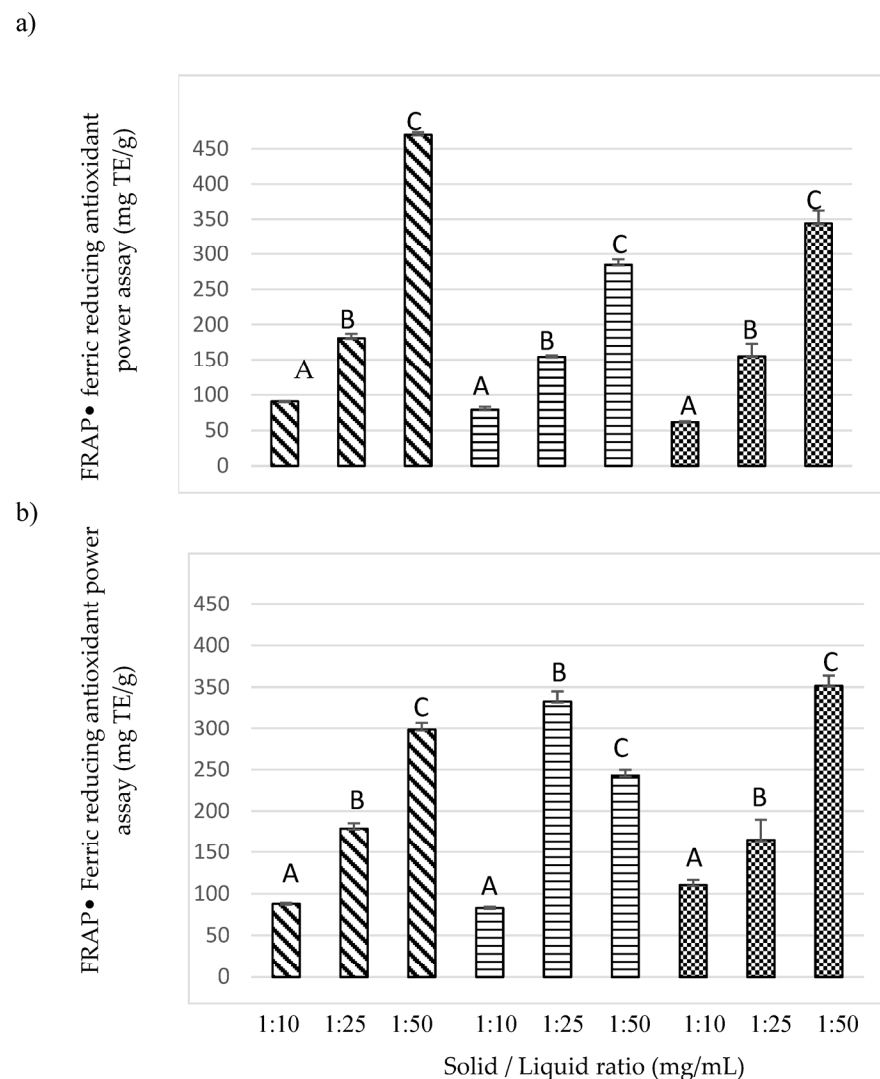


Figure 4. FRAP: ferric reducing antioxidant power assay of *T. diffusa*, *L. frutescens*, and *G. sarothrae* extracts obtained by (a) UAE and (b) maceration. The different letter indicates significant differences ($p < 0.05$) according to Tukey's multiple range test.

T. diffusa (in the solid/liquid ratio of 1:50 g/mL) presented the highest activity with the UAE extraction method (469 mg TE/g) compared to extraction by maceration (300 mg TE/g). On the other hand, in *G. sarothrae*, no difference was found between the extraction methods evaluated (350 mg TE/g). *L. frutescens* significantly presented the highest antioxidant activity with the maceration extraction method at a solid/liquid ratio of 1:25 g/mL (332 mg TE/g); this behavior was similar to the DPPH analysis. The results determined in the present study were superior to those determined for hydroalcoholic extracts from medicinal plants such as *Tephrosia purpurea* (L.) Pers (6 mg TE/g), *Lavandula stoechas* L. (9 mg TE/g), *Aesculus indica* (Wall. ex Cambess.) Hook. (47 mg TE/g), *Iris ensata* Thunb. (177 mg TE/g), *Kalanchoe pinnata* (Lam.) Pers. (206 mg TE/g) [3], and *Reum espiciforme* (83 mg TE/g) (*R. spiciforme* is known for its anticancer properties [21]).

ABTS Assay

The ABTS assay was also used to investigate the antioxidant activity of the plants *T. diffusa*, *G. sarothrae*, and *L. frutescens*. *T. diffusa* presented significantly higher antioxidant activity (300 mgTE/g) with a solid/liquid ratio of 1:50 (mg TE/mL) and using UAE (Figure 5). This behavior for this plant is similar to that presented previously with the DPPH and FRAP methods.

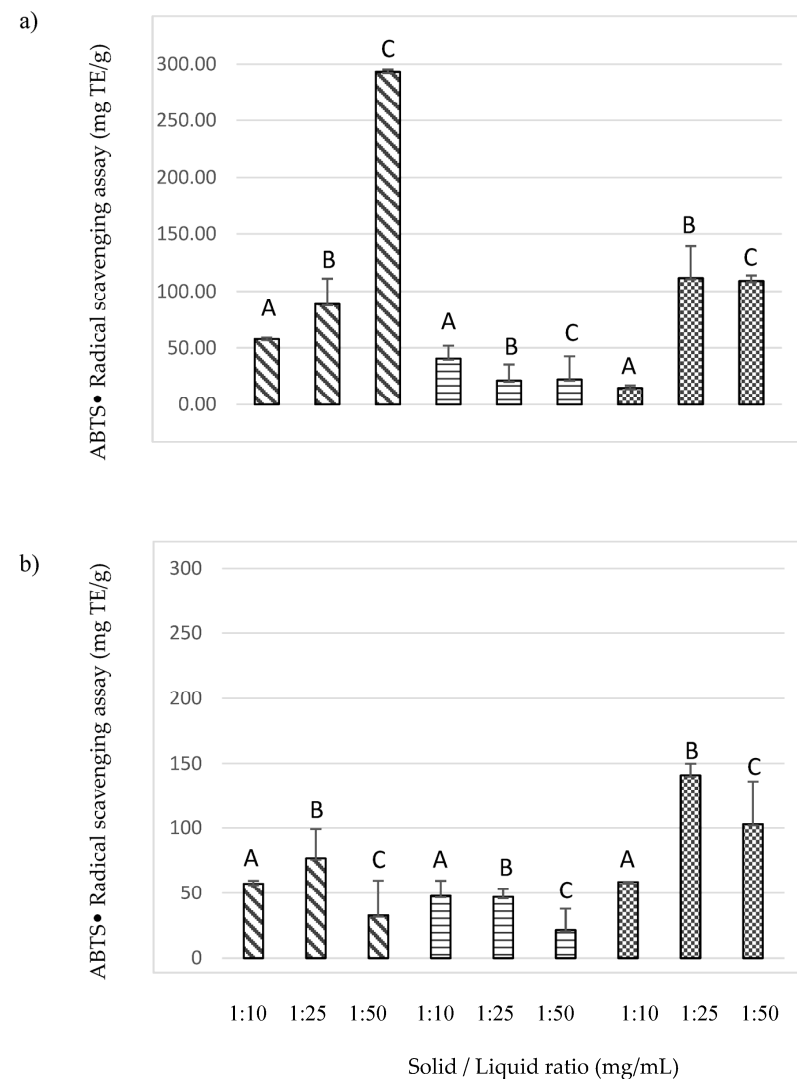


Figure 5. ABTS radical scavenging assay of *T. diffusa*, *L. frutescens*, and *G. sarothrae* extracts obtained by (a) UAE and (b) maceration. The different letter indicates significant differences ($p < 0.05$) according to Tukey's multiple range test.

L. frutescens and *G. sarothrae* showed greater antioxidant activity with the maceration extraction method. *L. frutescens* significantly presented the greatest activity at the solid/liquid ratio of 1:10 g/mL (50 mg TE/mL), and *G. sarothrae* significantly showed the greatest antioxidant activity at the solid/liquid ratio of 1:25 (141 mg TE/mL). For both plants, the values were lower than the maximum reached by *T. diffusa* (300 mg TE/g). The observed differential scavenging activities of the extracts against the DPPH• and ABTS•+ systems demonstrate the presence of different compounds and different chemical features (see HPLC-MS analysis). This phenomenon can also be explained by the different affinities of the evaluated methods. Unlike DPPH•, ABTS•+ has an affinity with water-soluble compounds and with lipophilic compounds since it is soluble in both aqueous and organic solvents.

3.3. Analysis of the Extraction Methods Evaluated

Table 2 shows a summary of the efficiency between the UAE and maceration methods. In the three antioxidant activity assays evaluated, *T. diffusa* with a solid/liquid ratio of 1:50 g/mL only showed a significant difference in the DPPH• assay and the highest activity was obtained for the maceration method. For *L. frutescens* with a solid/liquid ratio of 1:25 g/mL, maceration was the extraction method that significantly showed the highest antioxidant activity. Finally, for *G. sarothrae* using a solid/liquid ratio of 1:50 g/mL, the method that significantly presented greater antioxidant activity was UAE.

Table 2. Efficiency between extraction methods: UAE and maceration.

Plant	UAE	Maceration	UAE	Maceration	UAE	Maceration
	DPPH• (mg TE/g)		FRAP (mg TE/g)		ABTS•+ (mg TE/g)	
<i>T. diffusa</i>	95 ± 1.7 ^(1:50)	99 ± 4.1 * ^(1:50)	469 ± 3.5 ^(1:50)	300 ± 9 ^(1:50)	300 ± 2 ^(1:50)	32 ± 27.1 ^(1:50)
<i>L. frutescens</i>	55 ± 0.6 ^(1:25)	57 ± 0.57 * ^(1:25)	154 ± 2.4 ^(1:25)	332 ± 12.1 * ^(1:25)	21 ± 14.3 ^(1:25)	50 ± 16.0 * ^(1:25)
<i>G. sarothrae</i>	104 ± 4.3 * ^(1:50)	90 ± 0.1 ^(1:50)	350 ± 18.6 ^(1:50)	350 ± 12.2 ^(1:50)	111 ± 27.7 ^(1:25)	141 ± 8.9 ^(1:25)

Values are presented as means ± SD (n = 3). * Significantly different by *t*-test (*p* < 0.05). ^(1:25) Extraction carried out at a solid/liquid ratio of 1:25 g/mL. ^(1:50) Extraction carried out at a solid/liquid ratio of 1:50 g/mL. UAE: ultrasound-assisted extraction.

The maceration method was the most suitable for extracting antioxidant compounds from *T. diffusa* and *L. frutescens*, while UAE was more efficient for *G. sarothrae*.

3.4. RP-HPLC-ESI-MS Analysis

The bioactive compounds present in the plants *G. sarothrae*, *T. diffusa*, and *L. frutescens* were identified by RP-HPLC-ESI-MS (Table 3).

For *T. diffusa*, we identified 14 molecules that can be classified in different families, such as hydroxycinnamic acids, methoxycinnamic acids, and flavones. 24-Methylcholesterol ferulate, Caffeoyl tartaric acid, Sinensetin, 3-p-Coumaroylquinic acid, 4-p-Coumaroylquinic acid, and p-Coumaric acid 4-O-glucoside were the major component of polyphenolic extracts from *T. diffusa*.

Eleven molecules were identified for *L. frutescens* belonging to the hydroxycinnamic acids, methoxycinnamic acids, flavones, proanthocyanidin dimers, methoxyflavones, anthocyanins and tyrosols families. Apigenina 7-O-diglucurónido 3 and 4-DHPEA-EA were the major components of polyphenolic extracts from the *L. frutescens*. Apigenins were identified in similar studies [22]. Apigenin has been recognized in traditional medicine due to the fact that it has many pharmacologic activities, like 17-β-hidroxiesteroide deshidrogenasa enzyme inhibition [23,24].

Four molecules were found for *G. sarothrae*, and these molecules belong to flavonoids, lignans, and phenolic acids.

Table 3. Identification of phenolic compounds of hydroalcoholic extracts of *T. diffusa*, *L. frutescens*, and *G. sarothrae*.

Name	Retention Time (min)	[M-H] [−]	Compound	Family
<i>T. diffusa</i>	6.31	190.9	Scopoletin	Hydroxycoumarins
	6.9	365.8	Secoisolariciresinol	Lignans
	34.74	592.8	Apigenin 6,8-di-C-glucoside	Flavones
	34.95	370.9	Sinensetin	Methoxyflavones
	37.98	638.8	Malvidin 3-O-(6''-p-coumaroyl-glucoside)	Anthocyanins
	38.29	668.8	Spinacetin 3-O-glucosyl-(1->6)-glucoside	Methoxyflavonols
	41.06	590.7	Cyanidin 3-O-(6''-dioxalyl-glucoside)	Anthocyanins
	42.18	574.7	24-Methylcholesterol ferulate	Methoxycinnamic acids
	49.86	310.8	Caffeoyl tartaric acid	Hydroxycinnamic acids
	50.57	576.7	Procyanidin dimer B1	Proanthocyanidin dimers
	51.09	408.8	6-Geranylnaringenin	Alkylflavanones
	53.96	270.8	Naringenin	Flavanones
	55.51	324.9	Feruloyl tartaric acid	Methoxycinnamic acids
	57.15	336.8	3-p-Coumaroylquinic acid	Hydroxycinnamic acids
	57.55	336.7	4-p-Coumaroylquinic acid	Hydroxycinnamic acids
	57.97	325	p-Coumaric acid 4-O-glucoside	Hydroxycinnamic acids
<i>L. frutescens</i>	6.18	340.9	Caffeic acid 4-O-glucoside	Hydroxycinnamic acids
	6.57	376.8	3,4-DHPEA-EA	Tyrosols
	7.57	396.8	3-Sinapoylquinic acid	Methoxycinnamic acids
	7.7	376.9	3,4-DHPEA-EA	Tyrosols
	38.02	622.8	Apigenin 7-O-diglucuronide	Flavones
	40.78	576.8	Procyanidin dimer B1	Proanthocyanidin dimers
	41.49	622.8	Isorhamnetin 3-O-glucoside 7-O-rhamnoside	Methoxyflavonols
	42.45	542.8	3,4-Diferuloylquinic acid	Methoxycinnamic acids
	43.58	264.8	Formononetin	Methoxyisoflavones
	43.96	526.9	Malvidin 3-O-galactoside	Anthocyanins
	53.45	268.7	Apigenin	Flavones
	54.89	324.9	Feruloyl tartaric acid	Methoxycinnamic acids
<i>G. sarothrae</i>	28.97	330.3	Jaceosidin	Methoxyflavones
	44.53	358.7	Lariciresinol	Lignans
	47	772.1	Kaempferol 3,7,4'-O-triglucoside	Flavonols
	54.59	324.8	Feruloyl tartaric acid	Methoxycinnamic acids

4. Conclusions

To summarize our findings, we have reported for the first time the phenol contents of twenty-three medicinal plants used in the traditional medicine of Viesca, Coahuila, Mexico. *T. diffusa*, *L. frutescens*, and *G. sarothrae* are the richest sources of phenolic compounds and were selected to subsequently study their antioxidant potential by three different assays. Two extraction methods and three solid/liquid ratios were evaluated in the three plants. The three plants presented high antioxidant activity, the solid/liquid ratio presented a significant influence on their antioxidant activity, and the solid/liquid ratio of 1:50 g/mL presented the highest activity values for *T. diffusa* and *G. sarothrae*. *L. frutescens* presented the highest values of antioxidant activity with a solid/liquid ratio of 1:25 mg/mL. The maceration method was the most suitable for extracting antioxidant compounds from *T. diffusa* and *L. frutescens*, while UAE was more efficient for *G. sarothrae*. The HPLC-MS analysis showed the presence of 14 molecules for *T. diffusa*, 11 molecules for *L. frutescens*, and 4 molecules for *G. sarothrae*. The medicinal plants used in Viesca, Mexico, have a high antioxidant potential and can be a source of natural antioxidants with applications in medicine and the food industry. In conclusion, the plants used in traditional medicine from Viesca, Mexico, have great potential for the development of natural products.

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