A pharmacological and phytochemical study of medicinal plants used in Mexican folk medicine

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Received 13 October 2014, revised 26 February 2015

Sonora market is one of the most important places in Latin America for management of traditional medicine due to the convergence of knowledge from several different indigenous communities. For this reason we decided to apply the standardization methodology proposed by the World Health Organization (WHO) and adapted by our research group to a total of ten plants used in the Sonora market, because of their referred antioxidant properties. A fragment of 18S rDNA of four of these plants was registered in GenBank database 2014 (http://www.ncbi.nlm.nih.gov/genbank/). Quantitative analysis showed that the *E. angustifolia*® commercial extract (*Maryel Natura Gold*) contains higher levels of total flavonoids than the extracts of the plants used in the Sonora market. However, chlorogenic acid was found in *Castela erecta* Turpin ssp “chaparro amargo”, *Larrea tridentata* (Sessé & Moc. ex DC.) Coville “gobernadora”, *Opuntia ficus-indica* (L.) Mill. “Raíz de nopal”, *Tecoma stans* “tronadora”, and *Ibervillea sonorae* (S. Watson) Greene “Wereke”, but not in the *E. angustifolia*® extract. Antioxidant activity was higher in the *E. angustifolia*® extract compared to the extracts of evaluated plants. This information will serve as basis for progress in the recovery, standardization, pharmacological characterization and application of medicinal plants used in Mexican traditional medicine.

**Keywords**: Pharmacological characterization, Mexico, Folk medicine, *E. angustifolia* DC., Antioxidant activity, Molecular identification

**IPC Int. Cl.**: A61K 36/00, C09K 15/00, G06F 19/10, C07C 7/13, C01B 37/00, C01B 39/00

In order to meet their basic needs, man has always had a close relationship with plants. Folk or indigenous medicine is the result of this relationship¹ and still plays an important role in continents like Africa, Asia and America. The World Health Organization (WHO) has paid attention to the success achieved by Eastern countries like China, where incorporation of medicinal plants into official medicine resulted in their clinical assessment. In the last four years, the Food and Drug Administration (FDA) has approved three treatments based on herbal mixtures (antiallergic, anticancer, antipsoriatic)²; this fact could change the current view of traditional medicine in the world. In order to approve treatments based on medicinal plants, the establishment of protocols that allow the standardization of the process is required. This standardization consists of two main stages; in the first, safety and identity of the product must be guaranteed. In the second, chemical content of the extract must be guaranteed in order to achieve the desired pharmacological effect. To do so, it is necessary to measure some parameters of their chemical composition such as the presence of major chemical groups through qualitative analysis combined with the quantification of some secondary metabolites. Determination of metabolite fingerprints by using various analytical techniques such as HPLC, UV-Vis spectrum, GC-MS analysis, among others, is also part of the standardization process³. Finally, it is necessary to perform some *in vitro* or *in vivo* pharmacological essays.

Mexico is one of the five mega diverse countries of the world given that about 50% of the 22,000 vascular plant species are endemic⁴, and similarly to other countries, the use of native and incorporated medicinal plants is a common alternative to conventional medicine in some rural communities. Markets are the most common sites for obtaining these plants. However, their clinical use has not resulted in the development of scientific methodologies; for this

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reason, they cannot be incorporated into official medicine\(^5\). In this context, the objective of the present work was to apply the methodology to standardize medicinal plants to ten of the most used medicinal plants in the Sonora Market in order to contribute to the knowledge of Mexican medicinal plants.

**Methodology**

**Biological material**

Medicinal plants were selected by ethnobotanical study. In this study, the common name for each plant was obtained and then, scientific names were searched for in the catalogue of common and scientific names of Mexican plants as a first approach, and corroborated with the taxonomic classification of Mexican plants by Calderon and Rezdowski (2005)\(^6\). These studies were conducted by the taxonomist Susana Peralta of the Plantel Casa Libertad Herbarium. A commercial extract of *Echinacea angustifolia* manufactured by Maryel Natural Gold, Mérida, Yucatán, México, was obtained from a local merchandiser.

**DNA isolation and 18S gene amplification**

Total DNA was isolated from 50 mg of dry leaf from each plant using Axy Prep Multisource Genomic DNA Miniprep kit (Axygen Biosciences®, CA, USA). DNA was resuspended in 100 µL of DEPC-treated water. DNA purity was determined by A\(_{260}\)/A\(_{280}\) ratio and its concentration was quantified using a Nanodrop 2000 (Thermo Scientific, Wilmington, DE. USA). Integrity of all samples was verified by gel electrophoresis using 100 ng. For amplifying 18S gene, the following primers were used: Fwd: 5’–GTGGCCTAAACGGCCATAGTCCTC–3’ and Rv: 5’ –GGAAACTTACCAGGTCCAGAGAT AG–3’ based on the published maize sequences (Accession number AF168884)\(^7\). The amplicon was purified with the Wizard® SV Gel kit and the PCR Clean-Up System (Promega, Madison, USA) and sequenced by the Sanger method (Applied Biosystems ABI, Hitachi 3130XL Prism Genetic Analyzer, Mariland, USA).

**Preparation of extracts**

For obtaining the extracts, biological material (200 g) was dried and macerated starting with hexane (1000 mL). After a week of maceration, the solvent was recovered by filtering and it was concentrated under reduced pressure to obtain the solid extract. The filtered parts of the plant were macerated once again with ethyl acetate (1000 mL) and afterwards with methanol (1000 mL) using the same process described for hexane. Samples were refrigerated at 4°C until their further analysis\(^8\).

**Qualitative analyses**

**Determination of alkaloids**

An aliquot of 0.1 mL of each extract was applied on silica gel 60F\(_{254}\) plates (3 x 5 cm). Plates were eluted with chloroform-methanol 95:5 v/v and revealed with the Dragendorff reagent. Formation of red-brown spots indicates the presence of alkaloids\(^8\).

**Detection of tannins**

The extracts obtained (2 mg) were dissolved in 10 mL of distilled water. The solution was divided into 3 test tubes and treated with: a gelatin solution 1% (w/v) in test tube number 1; a gelatin-salt reagent (1 gm of gelatin and 10 gm of NaCl dissolved in 100 mL of distilled water) in test tube number 2; saline solution (NaCl 10% (w/v)) in test tube number 3. The appearance of a white precipitate in test tubes number 1 & 2 and the absence of such precipitate in test tube number 3 indicate the presence of tannins\(^8\).

**Determination of saponins**

The extracts obtained (2 mg) were placed in a tube containing 10 mL of distilled water and then, incubated in a water bath at 80 °C during 30 min. Afterwards, the tube was allowed to cool, stirred vigorously and left to stand for 15 to 20 minutes\(^8\). The presence and level of saponins was assessed by measuring the height of the foam formed.

**Determination of free anthracene derivatives**

Silica gel plates 60F\(_{254}\) of 3 x 5 cm were cut and an aliquot (0.1 mL) of each extract was applied. Plates were eluted with the same solvents used for alkaloids determination. Yellow or red fluorescent spots under UV-light indicate the presence of free anthracene derivatives\(^8\).

**Determination of volatile coumarins**

The extracts obtained (2 mg) were added to 10 mL of distilled water were covered with filter paper moistened in a caustic soda solution (1gm in 15mL) and heated until boiling point. After 5 minutes, the filter paper was removed from the tube, dried and exposed to UV-light. Blue fluorescence indicates the presence of volatile coumarins\(^8\).

**Quantitative analyses**

**Total phenolic compounds**

Total phenolic compounds were measured as described by Singlenton and Rossi (1965)\(^9\) using a
spectrophotometer (6705 UV/VIS JENWAY). Gallic acid was used as standard.

**Total flavonoids**

Total flavonoids were measured by the chloride aluminum method as described by Chang et al. (2002) using a spectrophotometer (6705 UV/VIS JENWAY). Quercetin was used as positive control (See, S1).

**Caffeic and chlorogenic acid determination**

Caffeic and Chlorogenic acid determinations were performed according to Pellati et al. (2011) with slight modifications. An HPLC Agilent 1200 series system consisting of a degasser, a quaternary pump, an autosampler, a thermostatted column compartment, and a UV detector was used for the determinations. A C_{18} (3.9 x 300 mm, W00671T018 serial number) column was used for the analyses; the column temperature was set at 30 °C. The mobile phase consisted of (A) 0.1% (v/v) acetic acid in H₂O and (B) acetonitrile. The gradient elution was as follows: initial, 15% B; 0-10 min, from 15 to 30% B; 10-18 min, from 30 to 65% B; 18-25 min, from 65 to 80% B; 25- 30 min from 80 to 90% B, 30- 35 min, linear gradient B. The post-running time was 3 min at a 1 mL min⁻¹ flow rate. Sample injection volume was 20 µL. The DAD acquisition range was 250-350 nm and peak integration was performed at 320 nm.

**Pharmacological analysis**

**Analysis of toxicity using Artemia salina bioassay**

Toxicity activity was measured using the A. salina bioassay described by Meyer et al. in 1982 and adjusted by our investigation group⁹.

**Antioxidant activity measured using DPPH**

Antioxidant activity was determined by diphenylpicrylhydrazyl (DPPH) assay described by Brand-Williams et al. in 1995 using a spectrophotometer JENWAY 6705 UV/VIS. Trolox was used as positive control (See, S1).

**Antioxidant activity measured using ABTS⁺**

Antioxidant capacity was measured according to the method described by Rivero-Pérez et al. in 2007 using a spectrophotometer JENWAY 6705 UV/VIS. Trolox was used as positive control (See, S1).

**UV/Vis Spectrum**

The solid extract (10 mg) was dissolved in 5 mL methanol and then the solution was filtered. Spectra were obtained using a spectrophotometer (6705 UV/VIS JENWAY) within an acquisition range from 190 to 1100 nm.

**Results and discussion**

The Sonora Market is one of the most important places in Mexico City for selling and buying medicinal plants, this market has sales of about 160 tons per month. There, the traditional knowledge of several indigenous communities of the Mexican Valley converges. Sonora Market was officially named in 1974 when this center of popular commerce took its current structure, made up of 10,262 m² distributed in two areas. In the first area, there are 11 aisles where ceramic, handcrafted decorative and herbal items, as well as Mexican toys, alebrijes (alebrijes are colored Mexican folk art sculptures of imaginary creatures) and seasonal items for any social event, animals and pet accessories can be found. The second area houses stalls selling saint images, herbs and medicinal plants. Regarding herbalism, plants availability in the market stalls depend on the demand for the product, as well as its natural availability in its native environment.

As a result of the ethnobotanical and bibliographic study a total of 10 plants used in the Sonora Market for its referred antioxidant properties for curing and preventing several illnesses were selected (Table 1); it is important to mention that these plants are among the most bought by consumers for several ailments. Once the plants were obtained in the market, they were identified as described in the section of materials and methods and a literature review was conducted to know the pharmacological properties reported in PubMed (http://www.ncbi.nlm.nih.gov/pubmed) for these plants. From the 10 selected plants, 5: Cecropia obtusifolia Bertol., Bryophyllum pinnatum (Lam.) Oken, Opuntia indica, Tecoma stans (L.) Juss. ex Kunth and Iberovia sonorae (S. Watson) Greene have been mentioned for their hypoglycemic properties, while Larrea tridentata (Sessé & Moc. ex DC.) Coville, Castela erecta Turpin ssp., Guazumia ulmifolia Lam., and Frangula purshiana Cooper syn Rhamnus purshiana DC. have been associated with other pharmacological properties as shown in Table 1. No pharmacological studies were found for Swietenia humilis Zucc.; however, there are some ethnobotanical studies reported in the Digital Library of Mexican Traditional Medicine developed by the National Autonomous University of México (see www.medicinaltradicionalmexicana.unam.mx) and in the Sonora Market (Table 1). This makes Swietenia
humilis Zucc. an interesting candidate for studying its antibiotic properties that have been empirically reported, but not pharmacologically validated.

In addition, a conserved region of the 18S ribosomal gene from each of the ten selected plants was amplified and sequenced as previously described in materials and methods (Table 2). The alignment of the obtained sequences to sequences previously reported allows having a molecular reference for identification of the analyzed biological material. However, the selected plants are not registered in the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/). The conserved fragment of 18S of 4 of the 10 plants studied, were registered by our group in GenBank with the following accession numbers: Cásca sa Sagrada (Frangula purshiana Cooper syn. Rhamnus purshiana DC., KJ937009), Guarumbo (Cecropia obtusifolia Bertol., KJ937006), Guazima (Guazuma ulmifolia Lam., KJ937008), and Wereke (Ibervillea sonorae (S. Watson) Greene, KJ937007) the others are shown in Table 2.

Qualitative and toxicity analyses were performed in the extracts obtained using the three solvents: hexane, ethyl acetate and methanol. In contrast, quantitative and pharmacological analyses were determined only in the methanolic extract, since this is the most similar to the Echinacea angustifolia DC. hydroalcoholic extract used for comparison. Also, the plants selected

<table>
<thead>
<tr>
<th>Table 1—Bibliographic review and empirical uses of plants used in the Sonora Market</th>
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<tbody>
<tr>
<td>a. Cásca sa Sagrada</td>
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<tr>
<td>a. Chaparro amargo</td>
</tr>
<tr>
<td>a. Guarumbo</td>
</tr>
<tr>
<td>a. Guazima</td>
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<tr>
<td>a. Prodigiosa</td>
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<tr>
<td>a. Raúz de nopal</td>
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<tr>
<td>a. Semilla de zopilote</td>
</tr>
<tr>
<td>a. Tronadora</td>
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<tr>
<td>a. Wereke</td>
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</tbody>
</table>
for the study are used as aqueous infusion for its therapeutic application.

Qualitative preliminary tests were carried out in the plants selected in the Sonora Market to determine the presence of different chemical groups shown in Table 3. Alkaloids, anthraquinones, tannins, saponins and coumarins are among the chemical groups associated to the therapeutic principles evaluated in each of the extracts. This qualitative evaluation represents an initial characterization. Since, it is a qualitative study we cannot obtain an absolute value for comparing to other plant species; however, it is possible to compare the plants used in the study among them. Results show that ‘prodigiosa’ ethyl acetate extract and ‘gobernadora’ methanolic extract contained the highest content of alkaloids; regarding saponins, hexane and ethyl acetate extracts of ‘wereke’ showed the highest levels. Coumarins were not detected in any of the analyzed species. Chemical groups of interest such as caffeic acid, chlorogenic acid, total phenols, and total flavonoids. Caffeic and chlorogenic acids are compounds with pharmacological properties related to antioxidant protection. Since, these compounds are reported in many plants with antioxidant properties, they represent a good chemical marker. It is important to mention that E. angustifolia was chosen as control because there are several studies that report high contents of these compounds in this plant and therefore, it represents a parameter for comparison to the plants used in Mexican traditional medicine. Content of these compounds is reported in Table 4. Caffeic acid was detected in Rhamnus purshiana “Cáscara sagrada”, Opuntia ficus “raíz de nopal” and Ibervillea sonorae “Wereke”, showing Cáscara sagrada much lower concentrations than the E. angustifolia® extract. Regarding chlorogenic acid, which was not detected in the Echinacea extract, we found concentrations of 32.3,
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21.4, 90.4, 34.6 and 15.7 ppm per gm of dry weight in the Mexican plants Castela erecta ssp “Chaparro amargo”, Larrea tridentata “Gobernadora”, Opuntia ficus “raíz de nopal”, Tecoma stans “Tronadora” and Iberovela sonorae “Wereke”, respectively (S2). In regards to total phenolic compounds and total flavonoids content, Rhamnus purshiana “Cáscara sagrada” and Semilla de zopilote showed a high content of total phenolic compounds (for statistical differences in the flavonoids and phenols content between plants see supporting information). Even though total phenols in these Mexican species were higher than in the E. angustifolia® extract, the Echinacea extract showed a stronger antioxidant activity, regarding the Mexican plants, we did not find statistical differences between groups (See supporting information S3).

Echinacea angustifolia® had the highest concentration of total flavonoids evaluated as milliequivalents of quercetin per gram of dry weight. We observed poor correlations between total phenolic compounds vs the antioxidant capacity and a better correlation between total flavonoid content and antioxidant capacity. The use of a wider range of plants is recommended in order to come to a better conclusion regarding the correlations. See supporting information S4-S7. There was a strong correlation (R² = 0.9998) between DPPH vs ABTS+, however, when we eliminated the value of Echinacea angustifolia® we obtained a bad correlation. These results suggest that for this correlation analysis it is necessary to have intermediate values (S8). It is important to mention that neither the extracts obtained from the Mexican plants nor the Echinacea angustifolia® extract were active against nauplii of Artemia salina, considering that values higher than 200 ppm of the crude extract are considered as toxic.

**Table 4—Phytochemical quantitative analyses and pharmacological assay**

<table>
<thead>
<tr>
<th></th>
<th>Caffeic acid</th>
<th>Chlorogenic acid</th>
<th>Total phenols</th>
<th>Total flavonoids</th>
<th>Antioxidant capacity (DPPH)</th>
<th>Antioxidant Capacity (ABTS)</th>
<th>Toxicity assay Artemia salina</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cáscara sagrada</td>
<td>0.022</td>
<td>n.d.</td>
<td>1133 ± 41</td>
<td>9.56 ± 0.08</td>
<td>0.28±0.05</td>
<td>0.09 ± 0.002</td>
<td>15.35</td>
</tr>
<tr>
<td>Chaparro amargo</td>
<td>n.d.</td>
<td>32.3</td>
<td>154 ± 17</td>
<td>6.77 ± 0.53</td>
<td>0.16±0.01</td>
<td>n.d.</td>
<td>1551</td>
</tr>
<tr>
<td>Gobernadora</td>
<td>n.d.</td>
<td>21.4</td>
<td>476 ± 11</td>
<td>0.88 ± 0.05</td>
<td>0.06±0.01</td>
<td>0.04 ± 0.002</td>
<td>1592</td>
</tr>
<tr>
<td>Guaranbo</td>
<td>n.d.</td>
<td>14.6</td>
<td>14 ± 0.03</td>
<td>0.63 ± 0.03</td>
<td>0.08±0.002</td>
<td>0.12 ± 0.002</td>
<td>276</td>
</tr>
<tr>
<td>Guazima</td>
<td>n.d.</td>
<td>58 ± 7</td>
<td>2.87 ± 0.59</td>
<td>0.05 ± 0.003</td>
<td>0.01 ± 0.001</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>Prodigiosa</td>
<td>n.d.</td>
<td>708 ± 29</td>
<td>0.31 ± 0.02</td>
<td>0.05±0.006</td>
<td>0.06 ± 0.001</td>
<td>254</td>
<td></td>
</tr>
<tr>
<td>Raíz de nopal</td>
<td>0.804</td>
<td>90.4</td>
<td>537 ± 31</td>
<td>0.19 ± 0.02</td>
<td>0.70±0.04</td>
<td>0.11 ± 0.005</td>
<td>260</td>
</tr>
<tr>
<td>Semilla de zopilote</td>
<td>n.d.</td>
<td>1040 ± 57</td>
<td>0.80 ± 0.02</td>
<td>0.31 ± 0.04</td>
<td>0.03±0.002</td>
<td>0.06 ± 0.004</td>
<td>1529</td>
</tr>
<tr>
<td>Tronadora</td>
<td>n.d.</td>
<td>34.6</td>
<td>411 ± 10</td>
<td>5.76 ± 0.47</td>
<td>0.03±0.002</td>
<td>0.12 ± 0.002</td>
<td>1550</td>
</tr>
<tr>
<td>Wereke</td>
<td>0.559</td>
<td>15.7</td>
<td>747 ± 42</td>
<td>5.41 ± 0.43</td>
<td>n.d.</td>
<td>0.04 ± 0.000</td>
<td>1536</td>
</tr>
<tr>
<td>Echinacea angustifolia®</td>
<td>0.368</td>
<td>n.d.</td>
<td>826 ± 11</td>
<td>15.70 ± 0.13</td>
<td>44.55±2.98</td>
<td>76.22 ± 3.81</td>
<td>1535</td>
</tr>
</tbody>
</table>

Only methanol extracts were analyzed

Expressed as: (a) ppm, (b) gallic acid milliequivalents/g dry weight, (c) quercetin milliequivalents/g dry weight, (d) trolox millimolar equivalents/ gm dry weight, (e) toxicity was expressed as lethal dose fifty DL₅₀ (ppm, mg/mL). n.d = not detected
Conclusion

This work presents the analysis of 10 plants used in the Sonora Market; 5 of them have been previously studied for their antiglycemic properties and the other 5 selected species have been related to diverse pharmacological properties. It is noteworthy that *Swietenia humilis* Zucc. “Semilla de zopilote” has extensive medicinal uses, as ethno botanical data indicate, but there are no pharmacological studies that validate them and therefore, it becomes an interesting species to study in order to find its active compounds or to develop the standardization process to obtain an herbal medicine. In the present study, it was shown that *Swietenia humilis* “Semilla de zopilote” has a moderate activity in the *Artemia salina* model and its antioxidant capacity was lower than that of *Echinacea angustifolia*.

None of the studied plants have a chemical and pharmacological profile that leads us to a standardize treatment, for this reason; the standardization procedure proposed by our research group in 2014 was applied. Main chemical components, antioxidant capacity, as well as the chemical UV spectrum of the analyzed plants are reported. These data will contribute to the knowledge required for their standardization to make possible their incorporation as herbal treatments in the official medicine.

Acknowledgement

This work was partially financed by Universidad Autónoma Metropolitana-Iztapalapa, Universidad Autónoma de la Ciudad de México and SECITI. We also thank CONACYT (No. 248821) for financial support to Rayn Clarenc Aarland during his Ph.D. studies (PNP Experimental Biology Program). This work is part of R. Clarenc Aarland PhD (UAM-I) dissertation and Cesar Morales Sánchez and Francisco Parra-Bustamante LPS (UACM) dissertations.

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