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Pharmacognostical studies of the plant drug *Mimosae tenuiflorae cortex*

E. Rivera-Arce [a,c], M. Gattuso [b], R. Alvarado [a], E. Zárate [a], J. Agüero [a], I. Feria [a], X. Lozoya [a,*]

[a] *Laboratory of Research and Technical Development of Phytodrugs, National Medical Center Siglo XXI, Mexican Institute of Social Security (IMSS), Av. Cuauhtémoc Av. # 330, Col. Doctores, 06720 México, D.F., Mexico*
[b] *Faculty of Biochemical and Pharmaceutical Sciences, National University of Rosario, Suipacha 531/2000 Rosario, Argentina*
[c] *Ph.D. Program in Biological Sciences, Autonomous Metropolitan University (UAM), Mexico City, Mexico*

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Abstract

The bark of the *Mimosa tenuiflora* (Willd.) Poiret (Leguminoseae) tree, known as *tepescohuite* in Mexico, is commonly used in this country and in Central America to elaborate different products for the treatment of skin burns and lesions. The cicatrizing properties of extracts obtained from this bark have been scientifically studied, attributing the main biological activity to its tannin and saponin content. Studies include clinical trials of phytodrugs based on *Mimosae tenuiflora* bark extracts for treatment of venous leg ulcerations. Recent commercialization of the plant drug *Mimosae tenuiflorae cortex* requires pharmacognostical information to develop quality-control methods for raw materials and extracts produced with this plant drug. The present paper reports a group of ethnobotanical, morphological, chemical, and molecular studies performed with *Mimosae tenuiflora* materials obtained by collection in the southeastern Mexican state of Chiapas. Macro- and micro-morphological parameters were established to authenticate the genuine drug that allowed detection of adulterants usually found in commercial samples of this plant material. These morphological characteristics can be used for rapid identification of the drug and are particularly useful in the case of powdered materials. The chemical studies performed demonstrated that tannins represent the major component group in the bark. Its content in genuine *tepescohuite* is 16% and is mainly composed of proanthocyanidins, a condition permitting a tannin-based chemical-control method for fingerprinting the plant drug. Contrariwise, the saponin concentration in *Mimosae tenuiflora* bark is extremely low, and its isolation and content evaluation represent a complex procedure that is unsuitable for routine control purposes. Finally, random amplified DNA (RAPD) analysis results a useful tool for obtaining DNA specific markers of *Mimosae tenuiflora* species which should be useful in future studies involving raw material authentication by molecular methods.

Keywords: *Mimosa tenuiflora*; Ethnobotany; Pharmacognosy; Plant drug raw materials

1. Introduction

Mimosa tenuiflora (Willd.) Poiret (Leguminoseae) is a tree known in Mexico under the popular name in Spanish of *tepescohuite*. The plant is distributed in areas of tropical deciduous forests in America, from the southeastern regions of Mexico to northern Brazil and Venezuela, growing as secondary opportunistic vegetation (Camargo, 2000). According to Mexican ethnobotanic sources, the bark of this plant – once dried, powdered, and directly applied to the lesion – is an effective remedy for treating skin burns and wounds (Camargo-Ricalde et al., 1994; Grether, 1988). In Mexico in 1984, this natural resource was utilized empirically to alleviate the sufferings of hundreds of victims of large natural-gas depot explosion; on that occasion, direct application of powdered *Mimosae tenuiflora* bark on patients' burns resulted in facilitation of skin regeneration and prevention of scarring in many of the patients. Subsequently, news of the existence of a miraculous Mexican tree skin was spread worldwide by the mass media, producing a rise in spotlighting commercial attention on this natural product and included the elaboration of several products with supposed medicinal properties (Lozoya et al., 1989).

During the 1990s pharmacological and phytochemical studies performed by Mexican research groups supported the existence of natural compounds with cicatrizing properties in *Mimosae tenuiflora* cortex. A series of pre-clinical experimental studies concluded that water and alcoholic extracts obtained from the dried bark of *Mimosae tenuiflora* are particularly rich in tannins and that these also contain steroidal saponins. The

* Corresponding author at: Estrecho 35, Prolongación Las Aguilas, 01759 México, D.F., Mexico.
E-mail address: 4509@igo.com.mx (X. Lozoya).

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biological activity of these extracts was defined as (a) possessing strong *in vitro* antimicrobial properties against a wide group of microorganisms, yeasts, and dermatophytes and (b) inducing the growth *in vitro* of fibroblasts and other human cells (Lozoya et al., 1989; Meckes et al., 1990a,b; Villarreal et al., 1991). Other studies, performed by French groups allowed for identification in the extracts of triterpenoidal saponins, designated as mimonosides A, B, and C, which according to *in vitro* observations induce proliferation of cultured human cells, possess an immunomodulation capacity, and were therefore attributed – at least in part – to extracts' potential cicatrizing properties (Jiang et al., 1991a,b; Antón et al., 1993).

Recently, clinical studies performed at Mexican Institute of Social Security (IMSS) hospitals with a standardized tannin content extract obtained from *Mimosae tenuiflora* bark showed excellent therapeutic properties on being applied in the treatment of skin venous leg ulcerations (Rivera-Arce et al., 2007).

Due to its efficacious application in the treatment of burns, wounds, and chronic skin lesions, the elaboration of diverse products from extracts obtained from *Mimosae tenuiflora* bark revealed the need of a formal scientific reference monograph on the identifying characteristics of the plant drug, *Mimosae tenuiflorae cortex*. Moreover, the increasing commercial interest in this plant drug and the lack of reference materials have stimulated the appearance in the herbal markets of Mexico of bark adulterants sold for the same purposes described popularly for *tepescohuite*. In the last years, quality-control procedures for the original plant drug are also demanded by regulatory authorities.

The present paper reports some pharmacognosy studies performed to establish morphological and chemical parameters to facilitate authentication of this plant drug.

2. Materials and methods

2.1. Ethnobotanical studies

Ethnobotanical studies were performed in the state of Chiapas in Mexico in several rural localities where this medicinal plant drug is obtained, and also at the National Medicinal Plants Market (also known as the Sonora Market) in Mexico City, where commercialization of the bark frequently occurs. Studies were performed following the methodology described by Cotton (1996) that includes semi-structured and open surveys, with the active participation of local bark collectors and vendors. Information was also obtained concerning main collection areas for *tepescohuite* and behavior of prices of the bark throughout its commercialization. In every case, vouchers were deposited at the IMSS Medicinal Plants Herbarium (IMSSM) and at the National Mexican Herbarium (MEXU) for references.

2.2. Morphological studies

Morphological studies of the plant drug were performed at macro- and microscopic levels. For macroscopic studies, dried *tepescohuite* bark fragments and several of its adulterants were employed. Characterization of the materials with respect to shape, the aspect of the internal surface, and types of bark fractures were performed according to the reference guides of Oliveira et al. (1991). For microscopic studies, fresh formaldehyde–acetic acid and alcohol (FAA) preserved materials were used. Dried materials from the herbarium were also employed for comparative studies. The materials were softened and hydrated with water and detergent and, if necessary, boiled slightly. Free hand sections were obtained and rendered transparent according to De Strittmatter (1973). Fast-green and diluted safranine was used for sample staining according to Johansen (1940). Definitive preparations for microscopy studies were mounted on Canada Balsam and rendered transparent with gelatin–glycerin.

2.3. Phytochemical studies

Phytochemical studies included thin-layer chromatography (TLC) profiles of extracts obtained by refluxing powdered barks in ethanol. Chromatograms were carried out on silica gel plates with two different mobile phases: $CHCl_3$/AcOH/CH_3OH/H_2O (32:16:6:4), and butanol, water and acetic acid (BAW) (50:10:4), visualized by spraying these with a mixture of anisaldehyde/sulfuric acid. Tannin content in bark water extracts was assessed by Folin–Denis method according to the methodology described in the Mexican Herbal Pharmacopeia (2001) against tannic acid as standard; absorbance was read on a spectrophotometer Perkin-Elmer MBA 2000. We added stoichiometric volumes of Folin–Denis reagent and Na_2CO_3 (35%, w/v) in 1-ml aliquots of the extract. After a 30-min reaction, absorbance was read at 700 nm. In an attempt to further correlate chemical composition with certain structures, the raw bark extract was fractionated by liquid–liquid partitioning and by chromatography on silica gel and Sephadex LH-20.

2.4. Genetic characterization

In an attempt to obtain DNA from *Mimosae tenuiflora*, RAPD markers for its discrimination among other species were used. Disease-free young leaves were washed in 70% ethanol and distilled water. After drying, the leaves were stored at −70 °C until use. Each 150-mg sample was cut into pieces and ground into powder with mortar and pestle. DNA was extracted from the sample according to Khanuja et al. (1999). The purity of the extracted DNA was determined by ultraviolet (UV) absorption. The DNA solutions were adjusted to a final 10 ng/μl concentration and used in RAPD reactions. Four nucleotide primers (10-mer) for RAPD analysis were purchased from Invitrogene (Carlsbad, CA, USA). DNA template (6 ng) was amplified in a 10-μl polymerase chain reaction (PCR) mixture consisting of 1× PCR buffer, 3 mM $MgCl_2$, 0.3 mM dNTP, 0.3 pmol primer, and 0.5 U Taq polymerase. DNA amplification was performed using a gradient thermocycler programmed for one 5-min step at 94 °C followed by forty-five 1-min cycles at 94 °C, 1 min at 36 °C, 2 min at 72 °C, and ending with 10 min at 72 °C. RAPD fragments were separated on 2% agarose gel by electrophoresis in 1× TAE buffer and stained with ethidium bromide. Amplified fragments were digitalized in a Bioimaging System and analyzed with the LabWorks Version 1.1.27 (UVP, Cambridge,

Table 1
Decamer primers used for RAPD amplification

Name	Sequence
A	5′-d[GGTGCGGGAA]-3′
B	5′-d[GTAGACCCGT]-3′
C	5′-d[CAATCGCCGT]-3′
D	5′-d[TCGGCGATAG]-3′

UK) software. RAPD bands were scored as 0 (absent) and 1 (present), and similarity index (SI) was calculated according to Lych (1990). For individuals x and y, where SI is the number of common fragments in the fingerprint profile (n_{xy}) were divided by the average number of fragments exhibited by both individuals and represented with the following formula: $SI = 2n_{xy}/n_x + n_y$, where n_{xy} is the number of bands shared between two samples, and $n_x + n_y$ is the total number of bands (Table 1).

3. Results

3.1. Ethnobotany

At the areas visited in southeastern Mexico, *Mimosae tenuiflorae cortex* is collected mainly from wild populations of live trees, nearly through the complete seasonal year with the exception of 2-month rainy season. In addition, collection is performed from fences constructed with the tree trunks of this plant. As a rule, *Mimosae tenuiflora* bark collection is a complementary activity in rural familial economy and it is carried out mainly by owners of a land used for other agricultural purposes. Nonetheless, due to the large demand for the product, collectors obtain already dried bark materials from the tree-trunk fences. Fresh bark obtained from trees is cut from the trunk in small, not always avoiding complete decortications and subsequent death of the plant. The strips are dried in a flat clay dish over a fire and later are chopped or torn into pieces with the aid of an ax or a hand mill, respectively (Fig. 1).

The majority of *Mimosa tenuiflora* bark traded in Mexico is provided from trees growing in the State of Chiapas (mainly from the Cintalapa and Jiquipilas regions). The commercial chain of the plant drug begins when land-owners of the trees sell the dried bark to local intermediaries who in turn sell it at the local market or directly to domestic medicinal plant-distributor companies. Afterwards, the latter introduce the cortex into the wholesale area of the National Herbal Market in Mexico City. Consequently, the price of the raw material grows disproportionately during each step of this commercial chain. The price paid to the producer by the gatherer is 0.15 USD dollars per kilo

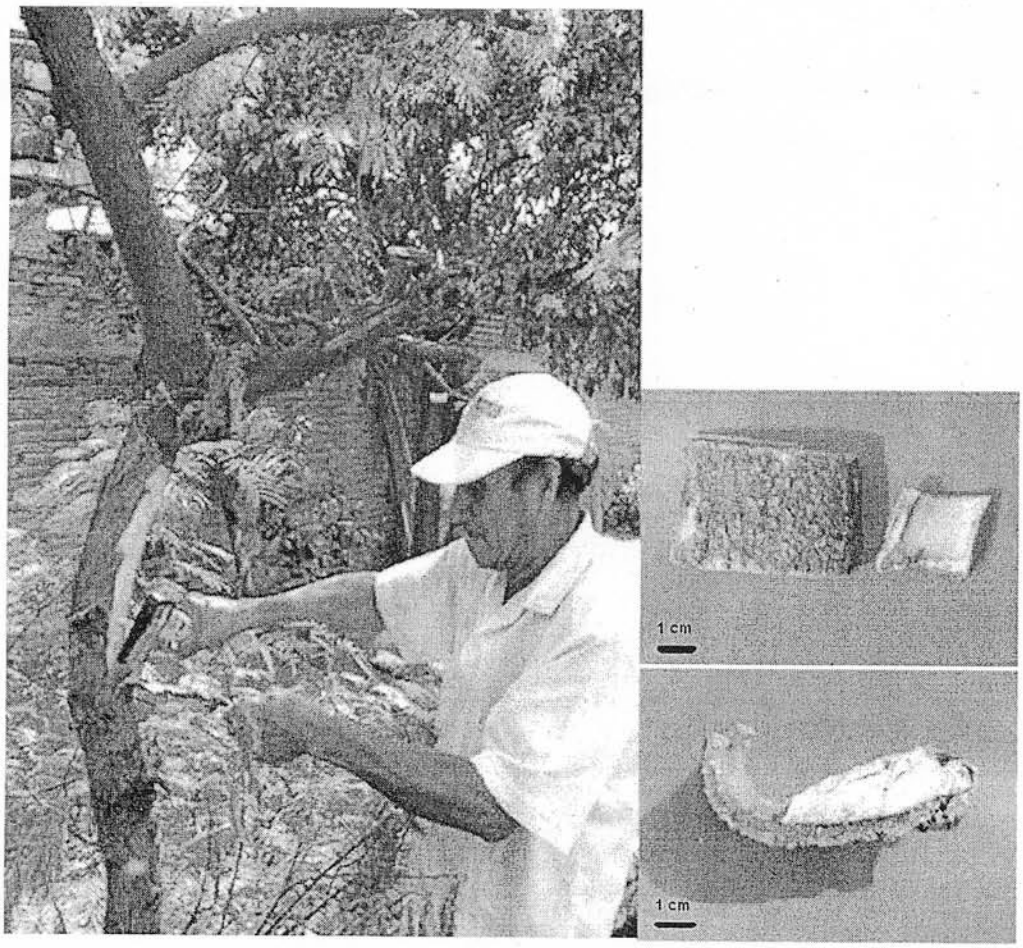

Fig. 1. Obtaining of *Mimosae tenuiflorae cortex* and aspect of the fragments of dried drug before packing.

of dried drug fragments of the cortex; the local gatherer obtains 0.60 dollars from the market intermediary and this receives 4.00 dollars for the same kilo from the consumer in the herbal market. Finally, the industry pays to the intermediary 100.00 USD dollars per kilo of powdered drug together with a certificate of quality that guarantees raw material authenticity.

3.2. The plant

The plant is taxonomically characterized by its leaves, which contain 5–10 pairs of pinnules and 10–30 pairs of linear leaflets that are 3–6-mm long and 0.72 mm wide. Its flowers are white and are grouped in thick spikes, 3–6.5-cm in length. The fruit is lanceolate, unarmed, compressed between the seeds, 2–4.5 cm in length and 5–7 mm wide, with two to six segments. Young branches, leaves, and ripe fruits contain trichomes and other glandular elements (Grether, 1988).

3.3. The plant drug

3.3.1. Denomination—Mimosae tenuiflorae cortex

3.3.1.1. Definition. The drug is composed of fragments of dried cortex of *Mimosa tenuiflora* (Willd.) Poiret (Leguminoseae), and is also the product of grinding it as a fine-textured powder, red-brown in color, opaque, and with a characteristic odor.

3.3.2. Macroscopic description—morphological characteristics

The drug comprises the bark obtained by decorticating the main stem and lateral branches in pieces or curved fragments that are 10–15-cm long, 5–7-cm wide, and 6–7-mm thick. The outer surface is brown in color and is cracked lengthwise into fissures separated by ridges (deep-fissured bark). The inner surface is fine, smooth, and sparking, red-brown in color, and fracture-laminated. The bark is odorless and possesses a slightly bitter astringent flavor (Fig. 1).

3.3.3. Microscopic description—anatomic characteristics

3.3.3.1. Outer bark. An imbricate rhytidome is present that is made up of three to five periderm, and collapsed phloem (Figs. 2A, f and s). In the periderm, the phellogen has a continuity-type shape, with phellem layers oriented toward the outside, while the phelloderm layers are oriented toward the inside. Phellogen cells are rectangular and flattened, appearing radial in the cross-section. The phellem is made up of two to five or more layers of cells in a clear radial position, but tangentially elongated due to the stress of secondary growth and largely filled with a yellow colored content. The phelloderm is composed of two to three layers of rectangular cells. The phloem is characterized by its structural disarray caused by sieve-cell collapse, parenchyma-cell expansion, and distortion of the rays (Figs 2A, f, s and 3B).

3.3.3.2. Inner bark. The cross-section enabled distinction of non-collapsed phloem containing sieve tubes, axial parenchyma cells, and rays. Sieve tubes occurred mainly in clusters of 3–10 sieve elements manifesting a polygonal shape with thin and unlignified cell walls (Figs. 2B, C and F). The phloem rays follow a less-straight course in the non-collapsed phloem. Some rays develop a funnel-shaped dilatation through tangential cell divisions. The axial parenchyma is filled with tannins (Fig. 2C).

Both the outer and inner bark contain tangential bands or clusters of sclerenchyma, which are composed of groups of isodiametric or tangentially elongated sclereids with thickened and pitted walls; they also contain groups of soft fibers, partly or wholly sheathed by a single row of short, thick-walled crystalliferous cells. The crystals are usually solitary rhombohedra (Figs. 3B, e; E and F).

3.3.3.3. Macerated bark. Analysis of the material yielded the following constitutive elements:

- *Phellem cells.* In the superficial view, these exhibit isodiametrical-mingled aspect with thick walls measuring 30 μm × 36 μm.
- *Secondary rays.* These are composed of procumbent-type cells. The height of the rays ranges from 3 to 12 cells in length and 2 to 4 cells in width (Fig. 2B).
- *Phloem fibers.* These are located in the outer and inner bark, and measure 800–1000 μm (Fig. 3F).
- *Sclereids.* These comprise brachysclereids measuring 30 μm × 32 μm and macrosclereids measuring 70 μm × 28 μm (Fig. 3E).

3.3.4. Powdered plant material

The powdered drug is red-brown in color and consists of a group of brachysclereids with pitted and thickened walls and in addition, there are numerous groups of fibers without lignin sheathed by a single row of short, thick-walled, crystalliferous cells. The crystals are usually solitary rhombohedra. Cork cells are polygonal, with a yellow content.

3.3.5. Adulterants

Bark adulterants have been detected in materials obtained from local collectors. Among these the following species were identified: *Mimosa arenosa* (Willd.) Poiret. (Leguminoseae); *Acacia pennatula* (Schltdl. & Cham.) Benth. (Leguminoseae); *Byrsonima crassifolia* (L.) Kunth. (Malpighiaceae); *Luehea candida* (Moc. & Sessé ex DC.) Mart. (Tiliaceae) and *Guazuma ulmifolia* Lam. (Sterculiaceae) (Table 2).

3.4. Chemical parameters

Results of TLC analysis are shown as R_f values for tannins, saponins and phenols, typical of genuine *Mimosae tenuiflora* bark in Table 3. Quantification of tannins using a linear tannic acid calibration graph for the analyzed concentration range (correlation coefficient of 0.994) showed that tannin content in all genuine bark samples was no less than 16%. Comparatively, tannins present in adulterants are different and in much higher concentrations (data not shown). As a result of the isolation procedures performed, one fraction (Fr. 7A) of the *Mimosae tenuiflora* extract was characterized by spectroscopic methods (nuclear magnetic resonance spectrometry, mass spectrometry,

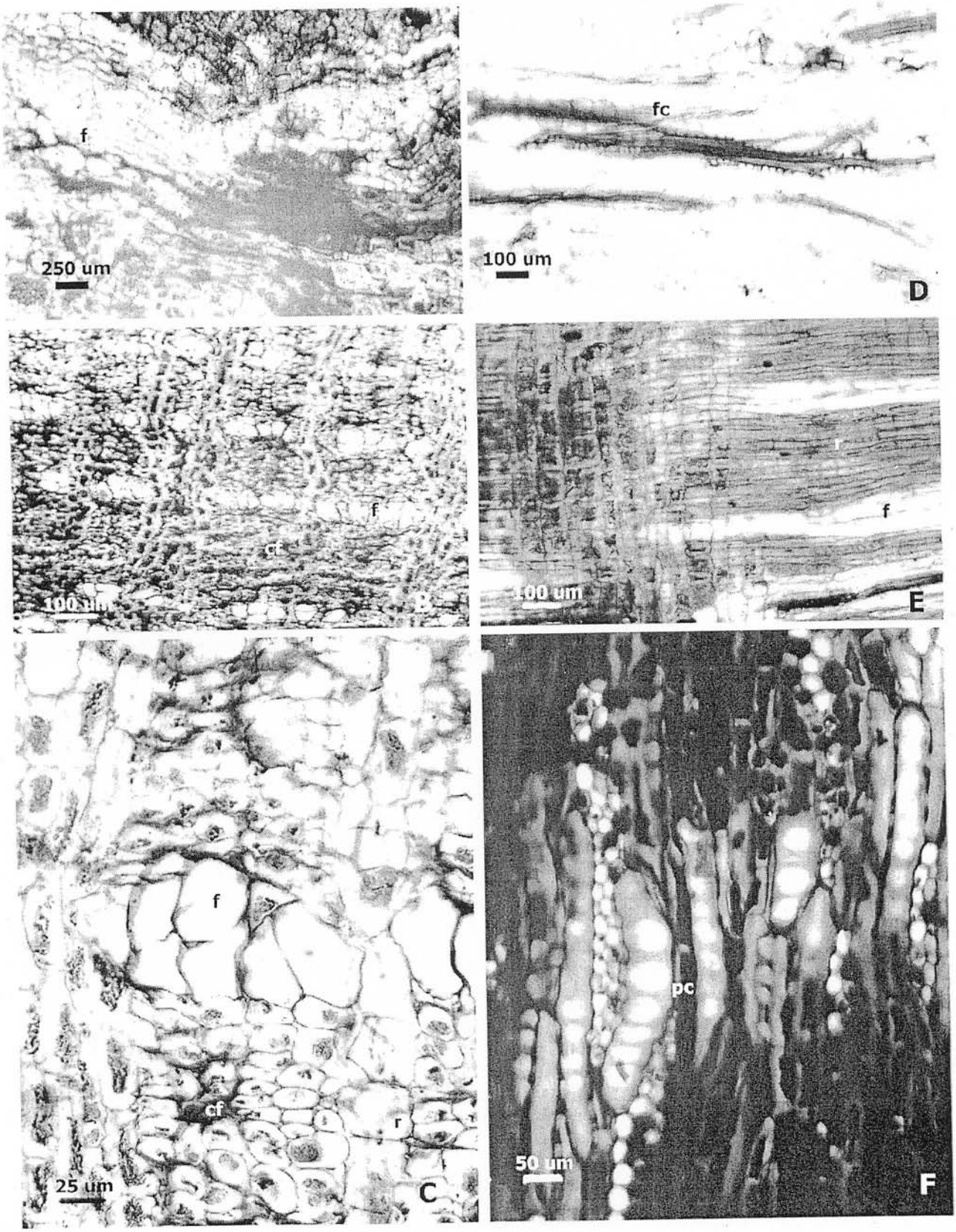

Fig. 2. Microphotography of *Mimosa tenuiflora* bark. A–F: cortex; A and D: cross-sections; A: external cortex; B: internal cortex; C: internal cortex (detail); D: external cortex showing collapsed phloem; E: longitudinal section, showing radial parenchyma; F: longitudinal section showing composed sieve plates. cf: parenchymatic cells of phloem containing tannins; f: phloem; fc: collapsed phloema; pc: composed sieve plates; r: parenchymatic ray; s: suber.

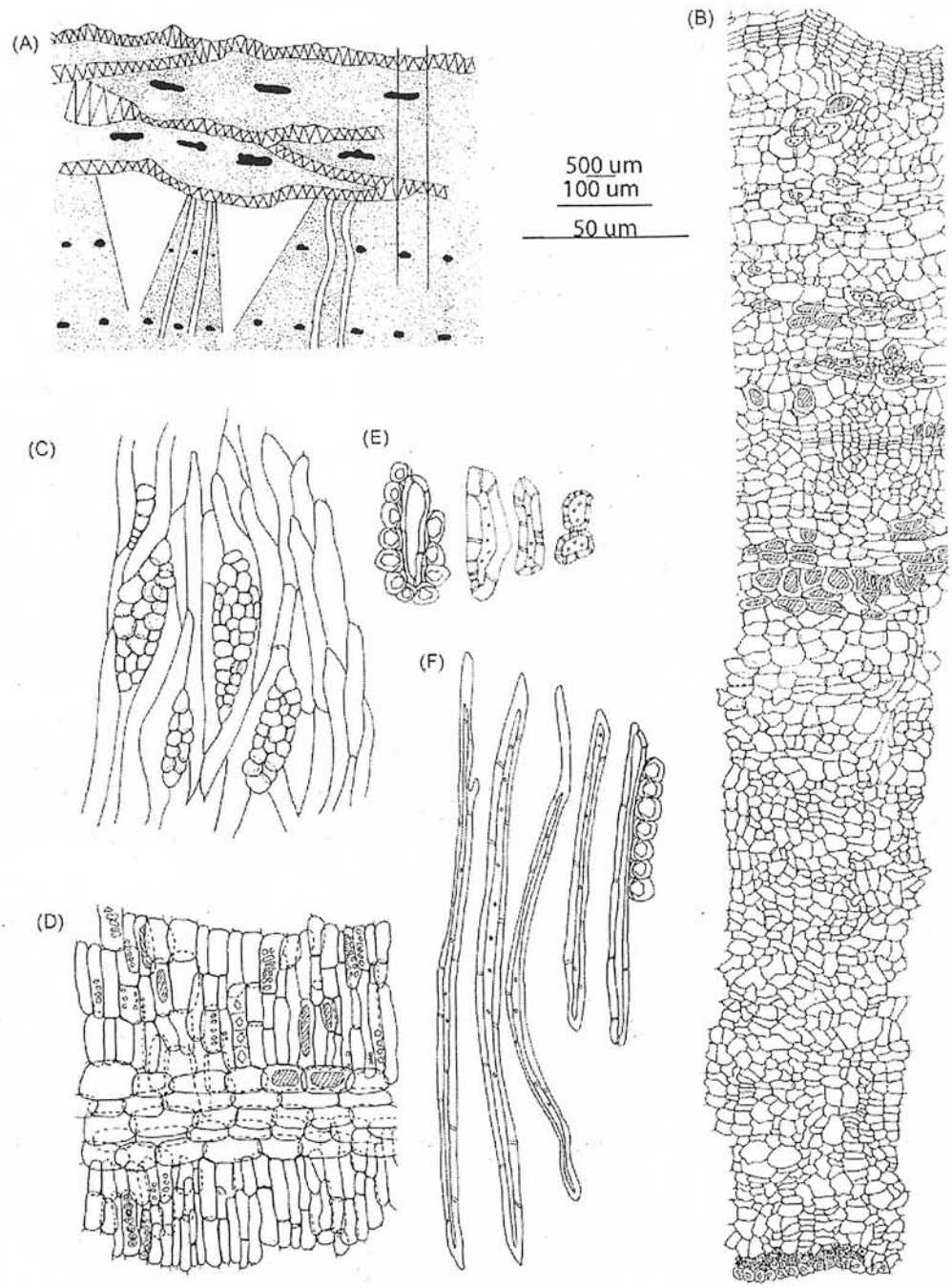

Fig. 3. Analytic micrograph of *Mimosa tenuiflora* bark. A: cross-section (schema); B: same as in A (detail); C: longitudinal radial section; D: longitudinal tangential section. E–F: powdered drug disociated, (E) sclereids, (F) fibers. Linemarks correspondence—1: A; 2: B; 3: C–F.

and infrared spectroscopy), showing that the extract was mainly composed of oligomeric condensed tannins (proanthocyanidins) and accounted for approximately 75% of the extract's total chromatographic peaks. On the other hand, a second fraction (Fr. D) containing major saponin component was also isolated and characterized by high performance liquid chromatography (HPLC), matching the retention time of the major peaks with those of the mimonosides A, B, and C standards. Total saponin concentration in the whole extract was ca. 0.15% (being 0.10% for mimonoside A; 0.032% for mimonoside B; and 0.016% for mimonoside C).

3.5. Genetic characterization

DNA obtained from fresh *Mimosae tenuiflora* leaves presented sufficient integrity for use in random amplified polymorphic DNA (RAPD) markers. The four decamer primers employed generated a total of 73 clear and visible RAPD fragments. Among these fragments, only 47 were specific for the species studied, and were differentiated by molecular weight. When compared with close species used as adulterants, 18 fragments of the total were specific for *Mimosae tenuiflora*, 18 for

Table 2
Comparative morphological characters of the original drug with some common adulterants of *Mimosae tenuiflorae* cortex

Species	Drug form	Color (external surface)	External surface	Internal surface	Fracture
Mimosa tenuiflora	Curved	Dark brown to red-brown	Longitudinal striae delimit small and deep fissures	Fine, smooth and sparking, red-brown colored	Clean
Mimosa arenosa	Curved	Chestnut-brown	Striated (longitudinal) with squamous rhytidome and abundant transversalloy elongated lenticels	Fibrous, deep brown-colored	Fibrous (long fibers)
Byrsonimia crassiflora	Curved	Ash greyish-brown	Curled striated (transversal and longitudinal) of variable deep and resinous	Very fibrous, refringent, red-brown-colored	Fibrous (short fibers)
Acacia pennatula	Flat	Rusty brown	Fissured (longitudinal and transversal) forming irregular plaques	Rusty and very fibrous, forming a characteristic teristic weft; resinous, with a refringent cinnabar color	Very fibrous (long fibers)
Luehea candida	Curved	Whitish bright brown	Rhytidome formed by easily detached plaques produciing a smooth surface	Refringent, hepatic brown-colored with transversal whitish striae	Very fibrous (long fibers)
Guazuma ulmifolia	Curved	Umber brown	Irregular surface with deep longitudinal fissures forming transversal plaques	Striated and resinous, with refringent, marbled, hepatic color	Fibrous (long fibers)

Table 3
R_f's for tannins in *Mimosae tenuiflorae* cortex

BAW	$CHCL_3$/AcOH/CH_3OH/H_2O
0.14	0.03
0.20	0.08
0.29	0.17
0.42	0.21
	0.23
	0.30
	0.52

Mimosa arenosa, and 11 for *Acacia pennatula*. Analysis of similar bands between these close species showed that primer A generated two similar RAPD fragments, primer B generated three, primer C generated five, and primer D only one, corresponding to 35% of all the amplified fragments. During analysis of all bands, *Mimosae tenuiflora* showed a group of seven similar bands as well as *Mimosa arenosa*, while *Acacia pennatula* showed a four-band group. Only primer D generated distinguishable characteristic band patterns different from the closely related species. Application of the similarity index (SI) in all band patterns demonstrated that *Mimosa arenosa* and *Acacia pennatula* had an SI of 0.30, while *Mimosae tenuiflora* and *Acacia pennatula* showed an SI of 0.22 and *Mimosae tenuiflora* and *Mimosa arenosa* an SI of 0.26. When statistical correlation analysis was applied to SI values of fragments generated with each decamer primer used, only primer D exhibited statistical significance ($p = 0.1$) among the three species. (Fig. 4)

4. Discussion

Ethnobotanical studies that have been conducted have shown that in Mexico, acquiring *Mimosa tenuiflorae cortex* is a profitable business for a group of commercial intermediaries, who obtain the plant drug from producers who in turn ignore procedures and strategies for the conservation of this natural resource. Materials obtained from fences are usually contaminated. Plant materials are obtained from non-cultivated tree populations that are permanently overexploited and at risk of rapid extinction; this situation elicits the utilization by these individual of the cortex of other plant species that are powdered and then mixed with the authentic species, resulting in a commercialized "*tepescohuite*" that is usually adulterated. There are studies published some time ago in which the propagation of this species by biotechnological procedures was suggested (Villarreal et al., 1993; Villarreal and Rojas, 1996). Intensive propagation and protection programs financed by the interested phytodrug industries are required to guarantee the commercial future of this plant drug at the long term.

The results obtained in morphological studies reported herein established the macro- and microscopic parameters that characterize the genuine plant drug *Mimosae tenuiflorae cortex*. These morphological characteristics can be utilized for quick identification of the drug and are particularly useful in the case of powdered materials. The chemical studies performed showed that tannins represent the major component group in the bark. Its content in genuine *tepescohuite* is ca. 16% and is principally composed of proanthocyanidins, a condition allowing for a tannin-based chemical-control method for fingerprinting the plant drug. Contrariwise, saponin concentration in the bark is extremely low and its isolation and content evaluation represent a complex procedure that is unsuitable for routine control purposes. Finally, the RAPD analysis utilized herein results in a useful tool to obtain DNA specific markers for *Mimosae tenuiflora* species that should be useful in future studies for authentication of raw materials by molecular methods.

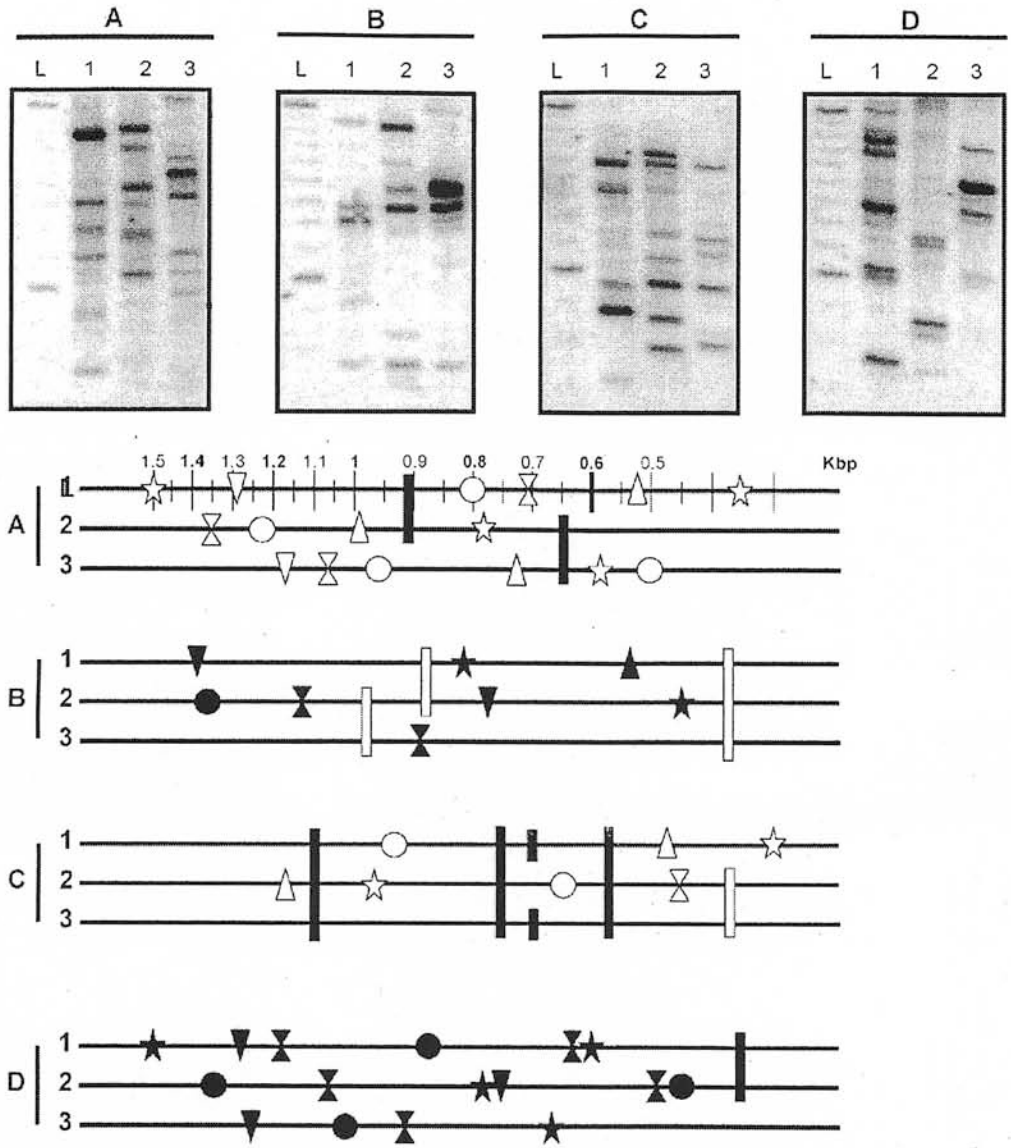

Fig. 4. DNA patterns and schematic representations of RAPD electrophoretic fragments of *Mimosa tenuiflora* and adulterants using the primers A–D. Each band between 0.3 and 1.5 kbp is represented by a symbol. The numbers in the gel and scheme (1–3) represent the samples used for amplification. L: 100 bp ladder, 1: *Mimosae tenuiflora*, 2: *Mimosa arenosa*, 3: *Acacia pennatula*.

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References

Antón, R., Jiang, Y., Weniger, B., Beck, J.P., Rivier, L., 1993. Pharmacognosy of *Mimosa tenuiflora* (Willd.) Poiret. Journal of Ethnopharmacology 338, 153–157.

Camargo-Ricalde, S., Grether, R., Martínez-Bernal, A., 1994. Uso medicinal del "tepescohuite", *Mimosa tenuiflora* (Leguminoseae) en México. Contacto 5, 29–34.

Camargo, S., 2000. Descripción, distribución, anatomía, composición química y usos de *Mimosa tenuiflora* (Fabaceae–Mimosoideae) en México. Revista de Biología Tropical 48, 939–954.

Cotton, C.M., 1996. Ethnobotany: Principles and Applications. John Wiley and Sons, Ltd., Chichester, UK, p. 140.

De Strittmatter, D., 1973. Nueva técnica de diafanización. Boletín de la Sociedad Argentina de Botánica 15, 126–129.

Farmacopea Herbolaria de los Estados Unidos Mexicanos, 2001. Secretaría de Salud, México. México, p. 22.

Grether, R., 1988. Nota sobre la identidad del tepescohuite en México. Boletín de la Sociedad Botánica de México 48, 151–152.

Jiang, Y., Massiot, G., Lavaud, C., Teulon, J.M., Guechot, C., Haag-Berrurier, M., Antón, R., 1991a. Triterpenoid glycosides from the bark of *Mimosa tenuiflora*. Phytochemistry 30, 2357–2360.

Jiang, Y., Haag-Berrurrier, M., Antón, R., 1991b. Structure of a new saponin from the bark of *Mimosa tenuiflora*. Journal of Natural Products 54, 1247–1253.

Johansen, D.A., 1940. Plant Microtechnique. McGraw-Hill, New York, p. 125.

Khanuja, S.P.S., Shasany, A.K., Darokar, M.P., Kumar, S., 1999. Rapid isolation of DNA from dry and fresh samples of plants producing large amounts of secondary metabolites and essential oils. Plant Molecular Biology Reporter 17, 1–7.

Lozoya, X., Navarro, V., Arnason, J.T., Kourany, E., 1989. Experimental evaluation of *Mimosa tenuiflora* (Willd.) Poiret (tepescohuite). I. Screening of the antimicrobial properties of bark extracts. Archivos de Investigación Médica 20, 87–93.

Lych, M., 1990. The similarity index and DNA fingerprinting. Molecular Biology and Evolution 7, 478–484.

Meckes, M., Lozoya, X., González, J.L., 1990a. Propiedades farmacológicas *in vitro* de algunos extractos de *Mimosa tenuiflora* (tepescohuite). Archivos de Investigación Médica 21, 163–170.

Meckes, M., Lozoya, X., González, J.L., Martinez, M., 1990b. Efecto producido por la fracción de alcaloides de *Mimosa tenuiflora* (tepescohuite) sobre el reflejo peristáltico del ileon del cobayo. Archivos de Investigación Médica 21, 171–178.

Oliveira, F., Akisue, G., Akisue, M.K., 1991. Farmacognosia. Atheneum, Sao Paulo, Brasil, p. 237.

Rivera-Arce, E., Chávez-Soto, M.A., Herrera-Arellano, A., Arzáte, S., Agüero, J., Feria-Romero, I.A., Cruz-Guzmán, A., Lozoya, X., 2007. Therapeutic effectiveness of a *Mimosa tenuiflora* cortex extract in venous leg ulceration treatment. Journal of Ethnopharmacology 109, 523–528.

Villarreal, M.L., Nicasio, P., Alonso-Cortes, D., 1991. Effects of *Mimosa tenuiflora* bark extracts on W138 and KB human cells in culture. Archivos de Investigación Médica 22, 163–169.

Villarreal, M.L., Rojas, G., Meckes, M., Nicasio, P., 1993. Studies on *Mimosa tenuiflora* callus culture. Interaction of kinetin and 2,4,dichlorophenoxy acetic acid in initiation and growth. Biotechnology Letters 15, 271.

Villarreal, M.L., Rojas, G., 1996. *In vitro* propagation of *Mimosa tenuiflora* (Willd.) Poiret, a Mexican medicinal tree. Plant Cell Reports 16, 80–82.