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Ethnobotanical, pharmacognostical, pharmacological and phytochemical studies on *Smilax domingensis* in Guatemala

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Abstract: *Smilax domingensis* Willd., Smilacaceae, known as zarzaparrilla, is a climbing shrub from Tropical America. The rhizome is popularly used in medicine as anti-inflammatory, antiseptic, and tonic. Since 1983 studies are being conducted in Guatemala for validation of the ethnobotanical uses, particularly *in vitro* demonstration of antimicrobial activity, using wild material, with high variability and some taxonomic problems. This article reports the taxonomic determination, cultivation of drug material, evaluation of use by *in vitro* and *in vivo* pharmacological assays, and phytochemical characterization. Extracts from cultivated material was evaluated by antimicrobial, anti-inflammatory, analgesic and immunomodulatory models, confirming the antimicrobial and immunomodulatory activities. Phytochemistry was done in the crude drug and extracts. Quality control parameters are described (micrographic drawings and phytochemical characteristics). Evidence is presented that cultivated rhizome has antimicrobial and immunomodulatory activities, validating the popular use and helping the industrial development of phytopharmaceutical products.

Article

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Introduction

Smilacaceae is a family of climbing shrubs represented by the single genus Smilax with close to 250 species worldwide, present with 26 species in Mesoamerica (Huft, 1994). Widely used since ancient times, the main species reported are *Smilax aristolochiaefolia* Mill., *S. febrifuga* Kunth, *S. ornata* Hook, and *S. regelii* Killip & Morton, characterized by roots and small rhizomes used as antiseptic and anti-pruritic drug (British Herbal Pharmacopoeia, 1983). *Smilax domingensis* Willd. is native from Tropical America, growing in lowlands, in humid forests of wide-leaved species (Standley & Steyermark, 1952). Although widely used, there are several taxonomic difficulties.

In traditional medicine, rhizomes are used as anti-inflammatory, antifungal, anti-pruritic, antiseptic, healing, diuretic and tonic (Cáceres, 2009). The main components found and shared by most species of the genus are the steroidal saponins, phytosterols, and triterpenoids (British Herbal Pharmacopoeia, 1983). The antimicrobial and anti-tumoral activities are attributed to parillin (Bérdy et al., 1982). Some pharmaceutical forms are available, such as infusion, tincture, elixir, lotion, and micropulverized powder (Cáceres, 2009).

After several ethnobotanical surveys conducted in Guatemala, it is evident that the most prevailing specie used is *S. domingensis*, which has a massive colored woody rhizome and show several differences in the botany, pharmacology and phytochemistry. The aim of this article is to review the specific literature and publish studies conducted in Guatemala for its scientific validation, the micrographical and phytochemical characteristics for quality control, and the results of agrotechnological and phytopharmaceutical developments for the standardization as drug.

Materials and methods

Botanical studies and distribution

Synonyms: *Smilax caudata* Lundell, *S. engleriana* Apt., *S. lanceolata* L., *S. microscola* (B.L. Robinson) Killip et C. Morton, *S. pseudo-china* A. Rich For Guatemala, Standley & Steyermark (1952) described thirteen species and twelve synonyms, where it is widely dispersed and often abundant; they indicated that *Smilax domingensis* Willd., Smilacaceae, is a synonym of *S. lanceolata*, while for Mesoamerica Huft (1994) described

25 species and suggested that at least eight species could be reduced to four after detailed analysis. After ten years collecting materials from Guatemala, Martínez & Cáceres (2001) confirmed thirteen species and proposed the botanical characteristics for its determination in the country.

S. domingensis is native from Mexico to Panama and the Antilles, it has been collected from 0-2000 masl; it grows on most soils over nearly all types of tutor tree in areas with yearly precipitation from 1400-3500 mm (Stevens et al., 2001).

Seven-year rhizomes from cultivated vines were collected in Ecoparcela El Kakawatal, Samayac, Suchitepéquez, Guatemala (14°33'37" N, 91°28'15" W, 450 masl) and voucher specimens deposited at Farmaya Laboratories herbarium (CFEH#662).

Ethnobotanical, ethnopharmacological and morphological studies

The most common name in Guatemala and the region is zarzaparrilla, but it is also known as cuculmeca, bejuco de canasta (Costa Rica), chiquihuite (Mexico), palo de la vida, corona de Cristo, tietie, China-root (Guatemala, Honduras, Belize), and bejuco de membrillo (Puerto Rico).

Historical publications since the XVI century indicate the ample use (Ximenez, 1967; Monardes, 1989), confirmed by publications form the early XX century, although botanical samples were not collected (Mejía, 1927; Roque, 1941; Aguilar, 1966; Mellen, 1974, Instituto Indigenista Nacional, 1978). Since 1980 field ethnobotanical surveys have been conducted in Guatemala, which showed that diverse names and ethnobotanical and ethnomedical uses are being attributed (Table 1).

Several ethnopharmacological studies have been conducted *in vitro* and *in vivo* based on wild materials, most of them in the framework of international cooperation networks, demonstrating interesting bioactivities, but with some degree of discrepancies (Table 2).

Few anatomic studies of American Smilax have been carried out, particularly for species from Argentina (Guaglianone & Gattuso, 1991) and Brazil (Andreata, 1997). For the microscopic evaluation fresh and dried samples from root and rhizome were analyzed by standard methodology Gattuso & Gattuso, 1999).

Agrotechnological studies

In 1990 zarzaparrilla collectors were convened to gather seeds from the vine, two main strains were detected, one from Cerro Gordo, Santa Rosa, and another from Cubilwitz, Alta Verapaz. Several methods for propagation were evaluated, including those from rhizome cuts, stalk, and seeds. Development of nursery plantlets was followed by experimentation on field transplantation and adaptation to tutors, design of cultural labor (Herrera et al., 2000), post-harvest technology (Ocampo et al., 2007), and extracts and products were developed for the phytotherapy industry (Cáceres et al., 2006).

Botanical and pharmacological studies

Antimicrobial, Leishmanicidal and Schisonticidal activities

Antibacterial and anti-yeast activities were determined by an agar plate dilution method according to Mitscher et al. (1972). For the screening, plates containing Muller-Hinton agar and 1 mg/mL of the extract were prepared; bacterial and yeast strains were inoculated in quadruplicate and incubated at 35 ºC for 24-48 h; results were expressed as positive (no growth) or negative

Table 1. Main ethnomedical information on the use of rhizomes of *Smilax domingensis* in Guatemala.

Name	Use	Reference
Tietie, China-root, Corona de Cristo	Basket making	Standley & Steyermark (1952)
Zarzaparrilla	Diaphoretic, abscess, tonic	Aguilar (1966)
Zarzaparrilla	Hepatic diseases, blood depurator	Mellen (1974)
Zarzaparrilla	Kidney, blood and venereal diseases, allergy	Instituto Indigenista Nacional (1978)
Zarzaparrilla, Diente de chucho	Leucorrhoea, urinary infection, ringworm	Cáceres et al. (1987a)
Zarzaparrilla	Diuretic, sudorific	Cáceres et al. (1987b)
Zarzaparrilla	Skin infections, vaginitis, ringworm	Girón et al. (1988)
Zarzaparrilla, Diente de chucho	Diarrhoea, stomach pain, inappetence, tonic	Cáceres et al. (1990)
Zarzaparrilla, Bejuco de la vida	Ringworm, skin infections	Cáceres et al. (1991a)
Zarzaparrilla	Leucorrhoea, ringworm	Cáceres et al. (1991b)
Zarzaparrilla	Venereal diseases	Cáceres et al. (1995)
Zarzaparrilla	Dysentery, fever	Cáceres et al. (1998)
Zarzaparrilla, Cak kul (Pocomchi)	Allergy, skin diseases	Nicolas (1999)
Chub Ixim (Qeqchi), Cocolmeca	Night sweat	Michel et al. (2007)

(growth). Minimal inhibitory concentration (MIC) was determined by the same procedure in quadriplates containing serial agar-extract dilutions (Cáceres et al., 1998).

Antifungal activity was screened by a dilution method in Sabouraud agar according to Brancato & Golding (1953). Four holes were opened, and 100 spores of 10 mycelial fungi (Table 3) were inoculated in quadruplicate, incubated at 27 °C for 21 days, and the colony diameter was measured. MIC was determined by the same procedure in quadruplicate containing agar-extract dilutions (Cáceres et al., 1998).

Activities against *Leishmania braziliensis, L. mexicana*, and *Trypanosoma cruz*i were studied *in vitro* using epimastigotes cultivated in LIT medium and trypomastigotes cultivated in tissue culture, suspension of both parasites were standardized and challenged in microwell against extracts as described in Cáceres et al. (1998).

Anti-inflammatory and analgesic activities

Anti-inflammatory activity was performed according to Winter et al. (1962). In three groups of five white rats both sex (165-180 g), the extract in 20% Tween 80 (25, 50, 100 and 200 mg/kg) was administered by orogastric cannula, with positive (125 mg/kg phenylbutazone) and negative controls; 30 min later an edema was induced in the sub-plantar aponeurosis in right foot pad with 0.05 mL of 1% carrageenan (E-407

Table 2. Results from bioassays performed in ethanolic extracts of *Smilax domingensis* rhizome from Guatemala.

Bioassay	Pharmacological results	Reference
Diuretic (decoction)	Urinary excretion increased 210±47% (hydrochlorothiazide 286±38%)	Cáceres et al. (1987)
Anticandidal	*C. albicans* (+, 40 mm in 0.15 mL/disk); *C. albicans* (+, 6.8±0.8 mm in 50 µL/disk)	Girón et al. (1988)
Antibacterial (tincture)	*E. coli* (-), *S. typhi* (+), *S. dysenteriae* (+), *S. flexneri* (+)	Cáceres et al. (1991a)
Antidermatophyte	*E. floccosum* (+), *M. canis* (-), *T. mentagrophytes* (+)	Cáceres et al. (1990)
Antigonorrhoeal	*N. gonorrhea* (negative, > 6.0 mm in 50 µL/disk)	Cáceres et al. (1991b)
Antibacterial	*S. aureus* (1 mg/mL), *P. aeruginosa* (5 mg/mL)	Cáceres et al. (1995)
Antiyeast	*C. neoformans (*0.5 mg/mL), *C. albicans* (5 mg/mL)	Cáceres et al. (1998)
Antiprotozoal/anti-*A. salina* nauplii	*T. cruzi* (>10 mg/mL), *A. salina* (>1 mg/mL)	Cáceres et al. (1998)
Cytotoxicity to cancer cells	MCF-7 (16 µg/mL), H-460 (12 µg/mL), SF-268 (27 µg/mL)	Calderón et al. (2006)
Estrogen binding	ERα (67 µg/mL), ERβ (69 µg/mL)	Michel et al. (2007)
Serotonin binding	5HTIA (27 µg/mL), 5HT5A (2 µg/mL), 5HT7 (16 µg/mL)	Michel et al. (2007)
Subchronic toxicity	Negative (2 g/kg, 90 days)	García-González et al. (2008)
Antifungal microdilution	*T. rubrum, T. mentagrophytes* (500 µg/mL)	Svetaz et al. (2010)

Table 3. Antimicrobial (MIC, mg/mL), leishmanicidal, schisonticidal, and larvicidal (LC50, mg/mL) activities of ethanol extract of cultivated *Smilax domingensis* rhizomes.

Bacteria		Yeast/fungi	
Bacillus subtilis	0.25	*Aspergillus flavus*	>1.0
Campylobacter jejuni	>1.0	*Aspergillus fumigatus*	>1.0
Escherichia coli	1.0	*Candida albicans*	>1.0
Mycobacterium smegmatis	0.25	*Candida tropicalis*	>1.0
Pseudomonas aeruginosa	0.25	*Cryptococcus neoformans*	0.50
Salmonella typhi	0.50	*Epidermophyton floccosum*	>1.0
Staphylococcus aureus	0.25	*Fonsecaea pedrosoi*	>0.1
Protozoa		*Microporum gypseum*	>1.0
Leishmania braziliensis	>1.0	*Microsporum canis*	>1.0
Leishmania mexicana	>1.0	*Saccharomyces cerevisiae*	>1.0
Trypanosoma cruzi (epimastigote)	>1.0	*Sporothrix schenckii*	0.25
T. cruzi (IC50)	>0.1	*Sporothrix schenckii* (yeast phase)	>1.0
Larvae and nauplii		*Trichophyton mentagrophytes*	0.50
Artemia salina	>1.0	*Trichophyton rubrum*	0.50
Aedes aegipti	>1.0		
Anopheles albimanus	>1.0		

from Rhodophyceae, CEAMSA, Spain) and measured by plethysmometer (Ugo Basile 7150) at 1, 3 and 5 h and analyzed by Dunnet test for significance.

Analgesic activity was determined by three methods according with internationally accepted principles for laboratory animal care as described by Saravia (2005). Data was analyzed by one-way ANOVA followed by Dunnet's multiple comparison tests for significance.

Weight resistance was performed according to Randall & Selitto (1957). Three groups of five white rats both sex (150-165 g) were submitted to weight resistance determination in right foot by analgesimeter (Ugo Basile 7200), selecting those supporting 2.5-3.5 g. The extract in 20% Tween 80 (25, 50, 100 and 200 mg/kg) was administered by orogastric cannula, with positive (125 mg/kg phenylbutazone) and negative controls. Edema was induced in sub-plantar aponeurosis in right foot pad with 0.05 mL of 1% carrageenan 30 min after administration of the drug. Weight resistance in grams was measured at 1, 2, 4, and 6 h.

Tail-flick was performed according to D'Amour & Smith (1941). Three groups of five white rats (185-200 g) were challenged with a constant intensity heat ray in the tail to determine the reaction time in a Tail-Flick apparatus (Panlab LE-7106), selecting those with a reaction time of 3-5 s. The extract and controls were administered as above. Reaction time was measured at 0.5, 1, 1.5, 2, 2.5, 3 and 4 h.

Hot plate was performed according to Malairajan et al. (2006): Three groups of five white male mice (20-23 g) were placed on a metallic hot plate at 58±2 ºC (Ugo Basile 7280) to determine the reaction time, selecting those with reaction time <5 s. The extract was administered as above, with positive (100 mg/kg, Tramadol) and negative controls. Reaction time was measured as above.

Immunomodulatory activity

Lymphoproliferation activity was performed according to Scudeiro et al. (1988). Lymphocytes were isolated by Histopaque from human peripheral blood, washed, resuspended in RPMI-FBS to $5x10^6$ cells/mL, and incubated with the extract (1 mg/mL), positive (ConA) and negative control (RPMI) for seven days at 36 ºC with 5% CO_2; 50 µL of XTT solution were added, incubated for 4 h, 50 µL of SDS were added and incubated for 1 h; absorbance was read in a plate spectrophotometer (Bio Tek EL 340) at 450 nm. Minimal effective concentration (MEC) was calculated comparing with ConA.

Hemolytic assay for complement activity was performed according to Klerx et al. (1983). Sheep erythrocytes ($4x10^8$ cells/mL) were sensitized with amboceptor for classic way; rabbit erythrocytes ($1.15x10^8$ cells/mL) were used for alternative way. Extract dilutions were made in microplate starting from 500 µg/mL, in buffer (VSB^{++}, classic way; EGTA-VSB, alternative way); hemolysis controls included buffer (0%), water (100%), inactivated and activated human serum with buffer. Plates were pre-incubated at 37 °C for 30 min, erythrocytes were added and incubated at 37 °C (alternative way 30 min, classic 60 min). Plates were centrifuged at 2500 rpm for 2 min and 50 µL of the supernatant transferred to a microplate containing 200 µL of demineralyzed water. Optical density was read at 405 nm in a plate spectrophotometer; mean inhibitory concentration (IC50) was calculated and compared with control.

Production of antibodies was performed according to Godhwani et al. (1988). White mice (22-24 g) eight week old were bled from the tail and plasma separated (day 0); then injected IP with 100 µL of a *Salmonella* suspension on days 1, 4 and 8. Extract dilutions were administered daily by orogastric cannula for 10 days adjusting the dose to the animal weight; blood samples were obtained at days 6 and 12, sera were frozen until use. Antibody production was evaluated by an agglutination test using Widal reagent.

Acute toxicity

Extract in 20% Tween 80 (200, 250, 300, 350 and 400 mg/kg) were administered to five groups of five mice (20-23 g) by orogastric cannula. Animal behavior was observed at 1, 4, 8, 12, 48 and 72 h, and every 24 h for eight days. Data was analyzed for death animals and LD50 calculated.

Chemical studies

Tinctures (1:5 and 1:10) and extracts (1:1, 2:1 and 3:1) were prepared from dry grind rhizomes by percolation with ethanol (35, 50, 70 and 95%) and concentrated in a rotary evaporator. Liquid:liquid partitions with solvents (hexane, chloroform, ethyl acetate, methanol) were done from the ethanol extract. For phytochemical screening and metabolites characterization, standard procedures were performed according to Lock de Ugaz (1994) and Solis et al. (2005), as standardized in our laboratories; for thin layer chromatography (TLC) procedures were done according to Wagner & Bladt (1996); and, quantification by spectrophotometric methods of steroidal sapogenins at 430 nm according to Baccou et al. (1977) and total flavonoids at 425 nm according to the Brazilian Pharmacopoeia (1996).

Results

Botanical characteristics

Plant description

It is an evergreen dioic woody vine, 2-4 m high with lignified rhizomes. Alternate leaves, ovate-acuminated short petiole with two lignified tendrils. Flowers are arranged in umbels. Staminated flowers usually have six stamens. Pistillated flowers with ovary superior. Fruit is a berry, red, purple, or black. Rhizome is partially lignified, voluminous, with tuberous swelling, reddish brown in color. Roots are adventitious, growing from the rhizomes.

Microscopic description

In cross section, it is circular (IA). The cortex is composed of an epidermis with thickened walls. Cortical parenchyma cells are elongated (Figure 1B), with idioblasts containing calcium-oxalate raphides, mucilage, and granular material; the most internal part, parenchymatic cells, also prosenchymatic. These cells are full of starch grains, 2, 3, and up to 6 or more compound, each grain being polyhedral, rather small and with central hilum (Figure 1B, H). Scattered in the parenchyma, there are closed collateral bundles rounded by a rather developed sheath of fibers.

Dissociated material shows fibers with strongly thickened-walls, approximately 400 µm long (Figure 1G); fibrotraqueids of 400 µm long (Figure 1D); long phloems measuring 36 µm wide, with appendage and simple terminal plate (Figure 1C); xylem axial parenchyma cells, (Figure 1E); external and internal cortical parenchyma cells (Figure 1F, H). The powder drug is reddish brown in color, and displays a characteristic flavor and smell. Analyzed individually, fragmented drug shows the anatomical features in Figure 2.

Ethnobotanical

Literature reviewed and surveys conducted during 1978-2005 demonstrated that several medicinal uses are attributed for *S. domingensis* in Guatemala. Main uses include: anti-infectious, anti-inflammatory, blood disorders, depurative, diaphoretic, diuretic, stimulant, and for venereal diseases (Table 1).

Agrotechnological

Research for 20 years indicates that reproduction of the plants is more pratical by seed propagation. Seeds are collected in November to February, fermented in water for a couple of days and dried; no data is known about its viability, but they must be planted the same year. Germination starts 50-90 days after seeding, with an average <50%. The most effective way is to collect natural seedlings from controlled individuals, which develop in the rainy season. Seedlings are grown for 2-3 years in plastic bags and then transfer to definitive field in the forest or intercropped in an agroforestry system, since it need a tutor to climb.

For two years, the cultural labor is to facilitate the climbing, cleaning the surroundings and consolidating the ground for rhizome development. Harvest is done in summertime when it is at least seven years old, the rhizome is digged to obtained the older part, leaving the younger sprout for propagation; the rhizomes are freed from soil and roots, washed, disinfected, sliced and dried. Fresh yield per plant is 25-45 kg, with a fresh:dry ratio of 2.5:1.

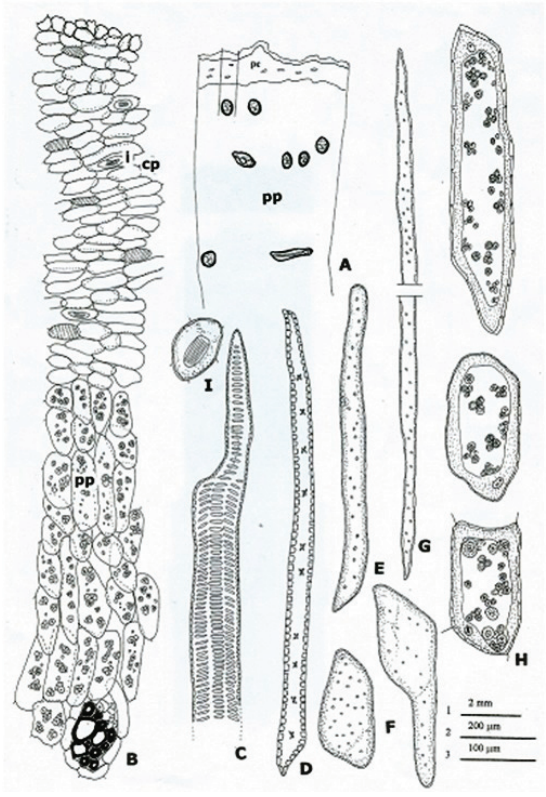

Figure 1. A-I: Rhizome of *S. domingensis*: A. cross sections portion, schema; B. detail of A; C-H: macerating: C. scalariformly thickening vessel; D. fibretracheid; E. axial parenchyma cells; F. cortical parenchyma cells; G. fibre; H. parenchyma of the pith cells; I. raphide of calcium oxalate. i, idioblast to raphides of calcium oxalate some contained in a parenchymatous cells; cp, cortical parenchyma; pp, pith parenchyma. Scale bars:1 to A; 2 to B; 3 to C, D, E, F, G, H.

Pharmacology

The evaluation of the published literature, initially as *Smilax lundelii* Killip and Morton from the wild, indicated that the rhizome extract was active against *Staphylococcus aureus, Candida albicans, Cryptococcus neoformans*, and *Microsporum gypseum* (Table 2), showing a MIC from 1-5 mg/mL (Girón et

al., 1988; Cáceres et al., 1998).

In this study we confirmed the inhibitory activity by the extract of cultivated rhizomes against *S. aureus* and *Pseudomonas aeruginosa* (MIC 0.25 mg/mL), *Sporothrix schenckii* (MIC 0.25 mg/mL), *C. neoformans* (MIC 0.50 mg/mL), and *Trichophyton rubrum* (MIC 0.50 mg/mL) (Table 3).

Immunomodulatory activity was detected by two methods. Inhibition of lymphocyte proliferation was shown by the rhizome and leaves extracts (MEC 62.3 µg/mL), but the stalk extract showed a stimulatory effect (MEC 31.2 µL/mL). The most potent activity was shown in the ethyl acetate partition. The ethanol extract from the rhizome showed inhibition of the classic pathway of the complement (IC50 12.88 µg/mL), and the ethyl acetate partition inhibited the alternative pathway (IC50 12.15 µg/mL). No analgesic or anti-inflammatory activity of the extracts was demonstrated by any of the bioassays. No acute toxicity was demonstrated (DL50 >400 mg/kg).

Chemistry

The chemistry of *Smilax* has been described primarily for the long roots and small rhizome type of species, which include steroidal saponins, flavonoides, polyphenols and stigmasterol (Bérdy et al., 1982). There is no published information on the chemical composition of *S. domingensis* rhizome. Data presented here refer to evaluations with cultivated material.

Phytochemical screening of the extract by macro, semi-micro and TLC demonstrated steroids by Salkowsky, Liebermann-Burchard and foam test; flavonoides and anthocyanins by Shinoda and TLC (Rf 0.24-0.89). Alkaloids, antraquinones and tannins were not detected, only phenolic compounds by $FeCl_3$ (Table 4).

Ethanol (50%) extracts and fractions showed flavonoids, saponins, sequiterpene lactones, coumarins and tannins. Rhizomes from female and male plants showed little difference, particularly the presence of sesquiterpene lactones in female plants, and the lack of these in male rhizomes. Flavonoids were present in the 1:1 extract at 0.02±0.01% and in the dry extract at 0.08±0.01%, expressed as quercetin; steroidal sapogenins in the 1:1 extract were 0.68±0.02%, and in the dry extract 1.63±0.02%.

In cooperation with the National Institute of Engineering, Technology and Innovation (INETI) from Lisbon, Portugal compounds were identified by HPLC in tinctures and extracts in order to select markers for extracts standardization. Proposed markers due to its abundance are: caffeic acid, quercitrin rhamnoside, and isorhoifolin. Comparing with standards it was not possible to detect caffeine, quercetin, kaempferol, theobromine, theophiline, catechin, epicatechin, epigallocatechin, epicatechin gallate, and gallocatechin. No anthocyanins were detected at 254 and 280 nm.

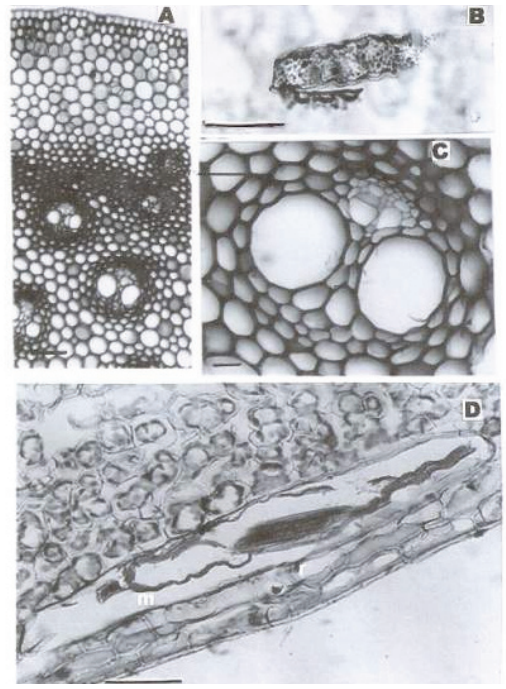

Figure 2. A-D: Rhizome of *S. domingensis*, cross sections, detail; A. portion; B. idioblasts containing granular material; C. closed collateral bundle; D. idioblast to raphides of calcium oxalate and mucilage. i, idioblast to raphides of calcium oxalate; m, mucilage. Scale bars: A 250 µm; B, 60 µm; C and D, 25µm.

Contrary to expected according to the literature, sarsapogenin, smilagenin or steroids (stigmasterol, β-sitosterol and cholesterol) were not detected by GC-MS analysis, according to the molecular masses of known saponins from *S. officinalis*. From 10 samples from different locations in Guatemala, chromatographic profiles were similar, with the exception of a sample from Carchá, which showed fragmentation patterns similar to sarsapogenin/smilagenin, which correspond to *S. kunthii.*

Discussion

Botanical characteristics

After several years of uncertainty about the taxonomy of native *Smilax* spp., we finally arrived to a clear description of *Smilax domingensis,* Smilacaceae. It is evident that two group of species are available in the market, one represented by a long whitish odorous roots (*S. aristolochiaefolia, S. regelii*, and others), and the other by a big woody red rhizome (*S. domingensis*). In this paper, the description by Huft (1994) is enriched by our field and botanical observations.

This is the first time that the micromorphological

Table 4. Phytochemical information on tinctures and extracts from *S. domingenis* rhizome analyzed by qualitative and TLC methods.

Sample	Anthocyanines		Flavonoids		Saponins		Tannins	Alkaloids
	Qualitat.	R_f	Qualitat.	R_f	Foam	R_f	Qualitat.	R_f
Tincture 1:5	++++	0.32,0.77,0.88	++++	0.27, 0.44, 0.88	+	0.8	−	−
Tincture 1:10	++++	0.32, 0.77, 0.88	++++	0.27, 0.36, 0.85	+	0.8	−	−
Ethanol 95%	++++	0.32, 0.77, 0.88	++++	0.27, 0.31, 0.81	+	0.8	−	−
Ethanol 70%	++++	0.32, 0.77, 0.88	++++	0.27, 0.32, 0.83	+	0.8	−	−
Ethanol 50%	++++	0.32, 0.77, 0.88	++++	0.27, 0.32, 0.83	+	0.8	−	−
Ethanol 35%	++++	0.32, 0.77, 0.88	++++	0.27, 0.33, 0.83	+	0.8	−	−
Extract 1:1	++++	0.32, 0.77, 0.88	++++	0.27, 0.44, 0.80, 0.87	+	0.72, 0.83	−	−
Extract 2:1	++++	0.32, 0.77, 0.88	++++	0.27, 0.44, 0.80, 0.87	+	0.56, 0.83	−	−
Extract 3:1	++++	0.32, 0.77, 0.88	++++	0.27, 0.31	+	0.72, 0.80	−	−
Sudan Red		0.94						
Methylene blue		0.39						
Rutine				0.27				
Quercetin				0.87				
Chlorogenic acid				0.45				
Cholesterol						0.88		
Saponin					++++	0.75		
Atropine								0.27
Ajmaline								0.36
Papaverine								0.48

− Negative; + slightly positive; ++++ strongly positive.

characteristics of *S. domingensis* rhizome are described, as well as the powder drug as it is used in industry. With these results and drawings we are contributing to the characterization and standardization of this crude drug and establishing parameters for quality control.

Ethnobotanical findings

According to Monardes (1989), several varieties of *Smilax* were among the most appreciated material exported since the XVI century from the New World to Europe, of particular interest is the reference of one specie very similar to the one obtained from China (*S. pseudo-china*), but "looking fresher and more potent". The work by Ximenez (1967) originally published in 1722, described the abundance of several classes of zarzaparrilla in the lowlands, indicating that this material is exported in large amounts to Europe.

The compilation of ethnobotanical surveys presented in Table 1, demonstrated the great variety of uses attributed by the population, particularly anti-inflammatory, immunomodulatory, antimicrobial, diuretic and tonic.

Agrotechnological

Agronomical data was published by our group (Herrera et al., 2000) as well as post-harvest technology. In this paper an integrated description is made to help in the sustainable technological handling of this specie in order to standardize the production and process of this woody rhizome.

Pharmacology

The preliminary publications on the pharmacology of this species demonstrated antimicrobial activity of the wild material. We confirmed the activity against bacteria, yeast and dermatophytes by cultivated material. This finding support one of the main ethnobotanical uses, in relation with infection control of the skin and mucosa.

Although no anti-inflammatory or analgesic activity was demonstrated, immunomodulatory activity attributed to the rhizome was demonstrated by inhibition of lymphocyte proliferation and of the classic pathway of complement, although some stimulatory effect was demonstrated in the stalk extract.

Other *Smilax* species have demonstrated immunomodulatory activity. *S. glabra* Roxb. inhibited inflammation of adjuvant-induced arthritis in rats (Jiang & Xu, 2003); and the active substances are the protein smilaxin (Chu & Ng, 2006) and the flavonoid astilbin,

which showed immunosuppressive activity against activated T lymphocyte, inhibited picryl chloride-induced ear swelling in mice and suppressed the expression of tumor necrosis factor-α and γ-interferon (Guo et al., 2007). The aqueous extract of *S. china* L. rhizome showed anti-inflammatory activity by egg-albumin-induced edema in rat and anti-nociceptive activity by hot-plate test and acetic acid-induced abdominal constriction in mice (Shu et al., 2006).

Phytochemistry

It is evident from this information that the botanical, chemical and pharmacological properties of *S. regelli* root are different from that of *S. domingensis* rhizome. It is clear that roots bearing species are rich in saponins, but the rhizome species are rich in flavonoids. Further studies are needed to establish the molecules responsible for the immunomodulatory activity.

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