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Pharmacognostic and Phytochemical Studies of *Smilax domingensis* Willd. in Cuba

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Abstract

A preliminary pharmacognostical and phytochemical evaluation of *Smilax domingensis* Willd., (Smilacaceae) was realized to determine the macromorphology and micromorphology characteristics, the physicochemical and phytochemical parameters from the rhizome of this spice that grows in Cuba. This crude drug showed the characteristic physicochemical values such as moisture content (13.11%), extractable matter in ethanol at 70% (13.53%), total ashes (3.45%), water soluble ashes (2.43%) and acid insoluble ashes (0.64%). Phytochemical screening revealed the possible presence of alkaloids, oils and/or fats, coumarins, saponins, flavonoids, pyrogallol-type tannins, quinones, catechins, reductants sugars, triterpens and steroids and absence of resins, aminoacids, cardiotonic glycosides, anthocyanidins and astringent and/or bitter principles, realized under WHO parameters.

Keywords

Smilax domingensis, Macromorphology, Micromorphology, Physicochemical Parameters, Phytochemical Screening

1. Introduction

Smilax domingensis Willd., Smilacaceae, known as bejuco chino or raíz de china, zarzaparrilla de la tierra (Cuba); bejuco de membrillo, dunguez blanco (Puerto Rico); chiquihuite (México), is a climbing shrub from Tropical America. The rhizome is popularly used in medicine as anti-inflammatory, antiseptic, depurative, sudorific, antasthmatic, antiherpetic, antirheumatic and for venereal diseases [1].

Smilacaceae is a family of climbing shrubs represented by the single genus *Smilax* with close to 250 species worldwide, present with 26 species in Mesoame-

rica [2].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 [3].

Smilax domingensis Willd. is native from Tropical America, growing in lowlands, in humid forests of wide-leaved species [4]. Although widely used, there are several taxonomic difficulties. Few anatomic studies of American Smilax have been carried out, particularly for species from Argentina [5] and Brazil [6].

In the scientific literature, there are some data of the phytochemical components and pharmacological actions while a small number of data of standards for identification and authentication about *Smilax domingensis* Willd. In Cuba, there is not available information for this spice.

The main components found and shared by most species of the genus are the steroidal saponins, phytosterols, and triterpenoids [3]. The antimicrobial and anti-tumoral activities are attributed to parillin [7]. Some pharmaceutical forms are available, such as infusion, tincture, elixir, lotion, and micropulverized powder [8].

Hence, the pharmacognostic and phytochemical investigations on *Smilax domingensis* Willd. has been carried out in this research, for the development and utilization of the promising medicinal plant.

2. Materials and Methods

2.1. Plant Material and Reagents

The *S. domingensis* Willd. rhizome was collected from Sierra Cristal, Sagua de Tánamo, Holguín Province, Cuba, 850 - 1000 masl, by Elio M. García Fargie in March, 2016. The authors are waiting for the Voucher specimen in the National Botany Garden in Havana, Cuba. The plant material is being authenticated by Dr. Jorge E. Gutiérrez Amaro. The harvested rhizomes were dried in the shade at room temperature (temperature 30˚C - 40˚C) on the Research Lab Table in the Faculty of Pharmacy and Foods (Havana University), ground into powdered form (1 mm) and stored in airtight containers. All reagents used were of analytical grade (Merck). All solvents were degassed prior to use in an ultrasonic bath without filtration.

2.2. Macroscopic and Organoleptic Studies

Three rhizomes were examined for morphological characters including size, shape, color, odor, taste, and extra features. The macro-morphological characteristic of the rhizome were observed under the magnifying lens (10×).

2.3. Microscopic Studies

Dried rhizomes were ground to coarse powder and packed, for microscopic identification, in a suitable container. No clearing agents were used. Photomicrographs of the powder section were taken with the help of Biomicroscopy

Primo Star (Zeiss Group, Germany) with 10x and 40x microscope objective lens (400 xs) and CX_{21} bio-microscopy unit (Canon-1000D EOS digital camera, Japan, coupled to PC software EOS-utility).

2.4. Phytochemical Studies

Dried rhizomes were ground to a coarse powder (grain size: 850 ± 29 μm) and packed in a suitable container for phytochemical identification. The powder was extracted with 70% ethanol, filtered and concentrated using vacuum distillation. The chemical constituents were screened according to Chhabra [9] to ascertain the presence of chemical components in diethyl ether, etanol and water. The UV spectrometric experiments were carried out on a UV-VIS JASCO V-530 (Japan). The scan range was 200 to 400 nm; absorbance 0.000-3.0000, band width 2.0 nm, spectral resolution 0.1 nm and the analyzed samples were diluted in methanol, into quartz cuvettes (d = 1 cm).

TLC conditions: TLCP (thin-layer chromatography plate) on silica gel with fluorescent indicator 254 nm on aluminum cards (layer thickness 0.2 mm) (10 × 20 cm) using *n*-butanol: acetic acid: water (BAW 65:25:10) as developing agent (v/v/v), concentrated sulfuric acid plus heat, $FeCl_3$ and $AlCl_3$ were the chromogenic agents. Rutin (R), quercetin (Q) and gossypitrin (G) were used as standard. The TLCP were examined under ultraviolet (254 nm and 365 nm) and ordinary light.

The total ash, acid insoluble ash, water soluble ash, extractable matter and moisture content were determined according to the standard procedures mentioned in the general rule of WHO [10].

3. Results and Discussion

3.1. Botanical Characteristics

3.1.1. Macroscopical Description

It is an evergreen dioic woody vine, 2 - 4 m high with lignified rhizomes. Rhizome is voluminous, with tuberous swelling, reddish brown in color, measuring 14 - 21 cm long, 3.925 cm wide and 3.175 cm high. The average weight is around 87.05 g. Roots are adventitious, growing from the rhizomes (**Figure 1**).

The powder drug is reddish brown in color, and displays a characteristic flavor and smell (**Figure 2**).

In this paper, the description by Huft [2] is enriched by our botanical observations. It is evident that the analyzed spice has a great coincidence by the description done by Ferrufino & Gómez-Laurito [11], and Cáceres [12]. We finally arrived to a clear description of *S. domingensis* in Cuba, represented by a big woody red rhizome.

3.1.2. Microscopical Description

Analyzed individually, Fragmented drug shows the anatomical features in **Figure 3**. Cortical parenchyma cells are elongated, with idioblasts containing calcium-oxalate raphides, mucilage, and granular material (3A); long phloems

Figure 1. Rhizomes of *S. domingensis* Willd.

Figure 2. Powder drug of *S. domingensis* Willd.

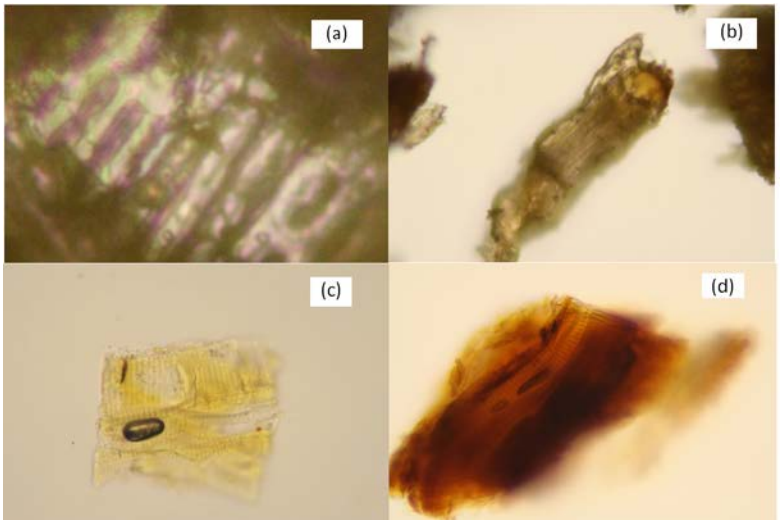

Figure 3. Fragmented drug of *S. domingensis* Willd.

measuring 36 μm wide, with appendage and simple terminal plate (3B) and scalariformly thickening vessels (3C and 3D).

This is the first time that the micromorphological characteristics of *S. domingensis* rhizome are described, as well as the powder drug in our country.

3.2. Phytochemical Studies

3.2.1. Phytochemical Screening

Preliminary phytochemical screening suggested the presence of flavonoids, alkaloids, coumarins, catechins, pyrochatecolic tannins, fat and/or volatile oils, saponins, triterpens and/or steroid, quinones and reducing sugars, and the absence of resins, amino acids or amines, cardiotonic glycosides, anthocyanidins and astringents and/or bitter principles (Table 1). Data presented here refer to evaluations with wild material. Further studies are needed to establish the molecules responsible for the real chemical composition.

There were three absorption peaks at 205 nm, 280 nm and 394 nm. There may be the presence of flavonoids in 70% ethanol extract [13]. This result corroborates the suggested presence of flavonoids after phytochemical screening (Figure 4).

TLCP (thin-layer chromatography plate) presented four obvious spots (Figure 5). Under ordinary light two spots were observed, the first one (1) with $R_f = 0.37$ and the second one with $R_f = 0.83$. Both spots are cream-yellowish in

Table 1. Phytochemical screening of *S. domingensis* Willd.

Test for constituents groups	Diethyl ether	Alcohol	Water
Dragendorff	+++	+++	+++
Wagner	+++	+++	+++
Sudan III	+		
Baljet	+++	++	
Lieberman-Burchard	+	+	
Catechins		+ (green)	
Resins		–	
Fehling		+	+
Foam test		+	+
$FeCl_3$		+ (intense green)	+ (intense green)
Ninhydrine		–	
Börntrager		+++	
Shinoda		+	+
Kedde		–	
Anthocyanidins		–	
Bitter principles and/or astringents			–

Legend: + (positive); ++ (very positive); +++ (highly positive); – (negative).

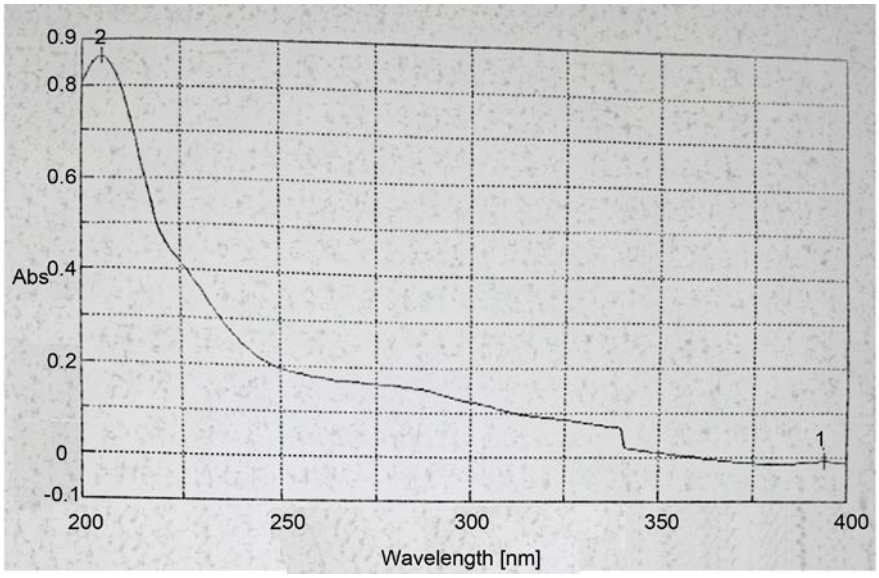

Figure 4. The UV absorption spectrum of *S. domingensis* Willd.

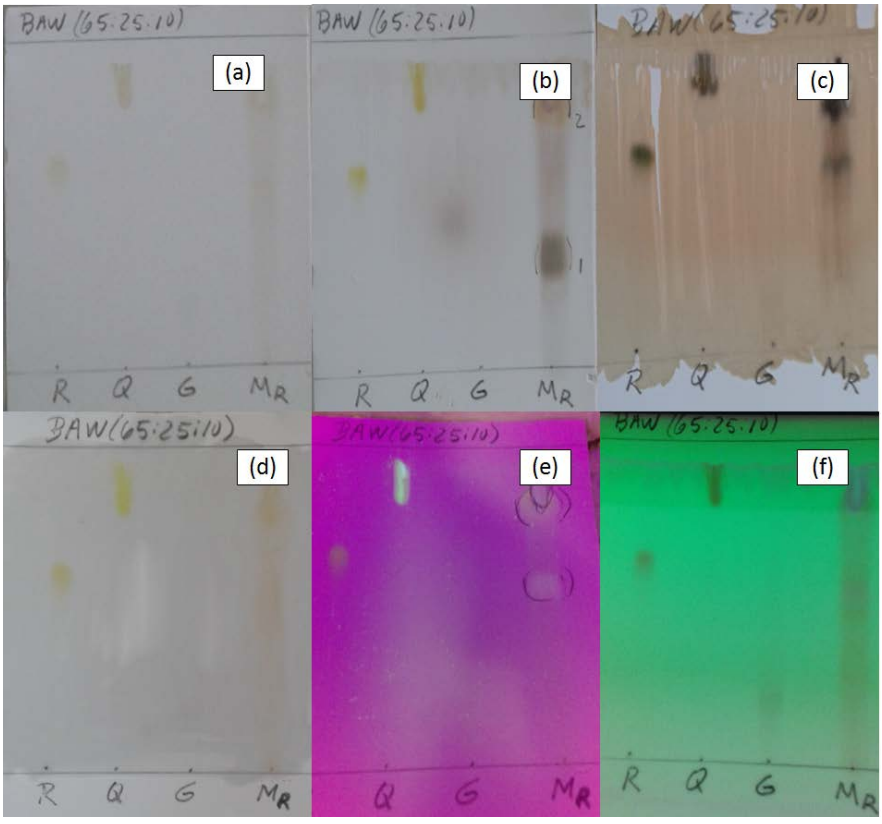

Figure 5. TLC of *S. domingensis* Willd.

color (5A). The colorimetric detection with concentrated sulfuric acid and heat presented the same two spots, with the identical R_f (0.37; 0.83), but, with different colors, the first one yellow-green and the second one yellow-orange, both of them representative of chemical compounds type flavonoids [14] [15] and another one appear near the solvent front red violet in color that could be re-

lated with terpenoids structures (5B). Colorimetric detection with $FeCl_3$ showed two spots (4) with R_f = 0.78 and another one near the solvent front (R_f = 0.90), both of them black green in color, indicating the presence of phenolic compounds derivates from catechol (5C). In colorimetric detection with $AlCl_3$ were observed two spots (3 R_f = 0.52; another one R_f = 0.90), both spots with few chromatography complexity at ordinary light (5D), but changing their colors under UV 365 nm to pale yellow (3) and the other one to more brilliant yellow, and inside this last one and near to the solvent front, another one clear blue (R_f = 0.94) (5E). Under UV 254 nm, the spots change in tonality, the three firsts with reddish orange colors, typically from chemical compounds like flavonoids and another one in the solvent front clear blue intense (5F).

The chemistry of Smilax has been described primarily for the long roots and small rhizome type of species, which include steroidal saponins, flavonoids, polyphenols and stigmasterol [5].

Phytochemical screening of the extract by macro, semi-micro and TLC demonstrated steroids by Salkowsky, Liebermann-Burchard and foam test; flavonoids and anthocyanins by Shinoda and TLC (Rf 0.24 - 0.89). Alkaloids, antraquinones and tannins were not detected, only phenolic compounds by $FeCl_3$. Ethanol (50%) extracts and fractions showed flavonoids, saponins, sesquiterpene 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%. 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 *Smilax 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 *Smilax kunthii* [12].

3.2.2. Physicochemical Characteristics

Moisture content (13.11%) was inside the limited index (8% - 14%). Extractable matter in ethanol at 70% (13.53%) suggests that this solvent is appropriated to the extraction of active components from the rhizomes. Total ash (3.45%) is according to the standard allowed (3% - 5%), while water soluble ash (2.43%) was relatively higher than the standard for medicinal plants (< 2%), the possible reason is that medicinal rhizome is not clean, containing soil and another inorganic impurity. Acid insoluble ash (0.64%) was lower than the standard (< 2%).

4. Conclusions

Several studies suggest that adulteration and misidentification of crude drugs can cause serious health problems to consumers and legal challenges for the

pharmaceutical industries. The macroscopic and microscopic characters of any plant drug are considered to be the preliminary steps for establishing their quality control profile. As per the guidelines of WHO, pharmacognostic standards should be proposed as a protocol for the diagnosis and authentication of the plants drugs.

Physicochemical parameters like ash values, moisture content are all indicators of quality plant medicine, which help to determinate the physiological and non-physiological ash, possibility of microbial growth or contamination and presence of impurities respectively. The relative high ratio of water soluble ash content of *S. domingensis* Willd. indicates that the crude drug contains plenty of physiological ash and the non-physiological content, it will affect the clinical efficacy of drugs, so it should pay attention to the control quality of medicinal materials in plants production.

This research showed that, *S. domingensis* Willd. can be identified by structural features or characteristics of rhizomes in Cuba. Specific cortical parenchyma cells with idioblasts containing calcium-oxalate raphides, mucilage, and granular material and scalariformly thickening vessels can be regarded as distinctive identification character.

Efforts have been made by the authors to bring out every detail on the macroscopical and microscopical characters of *S. domingensis* Willd. The study of pharmacognostical features had shown the standards, which will be useful for the detection of its identity and authenticity. It provides reference basis for formulating quality standard of *S. domingensis* Willd. authenticity of medicinal plant and resource utilization.

Conflict of Interest

The authors declare no conflict of interest.

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