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GC-MS Chemical Characterization of Main Components of *Smilax Domingensis* Wild. in Cuba

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Abstract

A preliminary chemical characterization of main components of ethanolic extract with dried rhizomes of *Smilax domingensis* Wid. that grow in Cuba was done using a GCMS-QP2010 Ultra Shimadzu and the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. After sample derivatization 125 chemical compounds were registered by the equipment and from them, 35 different chemical components were characterized and reported for the first time from this part of the plant in our country. The results demonstrate the developed method could be employed as a rapid and versatile analytical technique for identification of chemical constituents and quality control of *Smilax domingensis*.

Keywords: Smilax domingensis, chemical constituents, GC-MS, rhizomes, ethanolic extract.

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 (Roig, 2014).

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. Few anatomic studies of American Smilax have been carried out, particularly for species from Argentina (Guaglianone & Gattuso, 1991) and Brazil (Andreata, 1997).

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 (British Herbal Pharmacopoeia, 1983).

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).

Fig. 1. Macroscopical view of rhizomes from *S. domingensis* Willd. in Cuba.

The aim of this research was to characterize the chemical composition of rhizomes of *S. domingensis* from our country 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 Voucher No. HAJB 089193 was registered at National Botany Garden in Havana, Cuba. The plant material was 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.

The extracts were prepared with the ground material (60 g) without screen extracted in a Soxhlet apparatus with 675 mL of ethanol at 95% during 20 hours. The ethanolic extracts were concentrated and evaporated under vacuum to 200 mL at 120 rpm, a temperature of 70˚C and 500 mbar. All reagents used were of analytical grade (Merck). All solvents were degassed prior to use in an ultrasonic bath without filtration.

2.2. HPLC Analysis

HPLC analysis of ethanolic extract from the rhizomes was registered using an HPLC Knauer-Azura (Germany) equipped with an UV detector at 280 nm. Chromatographic conditions: Flow (1 mL/min); Column (RP-18e Knauer 250 x 4.6 mm Lichrospher 100-5); Manual injection (50.00 µL, twice); Running time (60 min); Pump pressure (11.7 MPa); Gradient (eluent A: water, eluent B: methanol, 15-85 % B during 30 min, follow by holding, increasing to 50 % A during 10 min, reversing to 0 % B during 5 min and equilibrating during 5 min).

2.3. GC–MS Analysis

For the identification of metabolites present in the rhizomes, the sample were subjected to chromatographic analysis in equipment GC/MS, brand Shimadzu QP2010, equipped with a splitter split/splitless. With a BP5 (30 m × 0.25 mm × 0.25 microns) capillary column under the following chromatographic conditions: Helium gas carrier obtained by electron impact fragments to a power of 70 eV rate of 1.2 mL/min, 1:50 split flow and the volume of injected

sample of 1 ul. Programmed oven temperature: initial temperature was 70˚C with a heating ramp of 10˚C/min to 300˚C and remained stable at this temperature for 10 minutes. Subsequently the temperature was increased at a rate of 10°C/minute to 300°C for a total time of 78 minutes with an injector temperature 250°C and the interface temperature 300°C.The compounds were analyzed using GC/MS NIST21 and NIST107 library and having into account the results obtained after phytochemical screening according with González *et al.* (2017). Silylation agent was N, O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) CAS 25561-30-2 Lot: 0901-1 Macherey-Nagel GmbH & C. KG.

2.4. Data Analysis

Comparison of the spectra with the NIST database using a probability-based matching algorithm was performed to achieve compound identification, along with comparison of relative retention indices (RI) to literature and standard reference values.

3. Results and Discussion

3.1. Chromatographic profile by HPLC

From rhizomes, 34 peaks in HPLC-UV chromatogram were identified as shown in figure 2. Their retention times were between 2.550 and 48.283 minutes, although the chromatogram resolution is not the most ideal after this retention time and up to 50 minutes, which allow us inferred that there are other components into analyzed sample. The most prominent peaks belongs to compounds **1** (2.550 min), **3** (3.333 min), **20** (26.517 min), **21** (27.383 min), **22** (28.267 min) and **24** (29.950 min), giving an idea that they are the majoritarian chemical components in the sample. Around peak **33** (45.400 min) and **34** (48.3 min) the chromatogram show another prominent area indicating the presence of several components but this zone have not a good resolution.

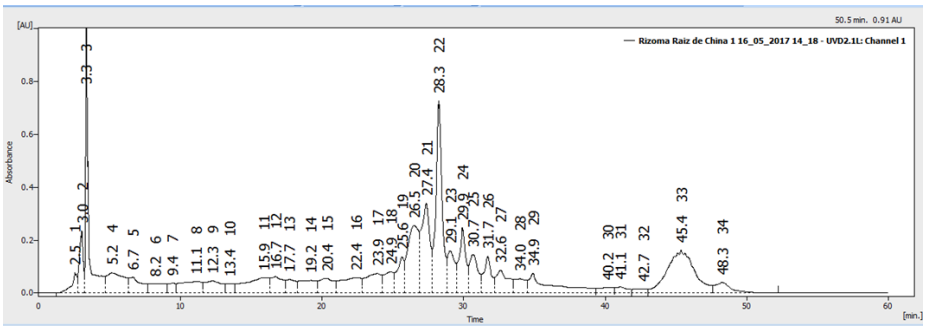

Fig. 2. Chromatographic profile by HPLC of rhizomes of *S. domingensis* Willd.

3.2. Chemical characterization by GC-MS

Preliminary phytochemical screening suggested the presence of flavonoids, alkaloids, coumarins, catechins, pirochatecolic 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 Data referred here refer to evaluations with wild material in our country (Yaque et al., 2017).

The peaks are marked with retention time in the GC-MS chromatogram of ethanolic extract of the rhizome from *S. domingensis*. Their retention times (RT) and their corresponding names are listed in Table 1. The qualitative analysis of the ethanolic extract showed the presence of 125 different kinds of chemical compounds according with the equipment database (Fig. 3) and among them; only 35 received the proposal of chemical characterization. The first 23 chemical compounds were discarded because they are related with the Silylation agent and their derivatives.

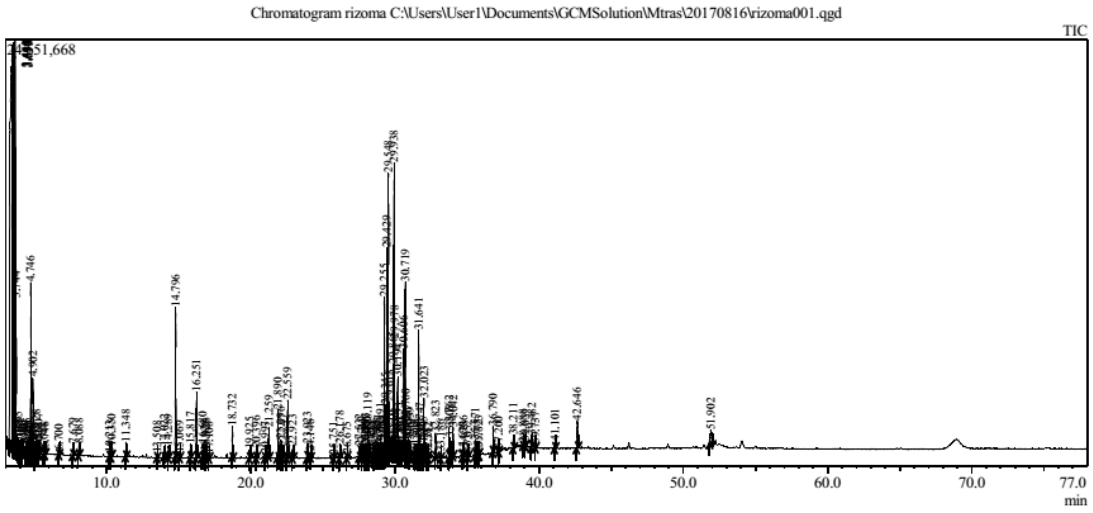

Fig. 3. GC chromatogram of rhizomes from *S. domingensis* Willd.

Most of the chemical components belong to organic acids, reductants sugars, and lactones and relative compounds. The chemical matches were around 80 % of coincidence with NIST21 and NIST107 libraries.

Table 1. Chemical components characterized by GC-MS in rhizomes of *S. domingensis*.

Peak #	R.Time	Molecular formula	Molecular weight	Base Peak	CAS	Library ID/Compound Name*
1	14.794	$C_{12}H_{32}O_3Si_3$	308	73.00	6787-10-6	Trimethylsilyl ether of glycerol
2	15.824	$C_{10}H_{22}O_4Si_2$	262	147	40309-57-7	Butanedioic acid (Succinic acid)
3	16.251	$C_9H_{20}O_4Si_2$	276	147	55557-24-9	Propanedioic acid
4	16.682	$C_{12}H_{30}O_4Si_3$	322	73	38191-87-6	Propanoic acid, 2,3-bis[(trimethylsilyl)oxy
5	15.815	$C_{10}H_{22}O_3Si_2$	246	147	55590-70-0	2-Butenoic acid
6	16.683	$C_9H_{22}O_3Si_2$	234	147	55162-32-8	Propanoic acid
7	18.735	$C_{13}H_{30}O_5Si_3$	350	73.00	65143-63-7	Malic acid
8	21.260	$C_{14}H_{32}O_5Si_3$	364	73	0 - 00 - 0	3-Hydroxy glutaric acid
9	22.097	$C_{12}H_{28}O_3Si_2$	276	73	54890-08-3	Pentanoic acid, 4-methyl-2-[(trimethylsilyl)oxy]
10	22.935	$C_{16}H_{42}O_4Si_4$	410	73	32381-52-5	Threitol
11	26.181	$C_{14}H_{32}O_5Si_3$	364	73	10589-37-4	Arabinoic acid, 2,3,5-tris-O-(trimethylsilyl)-, .gamma.-lactone, l
12	27.537	$C_{14}H_{32}O_5Si_3$	364	73	32384-55-7	D-Arabinonic acid, 2,3,5-tris-O-(trimethylsilyl)-, .gamma.-lactone
13	28.073	$C_{18}H_{42}O_6Si_4$	466	73	0-00-0	2-Methyl-2,4-bis(trimethylsilyloxy)bis(trimethylsilyl)glutarate
14	28.121	$C_{20}H_{52}O_5Si_5$	512	73	14199-72-5	Xylitol, 1,2,3,4,5-pentakis-O-(trimethylsilyl)
15	28.998	$C_{17}H_{42}O_5Si_4$	438	217	56271-69-3	D-Ribofuranose
16	29.251	$C_{19}H_{46}O_6Si_4$	482	217	6736-96-5	Glucofuranoside, methyl 2,3,5,6-tetrakis-O-(trimethylsilyl)
17	29.347	$C_{17}H_{42}O_5Si_4$	438	73	55555-45-8	D-Xylopyranose
18	29.434	$C_{21}H_{52}O_6Si_5$	540	217	19126-98-8	D-Fructose
19	29.547	$C_{22}H_{52}O_7Si_5$	568	217	27531-31-3	D-Glycero-L-manno-Heptonic acid, 2,3,5,6,7-pentakis-O-(trimethylsilyl)-, .gamma.-lactone
20	29.813	$C_{18}H_{44}O_5Si_4$	452	73	0-00-0	2-Deoxy-galactose
21	29.866	$C_{21}H_{52}O_6Si_5$	540	73	6736-94-3	D-Galactose
22	29.979	$C_{21}H_{52}O_6Si_5$	540	217	7045-52-5	beta.-D-Galactofuranose
23	30.193	$C_{21}H_{52}O_5Si_5$	524	73	114656-62-1	Myo-inositol, 5-deoxy-1,2,3,4,6-pentakis-O-(trimethylsilyl)
24	30.258	$C_{14}H_{30}O_5Si_3$	362	73	72361-20-7	Pentanedioic acid, 3-oxo-, tris(trimethylsilyl) ester
25	30.770	$C_{21}H_{52}O_6Si_5$	540	204	6736-97-6	D-Glucose
26	30.940	$C_{21}H_{50}O_6Si_5$	538	73	14251-19-5	Inosose
27	31.749	$C_{17}H_{42}O_5Si_4$	438	217	56271-64-8	beta.-DL-Arabinopyranose
28	31.982	$C_{20}H_{50}O_6Si_5$	526	73	57197-35-0	Ribonic acid
29	32.030	$C_{19}H_{40}O_2Si$	328	73	55520-89-3	Hexadecanoic acid
30	33.765	$C_{21}H_{40}O_2Si$	352	75	56259-07-5	9,12-Octadecadienoic acid
31	33.810	$C_{21}H_{42}O_2Si$	354	73	21556-26-3	Oleic acid
32	34.042	$C_{21}H_{44}O_2Si$	356	73	18748-91-9	Octadecanoic acid
33	35.574	$C_{21}H_{50}O_7Si_5$	554	73	55530-80-8	D-Glucuronic acid
34	36.789	C24H38O4	390	149	117-81-7	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester
35	42.644	$C_{16}H_{32}O_4Si_3$	372	255	0-00-0	4,6-Dioxohept-2-enoic acid, tri-TMS

*The nearest library standards.

Alkaloids, antraquinones, flavonoids, catechins, tannins or phenolic compounds, saponins and triterpenes or steroids were not detected. It is evident from this information that the chemical composition of *S. domingensis* in Cuba. Contrary to expected according to the literature, sarsapogenin, smilagenin or steroids (stigmasterol, β-sitosterol and cholesterol) were not detected by GCMS analysis, according to the molecular masses of known saponins from *S. officinalis* (Cáceres et al., 2012). It is evident from this information that the chemical composition of *S. domingensis* in Cuba is different from that of *S. domingensis* rhizome found in Guatemala.

These results by GC/MS are not agree with the results of phytochemical screening and TLC found by the authors in 2017 with the same sample in ethanolic extract when preliminary chemical characterization allow to 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.

TLC results evidenced the presence of chemical compounds type flavonoids, some structures related with terpenoids structures and the presence of phenolic compounds derives from catechol (Yaque et al., 2017). Further studies are needed to establish the molecules responsible for the chemical composition and the biological activities attributed to this rhizome, specially using HPLC-MS or LC-NMR. Toluene extract is still pendant to characterize by some appropriated method waiting to improve the real composition of chemical components of this promising medicinal plant.

4. Conclusions

GC-MS is frequently applied to characterize the chemical complexity of analytical samples based on its separation and identification capacity. *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. Following solvent extraction and derivatization, 35 metabolites from different chemical groups can be characterized in one analytical run. Besides sugars, acids, and polyols, diverse derivatives and other cyclic metabolites can be efficiently detected by metabolite profiling. The results from plant research to exemplify the applicability of GC-MS profiling and concurrent detection and identification of three principal groups of chemical components and other cyclic structures. Based on experimental data from own research, the present review has emphasized the capabilities of GC-MS to deduce chemical information on diverse compounds found in complex mixtures of plant metabolites. The compounds identified can be also used as biomarkers especially for *S. domingensis* due to little research has been published for this species. This is the first time that the chemical composition of *S. domingensis* rhizome in Cuba is described.

Conflict of Interest

The authors declare no conflict of interest.

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