New Phytochemical Profile by GC-MS of Toluene Extract of Rhizomes from *Smilax domingensis* in Cuba

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Abstract— *A preliminary chemical characterization of main components of toluene extract with dried rhizomes of Smilax domingensis Willd. 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) libraries. After sample derivatization 893 chemical compounds were registered by the equipment and from them, 30 different chemical components were characterized and reported for the first time from this part of the plant in our country. The results demonstrate that 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, toluene extract.

I. 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, anti-asthmatic, anti-herpetic, anti-rheumatic 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).

FIGURE 1: Macroscopical view of rhizomes from *Smilax domingensis* Willd. in Cuba.

Between 2017 and 2018, a Cuban research team reported a preliminary study about the pharmacognostic, physicochemical and phytochemical characteristics from this part of the plant, showing the values of 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 (Yaque, J.G. et al., 2017).

A preliminary chemical characterization of main components of ethanolic extract at 95 % with dried rhizomes of *Smilax domingensis* Willd. 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. 35 different chemical components were characterized and reported for the first time from this part of the plant in our country, 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 (Soledispa et al., 2018).

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.

II. 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 toluene during 16 hours. The toluene 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 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/split less. 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 (Figure 2).

FIGURE 2: GC-MS Shimadzu QP2010, BP5 capillary column and derivatization process of the sample used in the experiment.

2.3 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.

III. RESULTS AND DISCUSSION

3.1 Chemical characterization by GC-MS

Figure 3 shows the TIC chromatogram with the retention times of different kind of chemical components present in the sample, indicating that the main amount of chemical compounds in the extract are between 14.2 and 54 minutes of retention time. The most intense peaks are between 27.5 and 31.5 minutes of retention time.

After 78 minutes of running, 893 chemical components were automatically identified from the sample, and among them, the presence of 11 carboxylic acids, 13 reductants sugars and/or their derivatives, one coumarin derivative, 3 steroids and their derivatives, one catechin and one flavonoid were tentatively characterized.

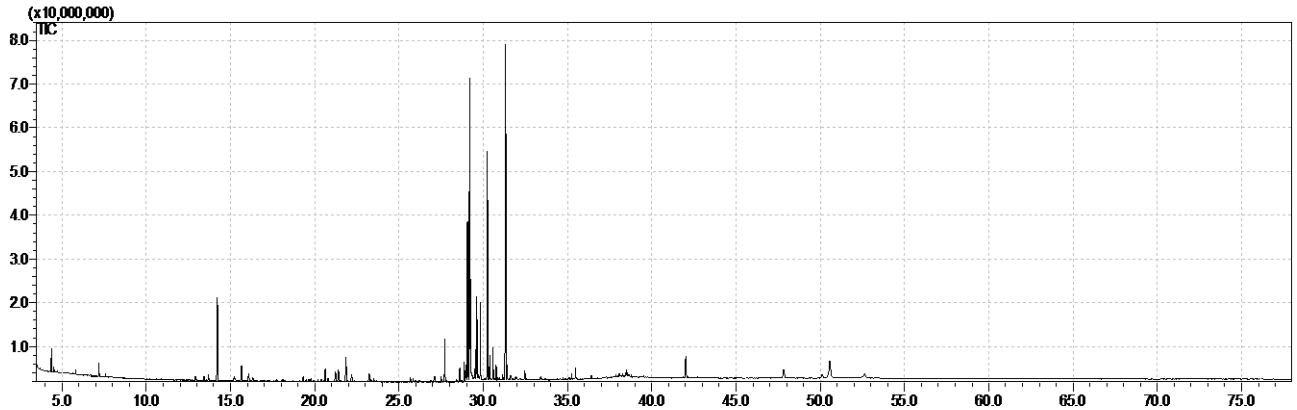


FIGURE 3: TIC chromatogram of rhizomes from *S. domingensis* Willd.

Table 1 summarize the peaks of the main components found in the extract with their retention times (Rt) and their corresponding names. The first 23 chemical compounds were discarded because they are related with the Silylation agent and their derivatives and water, as well as, the last 20 chemical components.

TABLE 1
CHEMICAL COMPONENTS CHARACTERIZED BY GC-MS FROM TOLUENE EXTRACT IN RHIZOMES OF *S. DOMINGENSIS*.

Peak #	R. time	Molecular formula	Molecular weight	Base Peak	Library ID/Compound name*
1	14.220	$C_{12}H_{32}O_3Si_3$	308	73	Trimethylsilyl ether of glycerol
2	15.646	$C_{10}H_{22}O_4Si_2$	262	147	Butanedioic acid (Succinic acid)
3	16.085	$C_{12}H_{30}O_4Si_3$	322	73	Propanoic acid, 2,3-bis [(trimethylsilyl)oxy
4	19.310	$C_{13}H_{30}O_5Si_3$	350	73	Malic acid
5	20.623	$C_{14}H_{32}O_5Si_3$	364	73	3-Hydroxy glutaric acid
6	20.795	$C_{11}H_{16}O_3Si$	224	209	Benzoic acid, 4-[(trimethylsilyl)oxy]-, methyl ester
7	21.405	$C_{12}H_{28}O_3Si_2$	276	73	Hexanoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester
8	23.231	$C_{11}H_{26}O_3Si_2$	262	73	Butanoic acid, 3-methyl-2 [(trimethylsilyl)oxy]-TMS
9	27.720	$C_{20}H_{52}0_5Si_5$	512	73	Ribitol, 1,2,3,4,5-pentakis-O-(trimethylsilyl)-
10	28.601	$C_{14}H_{32}O_5Si_3$	364	73	D-Arabinonic acid, 2,3,5-tris-O-(trimethylsilyl)-, .gamma.-lactone
11	28.875	$C_{19}H_{46}O_6Si_4$	482	217	Glucofuranoside, methyl 2,3,5,6-tetrakis-O-(trimethylsilyl)-, .alpha.-D-
12	29.061	$C_{21}H_{52}O_6Si_5$	540	217	D-Fructose
13	29.205	$C_{17}H_{42}O_5Si_4$	438	217	D-Ribofuranose, 1,2,3,5-tetrakis-O-(trimethylsilyl)
14	29.486	$C_{18}H_{44}O_5Si_4$	452	73	2-Deoxy-galactose, tetrakis(trimethylsilyl)
15	29.621	$C_{21}H_{52}O_6Si_5$	540	217	beta.-D-Galactofuranose
16	39.241	$C_{21}H_{52}O_6Si_5$	540	73	D-Galactose
17	30.391	$C_{21}H_{52}O_6Si_5$	540	73	Glucose
18	30.575	$C_{21}H_{50}O_6Si_5$	538	73	Inososе-2, 1,3,4,5,6-pentakis-O-(trimethylsilyl)-
19	30.764	$C_{24}H_{62}O_6Si_6$	614	73	Trimethylsilyl ether of glucitol
20	31.405	$C_{21}H_{52}O_5Si_5$	524	73	Myo-inositol, 5-deoxy-1,2,3,4,6-pentakis-O-(trimethylsilyl)
21	32.470	$C_{12}H_{28}O_3Si_2$	276	73	Pentanoic acid, 4-methyl-2-[(trimethylsilyl)oxy]-, trimethylsilyl ester
22	33.185	$C_{20}H_{40}O_2$	312	88	Octadecanoic acid, ethyl ester
23	33.400	$C_{21}H_{40}O_2Si$	352	73	9,12-Octadecadienoic acid (Z,Z)-, trimethylsilyl ester
24	33.450	$C_{21}H_{42}O_2Si$	354	73	Oleic acid
25	42.570	$C_{17}H_{14}O_4$	282	282	Coumaran-3-one, 6-methoxy-2-[4-methoxybenzylidene]
26	44.290	$C_{32}H_{56}OSi$	484	83	Stigmasterol trimethylsilyl ether
27	45.265	$C_{39}H_{54}OSi$	458	129	Cholesterol trimethylsilyl ether
28	46.555	$C_{34}H_{52}O_3$	508	327	Cholestan-7-ol, 8,14-epoxy-3-(phenylmethoxy)-
29	47.600	$C_{30}H_{54}O_6Si_5$	650	73	Catechin, penta-TMS-ether
30	48.280	$C_{23}H_{22}O_6$	394	192	Rotenone

The nearest library standards.

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).

Alanine (L-Alanine or alpha-Aminopropionic acid) with Molecular formula $C_3H_7NO_2$, Molecular weight 89 and Base peak 44 was detected at 12.315 min of retention time, but the coincidence index was low (only 39 %) (Figure 4). A Glycine derivative (Glycine, N-formyl-N-(trimethylsilyl)-, trimethylsilyl ester), with MF $C_9H_{21}NO_3Si$, MW 247 and BP 73, was detected at 19.425 min of Rt, with a coincidence index of 60 % (Figure 5). Although the percentage of coincidence are different in both cases, and are not above 85-90 %, we are not discarding the presence of aminoacids in the Cuban rhizomes of *S. domingensis.*

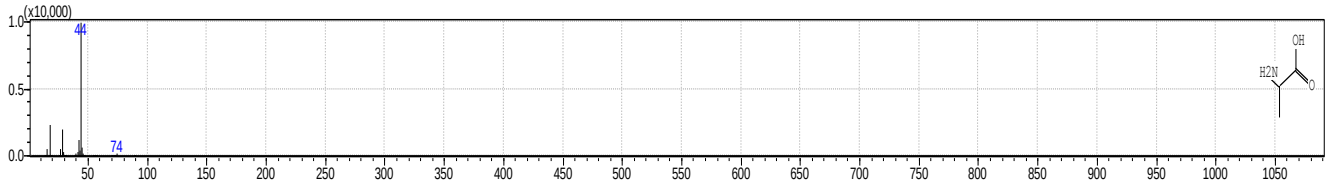


FIGURE 4: Mass spectrum detected by GC-MS of Alanine.

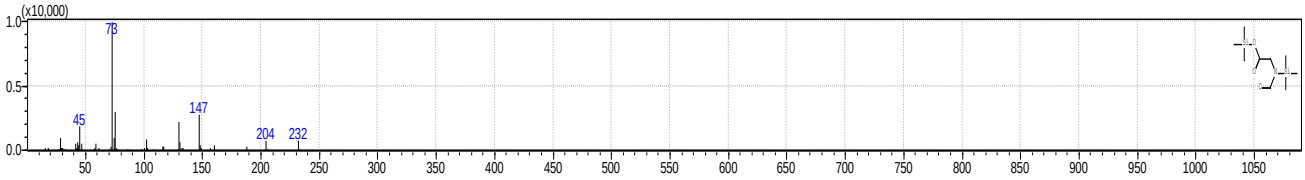
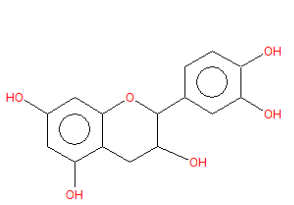


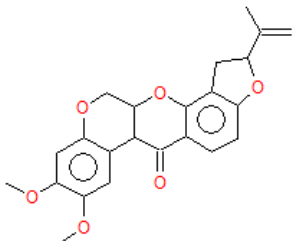
FIGURE 5: Mass spectrum detected by GC-MS of Glycine, N-formyl-N-(trimethylsilyl)-, trimethylsilyl ester.

Contrary to expected according to the literature, sarsapogenin and smilagenin were not detected by GCMS analysis, according to the molecular masses of known saponins from *S. officinalis* (Cáceres et al., 2012), but in this case, our results confirm the presence of different steroidal compounds like stigmasterol and cholesterol, corroborating the results found by Cáceres et al., 2012 in Guatemala.

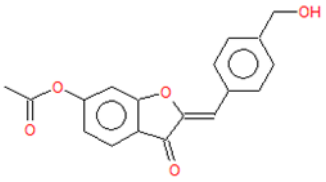
This new phytochemical profile shows the matches of 11 carboxylic acids and 6 reductants sugars or their derivatives (~34 %), and the presence of two new compounds that belongs to flavonoids group: Catechin and Rotenone, one coumarin derivative and three steroidal components or their derivatives comparing with the previous results reported by our research team in ethanolic extracts of this part of the plant (Figure 6).



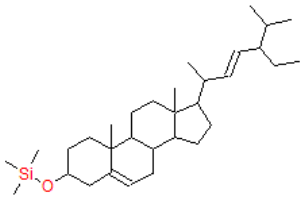
Catechin



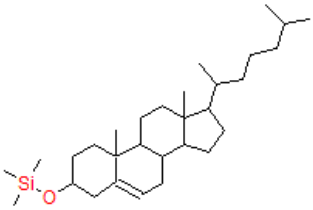
Rotenone



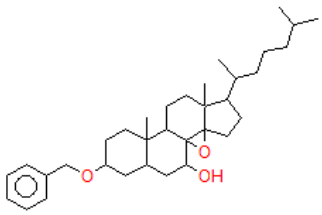
Coumaran-3-one-6-methoxy-2-[4-methoxybenzylidene]



Stigmasterol trimethylsilyl ether



Cholesterol trimethylsilyl ether



Cholestan-7-ol, 8,14-epoxy-3-(phenylmethoxy)

FIGURE 6: New chemical components found in *S. domingensis* by GC-MS in Cuba.

IV. CONCLUSIONS

GC-MS is frequently applied to characterize the chemical complexity of analytical samples based on its separation and identification capacity. Following solvent extraction and derivatization, 30 metabolites from different chemical groups can be characterized in one analytical run with a high percentage of coincidence of those compounds characterized by the same methodology in ethanolic extract from this part of the plant, principally sugars and/or derivatives and carboxylic acids. Six new phytochemical components were tentatively detected, although the authors not discarding the presence of two chemical compounds that belongs to aminoacids group. The results from plant research to exemplify the applicability of GC-MS

profiling and concurrent detection and identification of six principal groups of chemical components and other cyclic structures. Based on experimental data from own research, the present research 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. 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. Saponins still remain indeterminable in extracts from *S. domingensis* in our country.

CONFLICT OF INTEREST

The authors declare that they have not conflict of interest.

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