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Characterization by GC-MS of Chemical Components of Pistils with Pollen from *Taliparitti Elatum* Sw. (Fryxell) Malvaceae

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Abstract

Gas chromatography (GC) is an analytical technique widely applied to the analysis of mixtures of organic compounds. Ethanolic extract of pistils with pollen from the flowers of *Taliparitti elatum* Sw. was analyzed by GC-MS 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. Gas Chromatography Mass Spectrometry (GC-MS) analysis revealed the presence of 280 compounds and from them, 134 chemical components were characterized and reported for the first time from this part of the plant *Taliparitti elatum*. Our results demonstrate the developed method could be employed as a rapid and versatile analytical technique for identification of chemical constituents and quality control of *Taliparitti elatum* samples.

Keywords: Taliparitti elatum; GC-MS; Ethanolic extract; Pistils with pollen; Chemical components.

1. Introduction

Natural products (also known as secondary metabolites) have always been a significant source of new lead compounds in pharmaceutical industries. About half of the drugs currently in clinical use are natural products or synthetic molecules based on natural product scaffolds. The medicinal plants as sources of bioactive compounds continue to play a dominant role in the maintenance of human health [1].

The plant kingdom produces a vast number of different chemical structures, which is predicted to exceed 200,000 metabolites. Moreover, MS information of silylated natural products is insufficiently represented in available compound libraries for GC-MS platforms based on electron ionization (EI). More suitable chromatographic profiling platforms such as LC-MS are available for analysis of metabolites with higher polarity and molecular weights up to 2000 Da [2].

Mass Spectrometry (MS) has proved to be one of the most effective techniques in biomedical research, in special when complex matrixes of biological samples must be analyzed. The main advantages of MS are its high sensitivity, which allows analysis of compounds present in the µg scale, and high specificity, as it is able to separate molecules of the same molecular weight but different atom composition, and sometimes even to differentiate stereoisomeric compounds. Its easy coupling to separation techniques such as liquid and gas chromatography is also an excellent advantage [3].

Taliparitti elatum tree is quite attractive with its straight trunk, broad green leaves and hibiscus-like flowers. It grows quite rapidly, often attaining 20 meters (66 ft) or more in height. The attractive flower changes color as it matures, going from bright yellow to orange red and finally to crimson (Figure 1). The name mahoe is derived from a Caribe word. The 'blue' refers to blue-green streaks in the polished wood, giving it a distinctive appearance [4].

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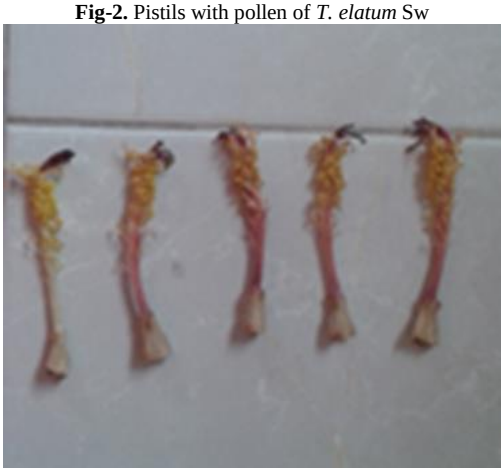
Fig-1. Flowers of *Taliparititi elatum* Sw

The external morphology of the pistil from the flowers of *T. elatum* present a stamina column measuring 4-10 cm long, filaments and anthers yellow in color, styles with red branches 8-12 mm long, stigmas red purple in color (Figure 2) [5].

Fig-2. Pistils with pollen of *T. elatum* Sw



Form this part of the flowers the toluene extract components characterized by GC-MS allows to inferred the presence of 16 acids or their derivatives, Threitol, several derivatives of D-Galactose and D-Xylopyranose, four esters and possibly a steroid [6]. GS-Mass of chemical components of ethanol extract from Bark of *Taliparititi elatum* Sw. (Fryxell) Malvaceae was reported [7].

The aim of the present study was to identify the phyto components of this plant and subjecting the ethanol extract of the plant flowers, specifically pistils with pollen, to Gas Chromatography–Mass Spectrum analysis. This work will help to identify the newer compounds, which may be used in drug design products and for its therapeutic values.

2. Material and Methods

2.1. Plant Material

Flowers were collected in January 2017 in the gardens of the Faculty of Pharmacy and Foods at Havana University, and identified at the herbarium of National Botany Garden of Havana, where the voucher specimen no. HAJB 82587 has been deposited. The collection of the flowers in Martinic was realizing at the same time. A voucher specimen is deposited and registered in French Pharmacopeia as Fournet 1752 (4232 Guad). Both, Cuban and Martinican specimens are registered as *Hibiscus elatus* S.w.

2.2. Extract and Samples Preparation

Dark red flowering types were collected daily. Pistil with pollen were separated from the rest of flowers components manually. The isolated pistils with pollen used were dried in an oven with controlled temperature, at 40˚C, during 5 days. 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.

2.3. Procedures, Instrumentation and Parameters

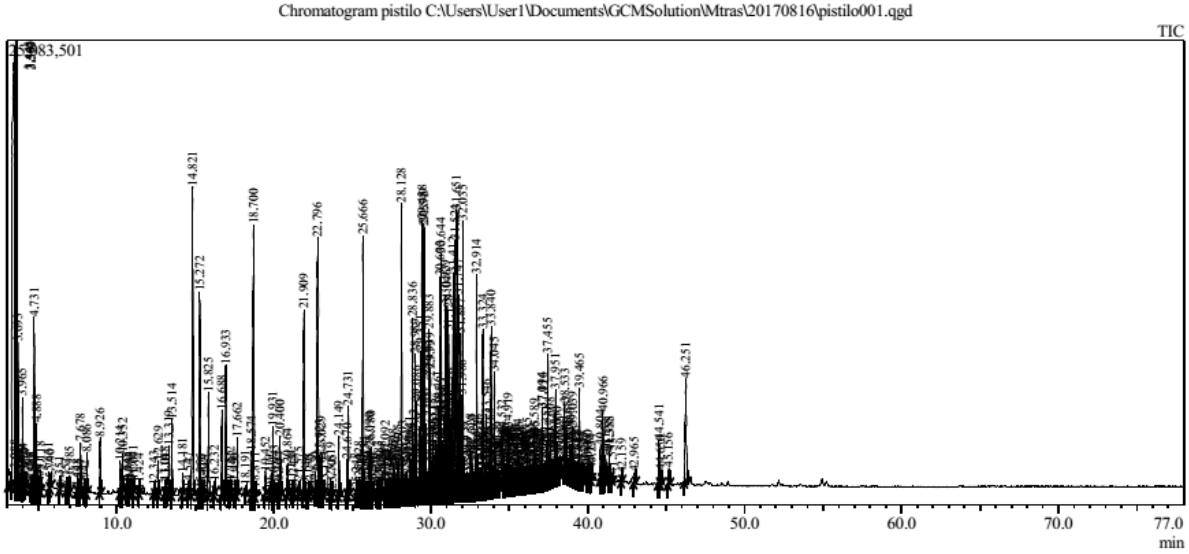
The sample were subjected to chromatographic analysis in equipment GC/MS, brand Shimadzu QP2010, equipped with a splitter split/splitless. With a BP5 (30 m × 0.25 mm × 0.25 microns) capillary column under the following chromatographic conditions: Helium gas carrier obtained by electron impact fragments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

3. Results and Discussion

Gas chromatography (GC) is an analytical technique widely applied to the analysis of mixtures of organic compounds [8]. The partition of the mixture takes place between a stationary liquid phase and a mobile gas phase. As analysis in the gaseous phase requires volatile derivatives, most of the GC procedures on non-volatile compounds use prechromatographic derivatization of the samples. Gas Chromatography (GC) is one of the key techniques for the separation of organics and, coupled to MS, one of the most common techniques of structural identification. However, flavonoids are largely nonvolatile, and need be derivatized; also, they are usually thermally unstable.

After 78 minutes of running the most concentrated zone of chemical components was between 3 and 47 minutes of retention time. From this time and up to the end of running lower peaks could be found in the chromatogram (Fig. 3) but all of them without importance because there are related with BSTFA derivatives. The last chemical component was recorded by the equipment at 46.251 minutes.

Fig-3. Current chromatogram of ethanolic extract of pistils with pollen from *T. elatum* Sw

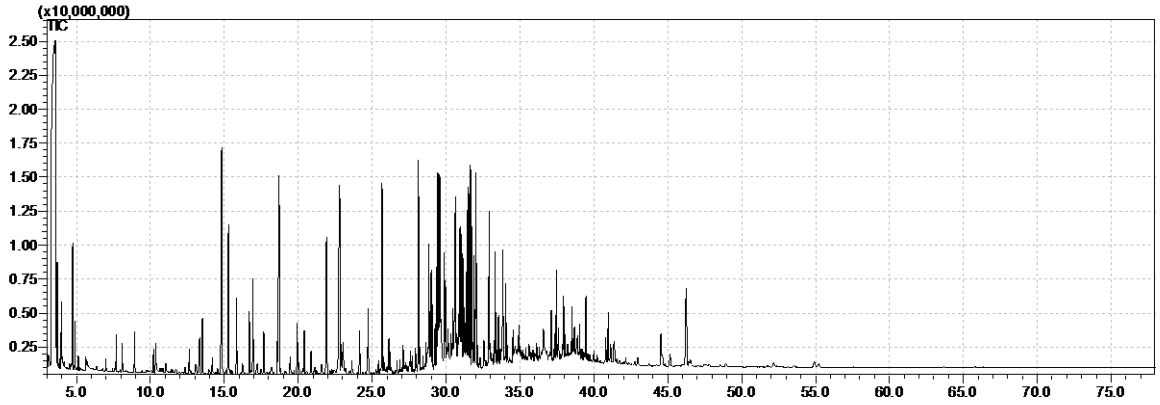


Sample processing for GC-MS-based metabolite profiling include solvent extraction, concentration to dryness and consecutive derivatization, often carried out in a two-step procedure. In the second step, extracted metabolites are derivatized with silylating reagents. The latter step is crucial for the adequate derivatization of non-volatile compounds, in order to capture a huge variety of metabolites with polar characteristics and high boiling points on a GC-MS system. Detectable compounds comprise sugars (mono-, di- and trisaccharides), sugar alcohols/acids, amino and fatty acids, phosphorylated intermediates and many plant secondary metabolites such as phenolics, terpenoids, steroids and alkaloids.

To have a better observation, the chromatogram was clarified eliminating the Peak Integrate for TIC (All Group). Figure 4 shows the current chromatogram of ethanolic extract of pistils with pollen from *T. elatum*. From the 280 different chemical compounds that were detected by the detector, only 134 were reported, and chosen as to be presenting into ethanolic extract of this part of the flowers.

Another important factor affecting the quality of metabolite data is the occurrence of artefacts of silylated compounds in GC-MS profiling [9]. Unexpected by-products might add to the complexity of peaks in a chromatogram and interfere with the identification process. This includes also conversion reactions of unstable intermediates, e.g., arginine to ornithine when using BSTFA or MSTFA, potentially leading to misinterpretation of metabolic data [10]. But more important, multiple peaks of one and the same metabolite, i.e., with different degree of TMS silylation of the original molecule, might be detected. This is particularly true for those metabolites with several functional groups such as amino acids (-COOH, -NH_2, -OH) and monosaccharides which carry a high number of hydroxy groups. The amino acid serine e.g., shows four active hydrogens which might be exchanged by TMS groups.

Fig-4. Current chromatogram of ethanolic extract of pistils with pollen from *T. elatum* Sw. without Peak Integrate for TIC (All Group)



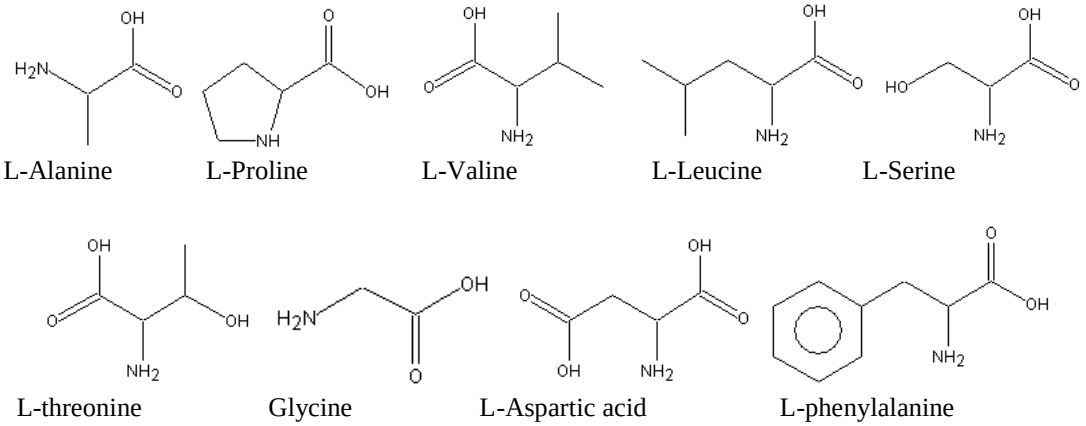
Taking into account the results of phytochemical screening done by our research team in 2017, we are proposing the presence of fat and oils, triterpenes and/or steroids, reductants sugars, coumarins, alkaloids and aminoacids or amines [5]. None other types of chemical constituents were reported to be presenting in ethanolic extracts from this part of the flowers such as flavonoids, anthocyanidins and tannins. Is very interesting the high amount of aminoacids (9), reductants sugars (28) and organic acids (78) in the sample. Table 1 list the name of nine aminoacids proposed to be presenting into ethanolic extracts of pistils with pollen from *T. elatum* (Sw.).

Table-1. Proposal aminoacids in the pistils with pollen's ethanolic extracts from *T. elatum*

Peak#	R. Time	I. Time	F. Time	Area	Area %	Height	Height %	A/H	Name
16	4.642	4.620	4.670	1045023	0.04	447904	0.06	2.33	L-Alanine, ethyl ester
38	11.041	11.010	11.110	1703252	0.07	679487	0.08	2.51	L-Proline, ethyl ester
41	12.629	12.570	12.680	4726012	0.19	1775229	0.22	2.66	L-Valine, N- (trimethylsilyl)-, trimethylsilyl ester
47	14.547	14.505	14.600	879365	0.04	352887	0.04	2.49	L-Leucine, N-(trimethylsilyl)-, trimethylsilyl ester
60	17.662	17.590	17.735	9011070	0.36	3062008	0.38	2.94	L-Serine, N,Obis(trimethylsilyl)-, trimethylsilyl ester
62	18.574	18.510	18.610	7057569	0.28	2440866	0.30	2.89	N,O,O-Tris(trimethylsilyl) -L-threonine
65	19.452	19.395	19.515	3658691	0.15	1190673	0.15	3.07	Glycine, N-formyl-N-(trimethylsilyl)-, trimethy
81	23.029	22.975	23.145	9937581	0.40	2235982	0.28	4.44	L-Aspartic acid, N-(trimethylsilyl)-, bis(trimeth
93	25.842	25.800	25.895	2393436	0.10	760477	0.09	3.15	N,O-Bis(trimethylsilyl)-L-phenylalanine

Another five aminoacids derivatives were found in ethanolic extracts of pistils with pollen, such as: l-Alanine, N-(trimethylsilyl)-, trimethylsilyl ester; L-Proline, 5-oxo-1-(trimethylsilyl)-, trimethylsilyl ester; L-Proline, 1-(trimethylsilyl)-, trimethylsilyl ester; L-Proline, 1-(trimethylsilyl)-4-[(trimethylsilyl) and L-Leucine, N-(tert-butyldimethylsilyl)-, tert-but. Figure 5 represents the chemical structure of the nine identified aminoacids.

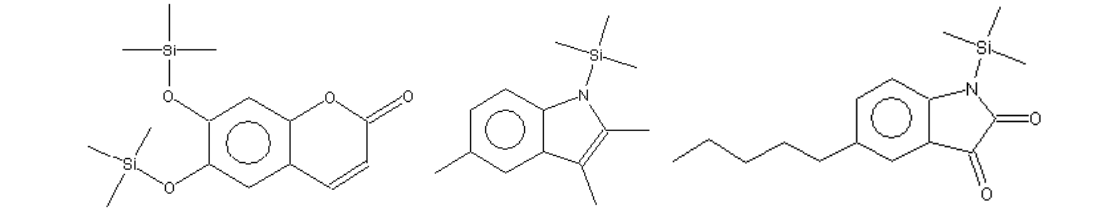
Fig-5. Chemical structures of nine proposal aminoacids in ethanolic extracts of pistils with pollen



In most cases phenolic structures are derived from aromatic amino acids such as phenylalanine and tyrosine. Detectable phenolic structures comprise monophenols such as thymol (an aromatic monoterpene), benzyl alcohols, phenylethanoids (e.g., tyrosol), and the coumarins (e.g., umbelliferone). The huge classes of aromatic acids include benzoic acid and cinnamic acid derivatives with different degree of hydroxylation and methoxylation.

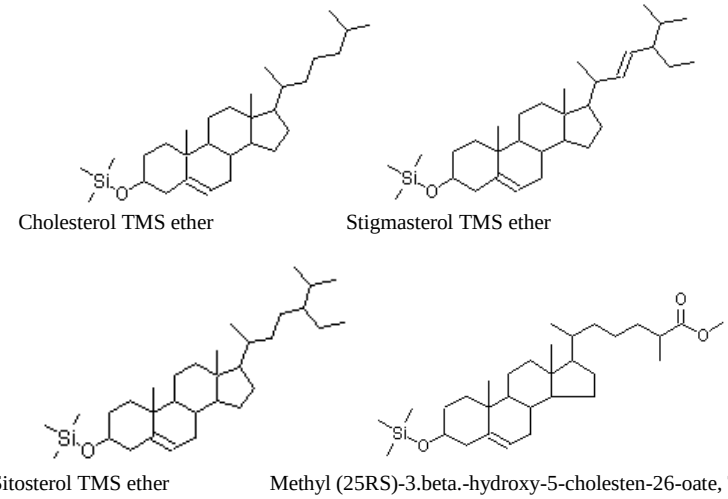
Only one coumarin and two compounds relatives with alkaloids backbone were present as chemical components in this kind of extracts from this part of the flowers: 6,7-Dihydroxycoumarin, di-TMS (Peak 176; Retention time 32.795 min); 1H-Indole, 2,3,5-trimethyl-1-(trimethylsilyl)- (Peak 109; Retention time 27.505 min) and 1H-Indole-2,3-dione, 5-pentyl-1-(trimethylsilyl)- (Peak 212; Retention time 35.320). Figure 6 shows the chemical structures of those components.

Fig-6. Chemical structures of 6,7-Dihydroxycoumarin, di-TMS, 1H-Indole, 2,3,5-trimethyl-1-(trimethylsilyl) and 1H-Indole-2,3-dione, 5-pentyl-1-TMS



Six structures corresponding to triterpenes or steroids were found in the ethanolic extracts. They were: Prosta-5,13-dien-1-oic acid, 15-methyl-9,11,15-tris[(trimethylsilyl)oxy]-, trimethylsilyl ester (Peak 193; Retention time 33.970); Prosta-10,13-dien-1-oic acid, 9-oxo-15-[(trimethylsilyl)oxy]-, trimethylsilyl ester (Peak 271; Retention time 41.133); Methyl (25RS)-3.beta.-hydroxy-5-cholesten-26-oate, trimethylsilyl ether (Peak 277, Retention time 44.541) Cholesterol trimethylsilyl ether (Peak 278, Retention time 44.650); Stigmasterol trimethylsilyl ether (Peak 279, Retention time 45.156); β- Sitosterol trimethylsilyl ether (Peak 280, Retention time 46.251). The structures of those last four mentioned components are representing in Figure 7.

Fig-7. Proposal steroidal structures identified in ethanolic extracts of pistils with pollen

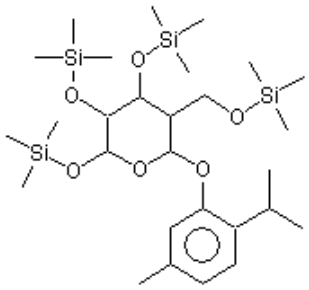


Twenty-eight different structures relatives with reductants sugars and their derivatives were tentatively identified in the sample matrix. Monosaccharides and Disaccharides, Pentoses and Hexoses were the most

representing examples of sugar constituents found in the analyzed extract. D-Glucose, D-Fructose, D-Galactose, Mannose, Xylulose, Altrose, D-Ribose, D-Turanose, Melibiose, Maltose and Saccharose were the common compounds tentatively suggested to be present in pistils with pollen ethanolic extracts of the flowers from *T. elatum*.

Thymol (an aromatic monoterpene), was detected like a glycosylated monoterpene (Peak 254, molecular mass 600, molecular formula $C_{28}H_{56}O_6Si_4$, Base Peak 73) at 38.687 min of retention time and identified as Thymol-.beta.-d-glucopyranoside, tetra(trimethylsilyl). The structure of such compound is shown in Figure 8.

Fig-8. Chemical structure of Thymol-.beta.-d-glucopyranoside, tetra(trimethylsilyl)



Another subgroup of chemical compounds or their respective derivatives that was identified into the extract's components belongs to the sugar-alcohols. Five different compounds were tentatively identified: Arabinitol, pentakis-O-(trimethylsilyl)-; Xylitol, 1,2,3,4,5-pentakis-O-(trimethylsilyl)-; Ribitol,1,2,3,4,5-pentakis-O-(trimethylsilyl)-; Pentitol, 1,3-didesoxy-tris-O-(trimethylsilyl)- and Myo-Inositol,1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-. Xylitol silylated compound was repeated five times with different retention times.

Among the acids or their derivatives, 78 different chemical constituents were tentatively identified in ethanolic extract of pistils with pollen in *T. elatum*. They include saturated and unsaturated acids, aromatic and aliphatic acids, esters of aromatic acids, hydroxy acids, and etc. Propanoic acid was the first acidic component detected at 3.752 min, while D-Erythro-Pentonic acid derivative was the last one at 42.159 min. Nonanoic acid, trimethylsilyl ester was found at 17.222 min of retention time. L-Ascorbic acid, 2-O-methyl-3,5,6-tris-O-(trimethylsilyl) was also detectable at 36.444 min.

These results are according to those reported by Gonzalez in 2016 when was suggested to be present into toluene extracts of this part of the flower the presence of 16 acids or its derivatives such as: propanoic acid, pentanoic acid, hydroxypropanoic acid, nonnanoic acid, dodecanoic acid, docosanoic acid, oleic acid, several derivatives from D-galactose and D-xylopyranose and some sterols.

The vast diversity of *N*-containing cyclic metabolites from plants, simple phenolics, aromatic acids and related structures, *O*-containing cyclic structures and other cyclic structures, ketones, aldehydes, alkanes, alkenes, alcohols, etc., were present as components into the extracts. To get a real idea of the phytoconstituents from this part of the flower Supplementary data associated with this article can be found in the online version.

4. Conclusions

The present study provides the most thorough qualitative investigation of chemical compounds and its derivatives in pistils with pollen of the flowers in *T. elatum* performed so far. For the very first time, qualitative data on these analytes in the drug were determined after detailed validation of a sensitive, cheap and reliable GC-MS method. The method enables the appropriate determination of such components, which obviously contribute to the activity, for the quality control of the drug and of herbal medicinal products containing *T. elatum*. Besides sugars, acids, and polyols, diverse phenolic and other cyclic metabolites can be efficiently detected by metabolite profiling. The paper describes own results from plant research to exemplify the applicability of GC-MS profiling and concurrent detection and identification of phenolics and other cyclic structures. Based on experimental data from own research, the present paper has emphasized the capabilities of GC-MS to deduce chemical information on phenolics and cyclic compounds found in complex mixtures of plant metabolites.

Appendix A. Supplementary Data

Supplementary data associated with this article can be found, in the online version, at http://

Conflict of Interest Statement

Authors declare not conflict of interest.

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