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Identification of compounds by GC-MS analysis of the ethyl acetate fraction of the hydroalcoholic extract of *Lippia multiflora leaves* before extraction of the essential oil

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Abstract: *Lippia multiflora*, commonly known as Gambian tea, is part of the Verbenaceae family, which classically includes more than 91 genera. *L. multiflora* is a plant used for its multiple medicinal properties in many traditional and phytotherapeutic cultures. This aromatic plant is very rich in minerals, notably potassium, calcium, fluorine, zinc, phosphorus, magnesium, silicon and iron. Phytochemical analysis revealed in its leaves the presence of polyphenols, flavonoids, sterols and terpenes, alkaloids, saponins and tannins. However, few structures of plant leaf molecules have been identified. Twelve (12) molecules were obtained by gas chromatography coupled with mass spectrometry analysis (GC-MS) of the ethyl acetate fraction of the hydroethanolic extract (70% ethanol) of *L. multiflora* leaves. before extraction of the essential oil. Within these detected compounds, there are seven (7) terpenes and five (5) polyphenols. Ten (10) of them have not yet been identified in *L. multiflora* leaves. This study shows that the leaves of *L. multiflora* contain new bioactive molecules of pharmacological interes.

Keywords: *Lippia multiflora*, Gas chromatography, Mass spectrometry, Hydroethanolic extract, Bioactive molecules

1. Introduction

For centuries, plants have played an instrumental role in traditional medicine, serving as a source of treatments for a wide range of illnesses. Today, with the rise of antibiotic resistance and the search for more sustainable and accessible therapies, plants continue to offer immense potential as a source of medicinal molecules. Indeed, aromatic plants and their essential oils constitute a precious treasure in the field of health and well-being (Taibi *et al.*, 2023). These concentrates of bioactive compounds extracted from plants offer a range of remarkable properties that make them essential in many fields. Far from being limited to their pleasant scent and their ancestral healing properties, plant aromas today reveal unsuspected potential in many fields, ranging from beauty to health to industry (Elbouzidi *et al.*, 2024). *Lippia multiflora* (Verbenaceae), grows in the Guinean and Sudano-Guinean savannahs (Mwangui *et al.*, 1991). The leaves of L. multiflora are strongly aromatic. Their shapes and colors differ from one variant to another. The leaves of the plant are used in the treatment of liver failure, fever, high blood pressure and malaria (Nsonde Ntandou *et al.*, 2010), gastrointestinal and enteric disorders (Bruneton, 1999) and also used in the treatment of diarrhea (Anonyme, 2013). Metabolites

known to belong to special classes of organic compounds possessing potent pharmacological activities have been reported in the literature, including essential oils, lignins, cellulose, tannins, starch, oxalates, flavonoids, saponins glycosides, peptides, caffeine, terpenes and alkaloids (Kunle *et al.*, 2003), (Jigam *et al.*, 2009), (Hamed *et al.*, 2021). However, very few of them have been isolated and characterized from the plant. Several phytochemical studies have revealed that the essential oil of *L. multiflora*, whose chemical composition is thymol/p-cymene/thymol acetate type (Durand *et al.*, 2011) contains 1,8-cineol, linalool, α -terpineol and citral (Kunle *et al.*, 2003), (Jigam *et al.*, 2009). With numerous pharmacological and biological effects (Gouollaly, 2010). Furthermore, hydroethanolic extracts and their ethyl acetate fraction have been shown to have antioxidant (Soumahoro *et al.*, 2020) and antifungal properties (Goly *et al.*, 2015).

The structures of the ethyl acetate fractions of plant leaves would justify these biological activities. The study aims to identify and characterize the structures of certain known compounds present in the leaves of Lippia multiflora before the extraction of the essential oil by application of gas chromatography analysis coupled with mass spectrometry (GC-MS).

2. Methodology

2.1 material

2.1.1 Plan material

Plant material consists of leaves. The fresh leaves of *L. multiflora* were harvested between 6:00 a.m. and 6:30 a.m. in Yamoussoukro (6° 54'24158" North and 5°19'25066" West) precisely in Djamalabo in the center of Côte d'Ivoire. *L. multiflora* was identified by Mr. Amani N'Guessan, botanist at the Institut National Polytechnique Félix HOUPHOUËT-BOIGNY (INP-HB) in Yamoussoukro. The leaves of *Lippia multiflora* were manually separated from the stem and transported in a bag to the Laboratory of Industrial Processes, Synthesis and New Energies (LAPISEN) of the INP-HB. The leaves were dried at room temperature of the laboratory (25°C ± 2) in the shade for 7 days. Dried leaves of *L. multiflora* were crushed using an electric grinder and the powder obtained was sifted using a sieve with a mesh diameter of 0.5 mm. The powder obtained was stored at 4°C in a dark bottle until use.

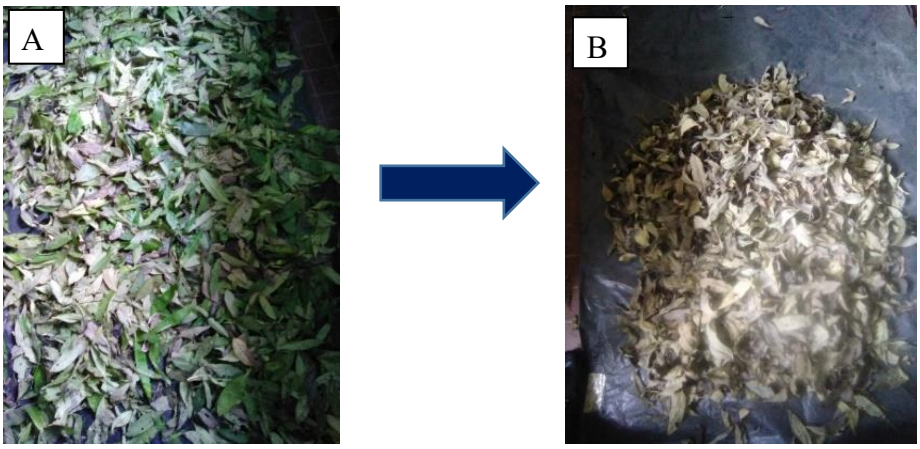


Figure 1. Photographs of *Lippia multiflora leaves* collected before drying (A) and after drying (B)

2.1.2 Experimental equipment

GC-MS analysis was performed according to the protocol described by (Kadda *et al.*, 2022) on a Perkin Elmer Clarus 680GC 600C MS equipped with a 60 m long Restek Rtx-5ms column, 0.25 mm

internal diameter and 0.25 µm stationary phase film thickness. Helium was used as carrier gas at a fixed flow rate of 1 mL/min. The oven temperature program was 80°C for 2 min, then a gradient of 5°C/min was applied up to 300°C. This latter temperature was maintained for 14 min for a total analysis time of 60 min. The injector temperature was set at 300°C. The injection was carried out in split mode with a ratio of 1:50. The mass spectrometer was set to electron impact mode with an ionization source temperature of 200°C with an electron energy of 70 eV, a scanning speed of 200 scans/min and a scanning range between 50 and 600 m/z.

2.2. Methods

2.2.1. Sample preparation

The hydroethanolic extract was prepared according to the method described by Yayé and colleagues (Guillaume et *al*., 2011). A mass of 100 g of dried leaf powder of *L. multiflora* is macerated in 1 L of an ethanol/water mixture (70/30: v/v) with magnetic stirring for 24 hours. After decantation, the mixture is filtered through hydrophilic cotton and Whatman paper N^{o} 2. The operation was repeated until the ground material was exhausted. The filtrate obtained was collected and concentrated at reduced pressure at a temperature of 40°C using a BUCHI 46 type rotary evaporator. The filtrate obtained is freeze-dried to give the hydroethanolic extract (E_{HA}) before extraction of essential oil. As part of our work, the fractions were obtained from the hydroethanolic extract using extraction by increasing polarity (Bouamama *et al*., 2006). Solvents used in order of increasing polarity are hexane, dichloromethane, ethyl acetate, ethanol and water. Thus 10 g of hydroethanolic extract were dissolved in 100 mL of distilled water and the homogeneous mixture was transferred to a separatory funnel. This mixture undergoes a liquid-liquid partition in a volume of 500 mL of solvent of increasing polarity. For each solvent, the operation is repeated 3 times. The organic phases were combined and dried over anhydrous magnesium sulfate. After double filtration on cotton and Whatmann paper N°2, the extraction solvent is eliminated under reduced pressure to give the fractions with hexane (F_{Hex}), dichloromethane (F_{DCM}), acetate d ethyl (F_{AE}). The aqueous phase is concentrated and extracted by maceration with ethanol. After filtration on cotton and Whatman paper N°2, the ethanol is eliminated under reduced pressure to give the ethanol fraction (F_{ETH}). The final aqueous phase is lyophilized to give the aqueous fraction (F_{AQ}). All these fractions were stored in the refrigerator at 4°C in a dark container. Then, a mass of 10 mg of the ethyl acetate fraction was derivatized by adding 250 µL of a mixture of N, O-(trimethylsilyl) trifluoroacetamide and Trimethylchlorosilane (BSTFA + TMCS, 99:1 (v/v)) and 250µL of pyridine. The mixture obtained was vortexed for 2 min then heated to 70°C in an oven for 30 min. One (1) µL of the solution obtained was injected into the chromatograph for analysis.

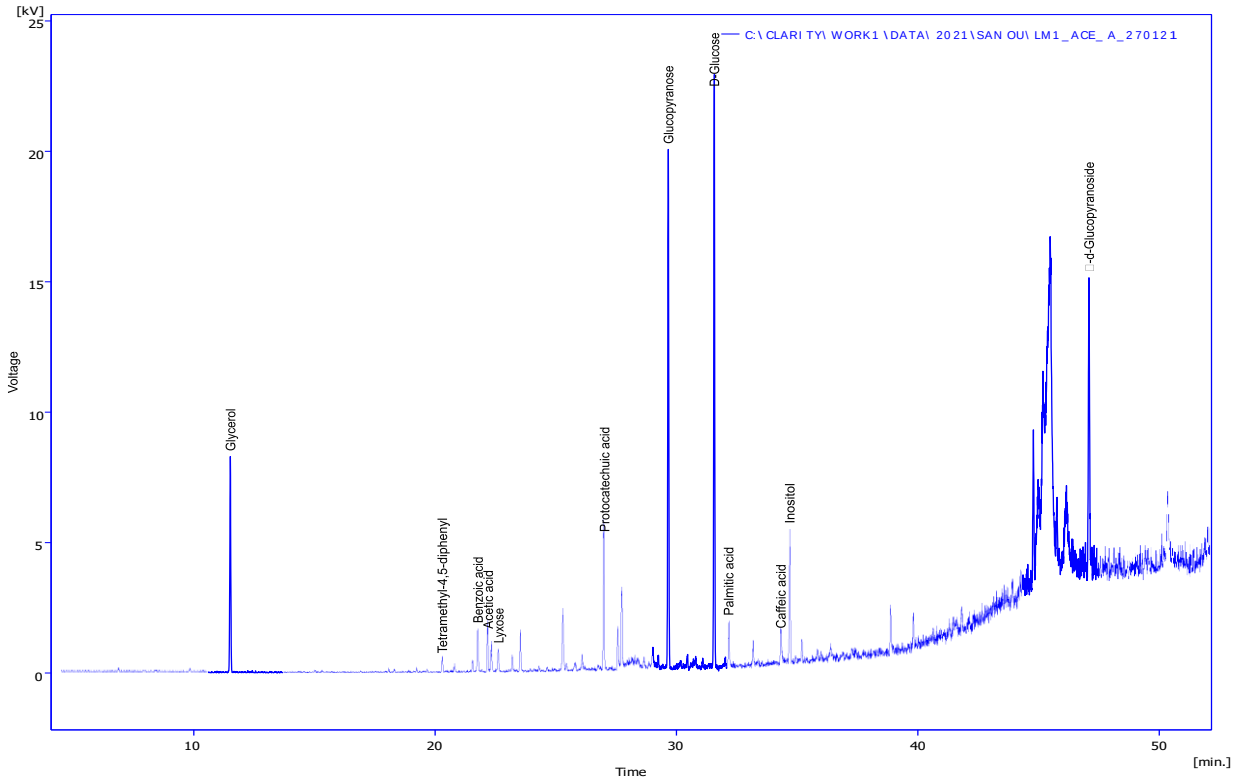
2.2.2. GC-MS analysis method

Gas chromatography coupled with mass spectrometry (GC-MS) allows the identification of some compounds. For plant extracts, this analysis requires acid hydrolysis of the different samples followed by silylation. Thus, non-volatile samples are transformed into more volatile silylated derivatives by derivatization. The identification of silylated compounds was possible by comparing the retention times with those of the standards. Spectral data were obtained from the Wiley and NIST libraries (Koné, 2018). The injection of these silylated derivatives into the chromatographic system leads to GC spectra and mass spectra which allow the sorting of compounds according to their retention index and the comparison of the mass spectra with those of the compounds in the mass spectrometer databases results in the identification of the different compounds in the sample.

3. Results and Discussion

3. 1. Identification by GC-MS

Identification of the compounds by GC-MS was possible, by comparing the retention times of the silylated compounds with those of the standards and the spectral data obtained from the Wiley and NIST libraries. **Figure 2** presents the GC chromatogram of the ethyl acetate fraction from the hydroethanolic extract of Lippia multiflora leaves before extraction of the essential oil. This chromatogram shows the presence of several peaks corresponding to several silylated compounds with retention times between 11.516 and 47.091min. The results obtained are recorded in **Table 1.**



Figures 2. GC chromatogram of the ethyl acetate fraction before extraction of the essential oil

Table 1. Compounds detected in the ethyl acetate fraction of the hydroethanolic extract of *Lippia multiflora leaves* before extraction of the essential oil

Peak N°.	Retention time (min)	Name of the molecule	%	Raw formula	Molar mass (g/mol)
1	11.516	Glycerol	8.1	$C_3H_8O_3$	92.05
2	20.292	1,2-diphenylethane-1,2-diol	0.7	$C_{14}H_{14}O_2$	214.01
3	21.773	3,4,5-trihydroxybenzoic acid	2.1	$C_7H_6O_5$	170.02
4	22.191	Hydroxy acetic acid	1.9	$C_2H_4O_3$	76.02
5	22.627	2, 3, 4, 5-tetrahydroxypental	1.2	$C_5H_{10}O_5$	150.05
6	27.015	3,4-dihydroxbenzoic acid	6.1	$C_7H_8O_4$	154.03
7	**29.663**	**Glucopyranose**	**21.6**	**$C_6H_{12}O_6$**	**180.06**
8	**31.562**	**D-glucose**	**23.3**	**$C_6H_{12}O_6$**	**180.06**
9	32.152	Palmitic acid	2.4	$C_{16}H_{32}O_2$	256.24
10	34.315	(E)-3-(3,4-dihydroxyphenyl)acrylic acid	2.7	$C_9H_8O_4$	180.04
11	34.684	Inositol	5.1	$C_6H_{12}O_6$	180.06
12	**47.091**	**β-d-Glucopyranoside**	**24.9**	**$C_{10}H_{20}O_7$**	**300.12**

There are twelve (12) compounds. Among these compounds, β-d-Glucopyranoside, D-glucose and Glucopyranose are the most abundant with proportions of 24.9%, 23.3%, and 21.6%.

3.2. Confirmation of the structures of the twelve (12) compounds detected from the ethyl acetate fraction

Compound 1

Compound 1 with a retention time of 11.401 min corresponds At Silylated glycerol with molecular mass m/z = 308 which does not appear on the mass spectrum **Figure 3.**

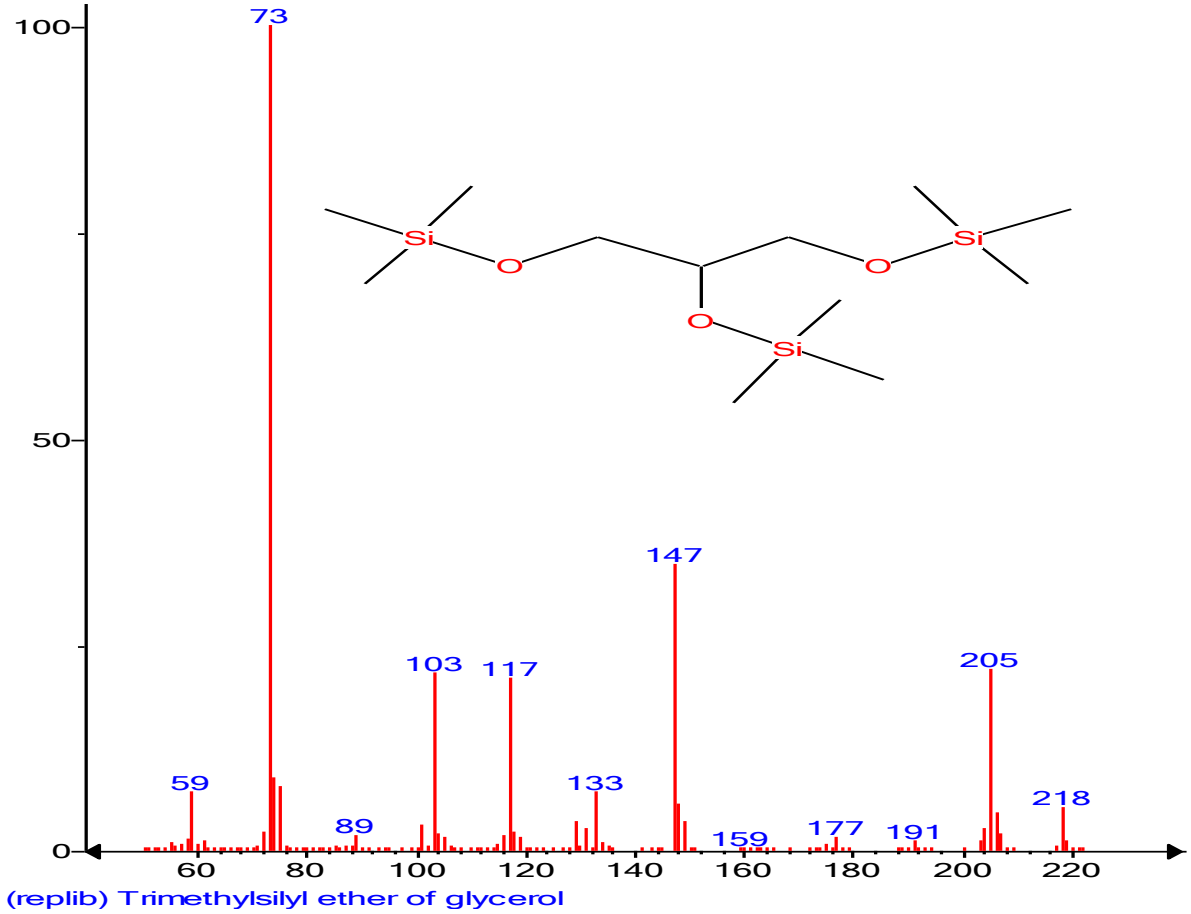
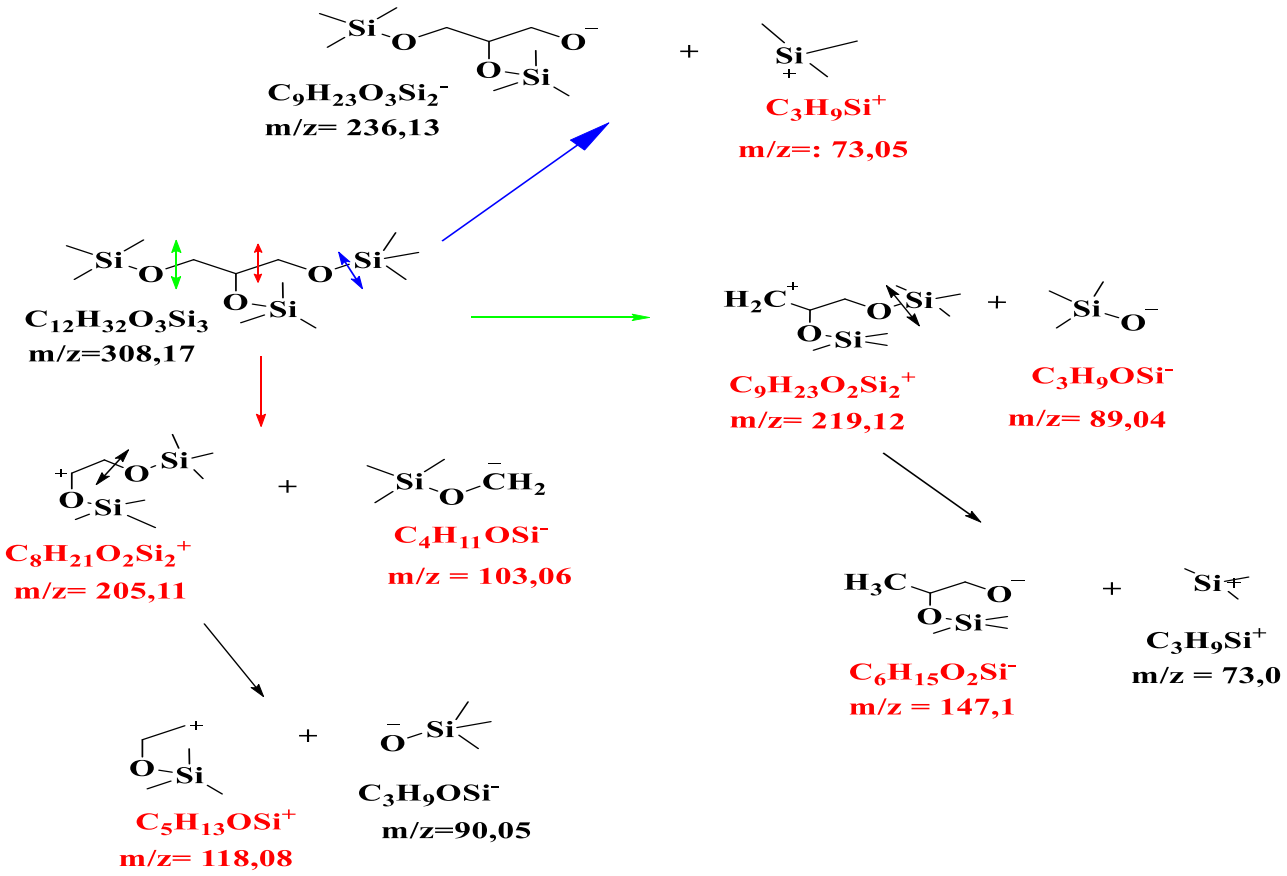


Figure 3. Mass spectrum of compound 1

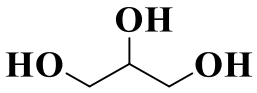
Analysis of the fragmentation spectrum of the silylated compound (**Figure 3**) shows the presence of major fragments at m/z = 218, m/z = 206, m/z = 147, m/z = 133, m/z = 117, m/z = 103, m/z = 73, m/z = 59. The disappearance of the molecular peak is due to total destruction of the molecule.

The fragment with mass m/z = 73 would be a trimethylsilyl group resulting from the breakage of the oxygen-silicon bond. Fragments with masses m/z = 219 (m/z = 218 on the spectrum, a difference that may be due to isotopes) and m/z = 89 would result from the breakage of an oxygen-carbon bond. The fragment of mass m/z = 147 would result from the breakage of the oxygen-silicon bond resulting

from the fragment of mass m/z = 219. The fragments of mass m/z = 205 and m/z = 103 would come from the breakage of a carbon-carbon bond (**Scheme 1**). Compound 1 is therefore glycerol which is classified in the terpene family. It was identified in the essential oil of the plant studied **(Bonou *et al.*, 2016**).



Scheme 1. Proposed fragmentation of compound 1



glycérol

$C_3H_8O_3$

m/z= 92.0

Figure 4. Structure of compound 1

Compound 2

Compound 2 with a retention time of 20.292 min corresponding to silylated 1,2-diphenylethane-1,2-diol with a molecular mass m/z=343.16.

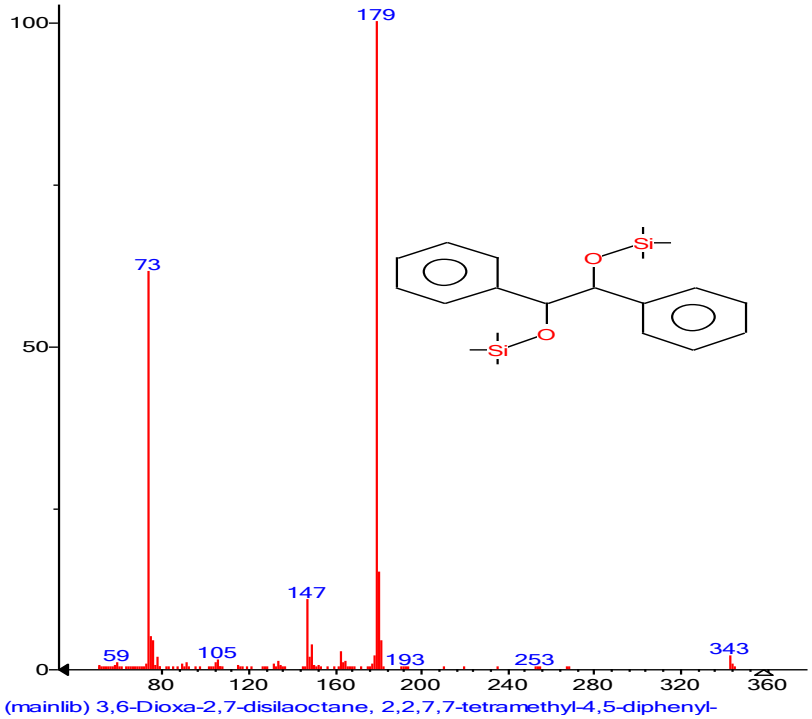
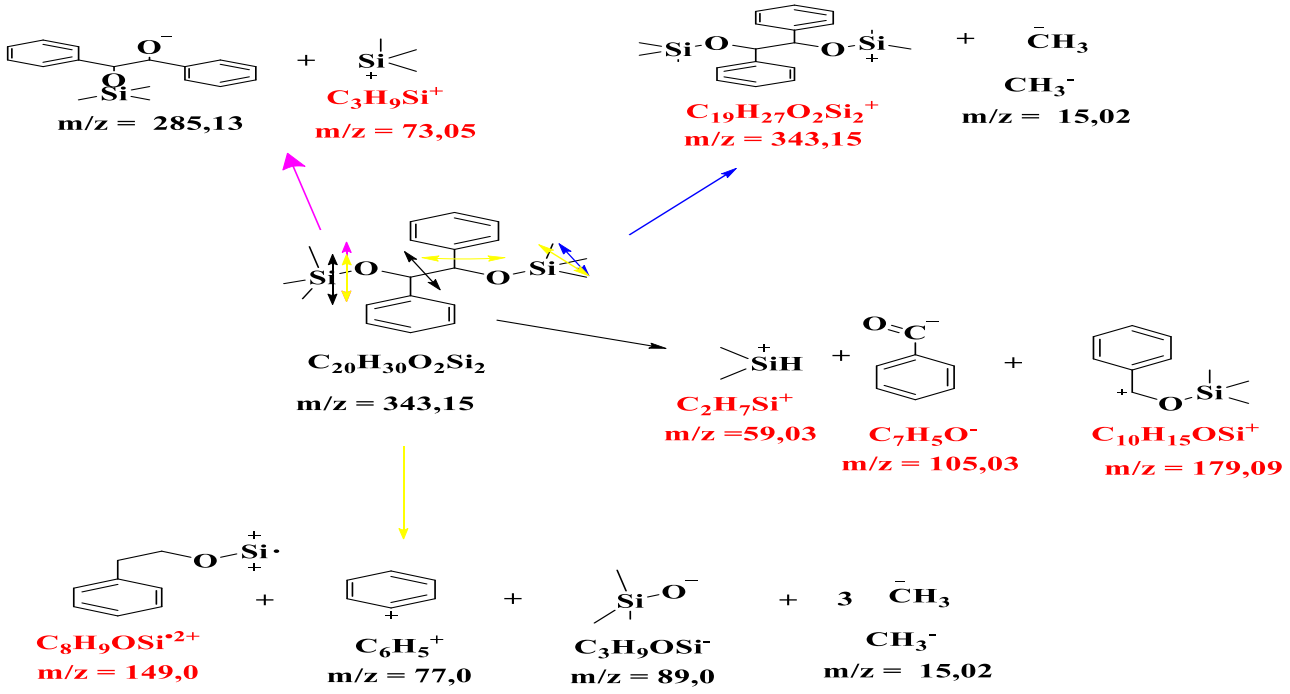


Figure 5. Mass spectrum of compound 2

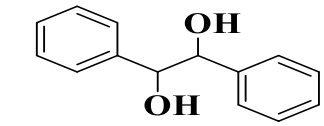
Analysis of the fragmentation spectrum of compound 2 (**Figure 5**) shows the presence of major fragments at m/z = 179, m/z = 147, m/z = 105, m/z = 73, m/z = 59.



Scheme 2. Proposed fragmentation of compound 2

The most abundant fragment has a mass of m/z = 179 (**Figure 5**). It would result from the loss of a methyl group on the silica and two other fragments of mass m/z = 59 m/z = 105. The fragment of mass m/z = 73 is the trimethylsilyl group which would have been obtained by the rupture of the oxygen-silicon bond. The fragment with mass m/z = 149 (m/z = 147 on the spectrum) would result

from the departure of three methyl groups, a C_3H_9OSi molecule $^-$ and a fragment with mass m/z = 77. The fragment with mass m/z = 343 results from the departure of a methyl group (**Scheme 2**). The structure sought is therefore that of 1,2-diphenylethane-1,2-diol (**Figure 6**) which is used in the synthesis of various organic compounds, notably drugs. This compound is from the family of phenolic compounds. It has not yet been identified in the plant studied.



1,2-diphenylethane-1,2-diol
$C_{14}H_{14}O_2$
m/z: 214.10

Figure 6. Structure of compound 2

Compound 3

Compound 3 with a retention time of 21.773 min corresponding to silylated 3, 4, 5-trihydroxy benzoic acid with a molecular mass m/z = 458 m/z which represents the molecular ion.

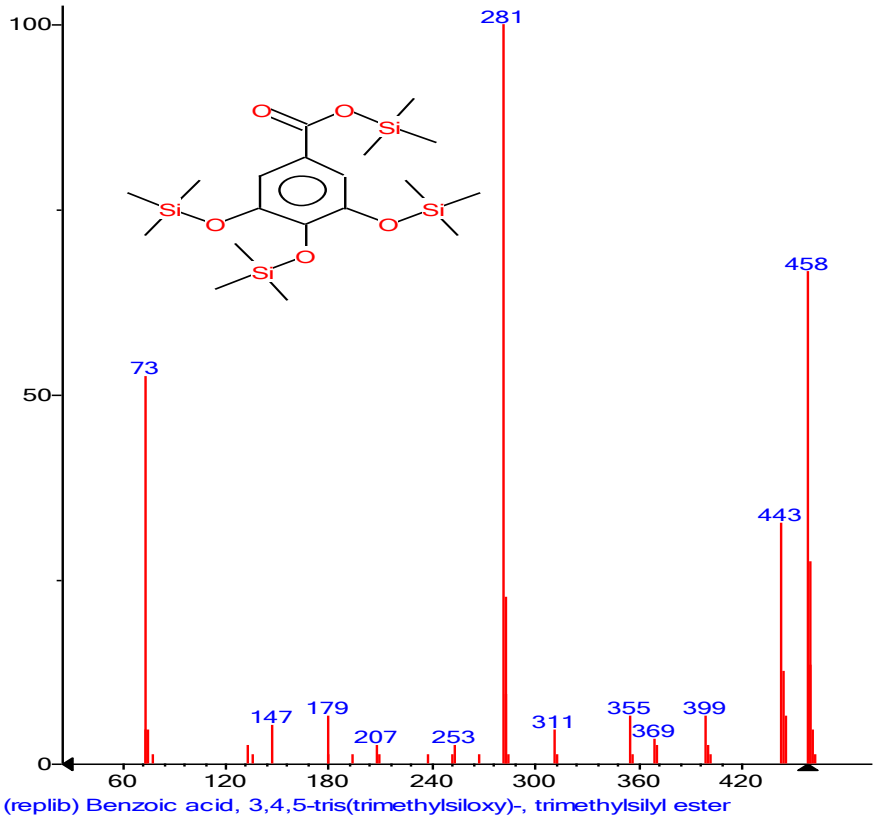
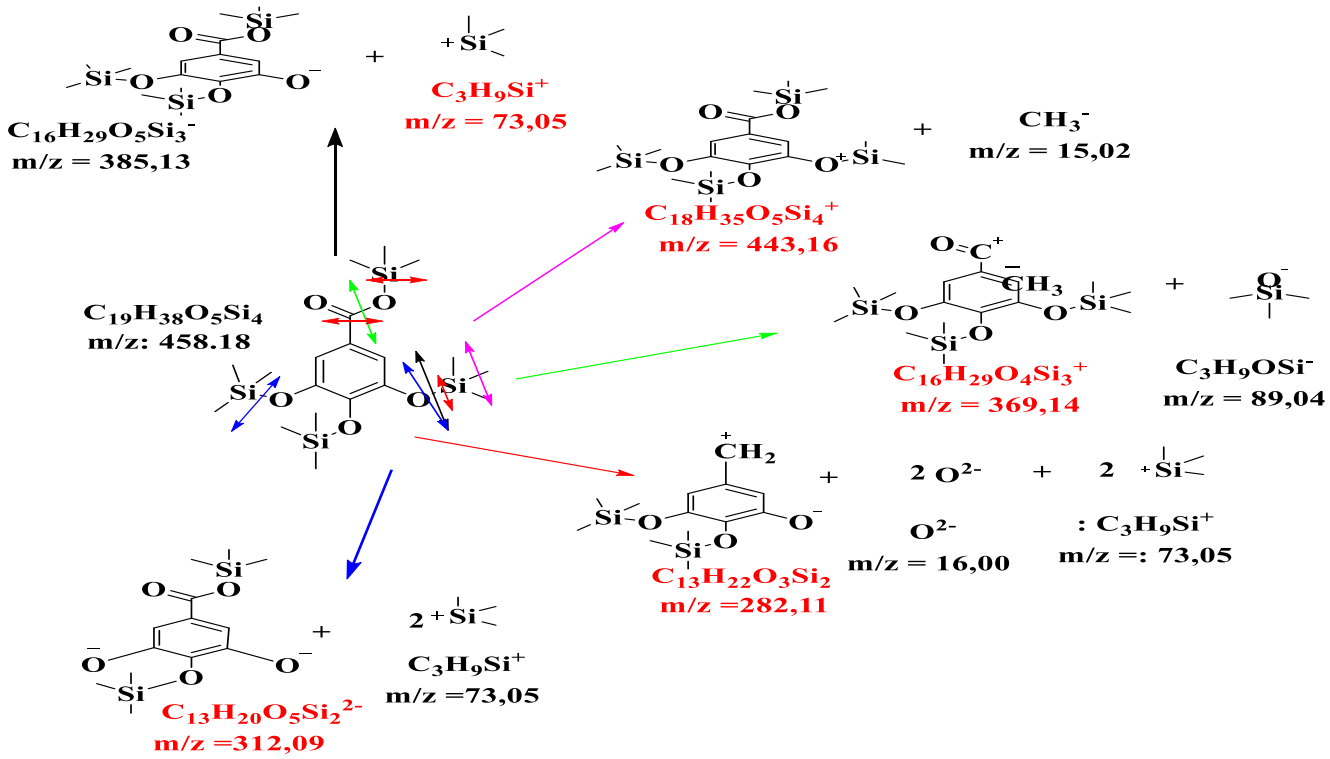


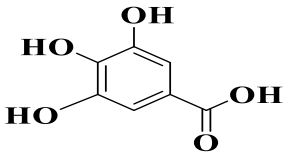
Figure 7. Mass spectrum of compound 3

Analysis of the fragmentation spectrum of the silylated compound (**Figure 7**) shows the presence of major fragments at m/z =458, m/z = 443, m/z =399, m/z = 369, m/z = 355, m/z = 311, m/z = 281(base peak), m/z = 253, m/z = 207, m/z = 179, m/z = 147, m/z = 73.



Scheme 3. Proposed fragmentation of compound 3

The observation of the molecular peak confirms the presence of the aromatic ring which stabilizes the structure of the molecule. The basic peak m/z = 282 (m/z = 281 on the spectrum) would result from the departure of two trimethylsilyl groups and two oxygens, the fragment with mass m/z = 443 would result from the departure of a methyl group while the fragment with mass m/z = 369 would come from the breakage of the carbon-oxygen bond. The fragment with mass m/z = 312 (m/z = 311 on the spectrum) would be due to the departure of two trimethylsilyl groups (**Scheme 3**). The structure sought is therefore 3, 4, 5-trihydroxy benzoic acid (**Figure 8**). It's a powerful antioxidant, which may have anti-inflammatory and antibacterial properties. Present in many plants. This compound can be classified in the phenolic family. It has not yet been identified in the plant studied.



acide-3, 4, 5-trihydroxybenzoique
$C_7H_6O_5$
m/z: 170.02

Figure 8. Structure of compound 3

Compound 4

Compound 35 with a retention time of 22.191 min corresponds to silylated 2-hydroxyacetic acid with a molecular mass m/z = 220.

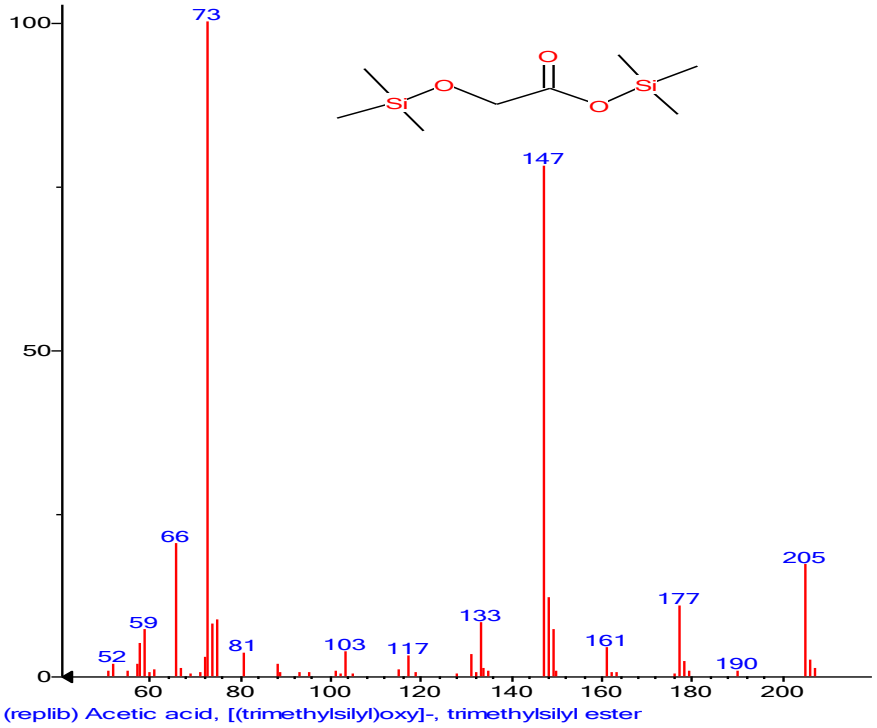
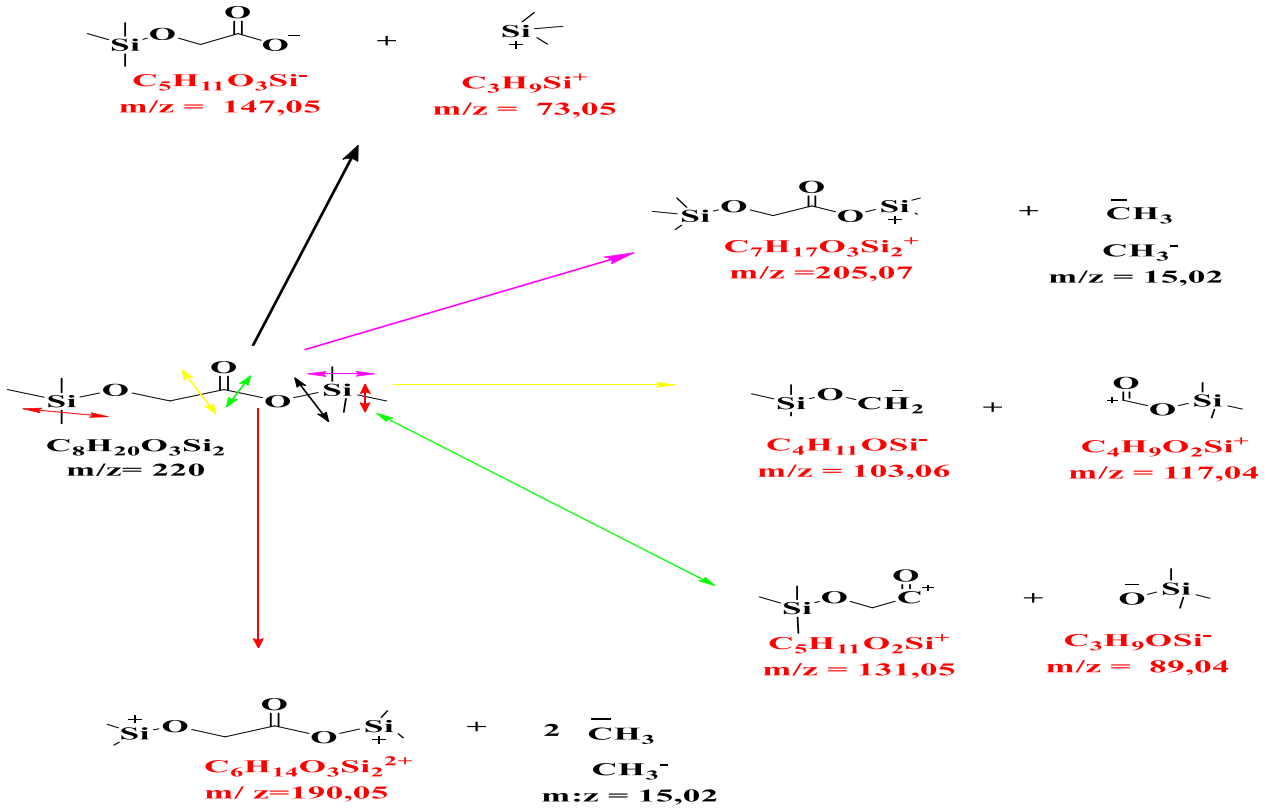


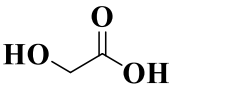
Figure 9. Mass spectrum of compound 4

Analysis of the fragmentation spectrum of the silylated compound (**Figure 9**) shows the presence of major fragments at m/z =205, m/z = 190, m/z =177, m/z = 161, m/z = 147, m/z = 133, m/z = 117, m/z = 103, m/z = 81, m/z = 73, m/z = 66, m/z = 59.



Scheme 4. Proposed fragmentation of compound 4

The fragment of mass m/z = 73 corresponding to the basic pis and the fragment of mass m/z = 147 would come from the cutting of the oxygen - silicon bond. The fragments of mass m/z = 103 and m/z = 117 would result from the cleavage of the carbon-carbon bond in the α position of the carboxyl group. The fragments have masses m/z = 131 (m/z = 132 on the spectrum, a difference that may be due to isotopes) and m/z = 89 (m/z = 91 on the spectrum, a difference that may be due to isotopes) would come from the carbon-oxygen bond in the α position of the carbonyl group. The fragments with masses m/z = 205 and m/z = 190 would result respectively from the breakage of one methyl group and two methyl groups (**Scheme 4**). The structure sought is therefore hydroxy acetic acid or glycolic acid (**Figure 10**). It is used in cosmetics for its exfoliating properties and involved in various metabolic processes. This compound can be classified in the terpene family. It would not yet have been identified in the plant studied.



Acide hydroxyacétique

$C_2H_4O_3$

m/z = 76,02

Figure 10. Structure of compound 4

Compound 5

Compound 36 with a retention time of 22.627 minutes is attributed to Lyxose or silylated 2, 3, 4, 5-tetrahydroxypentanal with a molecular mass m/z = 438.21.

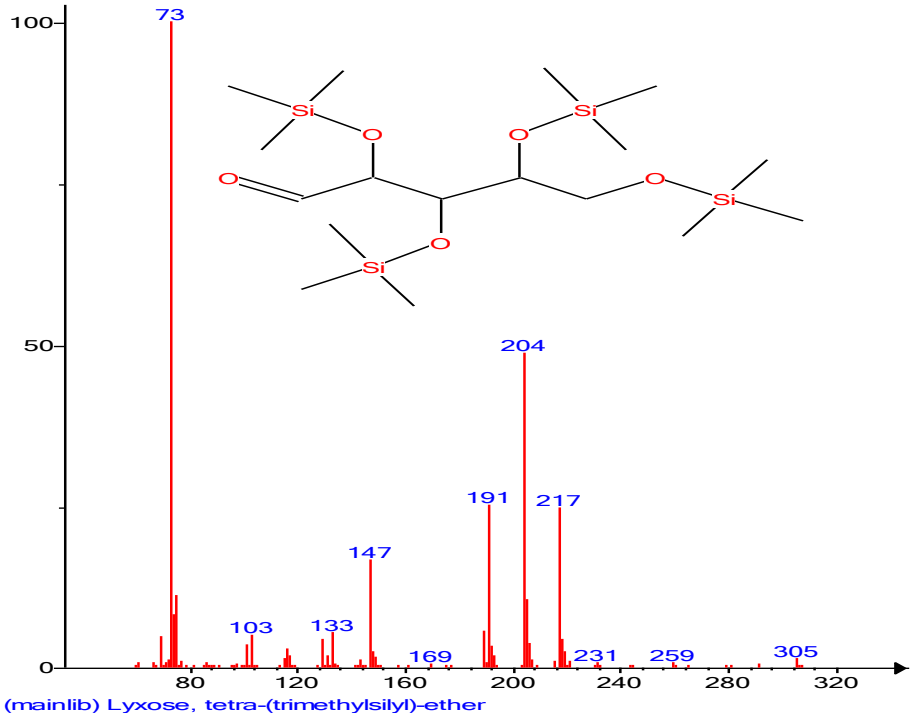
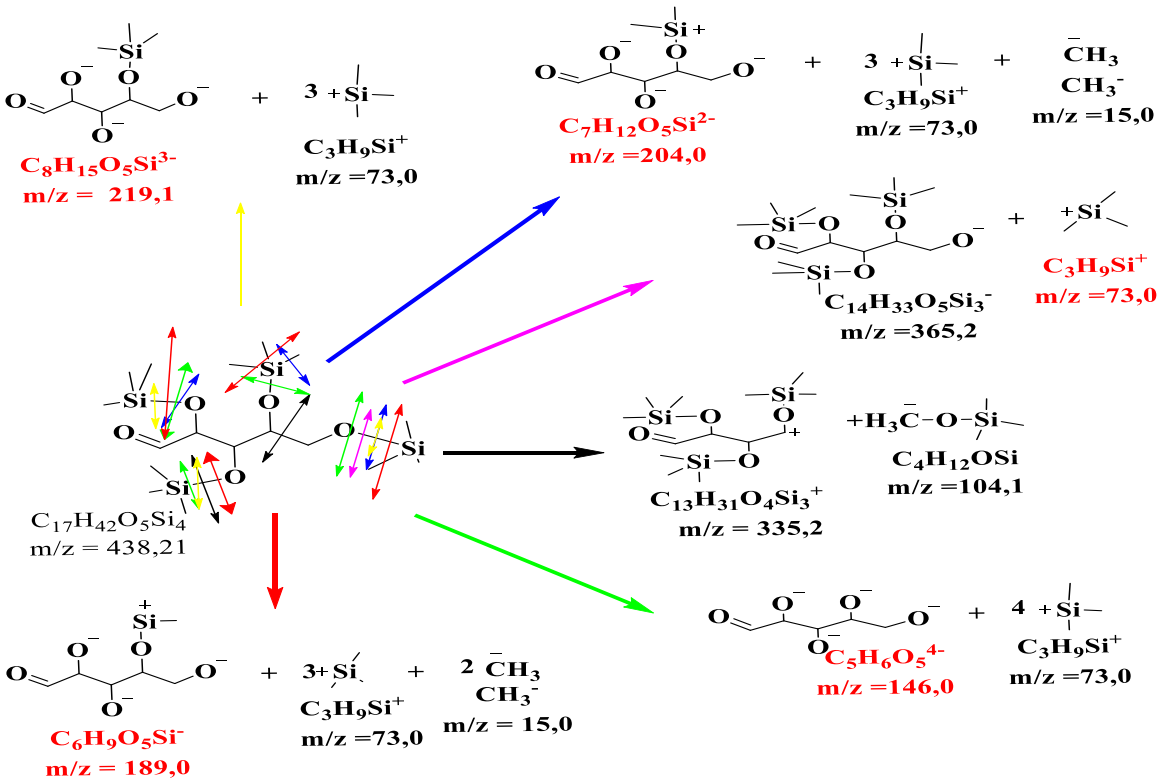


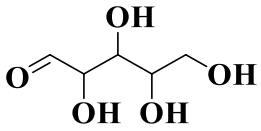
Figure 11. Mass spectrum of compound 5

Mass spectrum analysis of the silylated compound (**Figure 11**) reveals the presence of major fragments at m/z = 217, m/z = 204, m/z = 191, m/z = 147, m/z = 133, m/z =103, m/z =73.



Scheme 5. Proposed fragmentation of compound 5

The mass spectrum of its silylated derivative (**Figure 11**) reveals that the base peak with mass m/z = 73 would be a trimethylsilyl group resulting from the breakage of an oxygen-silicon bond. The fragment with mass m/z = 219 (m/z = 217 on the spectrum) would result from the loss of one of three trimethylsilyl groups. The fragment with mass m/z = 204 would result from the loss of one methyl group and three trimethylsilyl groups and the fragment m/z = 189 (m/z =192 on the spectrum) would result from the loss of two methyl groups and three trimethylsilyl groups. The fragment of mass m/z = 103 would come from the loss of the fragment of mass m/z = 335 and the fragment of mass m/z = 146 (m/z = 147 on the spectrum) would be due to the breakdown of the four groups trimethylsilyl (**Scheme 5**). This desired structure is therefore 2, 3, 4, 5-tetrahydroxypentanal (**Figure 12**). This compound can be classified in the terpene family. It has not yet been identified in the plant studied.



2, 3, 4, 5-tetrahydroxy pentanal

$C_5H_{10}O_5$

m/z= 150. ,05

Figure 12. Structure of compound 5

Compound 6

Compound 37 with a retention time of 27.015 min is attributed to silylated 3,4-dihydroxy benzoic acid with a molecular mass m/z = 370 which represents the molecular ion.

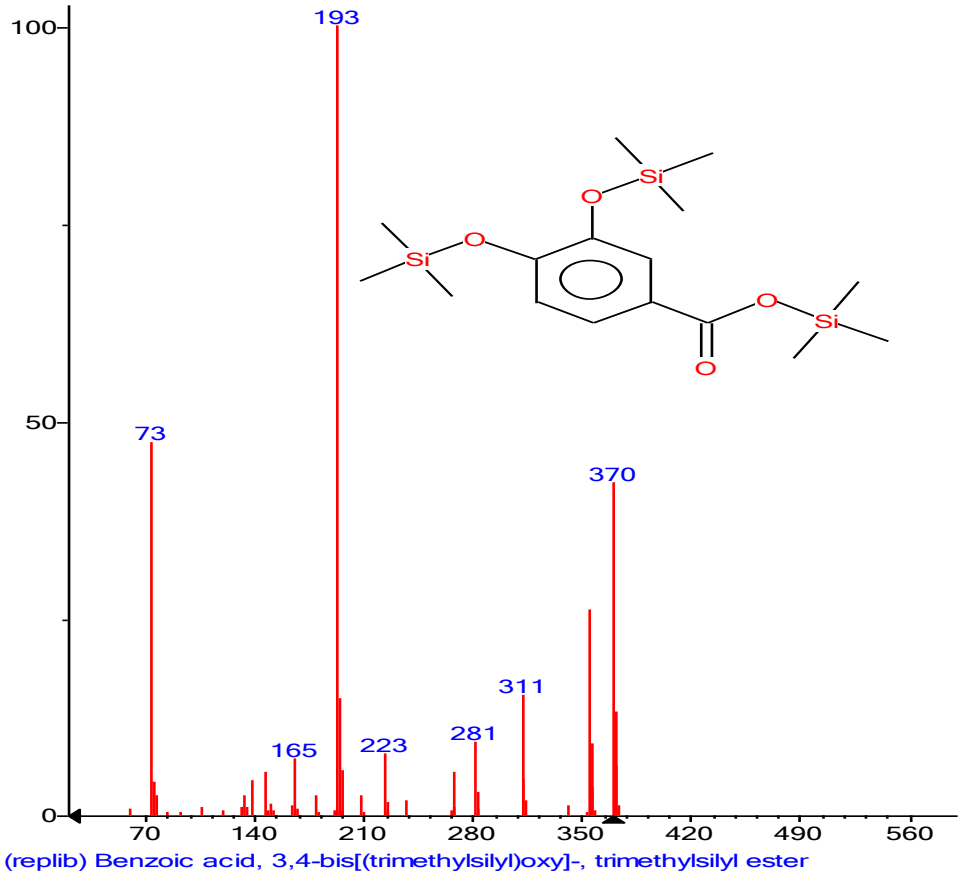
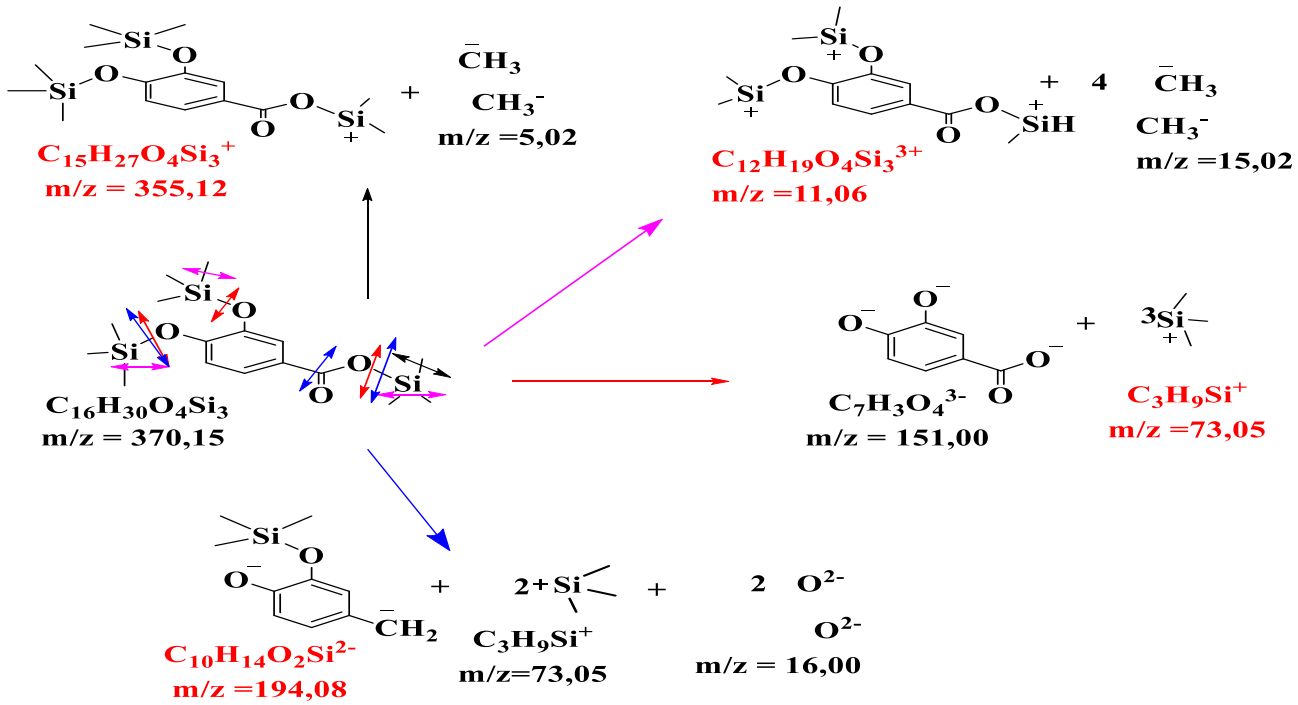


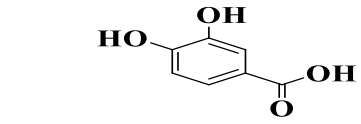
Figure 13. Mass spectrum of compound 6

Mass spectrum analysis of the silylated compound (**Figure 13**) reveals the presence of major fragments at m/z = 370, m/z = 331, m/z = 311, m/z = 281, m/z = 223, m/z = 193 (base peak), m/z = 165, m/z =73



Scheme 6. Proposed fragmentation of compound 6

The fragment with mass m/z = 73 would be a trimethylsilyl group resulting from the breakage of an oxygen-silicon bond. The most abundant fragment at m/z = 194 would result from the departure of two trimethylsilyl groups and two oxygens. The fragment with mass m/z = 73 would be a trimethylsilyl group resulting from the breakage of an oxygen-silicon bond. The fragments with masses m/z = 355 and m/z = 310 (m/z = 311 on the spectrum) result respectively from the departure of one (1) methyl group and four (4) methyl groups (**Scheme 6**). This desired structure is therefore 3,4-dihydroxy benzoic acid. This compound is in the family of phenolic compounds. It would not yet have been identified in the plant studied.



Acide 3, 4-dihydroxybenzoique

$C_7H_6O_4$

m/z=154,03

Figure 14. Structure of compound 6

Compound 7

Compound 7 with a retention time of 29.530 min corresponds to silylated Glucopyranose with a molecular mass m/z = 540.26.

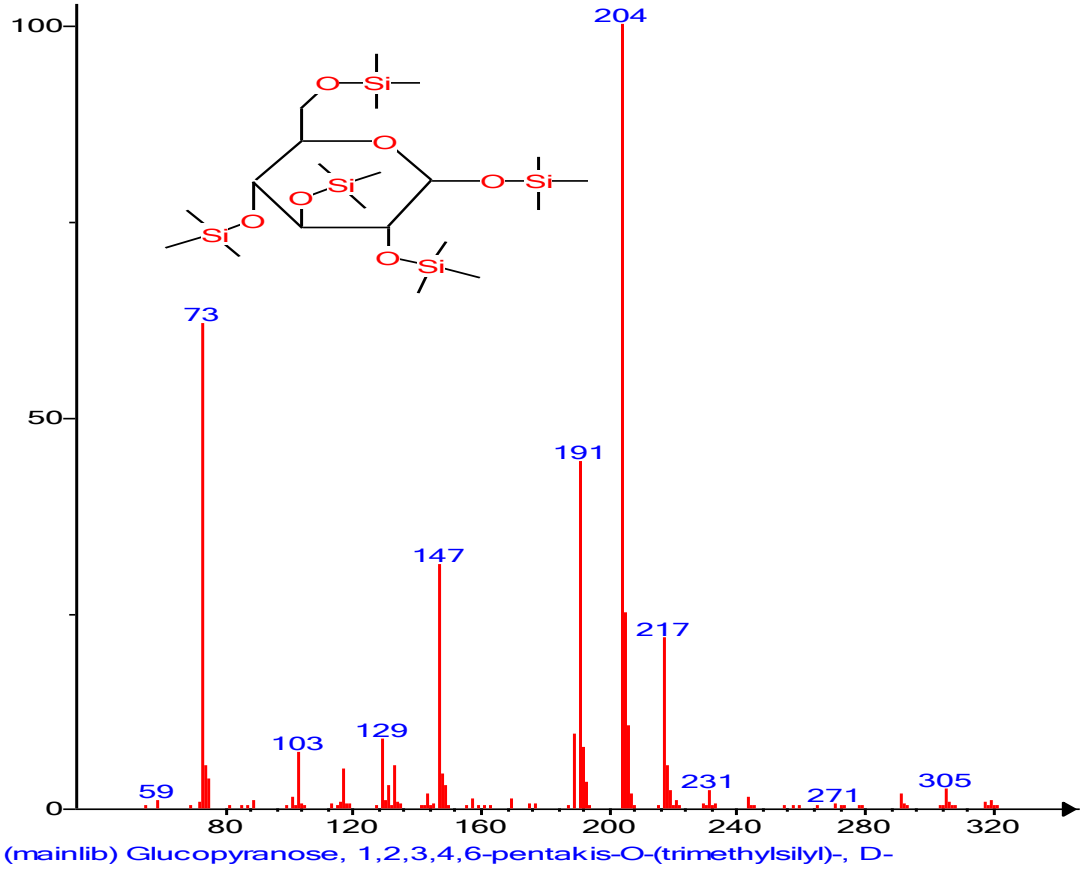
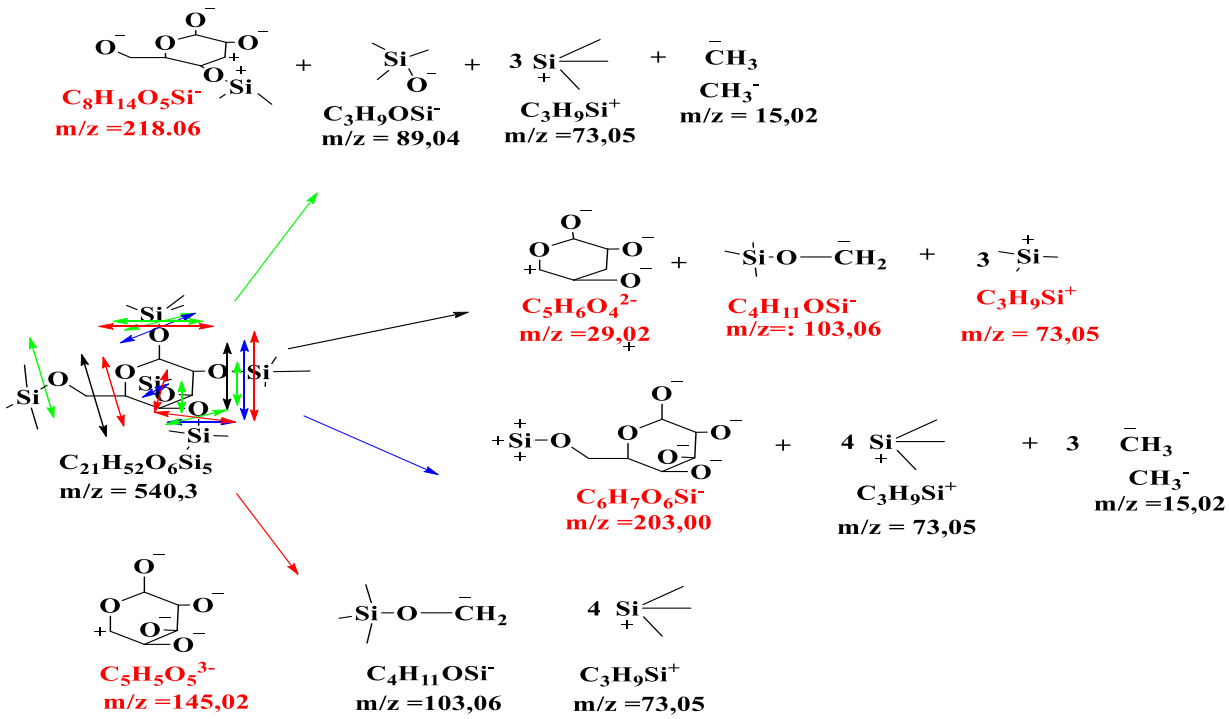


Figure 1. Mass spectrum of compound 7

Analysis of the fragmentation spectrum of compound 22 (Figure 15) shows the presence of major fragments at m/z = 305, m/z = 271, m/z = 231, m/z = 217, m/z = 204 (abundant peak), m/z=191, m/z=147, m/z=129, m/z=103, m/z=73, m/z=59.



Scheme 7. Proposed fragmentation of compound 7

The base peak with mass m/z = 204 (m/z = 203 on the spectrum) would result from the departure of four trimethylsilyl groups and three methyl groups. The fragments with masses m/z = 129, m/z = 103 and m/z = 73 would constitute the silylated compound. The fragment with mass m/z = 217 would come from the cleavage of a methyl group, four trimethylsilyl groups and a $C_3H_9OSi^-$ molecule. The fragment with mass m/z = 145 (m/z = 147 on the spectrum) would result from the cutting of fragments with mass m/z = 103 and four trimethylsilyl groups (**Scheme 7**). The structure sought would therefore be that of glucopyranose (**Figure 16**). It is a cyclic form of glucose, a simple sugar essential for cellular energy. It has reducing properties due to the free aldehyde group. This compound is from the family of terpene heterocycles (glucosides). It has not yet been identified in the plant studied.

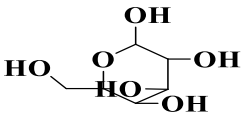


Figure 2 Structure of compound 7

Compound 8

Compound 8, with a retention time of 31.429 min, corresponds to silylated D-Glucose or silylated 2, 3, 4, 5, 6-pentahydroxyhexanal with a molecular mass m/z = 540.26.

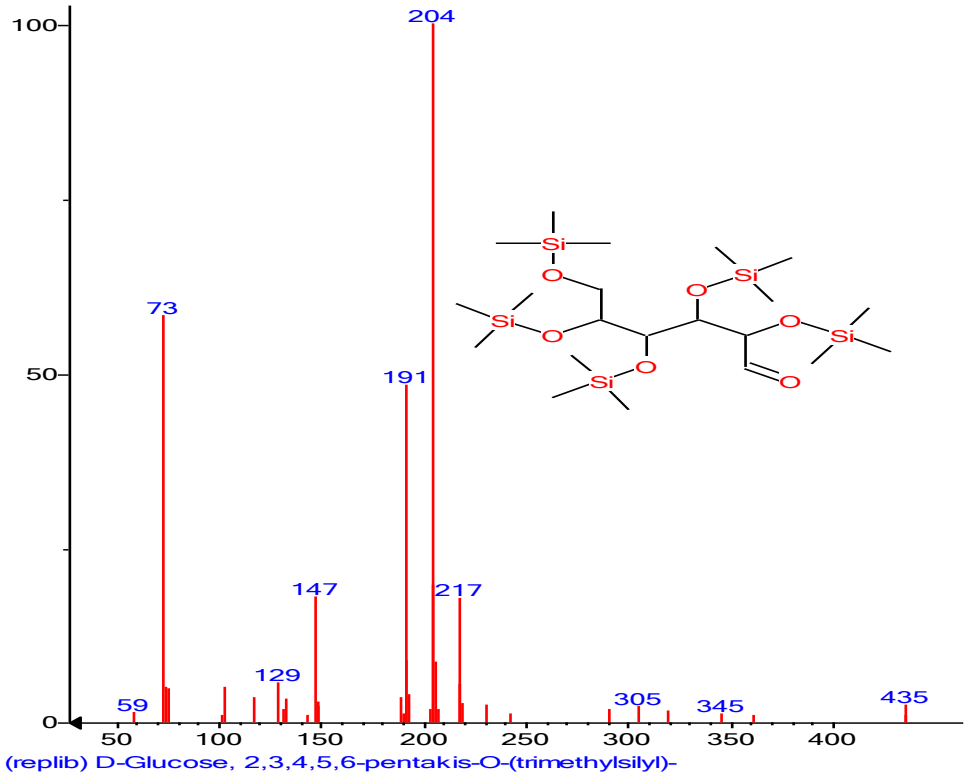
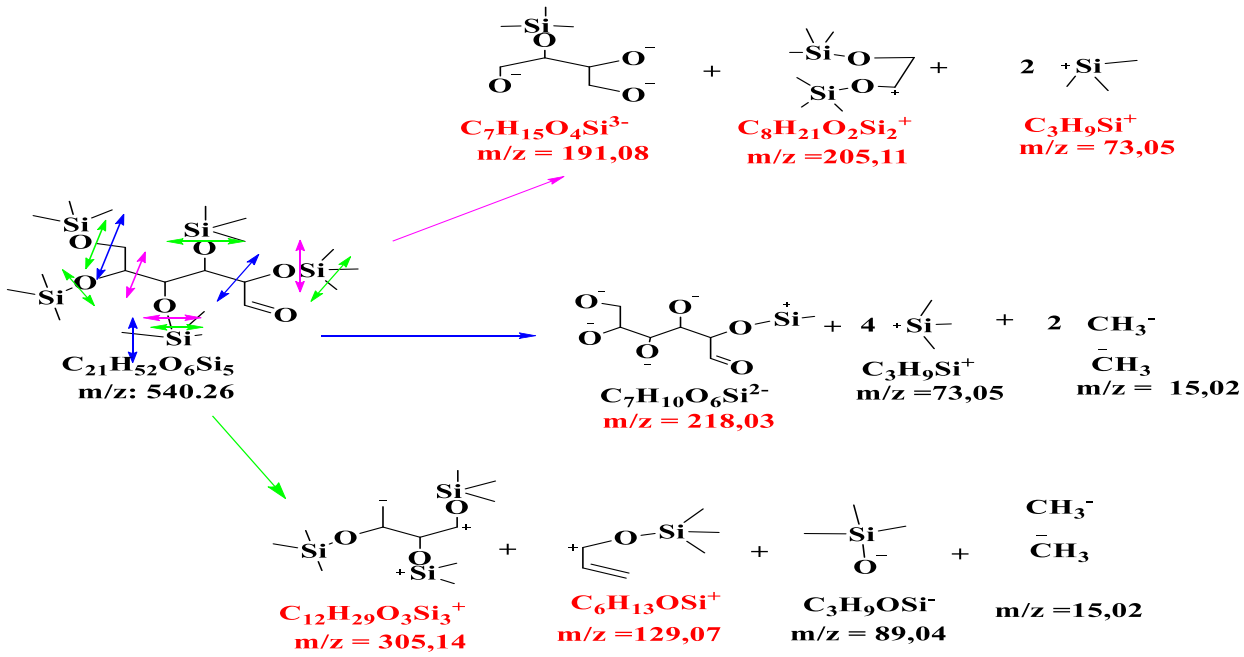


Figure 3 Mass spectrum of compound 8

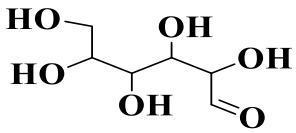
Analysis of the fragmentation spectrum of compound 8 (**Figure 17**) shows the presence of major fragments at m/z = 217, m/z= 204 (abundant peak), m/z=191, m/z = 147, m /z = 129, m/z = 103, m/z=73, m/z =59.



Scheme 8. Proposed fragmentation of compound 8

The fragment of the base peak with mass m/z = 205 (m/z = 204 on the spectrum) would come from the cutting of the fragments with masses m/z = 191 and m/z = 73. The fragment with mass m/z = 218 would result from the cleavage of four (4) trimethylsilyl groups and two (2) methyl groups of

silicon. The fragment of mass m/z = 305 would come from the breakage of the fragment of mass m/z = 129, by the loss of a silylated derivative [$(CH_3)_3SiO$] and a methyl group on the silicon (**Scheme 8**). The desired structure would therefore be that of D-glucose (**Figure 18**). It is a monosaccharide, primary energy source for most organisms with a specific rotational power. This compound can be classified in the terpene family. It has not yet been identified in the plant studied.



D-glucose
$C_6H_{12}O_6$
m/z: 180.1

Figure 4 Structure of compound 8

Compound 9

Compound 9 with a retention time of 32.061 min corresponds to silylated palmitic acid of molecular mass m/z: 328 which represents the molecular ion (**Figure 19**).

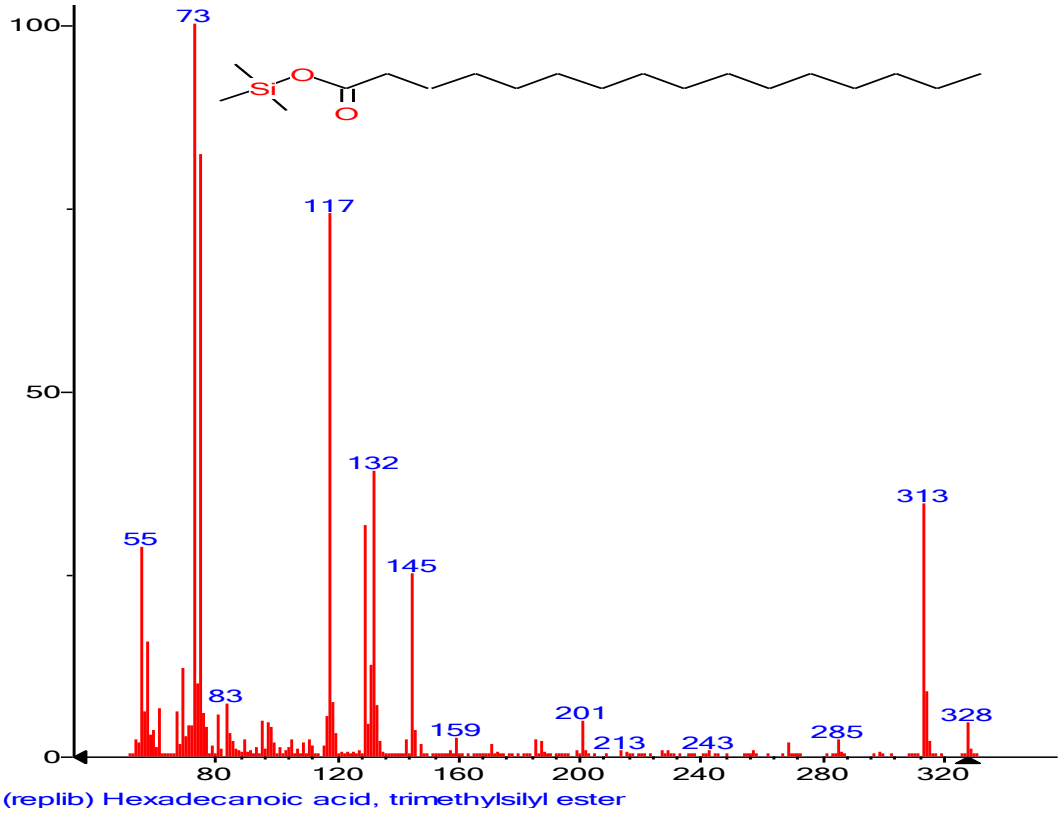
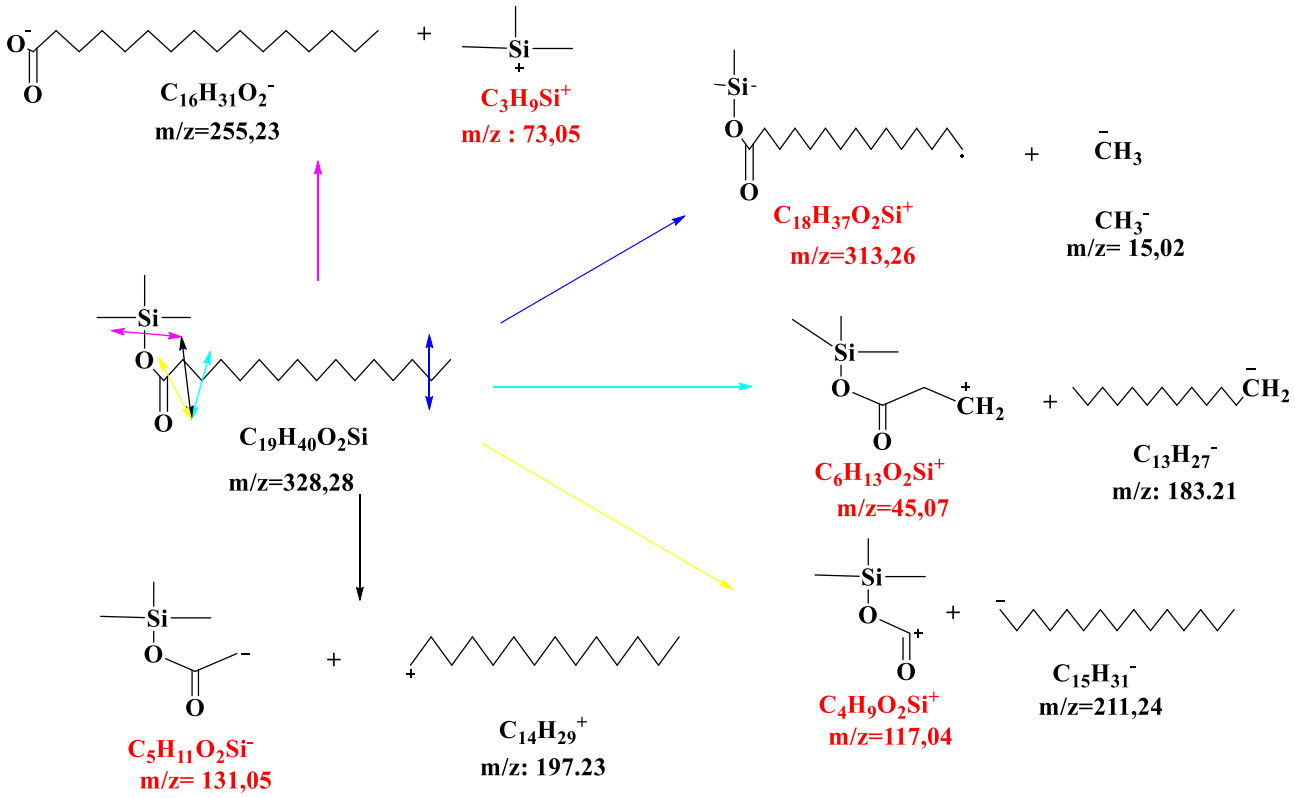


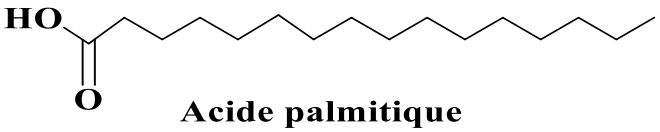
Figure 19. Mass spectrum of compound 9

Mass spectrum analysis of the silylated compound **(**Figure 19) reveals the presence of major fragments at m/z = 328, m/z = 313, m/z = 201, m/z = 145, m/z = 132, m/z = 117(base peak), m/z =73, m/z = 55.



Scheme 9. Proposed fragmentation of compound 9

The base peak with mass m/z = 73 would be a trimethylsilyl group resulting from the breakage of the oxygen-silicon bond. The fragments of mass m/z = 313, m/z = 145, m/z = 131 and m/z = 117 would come from the breakage of the carbon-carbon bond or the loss of the respective alkyl groups CH_3, $C_{13}H_{27}$, $C_{14}H_{29}$ and $C_{15}H_{31}$ (**Scheme 9**). Compound 9 is therefore palmitic acid which is classified in the terpene family. It was identified in the leaves of *Lippia multiflora* (Gouollaly *et al.*, 2019) .



Acide palmitique

$C_{16}H_{32}O_2$

m/z = 256

Figure 20. Structure of compound 9

Compound 10

Compound 41, with a retention time of 34.315 min, corresponds to (E)-3-(3,4-dihydroxyphenyl) silylated acrylic acid with a molecular mass of m/z = 396 which represents the molecular ion.

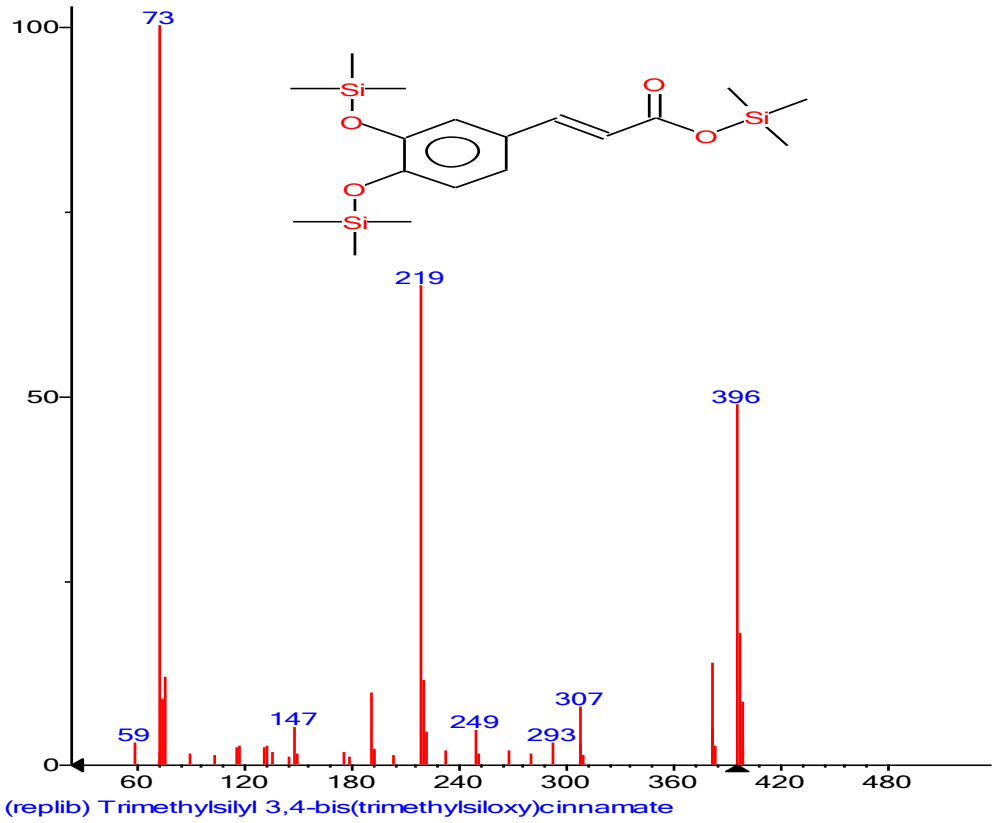
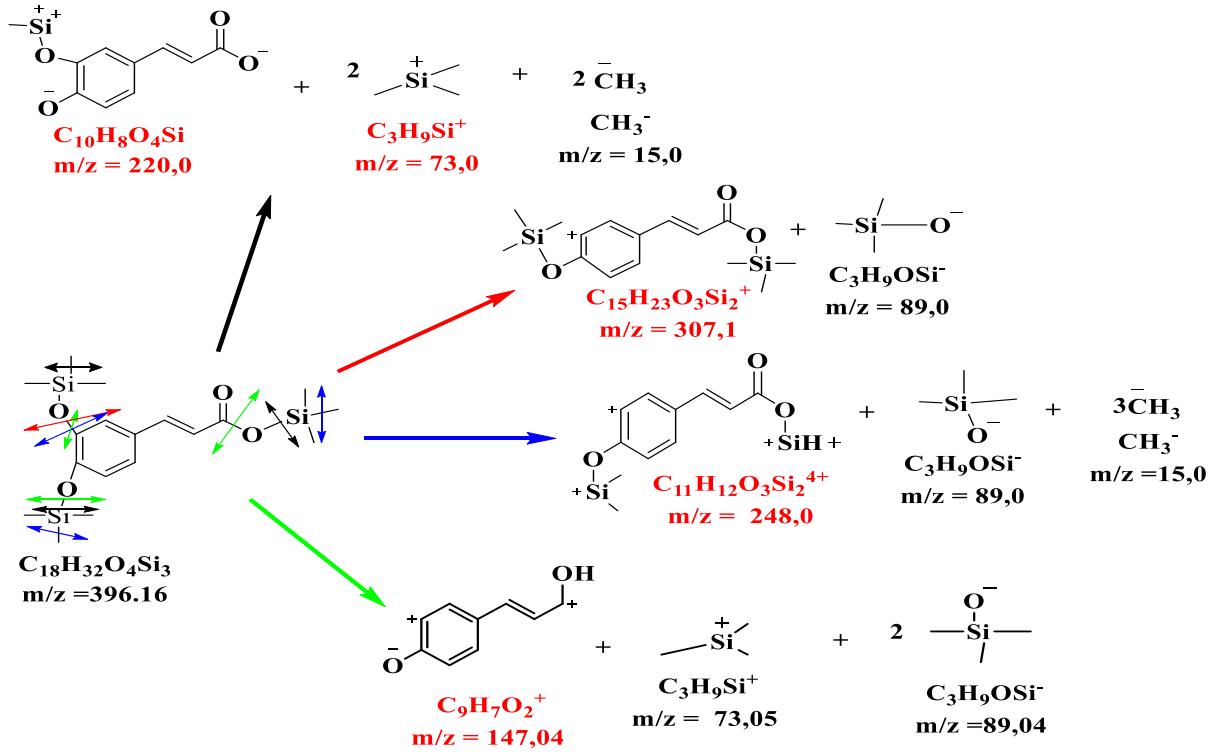


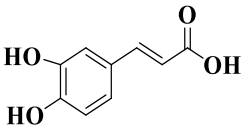
Figure 21. Mass spectrum of compound 10

Mass spectrum analysis of the silylated compound (**Figure 21**) reveals the presence of major fragments at m/z = 396, m/z = 307, m/z = 249, m/z = 219, m/z = 147, m/z =73, m/z =59.



Scheme 10. Proposed fragmentation of compound 10

The fragments with masses m/z = 73 and m/z = 220 (m/z = 219 on the spectrum) would result from the loss of a trimethylsilyl group and two (2) methyl groups. The fragment with mass m/z = 307 would result from the loss of the fragment with mass m/z = 89 and a methyl group. The fragment with mass m/z = 248 (m/z = 249 on the spectrum) would come from the departure of a trimethylsilanolate group and three methyl groups while the fragment with mass m/z = 147 would result from the loss of a trimethylsilyl group and two trimethylsilanolate groups (**Scheme 10**). The structure of the compound is therefore that of (E)-3-(3,4-dihydroxyphenyl)acrylic acid. It is a phenolic compound, precursor of lignin with antioxidant properties. It has not yet been identified in the plant studied.



Acide (E)-3-(3,4- dihydroxyphényl) acrylique

$C_9H_8O_4$

m/z=180,04

Figure 22. Structure of compound 10

Compound 11

Compound 25, with a retention time of 34.541 min, corresponds to silylated inositol with a molecular mass m/z = 612.30.

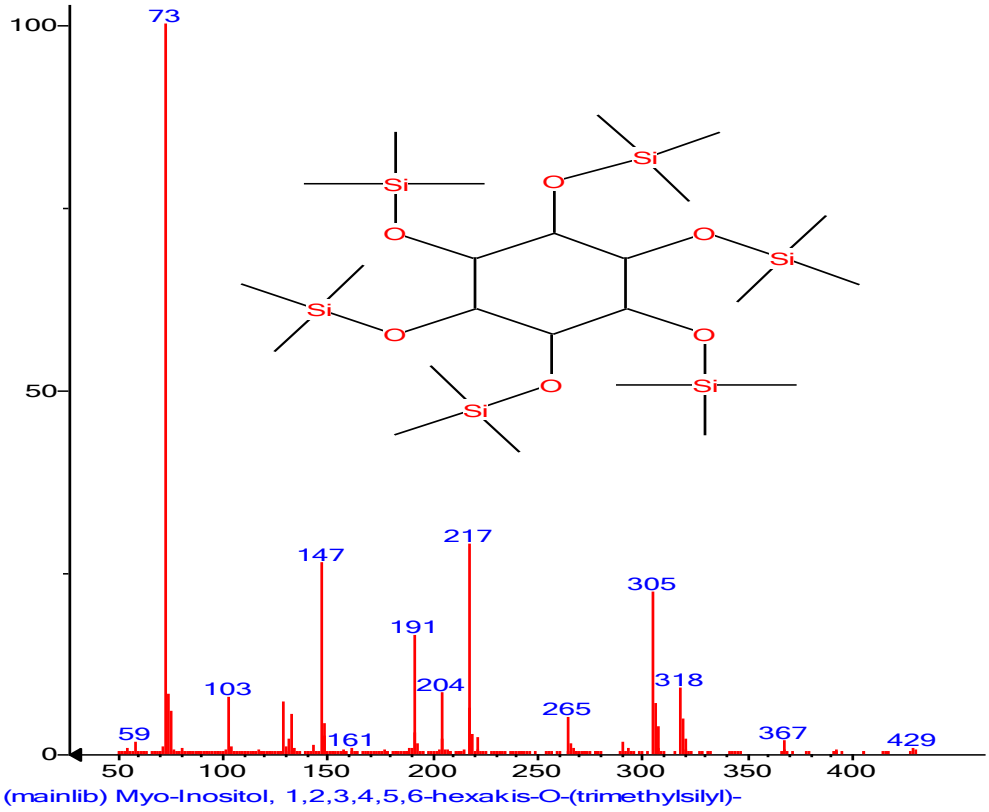
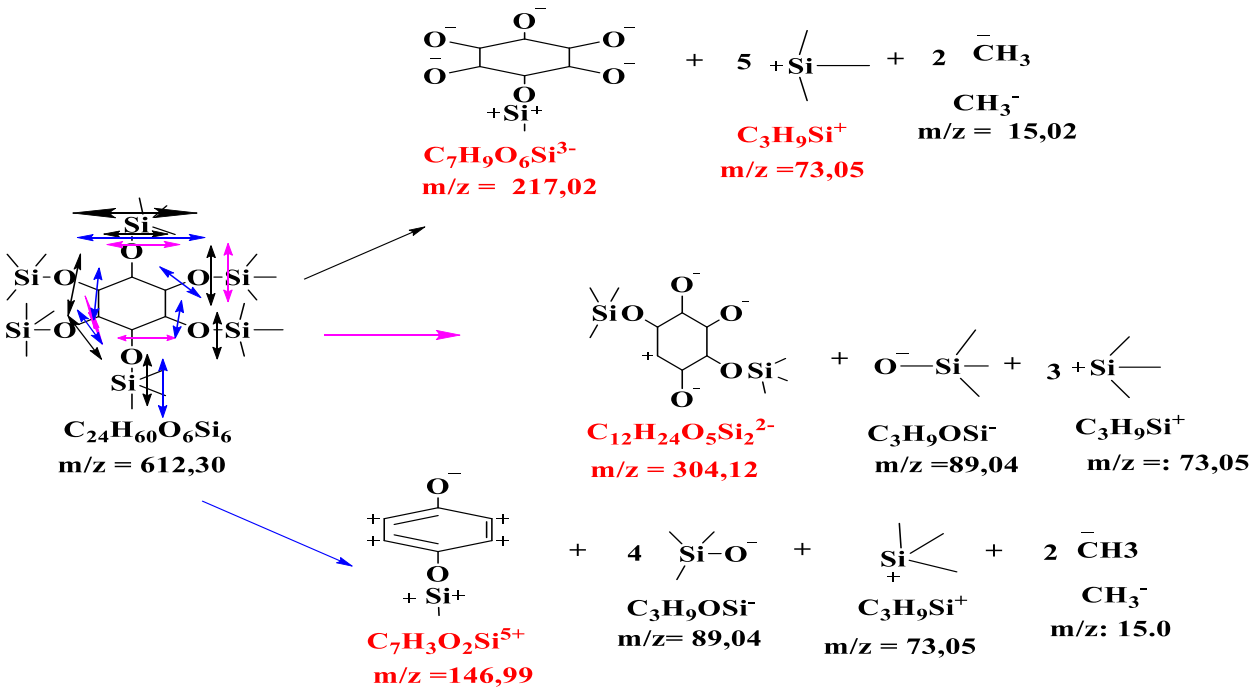


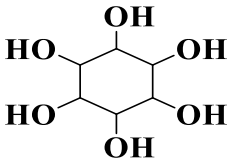
Figure 23. Mass spectrum of compound 11

Analysis of the fragmentation spectrum of compound 11 (**Figure 23**) shows the presence of major fragments at m/z = 318, m/z = 305, m/z = 265, m/z = 217, m/z = 204, m/z=191, m/z = 147, m/z = 103, m/z =73, m/z =59.



Scheme 11. Proposed fragmentation of compound 11

The base peak has a mass of m/z = 73 (**Figure 23**) and could be obtained by breaking the fragment with a mass of m/z = 217 and two (2) methyl groups on the silicon. The fragment with mass m/z = 304 (m/z = 305 on the spectrum) would come from the departure of one (1) trimethylsilanolate group and three (3) trimethylsilyl groups. The fragment with mass m/z = 147 would result from the cleavage of four (4) trimethylsilanolate groups, one trimethylsilyl group and two (2) methyl groups (**Scheme 11**). The structure sought would therefore be that of inositol (**Figure 24**). It is an isomer of glucose, important for cell signaling and membrane structure. This compound would belong to the cyclic terpene family. It has not yet been identified in the plant studied.



Cyclohexan-1, 2, 3, 4, 5,6-hexanol

$C_6H_{12}O_6$

m/z: 180.06

Figure 24. Structure of compound 11

Compound 12

Compound 12 with a retention time of 46.911 min corresponds to silylated β-D-glucopyranoside with a molecular mass m/z = 660.32.

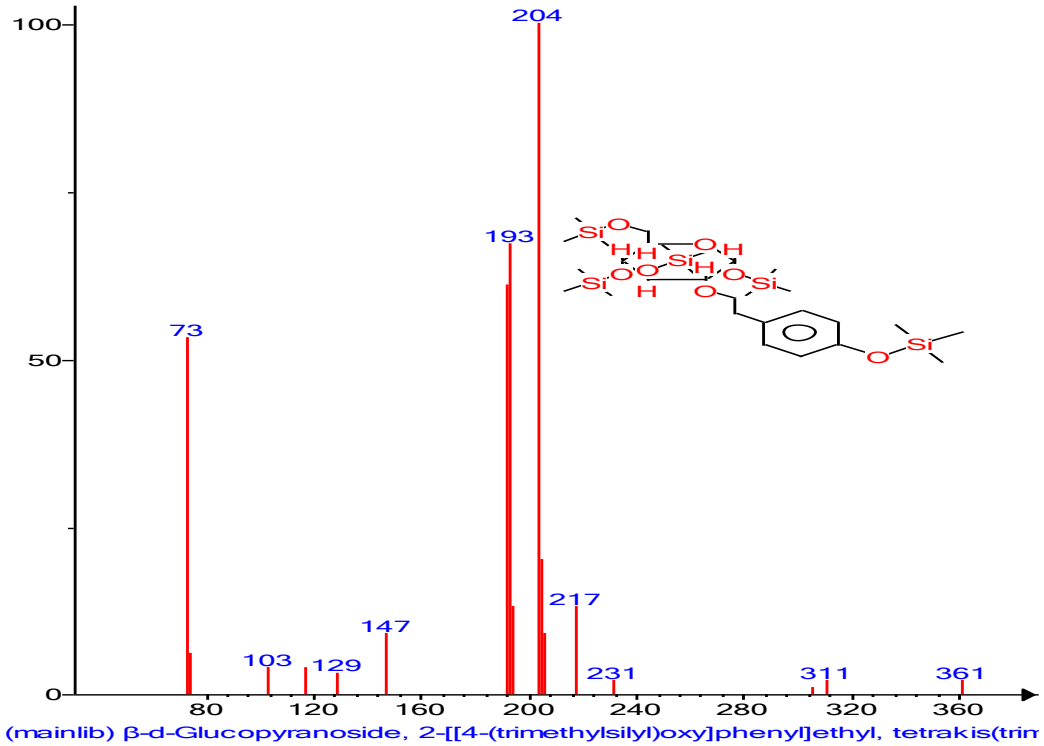
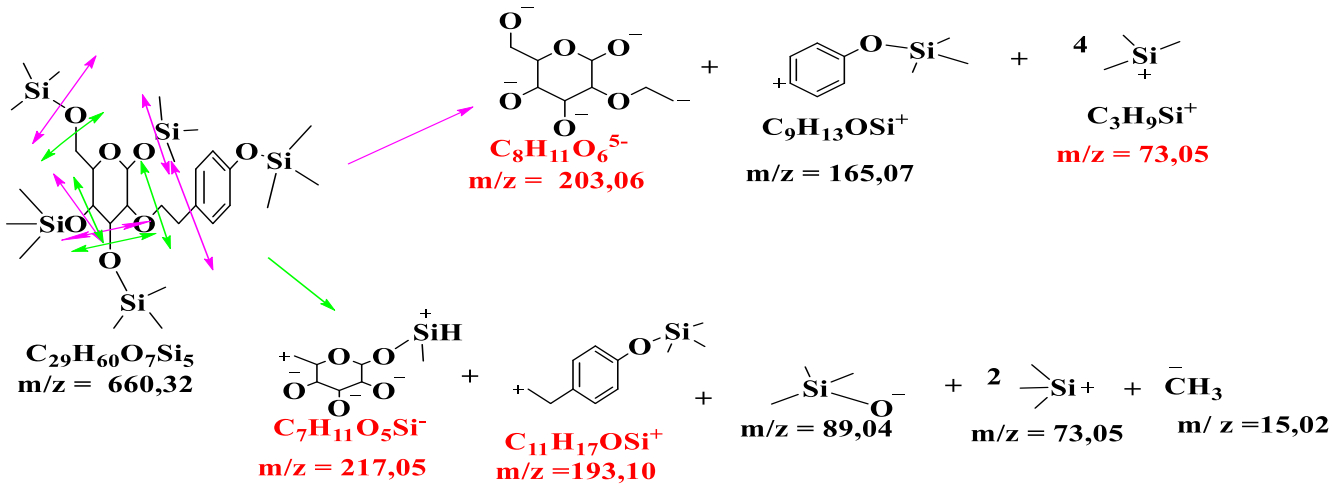


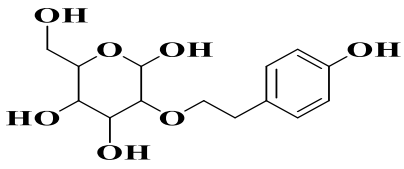
Figure 25. Mass spectrum of compound 12

Analysis of the fragmentation spectrum of compound 12 (**Figure 25**) shows the presence of major fragments at m/z = 217, m/z = 204, m/z = 193, m/z = 147, m/z = 129, m/z = 103, m/z =73.



Scheme 12. Proposed fragmentation of compound 12

The most abundant fragment which has the mass m/z = 203 (m/z = 204 on the spectrum) would come from the breakup of the fragments of mass m/z = 165 and m/z = 73. The fragments of mass m/z = 217 and m/z = 193 would result from the loss of a trimethylsilanolate group, a methyl group and two (2) trimethylsilyl groups (**Scheme 12**). The structure sought is that of β-D-glucopyranoside (**Figure 26**). This compound could belong to the terpene glucoside family. It's a glycoside formed from glucose. Its properties vary depending on the group attached to glucose. It has not yet been identified in the plant studied.



β-D-glucopyranoside
$C_{14}H_{20}O_7$
m/z = 300,12

Figure 26. Structure of compound 12

Conclusion

It appears from this analysis that the ethyl acetate fraction from the hydroethanolic extract of *Lippia multiflora leaves* contains several compounds. Among his proposed molecular structures, many molecules have not already been identified in the genus *Lippia.* The two (02) compounds identified in the leaves of the plant during this study, have already been detected in the study plant. Among the twelve (12) compounds obtained, seven (7) are terpenes and five (5) polyphenols.

The families of molecules corroborates the use of this plant in traditional medicine identification of these

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