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Study of the Chemical Constituents of the Hydroethanolic Extract of the Leaves of *Lippia Multiflora Moldenke* (Verbenaceae)

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Abstract

Lippia multiflora is an aromatic plant used in traditional medicine for its extracts (volatile and non-volatile) rich in bioactive molecules. In Africa, the infusion or decoction of the leaves is used orally to treat bronchial conditions. The objective of this study is to identify the phytochemical compounds present in the leaves of *L. multiflora*. The dichloromethane fraction, resulting from the hydroalcoholic extract, was obtained by maceration of the crushed dried leaves in an ethanol/water mixture (70/30: v/v) followed by liquid-liquid fractionations. After the phytochemical screening, the level of total polyphenols, total flavonoids and tannins were evaluated by colorimetric assay. Structural elucidation was carried out by gas chromatography coupled with mass spectrometry (GC-MS). The phytochemical screening results showed the presence of polyphenols, flavonoids, tannins, alkaloids and terpenoids then the dosage provided the level of total polyphenols, total flavonoids and total tannins. Linalool, linalool oxide, palmitic acid and N-(2-mehtylphenyl)-3-hydroxybut-2-en amide were identified as abundant compounds with respective proportions of 42.1 %, 22.1 %, 10.9 % and 10.6 %. The results could justify the traditional use of the plant in the treatment of various diseases.

Keywords: *Lippia multiflora;* Bioactive compounds; Gas chromatography; Mass spectrometry.

1. Introduction

Lippia multiflora (Moldenke), commonly known as "Savannah tea" is an aromatic plant of the Verbenaceae family which includes approximately 3295 species divided into 91 genera. It exists in the wild in the savannah zones of West Africa and gives off a heady odor. It is a woody, erect plant, with angular pubescent stems, and more or less smooth bark, reaching up to 2 or 4 m in height [1]**.** The leaves of *L. multiflora* are strongly aromatic. Their shapes and colors differ from one variant to another. The leaves, whorled in groups of 3 or 4, are oblong or elliptical with a long canate base, acuminate apex, finely toothed edges and whitish pubescence below. *L. multiflora* is an aromatic plant used for its multiple medicinal properties in many traditional and phytotherapeutic crops [2, 3]**.** It has been the subject of numerous studies which have made it possible to determine the chemical composition of both its essential oil and its non-volatile extracts. Its leaves are used in the African pharmacopoeia for the treatment of liver failure, fever, high blood pressure and malaria [4]**.** In Ivory Coast, the leaves of *Lippia multiflora* are used to relieve febrile attacks, bronchial conditions, conjunctivitis, and jaundice, gastrointestinal and enteric disorders [5]**.** A preliminary phytochemical screening of non-volatile extracts of *Lippia multiflora* carried out at the LAPISEN laboratory revealed the presence of sterols, saponins, alkaloids, tannins, flavonoids and terpenoids [6]. Many researchers have reported various pharmacological properties of the plant extract and its essential oil. The hydroethanolic extract of Lippia multiflora leaves has antibacterial activities [7] as well as positive inotropic and chronotropic effects on isolated toad heart [8]. The essential oil has insecticidal [5], antimicrobial [9], antioxidant and antiradical [10], sedative [11] , analgesic and antipyretic [12] and antimalarial [13] activities. The phytochemical study revealed the presence of n-tri triacontane, ursolic acid and salvigenin [12] in the leaves of Lippia multiflora. The evaluation of the different organic compounds of medicinal importance present in L. multiflora is currently not exhaustive. Therefore, this study aims to study in more detail the bioactive compounds present in the dichloromethane fraction from the hydroalcoholic extract of *L. multiflora* leaf because this fraction presents an interesting chromatographic profile.

However, one can generally expect to find essential oils, terpenoids, phenols, flavonoids and lipids such as phospholipids and triglycerides in this fraction.

2. Material and Method

2.1. Plant Material

The plant material consists of fresh leaves of *L. multiflora* (Figure-1) collected in the early morning (between 6:00 a.m. and 6:30 a.m.) in the center of Côte d'Ivoire in Yamoussoukro (6° 54'24158" North and 5°19' 25066" West) precisely in Djamalabo. *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 in the laboratory (25°C ± 2) in the shade for 7 days.

Figure-1. Photo of *Lippia multiflora* leaves

2.2. Methods

2.2.1. Preparation of the Hydroethanolic Extract

The dried leaves of *L. multiflora* were crushed using an electric grinder and the powder obtained (Figure-2) was sifted using a sieve with a mesh diameter of 0.5 mm. La poudre obtenue a été conservée au réfrigérateur à 4°C dans une bouteille sombre jusqu'à son utilisation.

Figure-2. *Lippia multiflora* leaf powder

2.2.2. Partition of the Hydroethanolic Extract

As part of our work, the fractions were obtained from the hydroethanolic extract followed by liquid-liquid fractionation [14]**.** Solvents used in order of increasing polarity are hexane, dichloromethane, ethyl acetate, ethanol and water. Thus, 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 Wattman N°2 paper. The operation was repeated until the ground material was exhausted. The filtrates obtained were 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. 10 g of hydroethanolic extract was 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 No. 2 paper, the extraction solvent is eliminated under reduced pressure to give the fractions with hexane (FHex), dichloromethane (FDCM), and ethyl acetate. (FAE). The aqueous phase is concentrated and extracted by maceration with ethanol. After filtration on cotton and Whatmann No. 2 paper, the ethanol is eliminated under reduced pressure to give the ethanol fraction (FETH). The final aqueous phase is lyophilized to give the aqueous fraction (FAQ). All these fractions were stored in the refrigerator at 4°C in a dark container.

2.2.3. Phytochemical Screening

The phytochemical screening carried out allows us to have a general idea of the different families of secondary metabolites present in plants. It is based on specific coloring or precipitate reactions characteristic of classes of secondary metabolites using appropriate reagents [11]**.**

2.2.3.1. Alkaloid Test

The residue obtained from 6 mL of evaporated extract is taken up in 6 mL of alcohol at 60° (alcoholic degree) and the alcoholic solution thus obtained is distributed in 2 test tubes. In the first tube, 2 drops of Dragendorff reagents are added. The appearance of a precipitate or orange color indicates the presence of alkaloids. In the second tube, 2 drops of Bouchardât reagent are added. The appearance of reddish-brown coloring indicates the presence of alkaloids [11, 15]**.**

2.2.3.2. Polyphenol Test

A drop of 2% alcoholic ferric chloride solution is added to 2 mL of extract. The appearance of a more or less dark blackish blue or green color reflects the presence of phenolic compounds [16]**.**

2.2.3.3. Flavonoid Test

A volume of 2 mL of extract is evaporated to dryness. After cooling, the residue is taken up with 5 mL of hydrochloric alcohol diluted twice in a test tube. Then 2-3 magnesium chips are added there. Finally, the addition of 3 drops of isoamyl alcohol, intensifying an orange or purplish color, indicates the presence of flavonoids [17]**.**

2.2.3.4. Tannin Test

One (1) mL of hydrochloric alcohol solution (mixture of 5 mL of alcohol, 5 mL of distilled water, 5 mL of concentrated hydrochloric acid) is added to 5 mL of infused tea. The solution obtained is then brought to a boil for 15 minutes. The formation of red precipitate soluble in amyl alcohol indicates the presence of catechic tannins [18]**.**

2.2.3.5. Leucoanthocyanins Test

Leucoanthocyanins are characterized by the reaction with cyanidin without addition of magnesium shavings with heating for 15 min in a water bath. In the presence of Leucoanthocyanins, a cherry-red or purplish color develops [11]**.**

2.2.3.6. Terpenoid Test

A volume of 5 mL of the solution to be analyzed is evaporated to dryness in a capsule on a sand bath. The residue obtained is dissolved hot in 1 mL of acetic anhydride (CH3CO)2O then transferred into a test tube to which 0.5 mL of concentrated sulfuric acid (H2SO4) is added. The reaction is positive if there is the appearance of a purple or purple ring turning blue then green [15]**.**

2.2.3.7. Saponosides Test

A mass of 1 g of vegetable powder is introduced into a 250 mL Erlenmeyer flask to which 100 mL of distilled water are added. The mixture is slightly heated, filtered, cooled and made up to 100 mL with distilled water. Into a test tube, 20 mL of the filtrate is introduced and shaken vigorously for 15 seconds. The tube is placed vertically for 15 minutes. After this period, if the foam persists, then the herbal drug contains saponins [11]**.**

2.2.4. Colorimetric Analyzes of Samples

2.2.4.1. Determination of Total Polyphenols

The determination of total polyphenols was carried out according to the method described by Wood, *et al.* [19]. To a volume of 30 µL of extract, 2.5 mL of Folin-Ciocalteu reagent diluted 1/10 are added. The mixture obtained is kept for 2 min in the dark at room temperature (27 ± 03°C) then 2 mL of a sodium carbonate solution (75 $g.L^{-1}$) are added. The solution obtained is then incubated at 50°C for 15 min. The absorbance is read with a UV-visible spectrophotometer at a wavelength of 760 nm against a blank consisting of 5 mL of Folin-Ciocalteu reagent diluted 1/10 and 4 mL of the carbonate solution of sodium (75 $g.L^{-1}$). Gallic acid is used as a reference standard for the quantification of total polyphenol contents expressed in mg of gallic acid equivalent per gram of extract (mg EAG/g of extract). The tests were carried out in triplicate for each sample. The polyphenol content is given by the formula in relation (1).

$$Q = (V \times C \times d)/m \quad (1)$$

With V the final volume of the extract (mL);
C: the concentration of the extract obtained with the calibration curve (g/L);
D: dilution
m: the mass of the test sample (g).

2.2.4.2. Assay of Total Flavonoids

The total flavonoid content was determined according to the method described by Marinova and colleagues [20]. Volumes of 0.75 mL of sodium nitrite at 5% (m/v) and Aluminum chloride at 10% (m/v) are added to 2.5 mL of extract with a ratio of 1/500 (m /V). After 6 min of incubation, the mixture is brought into contact with 5 mL of a 1 M sodium hydroxide solution. The volume obtained is adjusted to 25 mL then shaken vigorously. The absorbance is measured at a wavelength of 510 nm. Quercetin is used as a reference standard. The quantification of total flavonoid contents is expressed in milligrams of quercetin equivalent per gram of extract (mg $EQ.g^{-1}$ of extract). The tests were carried out in triplicate for each sample. The flavonoid content is given by the formula of relation (1)

2.2.4.3. Dosage of Tannins

2.2.4.3.1. Condensed Tannins

The condensed tannin content was determined according to the method described by Marinova, *et al.* [20]**.** To 500 µL of sample extract is added 1 mL of 1% vanillin solution with sulfuric acid. The tubes are placed in a water bath for 15 min at 20°C. The absorbance is measured at a wavelength of 500 nm. The condensed tannin content is given by the formula in relation (2).

$$T\ (\%) = \frac{5{,}2.10^{-2} \times DO \times V}{P} \quad (2)$$

With $5.2.10^{-2}$: Constant expressed in cyanidin equivalent;
DO: optical density;
V: volume;
P: test portion;
T%: condensed tannin content.

2.2.4.3.2. Hydrolyzable Tannins

The content of condensed hydrolyzable tannins was determined according to the method described by Mole and Waterman [21]**.**

To 500 µL of the extract is added 1.75 mL of a reagent consisting of a mixture of 1 mL of 0.01 M $FeCl_3$ solution in 1 mL of 0.001 M HCl solution. The absorbance is measured at a wavelength of 660 nm, 15 seconds after addition of the reagent. The hydrolyzable tannin content is given by the formula in relation (3).

$$T\ \% = DO \times (M \times V/Emole * P) \quad (3)$$

With DO the optical density;
Emole: 2169 gallic acid;
M: 300;
V: volume of extract used

2.2.5. GC-MS Analysis Methods

A mass of 10 mg of sample was derivatized by adding 250 µL of a mixture of N, O-(trimethylsilyl) trifluoroacetamide and Trimethylchlorosilane (BSTFA + TMCS, 99:1 (v/v)) and 250 µL 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.

The GC-MS analysis was carried out on a Perkin Elmer brand device, model Clarus 680GC 600C MS equipped with a Restek Rtx-5ms column 60 m long, with an internal diameter of 0.25 mm and 'a stationary phase film thickness of 0.25 µm. 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. The identification of silylated compounds was possible by comparison of retention times and mass spectra with those of the standards. Spectral data were obtained from the Wiley and NIST libraries [22].

2.2.6. Statistical Analyzes

All statistical values were expressed as mean ± standard error of mean (SEM). Linear correlation coefficients (R^2) are calculated using Microsoft Excel 2010 softwar.

3. Results and Discussion

Plants have been a source of organic compounds for centuries, and their potential to provide new and effective treatments for a wide range of diseases remains largely untapped. Despite the great diversity of plant species on Earth, our knowledge of their chemical constituents and their biological activities is still relatively limited.

Secondary metabolites such as phenolics, flavonoids, tannins, saponins, alkaloids, leucoanthocyanins, steroids, anthraquinones, terpenoids and carbohydrates are known to be present in plants. These phytochemicals (or secondary metabolites) are also known to possess biological activities such as anticancer, antioxidant, anti-inflammatory, antidiabetic, antimicrobial, hepatoprotective, antianthritic, hypoglycemic and hypocholesterolemic.

3.1. Qualitative Phytochemical Screening

Qualitative phytochemical screening of the dichloromethane fraction from the hydroalcoholic extract of *L. multiflora* leaf revealed the presence of alkaloids, polyphenols, flavonoids, sterols and terpenes, while saponins, tannins and leucoanthocyanins were absent (Table-1). Our results are slightly different from those reported in the literature [23-25].

Table-1. Results of phytochemical screening of the dichloromethane fraction from the hydroalcoholic extract of *Lippia multiflora* leaves

Secondary Metabolite Families	Results
Alcaloïdes	+
Polyphenols	+
Flavonoids	+
Gallic tannins	-
Catechical tannins	-
Leucoanthocyanins	-
sterols and terpenes	+
saponins	-

3.2. Quantitative Phytochemical Analysis

Table-2. Results of quantitative analysis of the dichloromethane fraction of the constituents from the hydroethanolic extract of *L. multiflora* leaves

Constituant phytochimique	Moyenne ± SEM
Polyphenols	2,4 ± 0,00 mg EQ/g dry extract
Flavonoids	3,5 ± 0,006 mg EQ/g dry extract
Condensed tannins	45,91 ± 0,11 %
Hydrolyzable tannins	14,1 ± 0,01 %

The contents of total polyphenols and total flavonoids were quantified from the preestablished standard curves of gallic acid and quercetin, unlike the tannin contents. The results show that the contents of total polyphenols and flavonoids are 2.4 ± 0.00 mg EAG/g of dry extract and 3.5 ± 0.006 mg EQ/g of dry extract respectively (Table-2). The dichloromethane fraction contains a small amount of polyphenols and flavonoids. These results which are in agreement with the phytochemical screening (Table-1) have been reported in the literature [23, 25]. The contents of condensed tannins and hydrolyzable tannins were also found to be 45.91 ± 0.11% and 14.1 ± 0.01%, respectively (Table-2). These results are not in agreement with those of the phytochemical screening (Table-1). Our results show the same trends as those in the literature [25].

3.3. 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.

3.4. GC-MS Analysis of the Dichloromethane Fraction

Figure-3. GC chromatogram of the dichloromethane fraction from the hydroethanolic extract of *L. multiflora* leaves

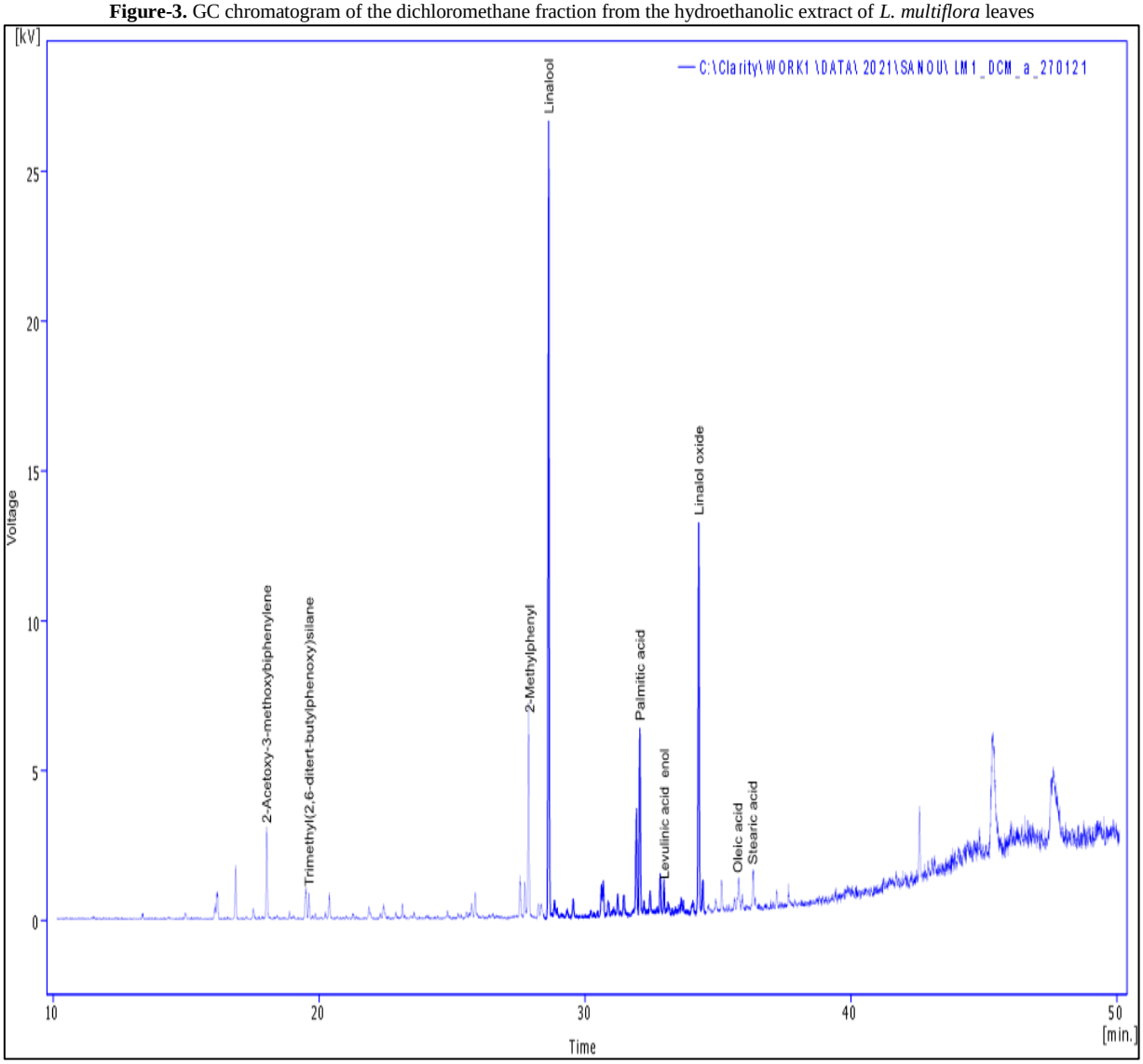


Figure-3 presents the GC timeline of the dichloromethane fraction from the hydroethanolic extract of *Lippia multiflora* leaves before extraction of the essential oil.

This chromatogram shows the presence of several peaks corresponding to several silylated compounds with retention times between 18.019 and 36.295 minutes.

The proposed structures of the different compounds in Figure-3 are summarized in Table-3. These are nine (9) compounds including six (6) terpenes and sterols, two (2) polyphenols and one (1) alkaloid. Among these compounds, linalool, linalool oxide, palmitic acid and N-(2-mehtylphenyl)-3-hydroxybut-2-en amide are the most abundant with proportions of 42.1 %, 22. 1 %, 10.9 % and 10.6 %.

Table-3. Compounds detected by GC-MS in the dichloromethane fraction from the hydroethanolic extract of *L. multiflora leaves*

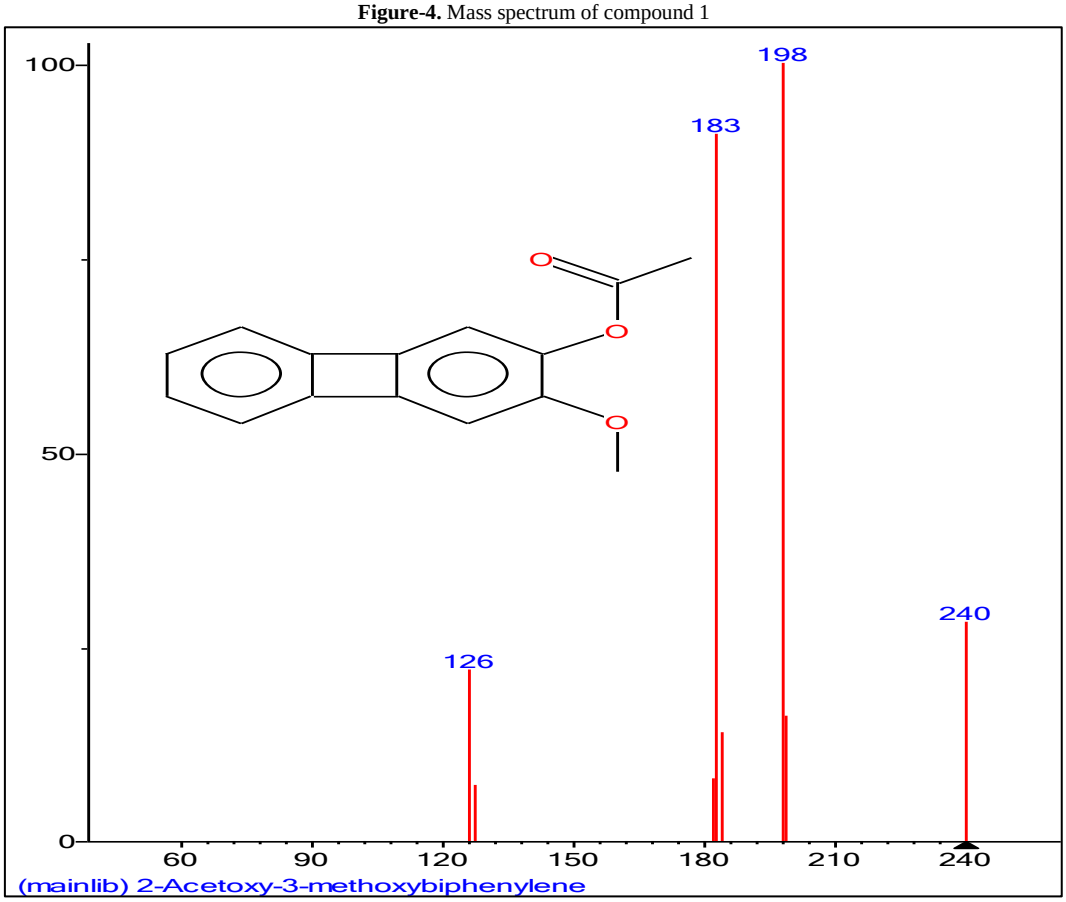
N°	Temps de rétention (min)	Nom de la molécule	%	Formule brute	Masse molaire (g/mol)
1	18,019	2-Acetoxy-3-methoxybiphenylene	5,4	$C_{15}H_{12}0_3$	240,08
2	19,612	2,6-di-*tert*-butylphenol	1,3	$C_{14}H_{22}0$	206,17
3	**27,913**	**N-(2-methylpheyl)-3-hydroxybut-2-en amide**	**10,6**	$\mathbf{C_{11}H_{13}NO_2}$	**191,09**
4	**28,630**	**Linalool**	**42,1**	$\mathbf{C_{10}H_{18}O}$	**154,14**
5	**32,061**	**Palmitic acid**	**10,9**	$\mathbf{C_{16}H_{32}O_2}$	**256,24**
6	32,974	Acide enol levulinique	1,6	$C_5H_8O_3$	116,05
7	**34,273**	**Linalool oxide**	**22,1**	$\mathbf{C_{10}H_{18}O_2}$	**170,13**
8	35,737	Oleic acid	2,8	$C_{18}H_{32}O_2$	282,26
9	36,295	Stearic acid	3,2	$C_{18}H_{36}O_2$	284,27

Confirmation of the structures of the nine (09) compounds detected in the dichloromethane fraction. In order to confirm or not the proposed structures, it is essential to study the fragmentation spectra of the different peaks.

Compound 1

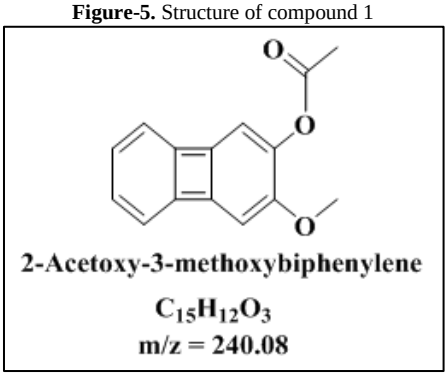
According to the gas chromatograph database coupled with mass spectrometry, compound 1 with a retention time of 18.019 minutes corresponds to 2-Acetoxy-3-methoxybiphenlene whose mass spectrum is given in Figure-4.

Figure-4. Mass spectrum of compound 1



The analysis of Figure-4 reveals that the molecular peak has the mass m/z = 240 which corresponds to the molecular mass of 2-Acetoxy-3-methoxybiphenlene (non-silylated compound). The most abundant fragment has a mass of m/z = 197 and the other fragments have masses of m/z = 183 and m/z = 126. This compound was synthesized by Kaza [26]**.** The mass spectrum data are similar to those in the literature. Compound 1 is therefore 2-Acetoxy-3-methoxybiphenlene (Figure-5).

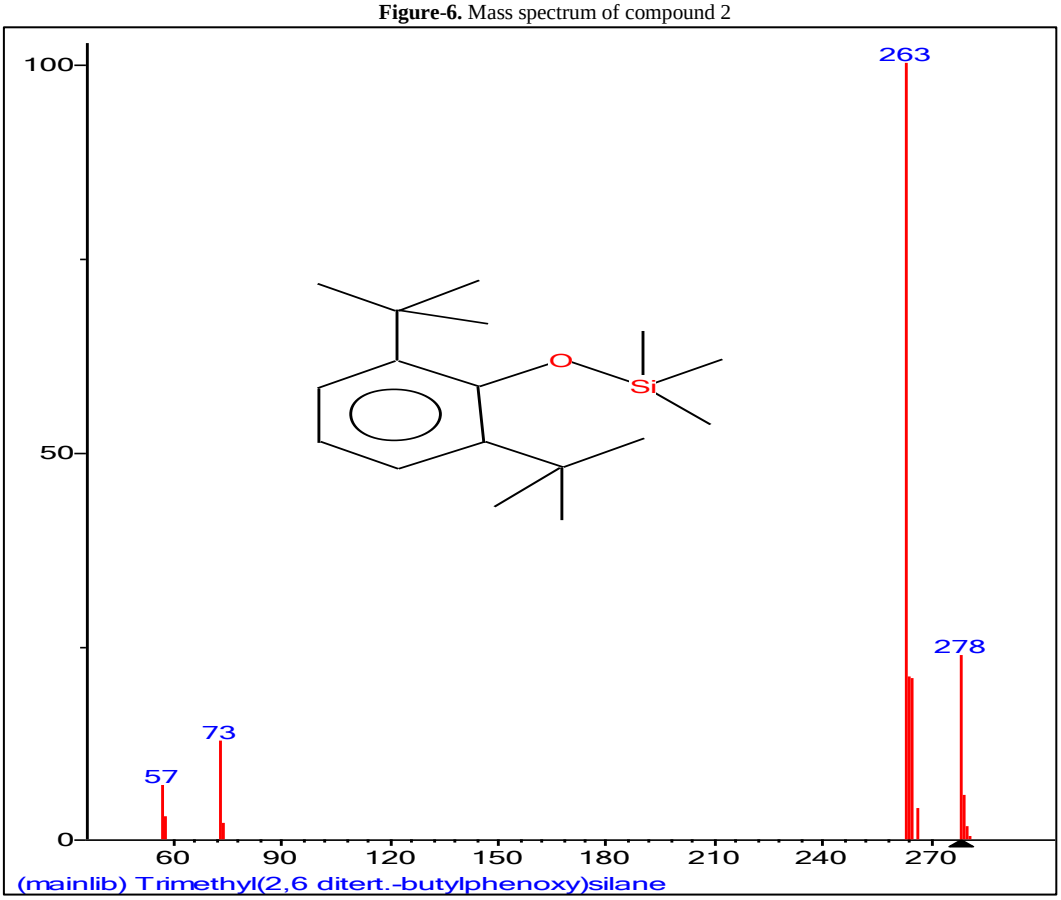
Figure-5. Structure of compound 1



Compound 2

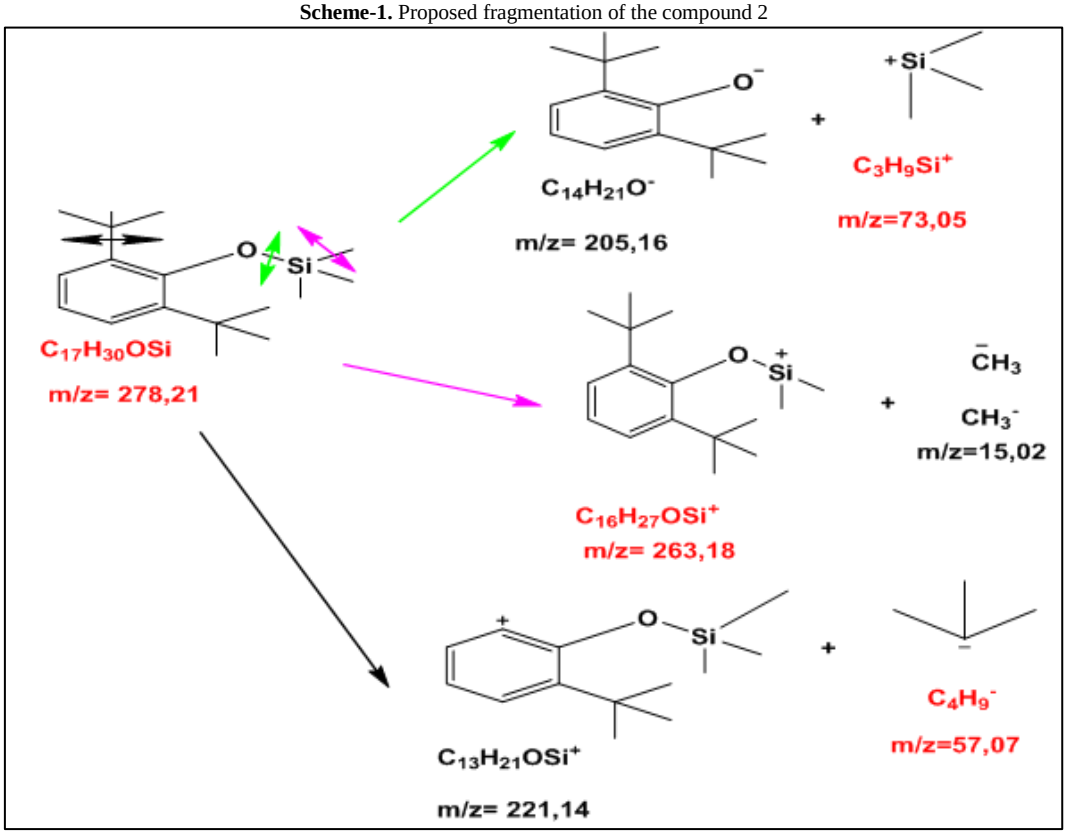
The device database indicates that compound 2 with a retention time of 19.612 minutes corresponds to Trimethyl (2,6-di-tert-butylphenoxy) silane whose mass spectrum of the silylated compound is given in Figure-6.

Figure-6. Mass spectrum of compound 2



Analysis of the mass spectrum in Figure-6 reveals that the molecular peak has the mass m/z = 278 which represents the molecular ion. The most abundant fragment has a mass of m/z = 263 and the other fragments have masses of m/z = 73 and m/z = 57.

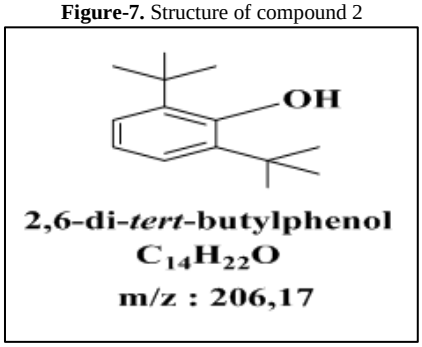
Scheme-1. Proposed fragmentation of the compound 2



The most abundant fragment at m/z =263 would result from the loss of a methyl group on silicon. The fragment at m/z =73 could be a trimethylsilyl group which results from the breakage of the oxygen-silica bond. The fragment at m/z =57 (C_4H_9) would come from the breakage of a carbon-carbon bond between a tert-butyl group and the

aromatic ring (Scheme-1). Compound 2 is therefore 2, 6-di-tert-butylphenol (Figure-7), belonging to the family of phenolic compounds. It plays the role of an antioxidant and has not yet been identified in the plant studied.

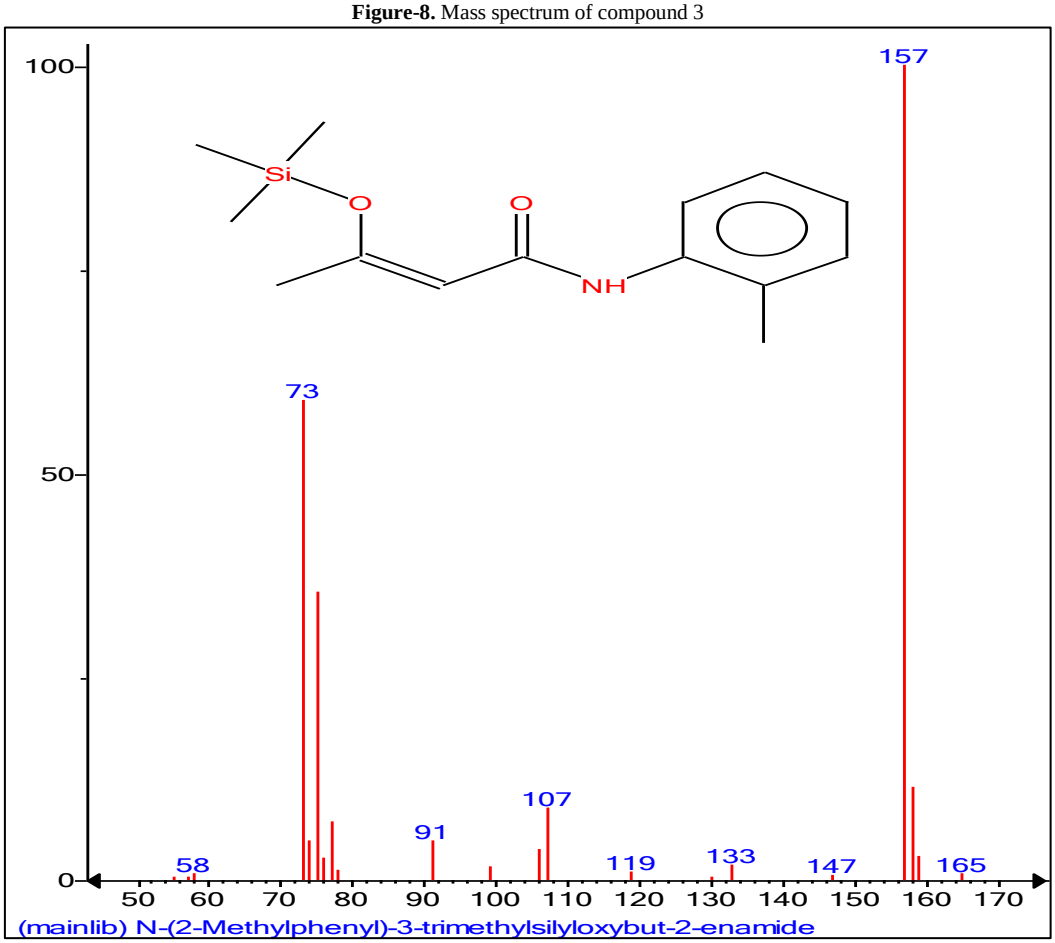
Figure-7. Structure of compound 2



Compound 3

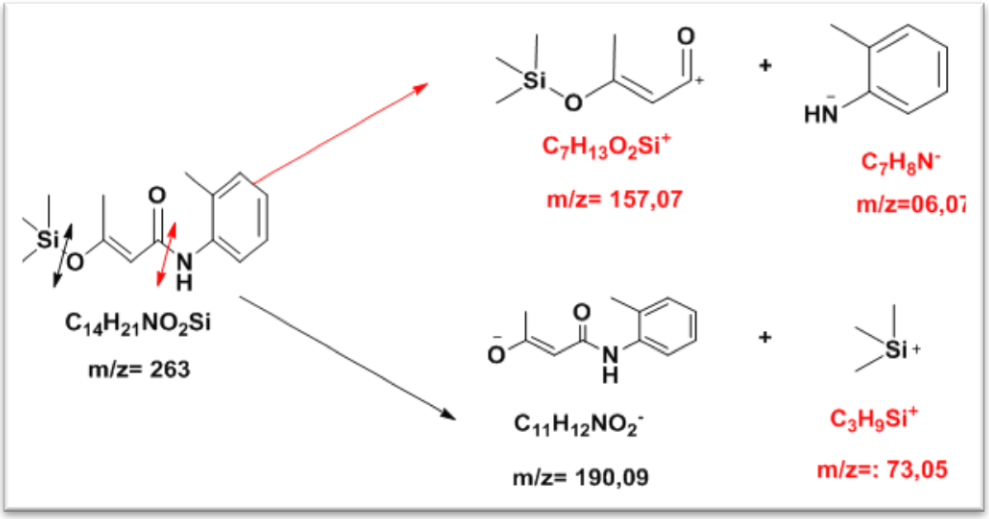
Compound 3, with a retention time of 27.913 minutes, corresponds to N-(2-methylphenyl) -3-hydroxybut-2-in silylated amide with molecular mass m/z = 263 which does not appear on the mass spectrum (Figure-8).

Figure-8. Mass spectrum of compound 3



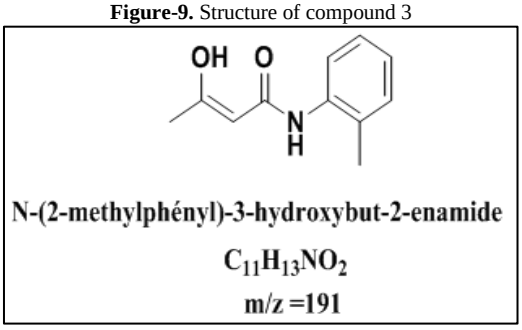
Mass spectrum analysis of the silylated compound (Figure-8) reveals the presence of major fragments at m/z = 157 (base peak), m/z =107, m/z =91, m/z =73. The disappearance of the molecular peak is due to total destruction of the molecule.

Scheme-2. Proposed fragmentation of the compound 3



The most abundant fragment at m/z = 157 and m/z = 106 (m/z = 107 on the Spectrum, a difference which may be due to isotopes) would result from the breakage of a carbon-nitrogen bond. The fragment with mass m/z = 73 could be a trimethylsilyl group which comes from the breakage of the oxygen-silicon bond (Scheme-2). Compound 3 is therefore N-(2-methylphenyl) -3-hydroxybut-2-en amide (Figure-9), belonging to the alkaloid family. It has not yet been identified in the plant studied.

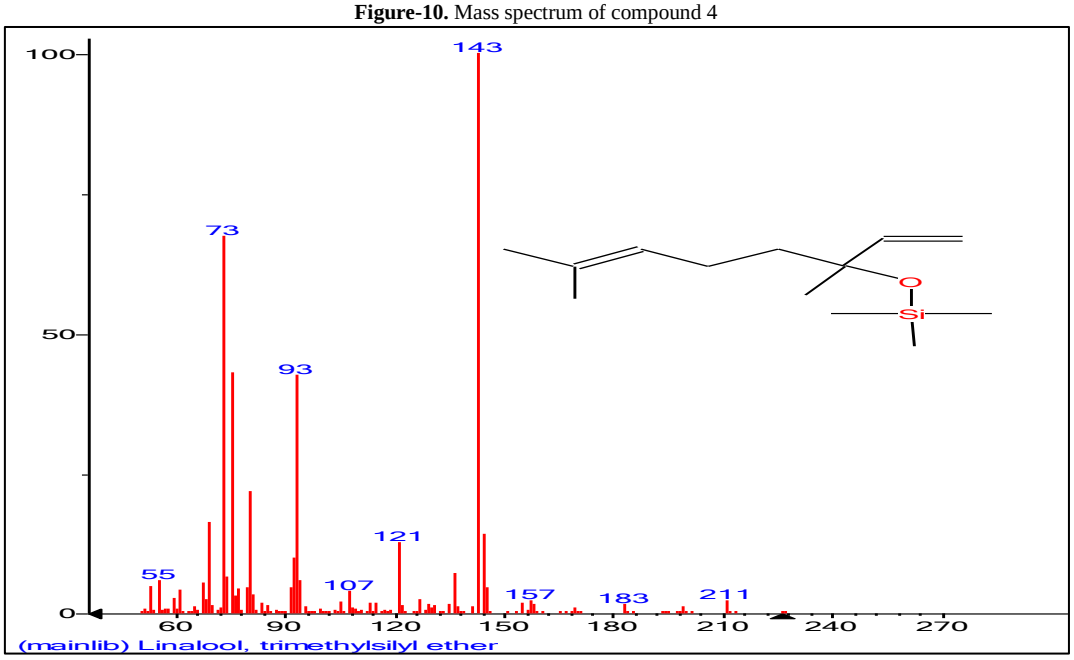
Figure-9. Structure of compound 3



Compound 4

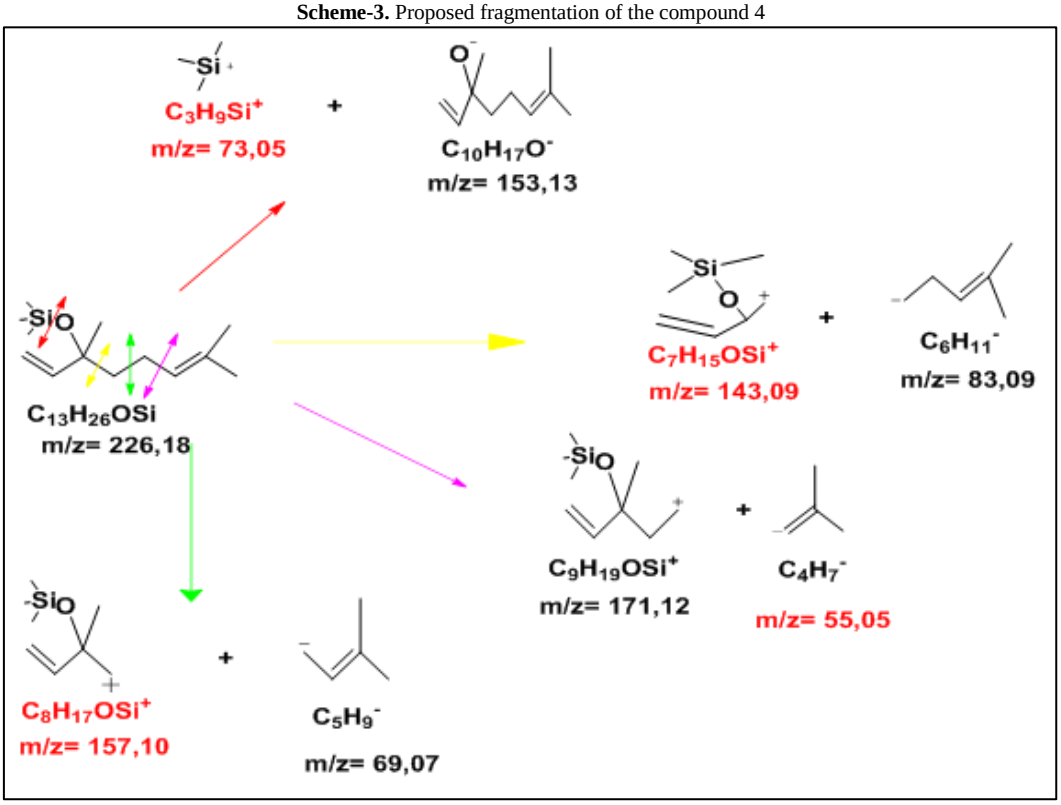
Compound 4, with a retention time of 28.630 minutes, corresponds to silylated Linalool with a molecular mass m/z = 226 (Figure-10).

Figure-10. Mass spectrum of compound 4



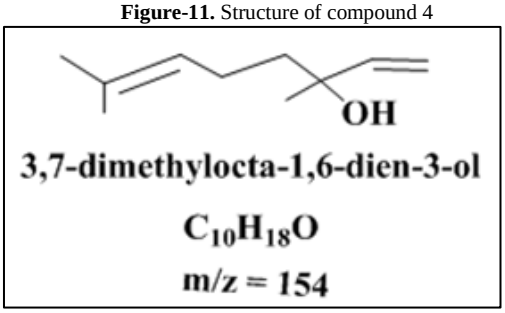
Mass spectrum analysis of the silylated compound (Figure-10) reveals the presence of major fragments at m/z = 157, m/z = 143 (base peak), m/z = 121, m/z = 107, m /z =93, m/z =73.

Scheme-3. Proposed fragmentation of the compound 4



The most abundant fragment at m/z = 143 (Figure-10) would result from the departure of the group of mass m/z = 83. The fragment of mass m/z = 73 would be a trimethylsilyl group resulting from the breakage of an oxygen bond -silicon. The fragments of mass m/z = 157 and m/z = 55 would result respectively from the breaking of the carbon-carbon bond at β and at α of the double bond of the iso group (Scheme-3). Compound 4 is therefore linalool belonging to the terpene family. It was identified in the essential oil of the plant [27].

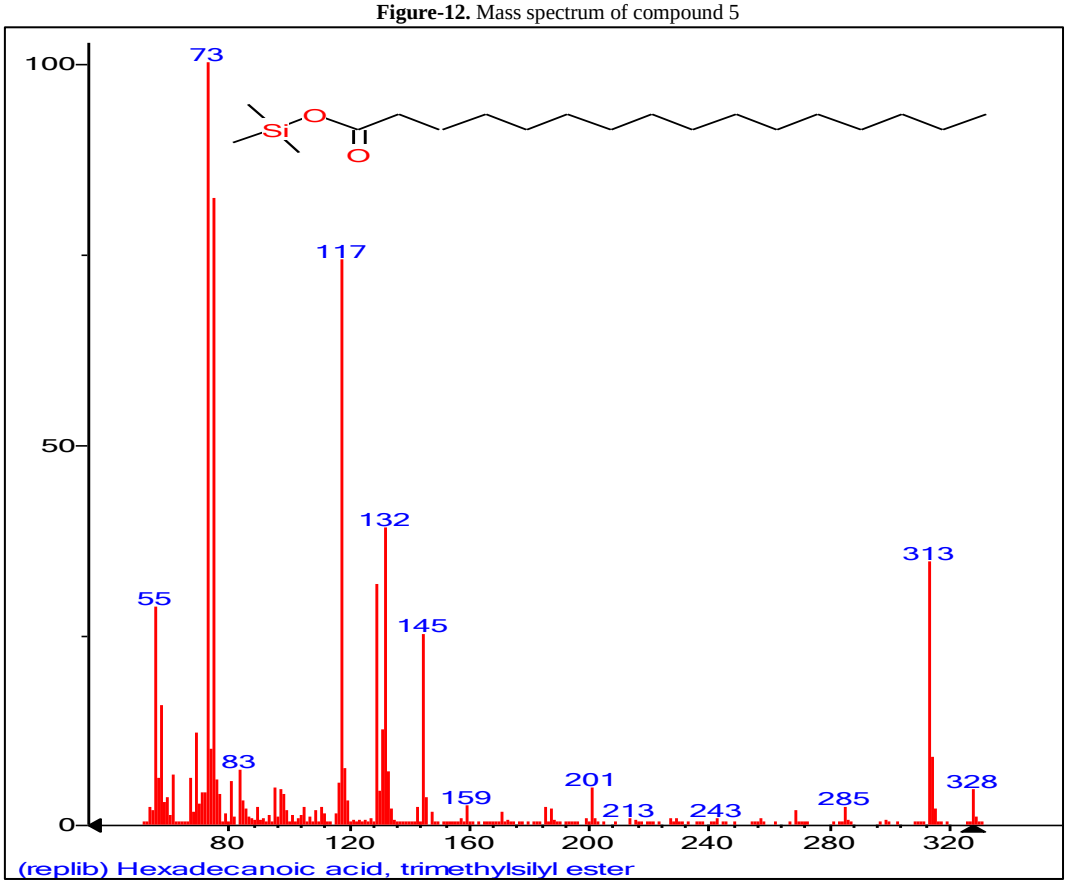
Figure-11. Structure of compound 4



Compound 5

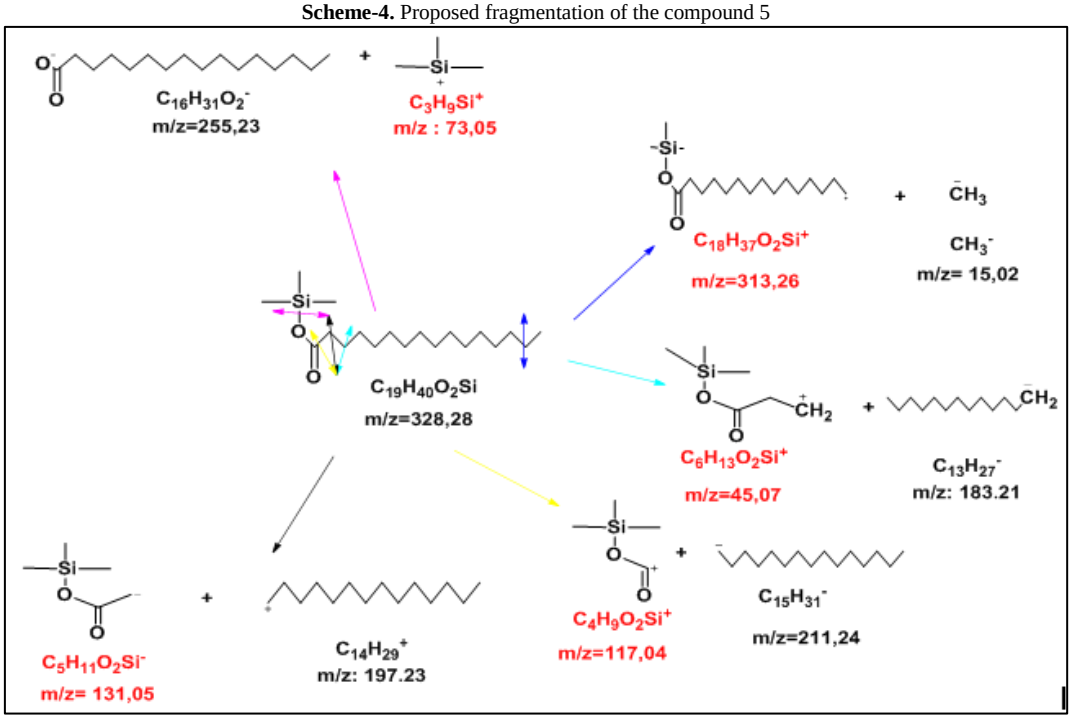
Compound 5 with a retention time of 32.061 minutes corresponds to silylated palmitic acid with a molecular mass m/z: 328 which represents the molecular ion (Figure-12).

Figure-12. Mass spectrum of compound 5



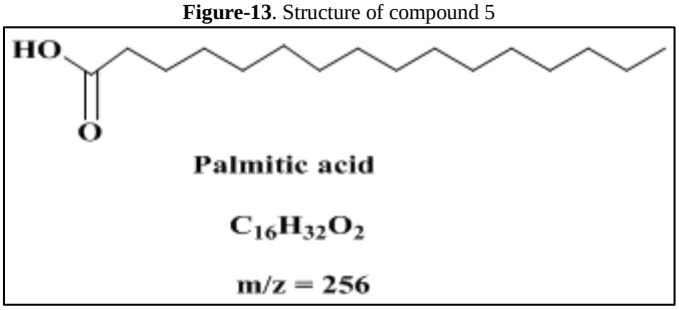
Mass spectrum analysis of the silylated compound (Figure-12) reveals the presence of major fragments at m/z = 328, m/z = 313, m/z = 201, m/z = 145, m/z = 132, m /z = 117(base peak), m/z =73, m/z = 55.

Scheme-4. Proposed fragmentation of the compound 5



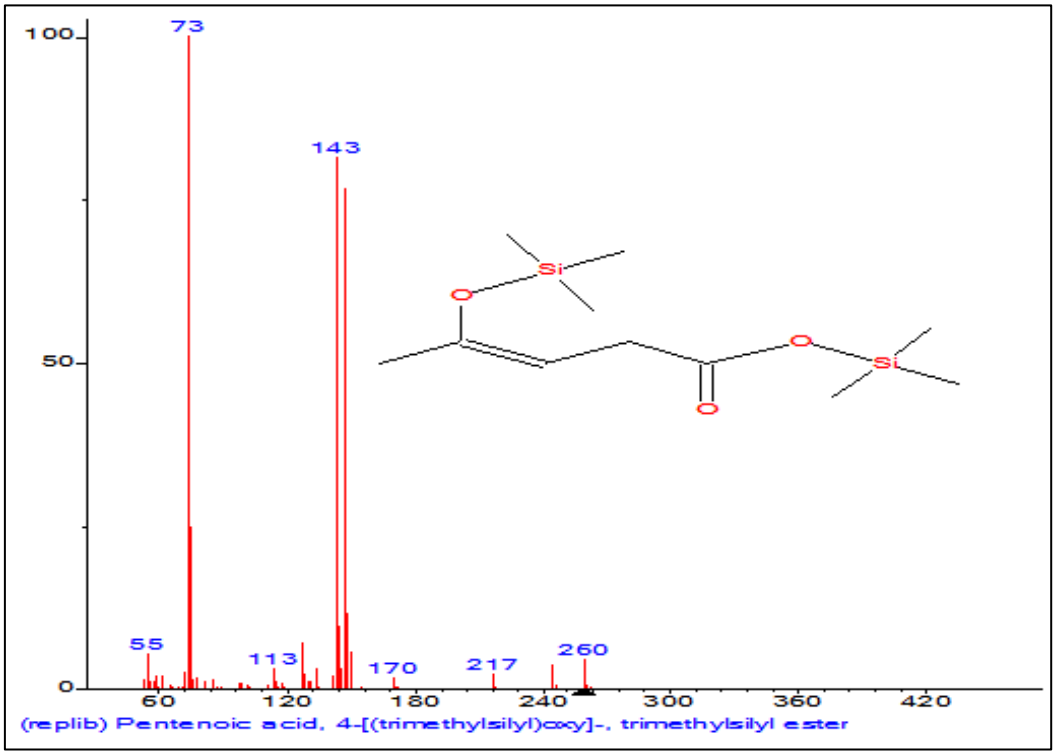
The base peak with mass m/z = 73 would be a trimethylsilyl group resulting from the breakage of the oxygen-silicon bond. The fragments with masses m/z = 313, m/z = 145, m/z = 131 and m/z = 117 would come from the breakage of the carbon-carbon bond or the loss of the respective alkyl groups CH_3, $C_{13}H_{27}$, $C_{14}H_{29}$ and $C_{15}H_{31}$ (Diagram-4). Compound 5 is therefore palmitic acid which is classified in the terpene family. It was identified in the leaves of Lippia multiflora [28].

Figure-13. Structure of compound 5



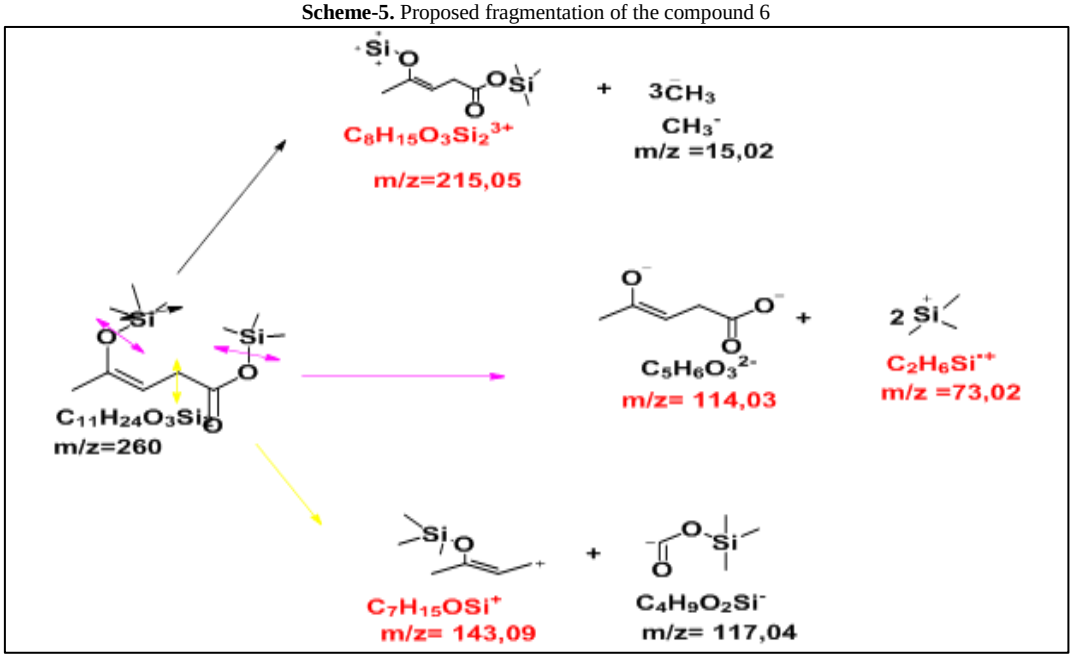
Compound 6

Compound 6, with a retention time of 32.974 minutes, corresponds to silylated levulinic acid (Figure-14) with a molecular mass m/z =260.



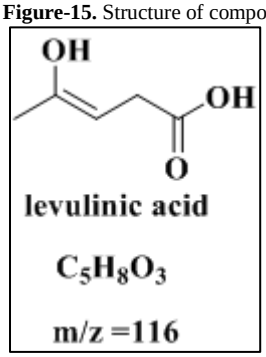
Mass spectrum analysis of the silylated compound (Figure-14) reveals the presence of major fragments at m/z = 260, m/z = 217, m/z = 170, m/z = 143 (base peak), m /z =73, m/z =55.

Scheme-5. Proposed fragmentation of the compound 6



The fragments at m/z = 73 and at m/z = 114 (m/z = 113 on the spectrum) would result from the breakage of two oxygen-silicon bonds. The fragment with mass m/z = 143 would come from the breakage of the carbon-carbon bond at α of the carboxylic group and the fragment with mass m/z = 215 (m/z = 217 on the spectrum, a difference which may be due to the isotopes) would result from the loss of three (3) methyl groups on silicon (Scheme-5). This compound would belong to the terpene family. It has not yet been identified in the plant studied.

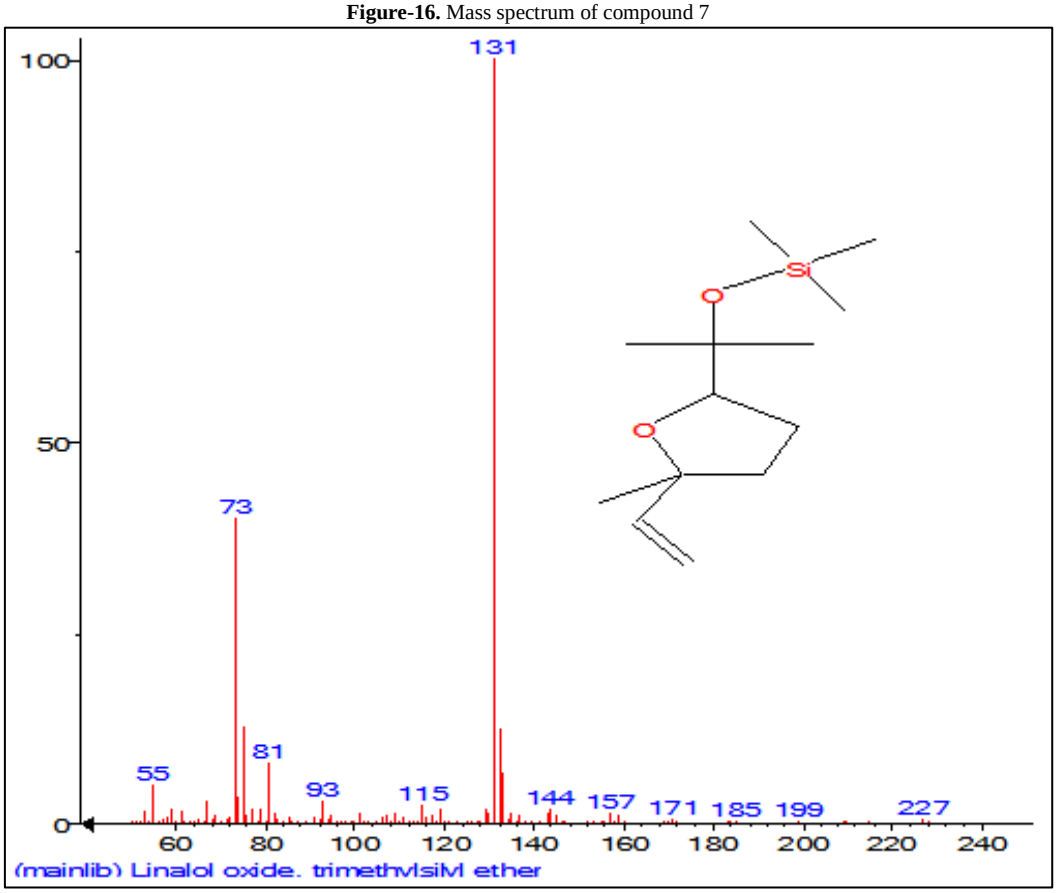
Figure-15. Structure of compound 6



Compound 7

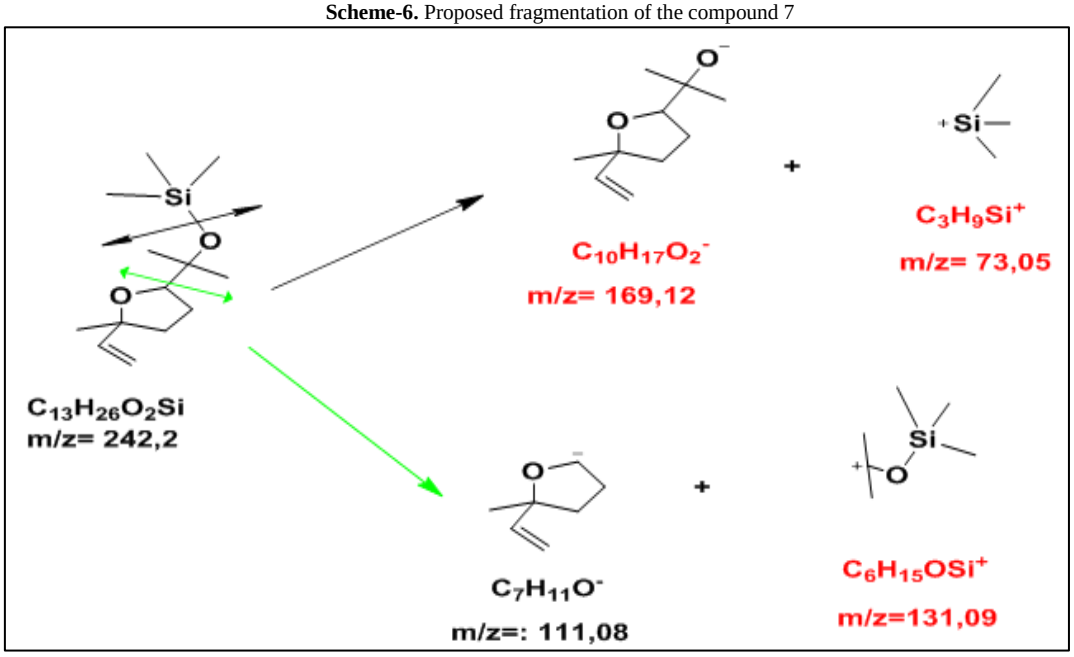
Compound 7, with a retention time of 34.273 minutes, corresponds to silylated linalool oxide with a molecular mass m/z = 242 which does not appear on the mass spectrum (Figure-16).

Figure-16. Mass spectrum of compound 7



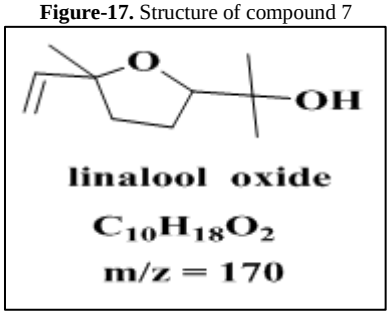
Mass spectrum analysis of the silylated compound (Figure-16) reveals the presence of major fragments at m/z = 171, m/z = 131 (base peak), m/z = 81, m/z =73, m /z = 55. The disappearance of the molecular peak is due to total destruction of the molecule.

Scheme-6. Proposed fragmentation of the compound 7



The mass spectrum of the silylated compound reveals that the base peak has the mass m/z = 131 (Figure-16) and could be obtained by the disruption of a group of mass m/z = 111. The fragments of mass m/ z = 73 and m/z = 169 (m/z = 171 on the spectrum, a difference which may be due to isotopes) would result from the breakdown of the oxygen-silicon bond (Diagram-6). Compound 7 is therefore linalool oxide, belonging to the family of terpene compounds (heterocycle). It was identified in the essential oil of the leaves of the plant studied [29].

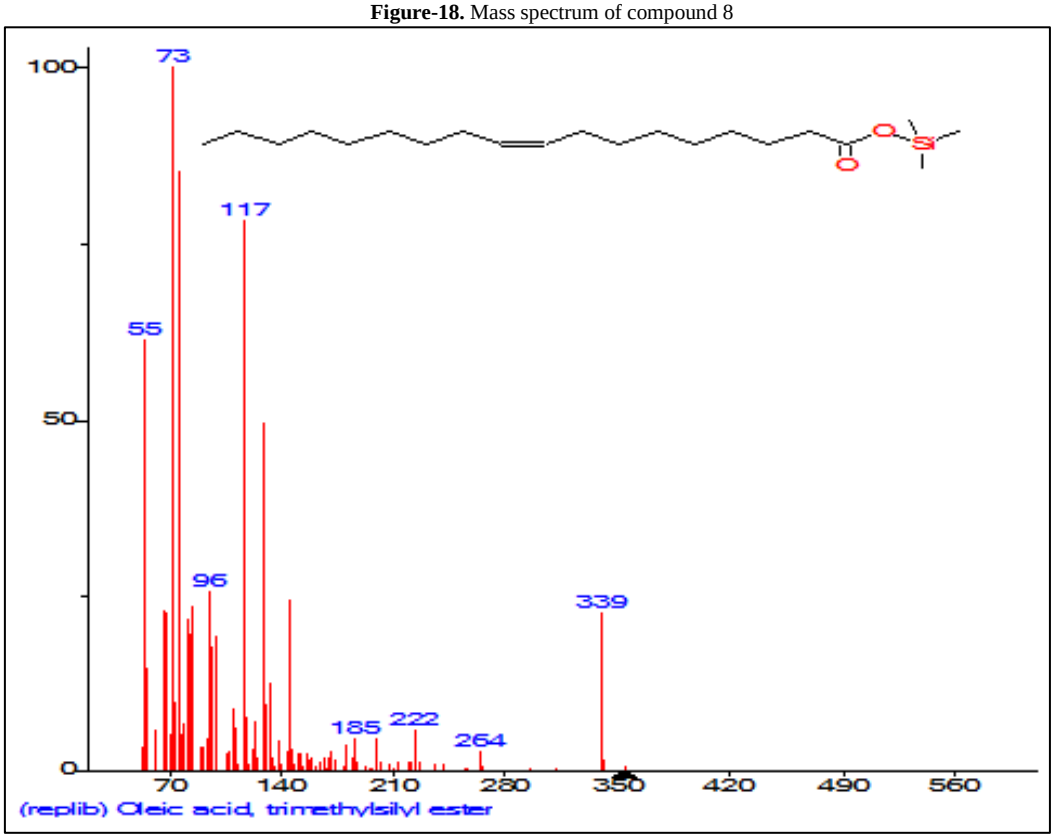
Figure-17. Structure of compound 7



Compound 8

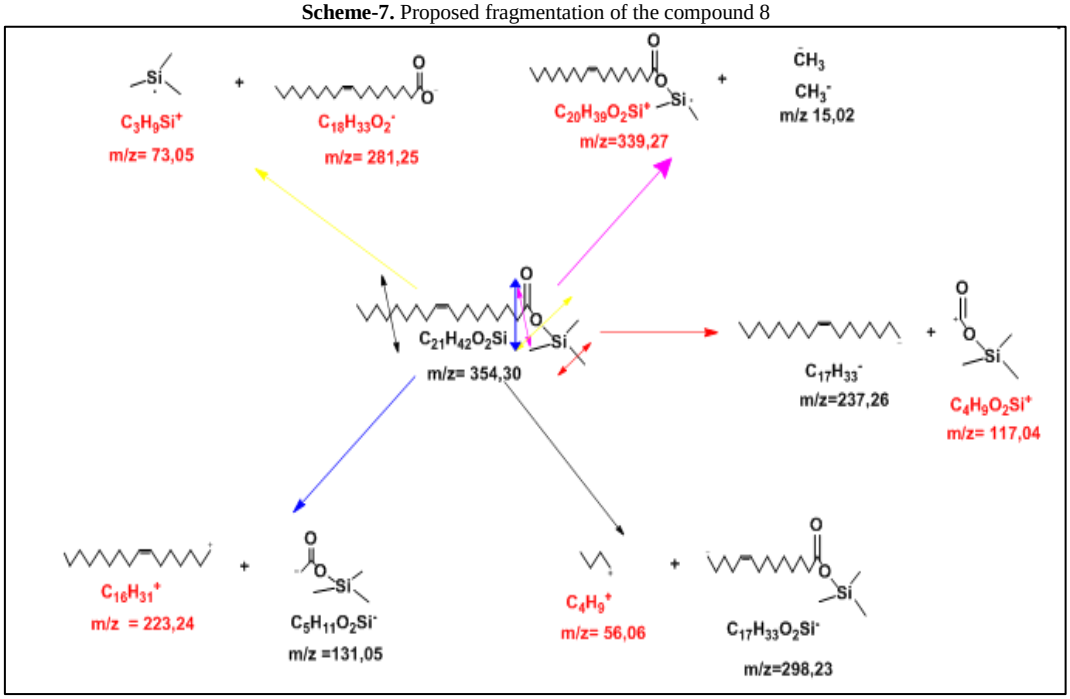
Compound 8, with a retention time of 35.737 minutes, corresponds to silylated oleic acid with a molecular mass m/z: 350 which represents the molecular ion (Figure-18).

Figure-18. Mass spectrum of compound 8



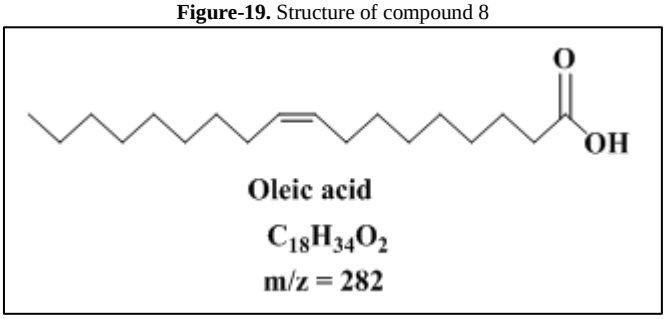
Analysis of the fragmentation spectrum of the silylated compound (Figure-18) shows the presence of major fragments at m/z = 339, m/z = 264, m/z = 222, m/z = 185, m/z = 117 (base peak), m/z = 96, m/z = 73, m/z = 55.

Scheme-7. Proposed fragmentation of the compound 8



The spectrum of the silylated compound (Figure-18) reveals that the fragment base peak m/z = 73 would be a trimethylsilyl group resulting from the breakage of the oxygen-silicon bond. The fragment with mass m/z =339 would be due to the loss of the methyl group (CH_3). The fragment with mass m/z = 56 (m/z = 55 on the spectrum) would be due to the breakage of the carbon-carbon bond at α of the group carboxylic. The fragment with mass m/z = 117 would come from the breakage of a carbon-carbon bond (Diagram-7). Compound 8 is therefore oleic acid, belonging to the terpene family. It was identified in the leaves of Lippia multiflora [28].

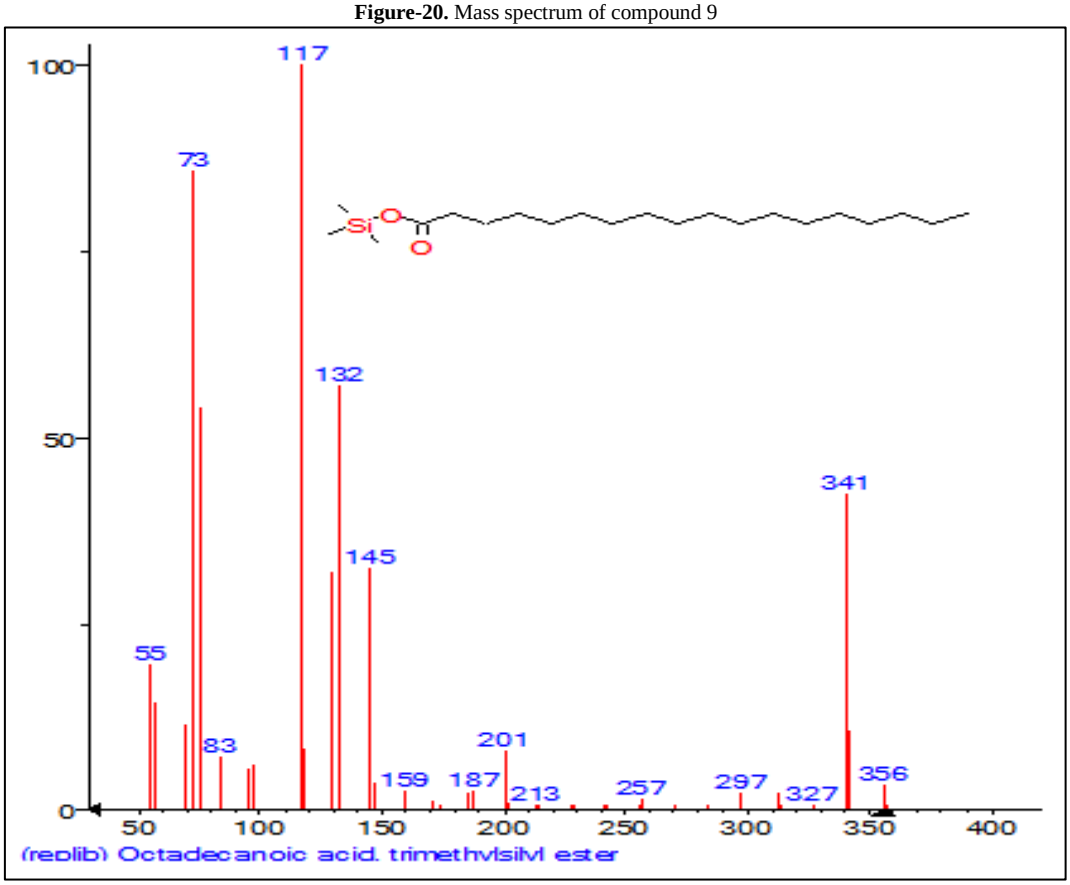
Figure-19. Structure of compound 8



Compound 9

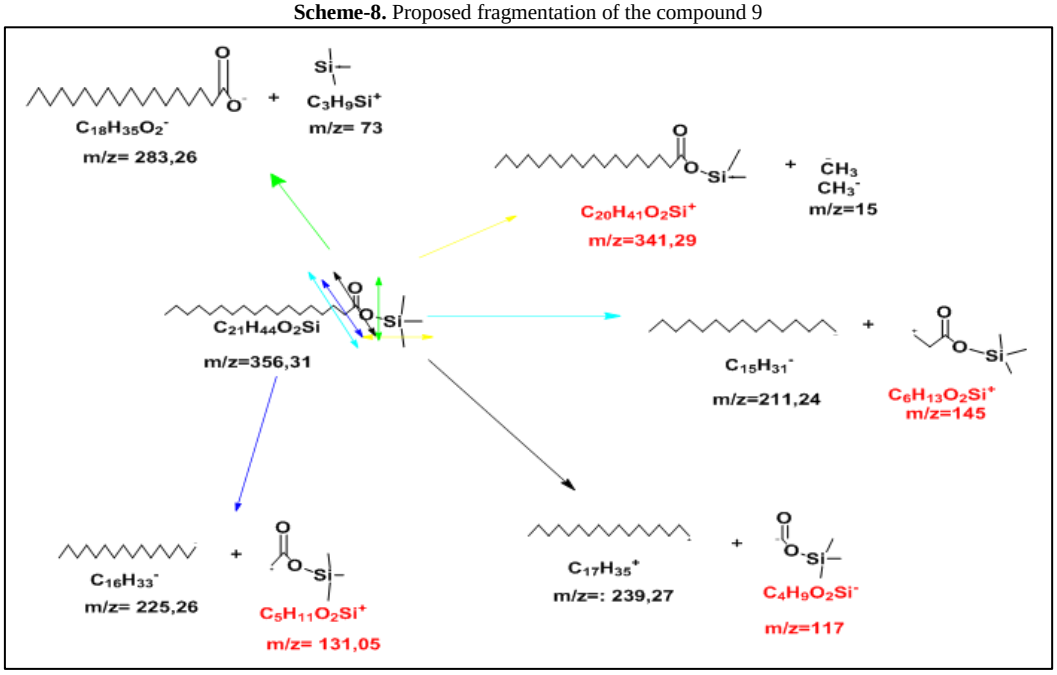
Compound 9, with a retention time of 36.295 minutes, corresponds to silylated stearic acid with a molecular mass m/z: 356 which represents the molecular ion (Figure-20).

Figure-20. Mass spectrum of compound 9



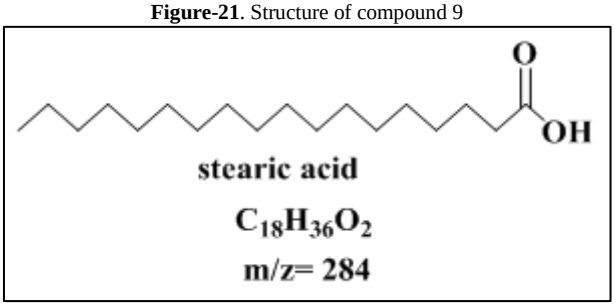
Analysis of the fragmentation spectrum of the silylated compound (Figure-20) shows the presence of major fragments at m/z = 356, m/z = 341, m/z = 145, m/z = 132, m/z = 117 (base peak), m/z = 83, m/z = 73, m/z = 55.

Scheme-8. Proposed fragmentation of the compound 9



The fragments of mass m/z = 117, m/z = 131 (m/z = 132 on the spectrum) and m/z = 145 would be due to the breaking of carbon-carbon bonds in α, β, γ respectively of the group carboxylic. The fragment with mass m/z = 341 would come from the breakage of the methyl group and the fragment with mass m/z = 73 would be a trimethylsilyl group resulting from the breakage of the oxygen-silicon bond. (Diagram-8). Compound 9 is therefore stearic acid belonging to the terpene family. It was identified in the leaves of Lippia multiflora [28].

Figure-21. Structure of compound 9



4. Conclusion

With a view to identifying the phytochemical compounds present in the leaves of Lippia multiflora, a phytochemical study and a structural analysis on the dichloromethane fraction of the leaves of Lippia multiflora were carried out. This study allowed us to obtain for phytochemical screening the presence of alkaloids, polyphenols, flavonoids, sterols and terpenes as well as tannins. The spectrophotometric determination of these secondary metabolites showed that the contents of flavonoids and total polyphenols are low. The GC-MS structural analysis method allowed the detection of nine (09) compounds including six (06) terpenes and sterols, two (02) polyphenols and one (01) alkaloid. Among the proposed molecular formulas, five (05) correspond to molecules already identified in the Lippia genus. The identification of these families of molecules corroborates the use of this plant in traditional medicine.

Competing Interests

Authors have declared that no competing interests exist concerning this manuscript.

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