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# Compound Identification by HPLC-ESI-Q-TOF-MS/ MS Analysis of the Dichloromethane Fraction of *Hyptis suaveolens* Leaves After Extraction of the Essential Oil

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**Abstract:** *Hyptis suaveolens (Lamiaceae*), widespread in tropical areas of America, Asia, and Africa, is used in more than twenty-two countries for its medicinal properties. The plant's leaves contain polyphenols, tannins, sterols and terpenes, saponins, flavonoids, quinones and anthraquinones before and after extraction of the essential oil. However, no molecular structures were identified in the leaves of the plant after extraction of the essential oil. So, after hydroethanolic (70/30; v/v) maceration of 100g of powder front and after extracting essential oil taken separately, a successively fractionate extract was obtained from solvents of increasing polarities. Next, the compounds of the dichloromethane fraction after extracting essential oil have been identified by HPLC-ESI- Q-TOF -MS/ MS method. As a result, the determination of the structures of nine (9) known compounds of the *Hyptis* genus was carried out by dereplication on the dichloromethane fraction from the hydroalcoholic extract (70%) of the plant's leaves after extraction of the essential oil. Of these nine (09) compounds, four (04) are phenolics and the remaining five (05) are terpenoids. Five (05) of these molecules had not yet been identified in *Hyptis suaveolens* leaves. This study shows that *Hyptis suaveolens* leaves after extraction of the essential oil are new source of bioactive compounds of pharmacological interest.

**Keywords:** *Hyptis suaveolens*, Dereplication, Molecular Structure, After Extraction of Essential Oil

## 1. Introduction

*Hyptis suaveolens* (Lamiaceae) is a species of tropical flora with multiple medicinal properties [1]. All parts of the plant are used in traditional medicine to treat a wide range of ailments, including respiratory ailments, gastrointestinal infections, antirheumatics, antisuporifics, colic, colds, indigestion, fever, abdominal pain, burns, sores, cramps, and many others.

Skin complications [2]. Several phytochemical studies have revealed that *Hyptis suaveolens* leaves contain essential oils containing menthol, limonene and sesquiterpenes [3] with numerous pharmacological and biological properties [4]. Leaf extracts have revealed the presence of alkaloids, steroids, terpenoids, tannins, flavonoids, anthraquinones and phenols before and after extraction of the essential oil [4-6]. In addition, hydroethanolic extracts and their dichloromethane fraction have shown antioxidant [7] and antibacterial properties [6]. However, the determination of the molecular structures of compounds in *Hyptis suaveolens* leaves after extraction of the essential oil has not been studied to date. This is the background to the present study.

The aim of this work is to determine the structures of several known and unknown compounds that may be present in *Hyptis suaveolens* leaves after extraction of the essential oil, using HPLC-ESI- Q-TOF -MS/ MS analysis.

# 2. Material and Methods

## *2.1. Material*

### *2.1.1. Plant Material*

The leaves of Hyptis suaveolens were collected in July 2017 in Yamoussoukro (6047'18.762" North and 5015'25.9992" West) in the center of Côte d'Ivoire and identified by Mr. Amani N'Guessan, botanist at the National Polytechnic Institute Félix HOUPHOUËT-BOIGNY (INP-HB) from Yamoussoukro. A specimen of Hyptis suaveolens is listed in the CSRS herbarium under the number: Coll n°C: 18027/bdcsrs: 65599. The leaves were divided into two (2) lots. The 1st batch dried directly in the shade at room temperature in the laboratory (26 to 30°C) for 7 days and the 2nd batch also dried under the same conditions after extraction of essential oil by hydrodistillation. The various dry leaves of the 1st and 2nd batches were then ground using an electric grinder of the IKA M20 brand. The various ground materials obtained were sieved using a sieve of 0.5 mm mesh. The various powders obtained were stored at 4°C. until their subsequent use.

### *2.1.2. Experimental Equipment*

For dereplicative analysis, an Agilent 1260 Infinity HPLC system coupled to an Agilent 6530 Q-TOF-MS mass spectrometer, equipped with an ESI source, was used. Analyses were performed in positive mode. A Sunfire® C18 analytical column (150×2.1 mm 3.5 µm, Waters) is used. In the positive ion mode, purine $C_5H_4N_4$ (ion at *m/z* 121.050873 g/mol) and phosphagen $C_{18}H_{18}F_{24}N_3O_6P_3$ (ion at *m/z* 922.009798) were used as internal locking masses. Full scans were acquired at a resolution of 11,000 (at *m/z* 922).

## *2.2. Methods*

### *2.2.1. Sample Preparation*

The total hydroalcoholic extract was prepared according to the method described by Soumahoro *et al* [5]. A 100g mass of sample crushed material was macerated in 1 L of an ethanol/water mixture (70/30: v/v) under magnetic stirrer for 24 hours. After settling, the mixture was successively filtered through absorbent cotton and Watman No. 2 paper. The operation was repeated three (3) times until the crushed material was exhausted. The filtrate obtained was concentrated under reduced pressure at a temperature of 40°C using a BUCHI 461 rotary evaporator, then freeze-dried to give the total hydroalcoholic extract after ($EHA_2$) extraction of the essential oil. The total hydroalcoholic extract obtained was fractionated successively using solvents of increasing polarity (hexane, dichloromethane, ethyl acetate, ethanol, and water) following the method reported by Bouamama et *al* [8]. The hydroalcoholic extract (10 g) was dissolved in 100 mL water and successively partitioned with hexane (3x 100mL), dichloromethane (3x 100 mL) and ethyl acetate (3x 100 mL). The various organic phases obtained were separately dried over anhydrous sodium sulfate. After filtration and removal of solvents under reduced pressure, the fractions of hexane (FHEX), dichloromethane (FDCM) and ethyl acetate (FAE) were obtained. Next, 5 mg of dichloromethane fraction was dissolved in 1 mL of analytical methanol, then 1mL of this solution was syringed into 1mL of methanol. This extract is filtered again using a 0.5 µm filter syringe. Finally, 300 µL are collected for storage in a case prior to HPLC-QTOF-MS/MS analysis.

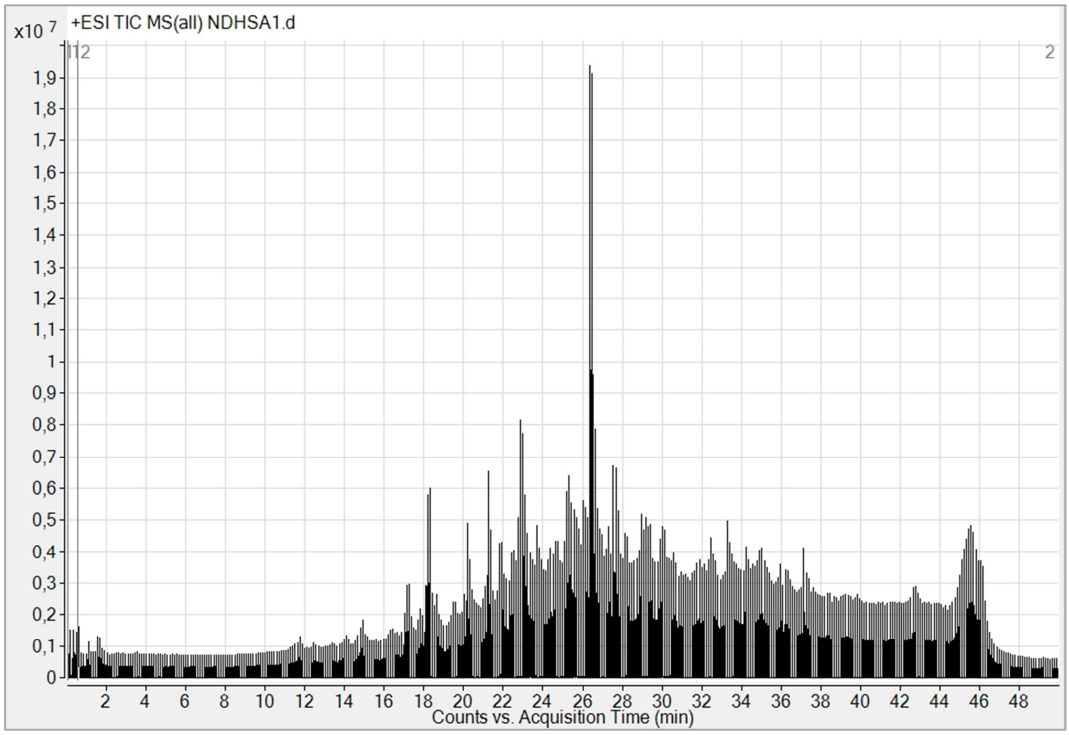



***Figure 1.*** *Total ESI/MS chromatographic profile.*

#### *2.2.2. Comparative HPLC- ESI- Q-TOF-MS/MS Analysis of the Dichloromethane Fraction Using the Dereplicative Method*

Dereplicative analysis is a new method for rapidly identifying known molecules in a complex mixture [9]. It is based on the coupling of High-Performance Liquid Chromatography (HPLC) and Tandem Mass Spectrometry (MS/MS or $MS^2$)/Q-TOF [10]. A Sunfire® C18 analytical column (150×2.1 mm; 3.5 µm, Waters) is used with a flow rate of 250 µL/min and a two-way linear gradient: Lane A (95-0% H2O plus 0.1% formic acid), Lane B (5-100% ACN) for 30 minutes. ESI conditions were defined with a temperature of 320°C, a source voltage of 3.5 kV, and a gas flow rate of 10 µL/min. In the positive ion mode, purine $C_5H_4N_4$ (ion at *m/z* 121.050873 g/mol) and phosphagen $C_{18}H_{18}F_{24}N_3O_6P_3$ (ion at *m/z* 922.009798) were used as internal locking masses. Full scans were acquired at a resolution of 11,000 (at *m/z* 922). Sample injection volume was set at 5 µL.

Analysis of the dichloromethane fraction from the hydroalcoholic extract of *Hyptis suaveolens* leaves after extraction of the essential oil was carried out using the HPLC-ESI-Q- TOF-MS/MS method. An automated integration of the chromatogram obtained, using MassHunter® (Agilent) Qualitative Analysis B.07.00 software, was then used to obtain the peaks of the various main compounds derived from this fraction (figure 1).

By clicking on a given peak, the software generates a set of formulas corresponding to the single molecular ion [M+H]+ (Table 1.).

***Table 1.*** *Raw formulas suggested by MassHunter software.*

| ID Source | Formula | Species | m/z | Score | Diff (ppm) | Score (MFG) | Mass (MFG) | DBE |
|---|---|---|---|---|---|---|---|---|
| MFG | $C_{20}H_{30}O_2$ | (M+H)+ | 303.2318 | 98.33 | -0.24 | 98.33 | 302.2246 | 6 |
| MFG | $C_{18}H_{28}N_3O$ | (M+H)+ | 303.2318 | 89.9 | -4.92 | 89.9 | 302.2232 | 6.5 |
| MFG | $C_{13}H_{30}N_6S$ | (M+H)+ | 303.2318 | 83.53 | 0.58 | 83.53 | 302.2253 | 2 |
| MFG | $C_{15}H_{32}N_3OS$ | (M+H)+ | 303.2318 | 78.37 | 5.35 | 78.37 | 302.2266 | 1.5 |
| MFG | $C_{16}H_{26}N_6$ | (M+H)+ | 303.2318 | 72.65 | -9.64 | 72.65 | 302.2219 | 7 |
| MFG | $C_{17}H_{34}O_2S$ | (M+H)+ | 303.2318 | 65.35 | 10.08 | 65.35 | 302.228 | 1 |
| MFG | $C_{19}Cl_3O_9S_4$ | (M+2H)+2 | 303.3823 | 46.84 | -1.57 | 46.84 | 604.7491 | 18.5 |
| MFG | $C_{14}H_2Cl_3N_3O_8S_5$ | (M+2H)+2 | 303.3823 | 46.61 | 1.79 | 46.61 | 604.7511 | 14 |
| MFG | $C_{15}Cl_3O_{14}S_3$ | (M+2H)+2 | 303.3823 | 45.56 | 2.57 | 45.56 | 604.7516 | 14.5 |
| MFG | $C_{14}HCl_2NO_{12}S_5$ | (M+2H)+2 | 303.3823 | 43.98 | -3.45 | 43.98 | 604.7479 | 14 |
| MFG | $C_{16}H_4Cl_3O_9S_5$ | (M+2H)+2 | 303.3823 | 42.77 | 4.01 | 42.77 | 604.7524 | 13.5 |
| MFG | $C_{23}Cl_3O_4S_5$ | (M+2H)+2 | 303.3823 | 38.31 | -5.7 | 38.31 | 604.7466 | 22.5 |
| MFG | $C_{17}Cl_3N_4O_5S_5$ | (M+2H)+2 | 303.3823 | 36.77 | 6.22 | 36.77 | 604.7538 | 18.5 |

# 3. Results and Discussion

### *3.1. Dereplicative HPLC-MS/Q-TOF Analysis of the Dichloromethane Fraction After Essential Oil Extraction*

The HPLC-MS/Q-TOF chromatographic profile of the dichloromethane fraction from the hydroalcoholic *Hyptis suaveolens* leaves after extraction of the essential oil is shown in figure 2.

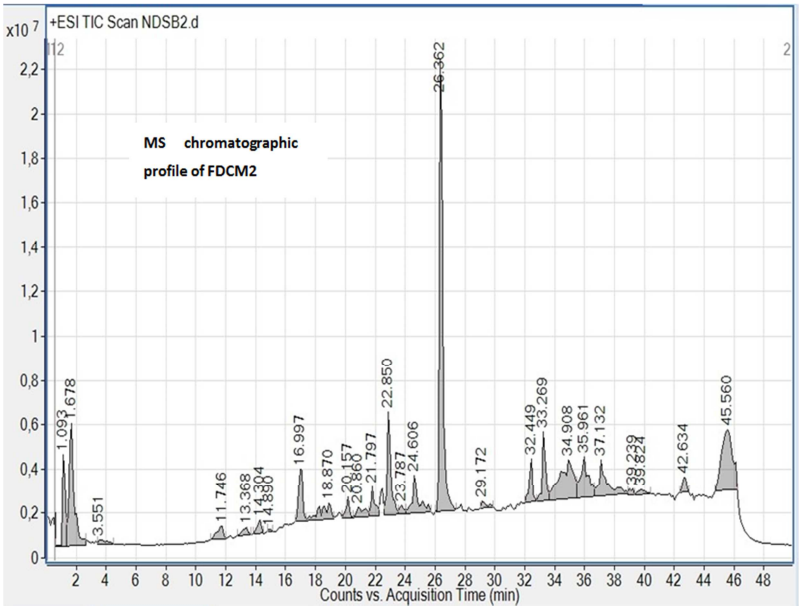



***Figure 2.*** *ESI/MS chromatographic profile of the majority compounds in the dichloromethane fraction after HE extraction.*

Figure 3 shows the presence of numerous compounds of different polarities, with retention times ranging from 1.093 min to 45.56 min. Analyses carried out in positive mode enabled us to determine the molecular masses and gross formulae of the compounds revealed by chromatography (Table 2).

***Table 2.*** *Compounds detected in the dichloromethane fraction after HE extraction ($_{FDCM2}$).*

| PIC number | Retention time (min) | Gross formula | Molecular mass (g/mol) | Score (%) |
|---|---|---|---|---|
| 1 | 1.678 | $C_9H_{20}N_2O_{12}S$ | 380 | 84.77 |
| 2 | 3.551 | $C_5H_7NO_3$ | 129 | 95.6 |
| 3 | 11.746 | $C_9H_6O_4$ | 178 | 85.96 |
| 4 | 13.368 | $C_7H_6O_2$ | 122 | 82.14 |
| 5 | 14.304 | $C_{11}H_{18}N_6O_7$ | 346 | 96.58 |
| 6 | 14.89 | $C_{16}H_{32}N_{12}O_3S_2$ | 504 | 86.08 |
| 7 | 16.997 | $C_{15}H_{23}N_{11}O_3$ | 405 | 93.53 |
| 8 | 18.402 | *$C_{10}H_{16}O$ | 152 | 86.09 |
| 9 | 18.87 | $C_{23}H_{34}O_{11}$ | 456 | 77.25 |
| 10 | 20.157 | $C_7H_6O_2$ | 122 | 87.5 |
| 11 | 20.86 | $C_{25}H_{22}O_2$ | 354 | 74.45 |
| 12 | 20.860 | *$C_{21}H_{28}O_7$ | 392 | 56.54 |
| 13 | 21.454 | *$C_{10}H_{18}O_2$ | 170 | 77.85 |
| 14 | 21.680 | *$C_{15}H_{10}O_7$ | 302 | 99.67 |
| 15 | 21.797 | $C_{13}H_{22}O_2$ | 210 | 99.25 |
| 16 | 21.876 | *$C_{21}H_{20}O_{12}$ | 464 | 97.55 |
| 17 | 22.031 | *$C_{10}H_{12}O_2$ | 164 | 63.19 |
| 18 | 22.85 | $C_9H_6O_3$ | 162 | 99.39 |
| 19 | 22.886 | $C_9H_{18}N_2S$ | 360 | 96.53 |
| 20 | 24.606 | *$C_{30}H_{40}O_{11}$ | 576 | 98.39 |
| 21 | 26.362 | $C_8H_4O_3$ | 148 | 83.98 |
| 22 | 29.172 | $C_{22}H_{44}$ | 308 | 86.03 |
| 23 | 32.449 | $C_4H_6NO_5$ | 148 | 72.76 |
| 24 | 33.035 | *$C_{19}H_{28}O$ | 272 | 71.41 |
| 25 | 33.269 | $C_{12}H_{22}O_2$ | 198 | 69.16 |
| 26 | 34.556 | *$C_{20}H_{34}O_2$ | 306 | 98.26 |
| 27 | 34.908 | $C_{30}H_{57}NO_3S$ | 511 | 67.55 |
| 28 | 35.961 | $C_{18}H_{35}NO$ | 281 | 90.54 |
| 29 | 37.132 | $C_{24}H_{38}O_4$ | 390 | 98.82 |
| 30 | 39.239 | $C_{26}H_{41}Cl$ | 388 | 97.82 |
| 31 | 39.824 | $C_{28}H_{43}N$ | 393 | 81.05 |
| 32 | 42.634 | $C_{44}H_{58}N_2O_3$ | 662 | 94.04 |
| 33 | 45.56 | (ND) | 109 | 97.14 |

*: Compounds already identified in the Hyptis genus

Analysis of the dichloromethane fraction from the hydroalcoholic extract of *Hyptis suaveolens* leaves after extraction of the essential oil shows that the plant is rich in thirty-two molecules (32) (table 2).

Of the compounds detected in the dichloromethane fraction, nine (09) have already been isolated from the *Hyptis* genus [1, 11, 12] (Table 3).

***Table 3.*** *Known compounds detected in the dichloromethane fraction after essential oil extraction.*

| **Known compounds detected in the dichloromethane fraction** | | | **After extraction of essential oil** | |
|---|---|---|---|---|
| **Name** | **Brute formula** | **Molecular mass (g/mol)** | **Retention Time (ms)** | **Score (%)** |
| 2-méthyl-3-méthylène-2-(4-méthylpent-3-èn-1-yl)oxirane | $C_{10}H_{16}O$ | 152.24 | 18.405 | 75.46 |
| 1-(5-(hydroxy(2-oxotetrahydro-2H-pyran-3-yl)methyl)tetrahydrofuran-2-yl)ethyl-3-(4- hydroxyphenyl) propanoate | $C_{21}H_{28}O_7$ | 392.45 | 20.859 | 65.20 |
| (3-methyl-3-(4-methylpent-3-en-1-yl) oxiran-2-yl) methanol | $C_{10}H_{18}O_2$ | 170.12 | 21.454 | 77.85 |
| Quercetin | $C_{15}H_{10}O_7$ | 302.24 | 21.652 | 92.89 |
| 3-O-β-D-glucopyranoside quercetin | $C_{21}H_{20}O_{12}$ | 464.38 | 21.842 | 85.84 |
| 4-allyl-2-methoxyphenol | $C_{10}H_{12}O_2$ | 164.20 | 22.872 | 84.98 |
| β-sitosterol glycoside | $C_{30}H_{40}O_{11}$ | 576.86 | 24.65 | 80.12 |
| 5α-androst-9(11)-èn-12-one | $C_{19}H_{28}O$ | 272.43 | 33.034 | 56.21 |
| Suaveolol | $C_{20}H_{34}O_2$ | 306.49 | 34.907 | 73.87 |

### *3.2. Confirmation of the Structures of Known Compounds (09) Detected in the Dichloromethane Fraction*

Compound structures were determined by interpreting data from HPLC-ESI-MS/Q- TOF analyses of the dichloromethane fraction from the hydroalcoholic of *Hyptis suaveolens* leaves after extraction of the essential oil. This analysis yielded mass and fragmentation spectra, as well as the crude formulae of several major compounds. Among the crude formulas obtained, the NIST, ChemSpider and PubChem databases provided structures. We are interested in the structures corresponding to those of certain compounds already isolated from the *Hyptis* genus.

*Structure of compound 1*

Compound 1, which appears at a retention time of 18.40 min (Figure 3), gives the molecular ion peak [M+H]+ at m/z: 153.127 corresponding to a molecular weight of 152.120 g/mol. The most probable molecular formula is $C_{10}H_{16}O$ (cal. 152.24).

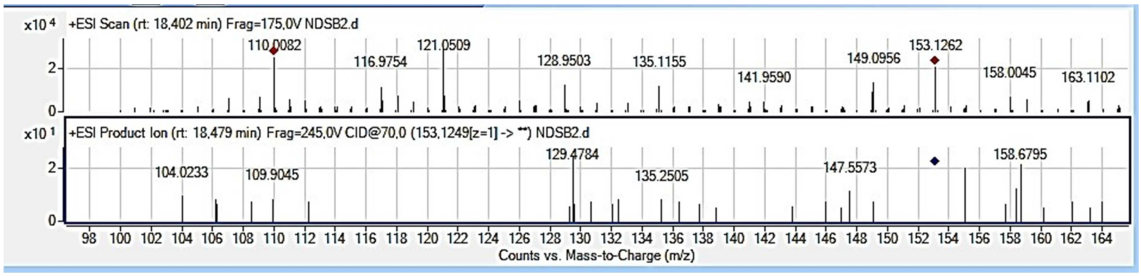
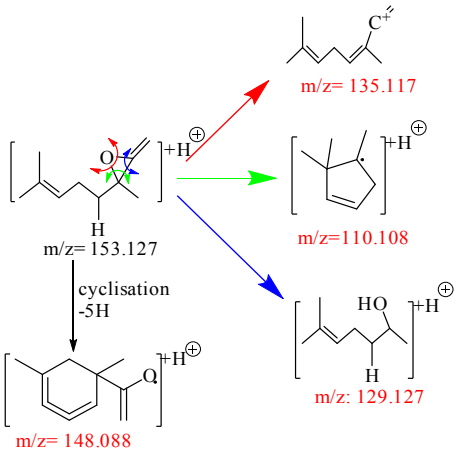



***Figure 3.*** *LC-ESI/MS mass spectrum and ESI/MS fragmentation spectrum of compound 1.*

Analysis of the fragmentation spectrum of compound 1 (Figure 4) shows the presence of major fragments at m/z: 148 [M+H-5H], m/z: 135 [M+H-18], m/z: 129 [M+H-24] (base peak), m/z: 110 [M+H-43], m/z: 104 [M+H-49].

Among the structures proposed by the ChemSpider and PubChem databases, only 2- methyl-3-methylene-2-(4-methylpent-3-en-1-yl) oxirane gives a fragmentation mode similar to that of the desired compound (Scheme 1).




***Scheme 1.*** *Proposed fragmentation of compound 1.*

The fragment at m/z: 148 (even mass) would be due to rearrangement (cyclization) with loss of hydrogen atoms. The fragment at m/z: 110 with an even mass would result from cyclization followed by removal of an ethoxy group ($CH_3CO$-). The fragments at m/z 135 and 129 result from the loss of a water molecule and a $C_2$ group respectively.

Compound 1 (Figure 4) is therefore 2-methyl-3-methylene-2-(4-methylpent-3-en-1-yl) oxirane. This molecule could be classified in the terpene family. The structure of this compound is in line with the literature [13].

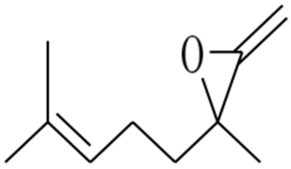


***Figure 4.*** *Structure of compound 1.*

Structure of compound 2

Compound 2, which appears at a retention time of 20.859 min, corresponds to the molecular ion [M+H]+ m/z: 393.1879 with a molecular weight of 392.1835 g/mol. The most probable molecular formula is $C_{21}H_{28}O_7$ (cal. 392.45) (figure 5).

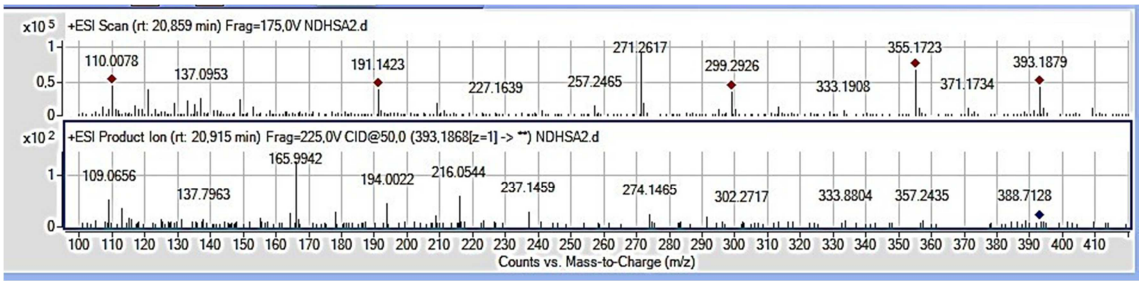
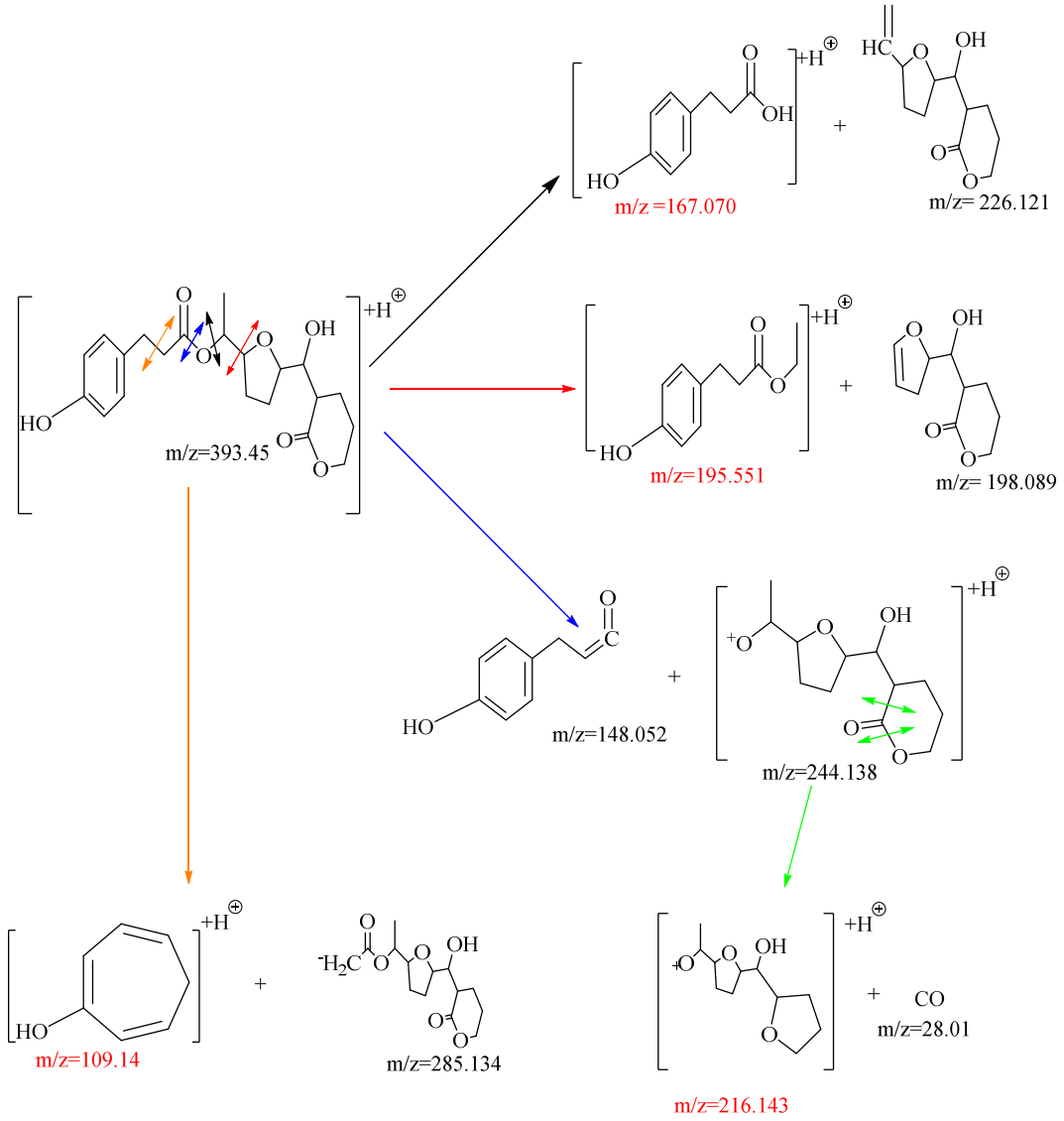



***Figure 5.*** *LC-ESI/MS mass spectrum and ESI/MS fragmentation spectrum of compound 2.*

Analysis of the fragmentation spectrum of compound 2 (Figure 5) shows the presence of characteristic fragments at m/z: 216[M+H-177], m/z: 194[M+H-177-22], m/z: 166[M+H- 177-22-28] (base peak), m/z: 109[M+H-177-22-29-57].

Among the structures proposed by the ChemSpider and PubChem databases, only 1- (5-(hydroxy(*2-oxotetrahydro-2H-pyran-3-yl*) methyl)tetrahydrofuran-2-yl)ethyl-3-(4-hydroxyphenyl) propanoate gives a fragmentation mode similar to that of the desired compound (Scheme 2).




***Scheme 2.*** *Proposed fragmentation of compound 2.*

The basic molecular weight peak m/z: 167 (166 on the spectrum following nitrogen- rule rearrangement), would be due to a Mc Lafferty-type rearrangement with cleavage of the carbon-oxygen bond in α (Fragmentation α). The fragment m/z: 195 (194 on the spectrum after a rearrangement, nitrogen rule), would result from cleavage of the carbon-carbon bond at α of the methyl group. The fragment m/z: 109 would result from cleavage of the β-carbon bond of the aromatic ring followed by formation of the tropylium ion. Compound 2 (Figure 6) is therefore 1-(5-(hydroxy(*2-oxotetrahydro-2H-pyran-* 3-yl) methyl) tetrahydrofuran-2-yl) ethyl-3-(4-hydroxyphenyl) propanoate and belongs to the phenolic compound family. *Hyptis brevipes* leaves have also been identified in the same family as the study plant [14].

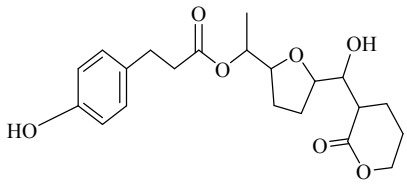


***Figure 6.*** *Structure of compound 2.*

Structure of compound 3

Compound 3 with a retention time equal to 21.445 min corresponds to the molecular ion [M+H]+ at m/z: 171.139

with a molecular weight equal to 170.131g/mol. The most probable molecular formula is $C_{15}H_{10}O_7$ (cal. 170,120) (figure 7).

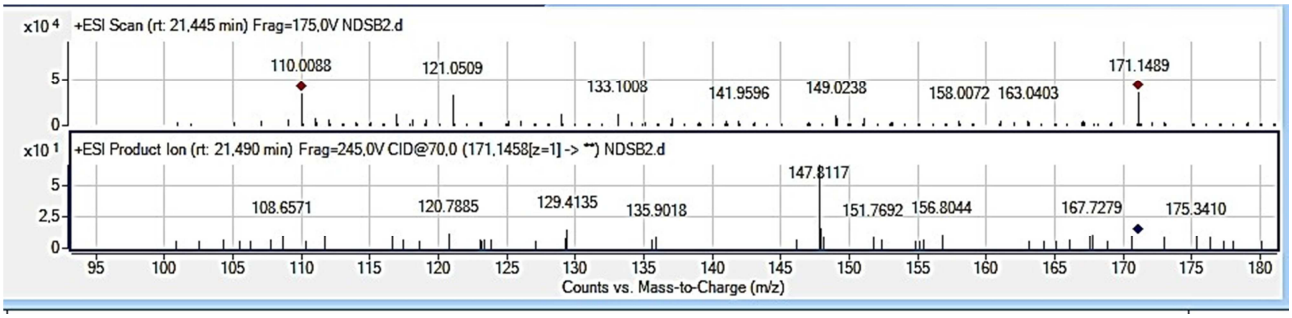
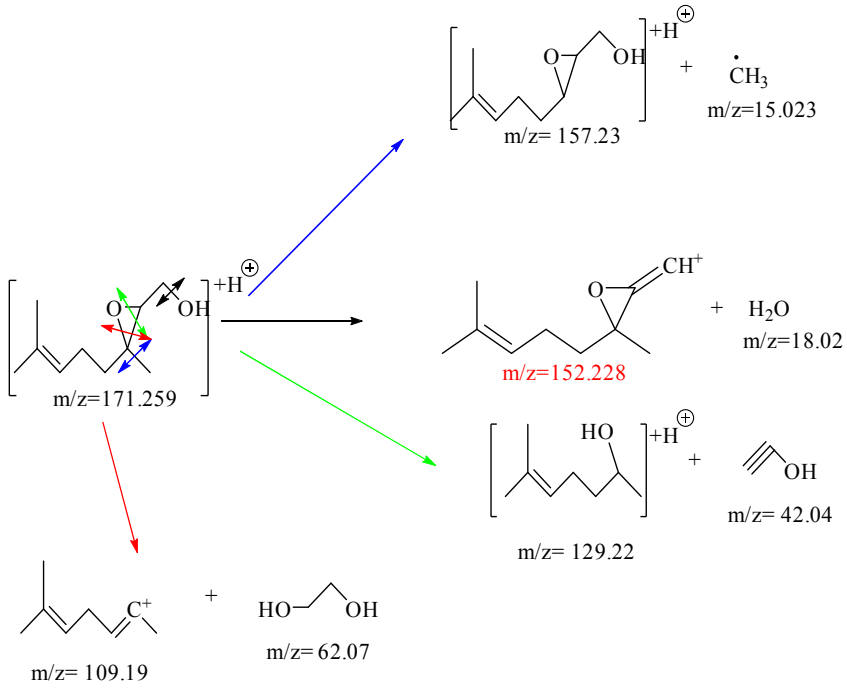



***Figure 7.*** *LC-ESI/MS mass spectrum and ESI/MS fragmentation spectrum of compound 3.*

Analysis of the fragmentation spectrum of compound 3 (Figure 7) shows the presence of major fragments at m/z: 157[M+H-14], m/z: 152[M+H-19], m/z: 148[M+H-23] (base peak), m/z: 129[M+H-42], m/z: 121[M+H-50], m/z: 109[M+H-62].

Among the structures proposed by the ChemSpider and PubChem databases, only (3- methyl-3-(4-methylpent-3-en-1-yl) oxiran-2-yl)methanol gives a fragmentation mode similar to that of the desired compound (Scheme 3).




***Scheme 3.*** *Proposed fragmentation of compound 3.*

The fragment at m/z: 157 would be due to the loss of a methyl group from the epoxide by α-fragmentation. The fragment at m/z: 152 would result from the loss of a water molecule. As for the fragment at m/z: 129, would result from a double cleavage on the epoxide in the γ position of the hydroxyl group. Similarly, the fragment at m/z: 109 would result from a double cleavage of the epoxide at β of the methyl group. Compound 3 (Figure 8) is therefore (3-methyl-3-(4- methylpent-3-en-1-yl)oxiran-2-yl)methanol and belongs to the sterol and terpene family. It has already been isolated from *Hyptis suaveolens* leaves [13].

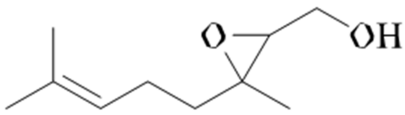


***Figure 8.*** *Structure of compound 3.*

Structure of compound 4

Compound 4 with a retention time of 21.814 min corresponds to the molecular ion [M+H]+ at m/z: 303.0494. Its molecular weight is 302.0427g/mol. The most probable molecular formula is $C_{15}H_{10}O_7$ (cal. 302.238) (figure 13).

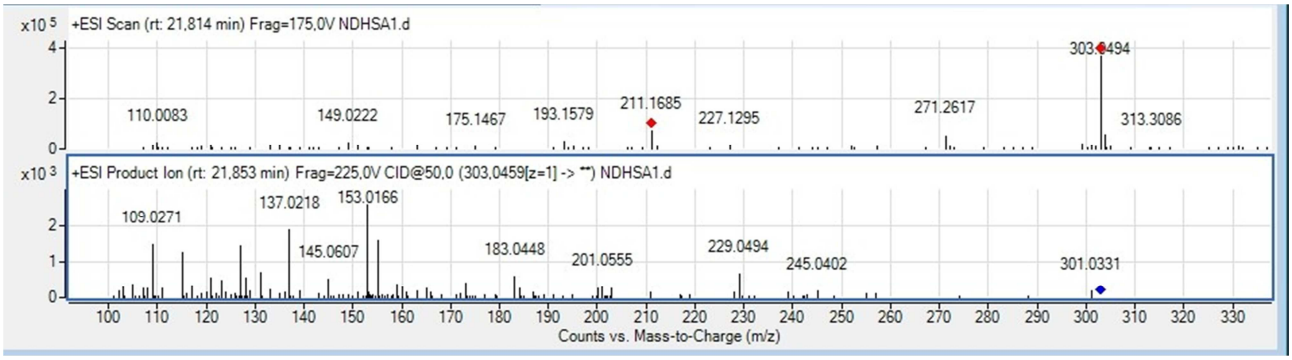
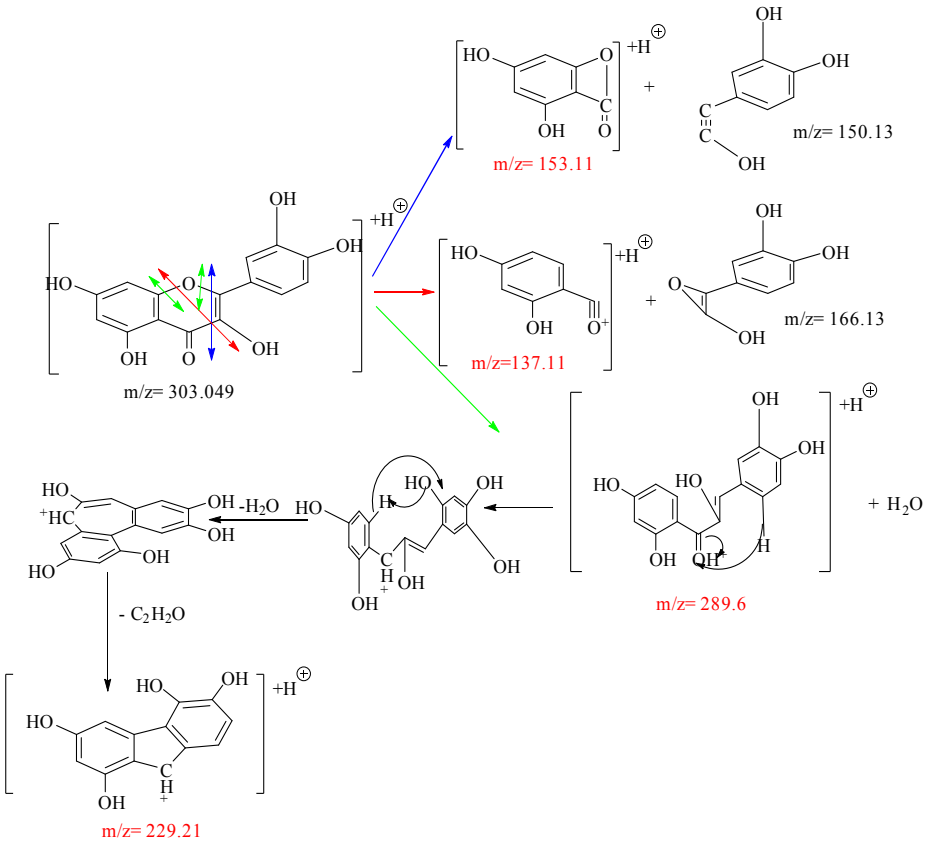



***Figure 9.*** *LC-ESI/MS mass spectrum and ESI/MS fragmentation spectrum of compound 4.*

Analysis of the fragmentation spectrum of compound 4 (Figure 13) shows major fragments at m/z: 229 [M+H-74], m/z: 153 [M+H-150] (base peak), m/z: 137 [M+H-166].

Among the structures proposed by the ChemSpider and PubChem databases, only quercetin gives a fragmentation mode like that of the desired compound (Scheme 4).




***Scheme 4.*** *Fragmentation of compound 4.*

The base peak at m/z: 153 derives from a double cleavage on the intermediate C ring of the carbon-oxygen and carbon-carbon bonds respectively in the γ and β position of its hydroxyl group. Also, the fragment at m/z: 137 derives from a double cleavage on the intermediate C ring of the carbon-oxygen and carbon-carbon bonds respectively in the α position of the aromatic ring and β position of its hydroxyl group.

As for the fragment at m/z: 229, it results from the elimination of the oxo group, followed by cyclization after the loss of a water molecule and an epoxide group on the intermediate C ring (Scheme 4). Compound 4 (Figure 10) is therefore quercetin, a member of the flavonoid family. This compound is present in the leaves of *H. suaveolens* [1].

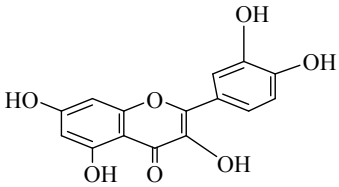


***Figure 10.*** *Structure of compound 4 (quercetin Structure of compound 5.*

Compound 5 with a retention time of 21.814 min

corresponds to the molecular ion [M+H]+ at m/z: 465.1028 with a molecular weight of 464.0955g/mol. The most probable molecular formula is therefore $C_{21}H_{20}O_{12}$ (cal. 464.3790) (Figure 11).

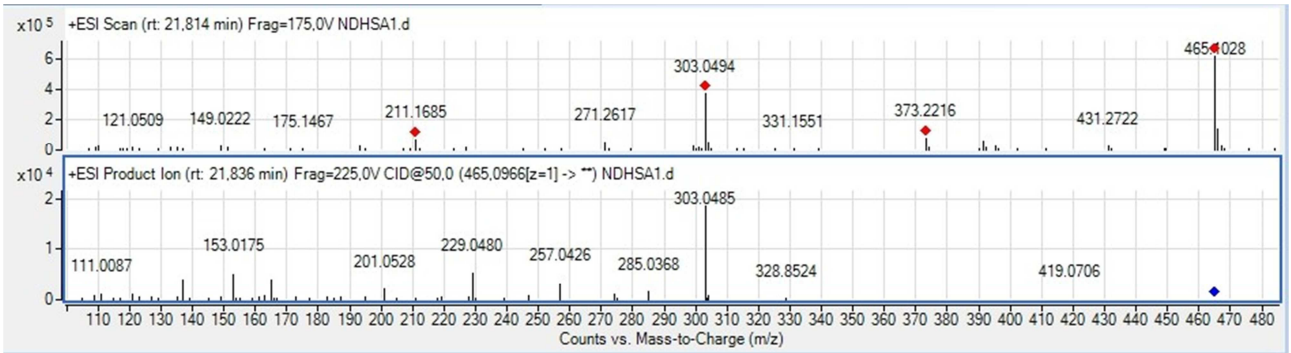
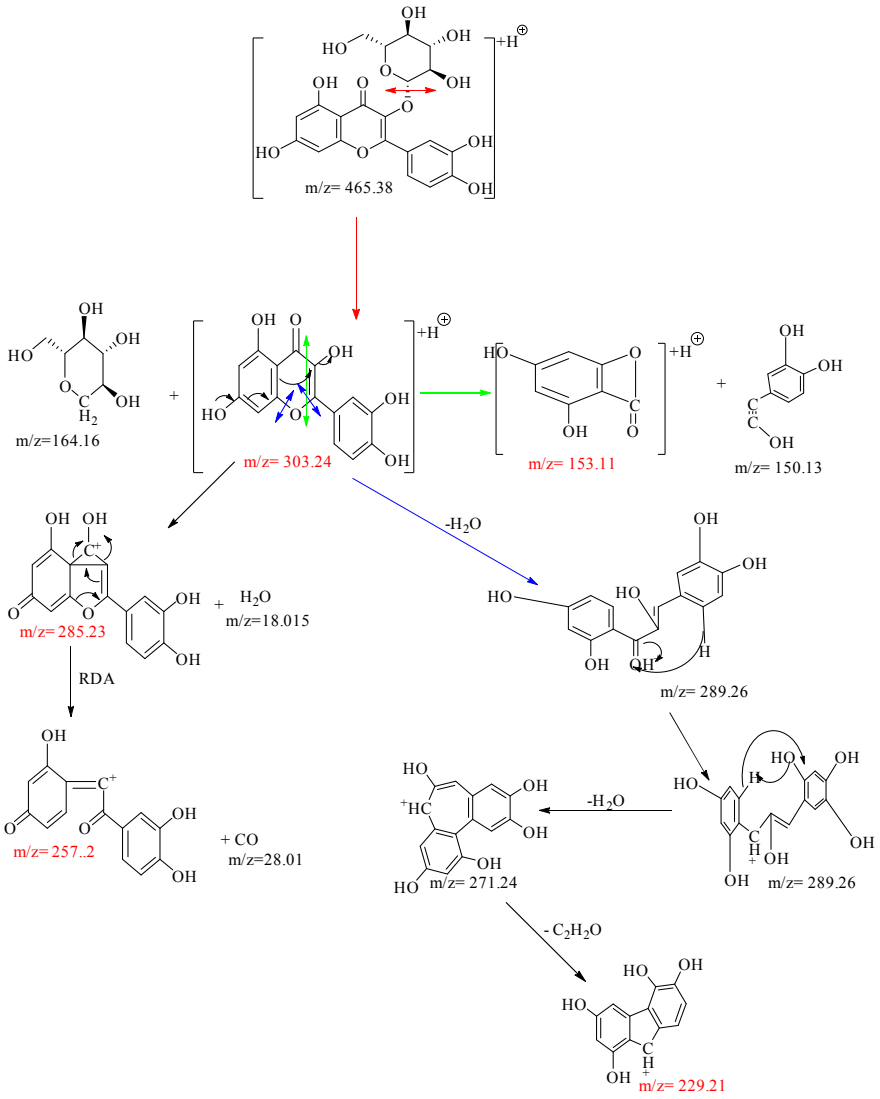



***Figure 11.*** *LC-ESI/MS mass spectrum and ESI/MS fragmentation spectrum of compound 5.*

Analysis of the fragmentation spectrum of compound 5 (Figure 11) indicates the presence of major fragments at m/z: 303[M+H-162] (base peak), m/z: 285[M+H-162-18], m/z: 257[M+H-162-46], m/z: 229[M+H-162-74], m/z: 153[M+H-162-150].

Among the structures proposed by the ChemSpider and PubChem databases, only quercetin 3-O-β-D-glucopyranoside gives a fragmentation mode similar to that of the desired compound (Scheme 5).




***Scheme 5.*** *Fragmentation of compound 5.*

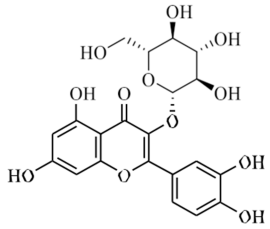


***Figure 12.*** *Structure of compound 5.*

The base peak at m/z: 303 results from the loss of the glucosyl group. The fragment at m/z: 153 results from the base peak after a double cut on the intermediate C ring of the carbon-oxygen and carbon-carbon bonds respectively at β and α of the hydroxyl group of this ring. Similarly, the fragment at m/z: 285 is derived from the base peak by loss of a water molecule on the intermediate C ring. As for the fragment at m/z: 257, it results from a Retro Diels-Alder (RDA) mechanism on the intermediate C ring with loss of the carbonyl group (Scheme 5).

Compound 5 (Figure 12) is therefore the flavonoid quercetin 3-O-β-D- glucopyranoside. It is present in the plant studied [12].

Structure of compound 6

Compound 6 with a retention time of 22.165 min corresponds to the molecular ion [M+H]+ at m/z: 165.0901 with a molecular weight of 164.0837g/mol. The most probable molecular formula is $C_{10}H_{12}O_2$ (cal. 164.20) (Figure 13).

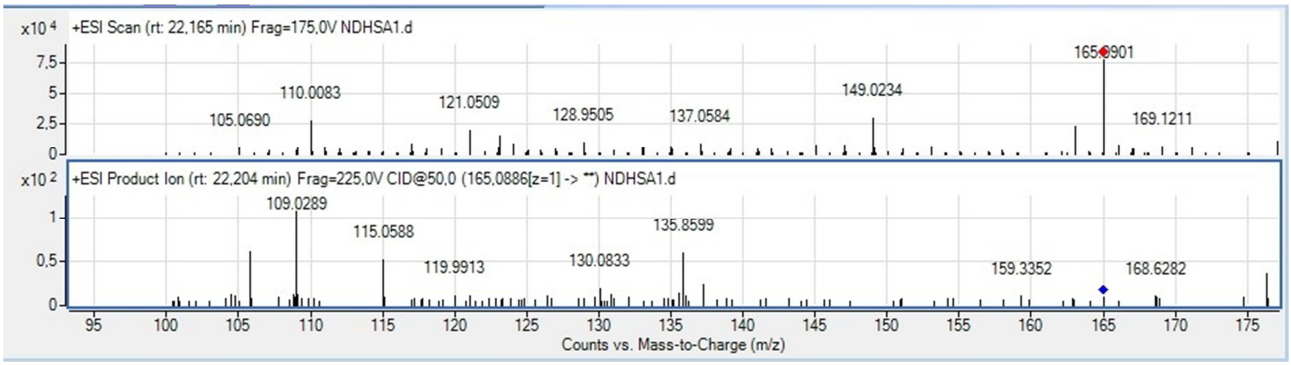
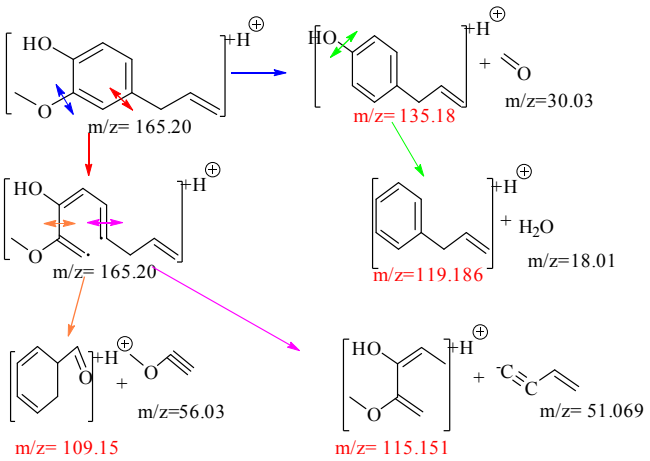



***Figure 13.*** *LC-ESI/MS mass spectrum and ESI/MS fragmentation spectrum of compound 6.*

Analysis of the fragmentation spectrum of compound 6 (Figure 13) indicates the presence of major fragments at m/z: 136 [M+H-29], m/z: 120 [M+H-45], m/z: 115 [M+H-50], m/z: 109 [M+H-56] (base peak).

Among the structures proposed by the ChemSpider and PubChem databases, only 4- allyl-2-methoxyphenol gives a fragmentation mode like that of the desired compound (Scheme 6).




***Scheme 6.*** *Proposed fragmentation of compound 6.*

The fragment at m/z: 135 results from the loss of the methoxyl group in formaldehyde form. The fragment at m/z: 119 would be derived from the preceding fragment after dehydration. The majority peak at m/z: 109 results from double cleavage on the aromatic ring of the carbon-carbon single bonds in the β and γ positions of the methoxyl group. Similarly, the fragment at m/z: 115 would be due to a double cleavage on the ring of the single and double bonds in α position of the propenyl group (Scheme 6). Compound 6 (Figure 14) is therefore 4-allyl-2- methoxyphenol from the phenolic compound family. It has been identified in the essential oil of *H. suaveolens* leaves harvested in Tanzania [15].

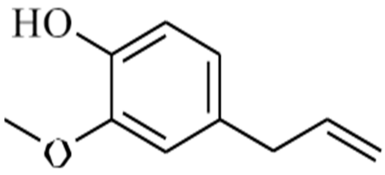


***Figure 14.*** *Structure of compound 6.*

Structure of compound 7

Compound 7 with a retention time of 24.605 min corresponds to the molecular ion [M+H]+ at m/z: 577.262 with a molecular weight of 576.259 g/mol. The most probable molecular formula is $C_{16}H_{12}O_6$ (cal. 576.859) (figure 15).

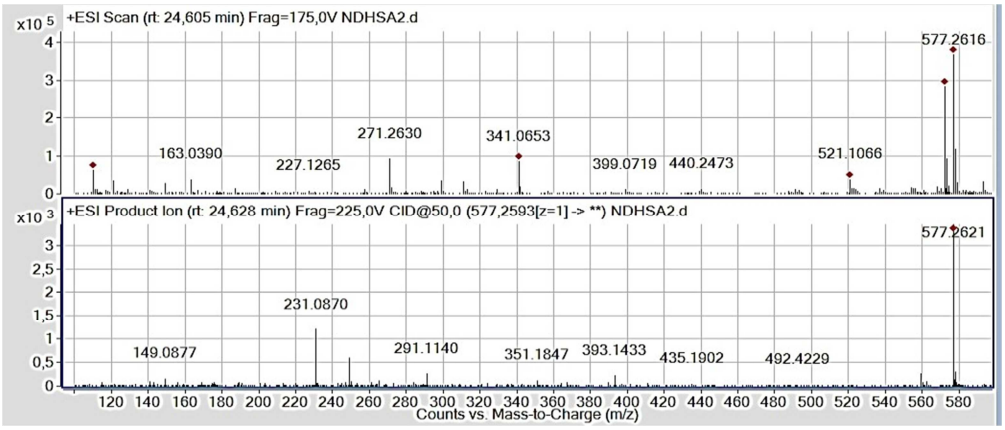
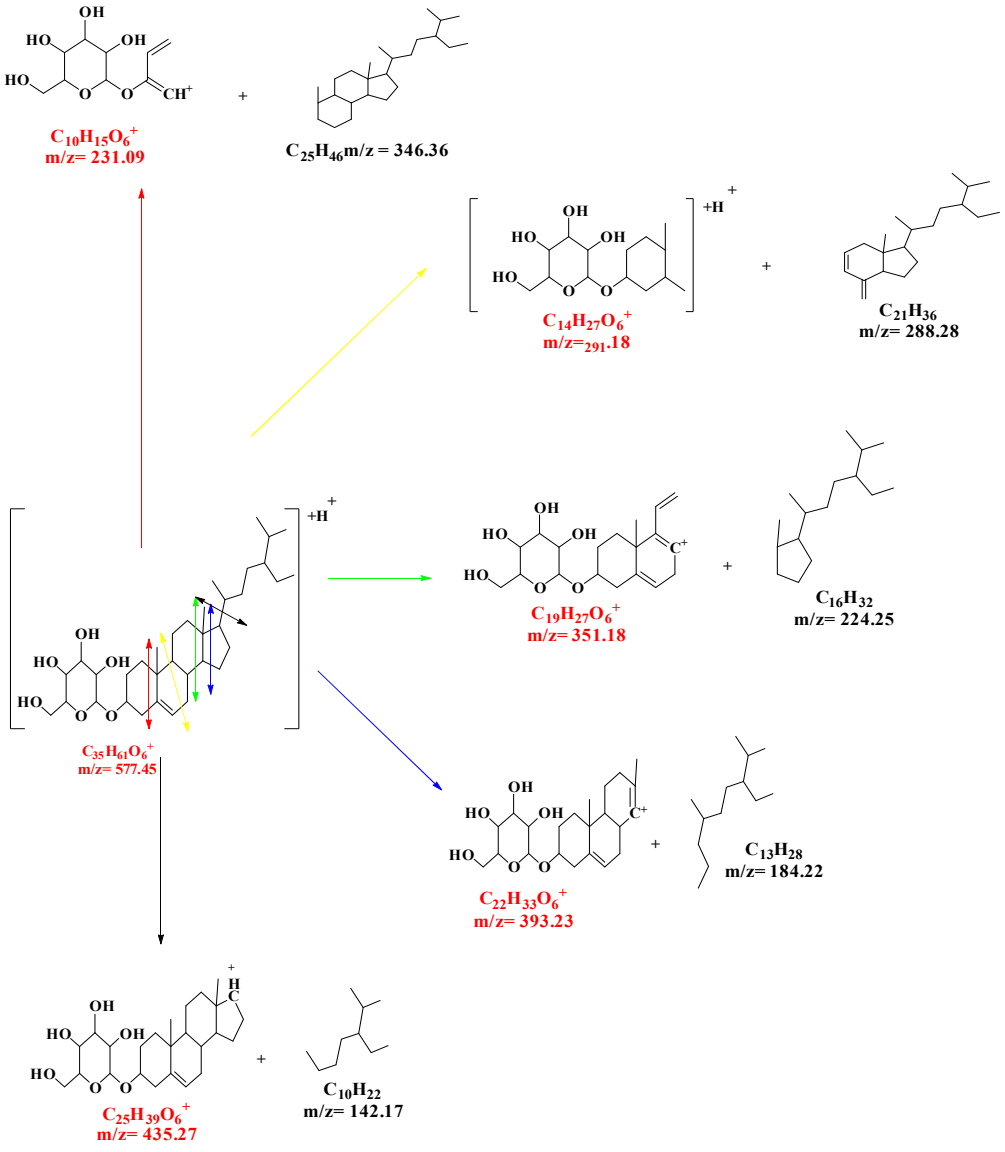



***Figure 15.*** *LC-ESI/MS mass spectrum and ESI/MS fragmentation spectrum of compound 7.*

Analysis of the fragmentation spectrum of compound 7 (Figure 15) indicates the presence of major fragments at m/z: 577[M+H] (base peak), m/z: 492[M+H-85], m/z: 393[M+H-184], m/z: 351[M+H-226], m/z: 291[M+H-286], m/z: 231[M+H-346], m/z: 149[M+H-428]. ubChem databases, only β- sitosterol glucose gives a fragmentation mode like that of the desired compound (Scheme 7)




***Scheme 7.*** *Proposed fragmentation of compound 7.*

The molecular peak is the base peak, indicating the stability of the molecule. The fragment at m/z: 231 would result from the double cleavage on the ring contiguous to the glucosyl group of the two α-carbon bonds of the following ring. The fragment at m/z: 291 would also result from a double split on the unsaturated ring of the carbon-carbon bonds between the preceding and following ring and that between the unsaturation and the ($CH_2$) group. The fragment at m/z: 351 would be due to another double cleavage on the third $_{C6}$ ring of the carbon-carbon bonds adjacent to the ($C_5$) ring. The fragment at m/z: 393 would result from the cleavage of carbon-carbon bonds on the ($C_5$) ring at α of the adjacent ring. As for the fragment at m/z: 435, it would result from the loss of the substituent 1-methyl,4-isopropylhexane ($C_{10}H_{21}$) (Figure 16).

Compound 7 (Figure 16) is therefore β-sitosterol glucose, which belongs to the class of sterols and terpenes. It is indeed present in the plant studied [16].

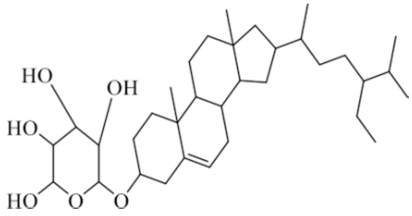



***Figure 16.*** *Structure of compound 7.*

Structure of compound 8

Compound 8 with a retention time of 33.106 min corresponds to the molecular ion [M+H]+ at m/z: 273.2210 with a molecular weight of 272.2140 g/mol. The most probable molecular formula is $C_{19}H_{28}O$ (cal. 272.43) (Figure 17).

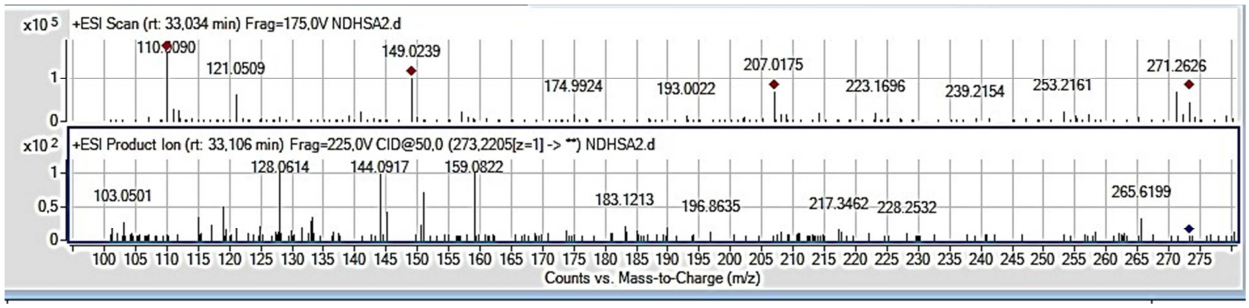
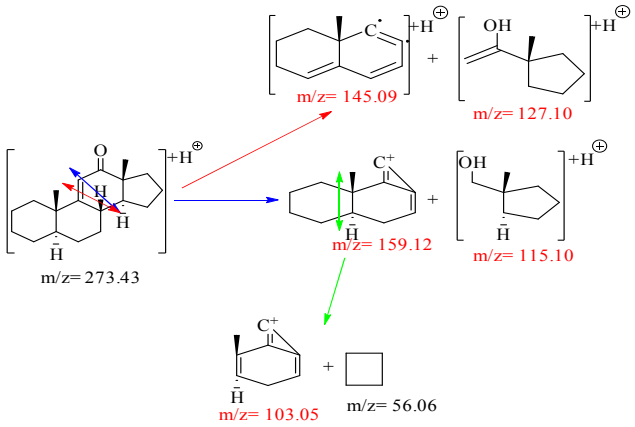



***Figure 17.*** *LC-ESI/MS mass spectrum and ESI/MS fragmentation spectrum of compound 8.*

Analysis of the fragmentation spectrum of compound 8 (Figure 17) shows the presence of major fragments at m/z: 159[M+H-114] (base peak), m/z: 144[M+H-129], m/z: 128[M+H- 145], m/z: 115[M+H-158], m/z: 103[M+H-114-56].

Among the structures proposed by the ChemSpider and PubChem databases, only 5α- androst-9(11)-en-12-one gives a fragmentation mode like that of the desired compound (Scheme 8).




***Scheme 8.*** *Proposed fragmentation of compound 8.*

The base peak at m/z: 159 derives from the double cleavage on the unsaturated ring of the carbon-carbon bond between the unsaturation and the carbonyl group and that between the ($C_5$) ring and the saturated ($C_6$) ring. The fragment at m/z: 103 is thought to have arisen from the base peak because of the loss of a cyclobutane following the cleavage on the saturated ring of the carbon-carbon bonds adjacent to the second ring at ($C_6$). Fragments at m/z: 145 (144 on the spectrum) and m/z: (128 on the spectrum) would result from the breakage on the unsaturated ring of the carbon-carbon bonds contiguous to the intermediate ring at ($C_6$) (Scheme 8). Compound 8 (Figure 18) would therefore be the 5α-androst-9(11)-en-12-one of the terpene and sterol family. It has been identified in the plant's leaves [17].

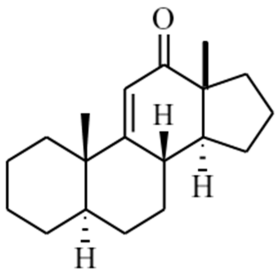



***Figure 18.*** *Structure of compound 8.*

Structure of compound 9

Compound 9 with a retention time of 34.691 min corresponds to the molecular ion [M+H]+ at m/z: 307.262 with a molecular weight of 306.256 g/mol. The most probable

molecular formula is $C_{20}H_{34}O_2$ (cal. 306.49) (Figure 19).

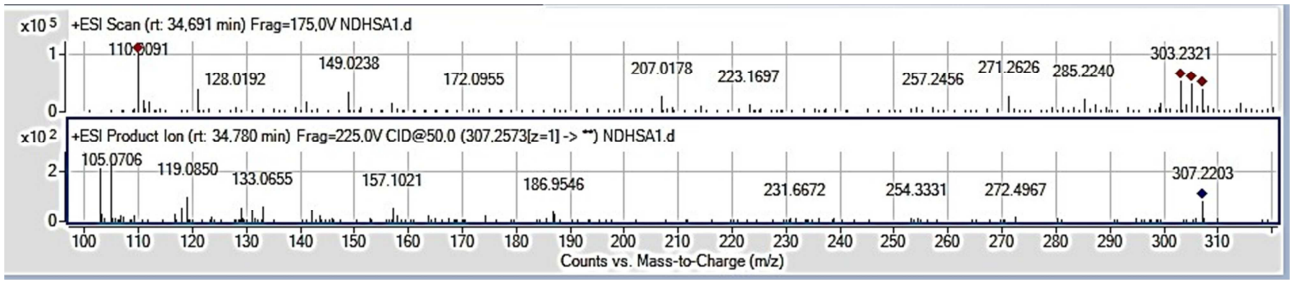
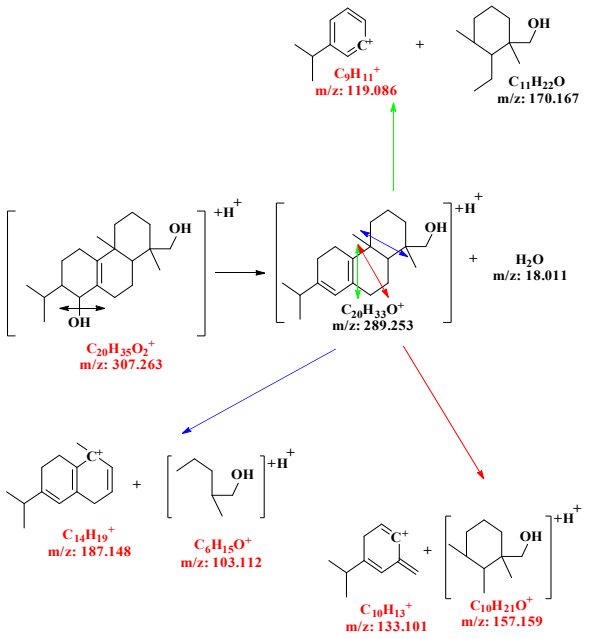



***Figure 19.*** *LC-ESI/MS mass spectrum and ESI/MS fragmentation spectrum of compound 9.*

Analysis of the fragmentation spectrum of compound 9 (Figure 19) indicates the presence of major fragments at m/z: 187[M+H-18-102], m/z: 157[M+H-18-132], m/z: 133[M+H-18- 156], m/z: 119[M+H-18-170], m/z: 105[M+H-18-184] (base peak).

Among the structures proposed by the ChemSpider and PubChem databases, only Suaveolol shows a fragmentation mode similar to that of the compound in question (Scheme 9).




***Scheme 9.*** *Fragmentation of compound 9.*

The observation of the molecular peak at m/z: 307 on the spectrum denotes the stability of the molecule, which would be due to the presence of unsaturation in the structure. Almost all the fragments are obtained after dehydration of the unsaturated ring. The majority fragment at m/z: 103 (105 on the spectrum) and the mass fragment at m/z: 187, originate from cleavage on the saturated ring of the α-carbon bonds of the adjacent ring. The fragment at m/z: 119 is due to cleavage on the intermediate ring of the α-carbon bonds of the unsaturation. Concerning the fragments at m/z: 157 and at m/z: 133, they result from the double cleavage on the intermediate ring of the carbon-carbon bonds in α and β position of the other two rings (Scheme 9). Compound 9 (Figure 20) is therefore Suaveolol, which belongs to the terpene and sterol family. It has already been reported in the literature [18].

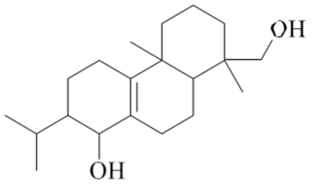


***Figure 20.*** *Structure of compound 9.*

## 4. Conclusion

This analysis shows that the dichloromethane fraction from the hydroethanolic extract of *Hyptis suaveolens* leaves after extraction of the essential oil, contains several compounds (thirty-two (32)). Many of the molecular formula proposed do not correspond to molecules already identified in the *Hyptis* genus. On the other hand, nine (09) correspond to structures already isolated from the genus. Of the nine (09) already known compounds detected in the plant's leaves after extraction of the essential oil during this study, five (05) had not yet been identified in *Hyptis suaveolens* leaves. Moreover, four (04) of the nine (09) compounds identified are phenolic compounds and the other five (05) are terpenoids.

This study shows that, in addition to known compounds, *Hyptis suaveolens* leaves contain new bioactive compounds after extraction of the essential oil. The presence of these compounds would justify the biological and pharmacological activities of the plant's leaves after extraction of the essential oil.

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## Compliance with Ethical Standards

This article does not contain any studies involving human or animal subjects.

## Conflicts of Interest

The authors declare no conflicts of interest.

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