Journal of Materials and Environmental Science
ISSN : 2028-2508
e-ISSN : 2737-890X
CODEN : JMESCN

J. Mater. Environ. Sci., 2023, Volume 14, Issue 12, Page 1529-1550

http://www.jmaterenvironsci.com

Identification of compounds by HPLC-ESI-Q-TOF-MS/MS analysis of the dichloromethane fraction from the hydroalcoholic extract of *Hyptis suaveolens* leaves before extraction of the essential oil

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Received *23 Nov 2023,*
Revised *12 Dec 2023,*
Accepted *13 Dec 2023*

Citation: *Soumahoro B., Kone K. P. F. O., Douhore G. Y. T., Soro Y. (2023) Identification of compounds by HPLC-ESI-Q-TOF-MS/MS analysis of the dichloromethane fraction from the hydroalcoholic extract of Hyptis suaveolens leaves before extraction of the essential oil, J. Mater. Environ. Sci., 14(12), 1529-1550*

Abstract*: Hyptis suaveolens* is a plant used for its many medicinal properties. the leaves of the plant contain polyphenols, tannins, sterols and terpenes, saponins, flavonoids and quinones and anthraquinones both after and before extraction of the essential oil. However, few molecular structures of plant leaves have been identified. The determination of the structures of ten (10) known compounds of the Hyptis genus was carried out by dereplication on the dichloromethane fraction from the hydroalcoholic extract (70%) of the leaves of the plant before extraction of the essential oil. Of these 10 compounds, five (05) are phenolic compounds and the other five (05) are terpenoids. Five (05) of these molecules had not yet been identified in the leaves of *Hyptis suaveolens*. This study shows that *Hyptis suaveolens* leaves contain new bioactive compounds of pharmacological interest.**:**

Keywords: *Hyptis suaveolens; Dereplication; identification ; molecular structures;*

1. Introduction

Plants are a priority and inexhaustible source of molecules for the treatment of various diseases of populations and in particular those in developing countries (Soro et *al*., 2012; Elmsellem *et al.*, 2019). Among these medicinal plants are aromatic plants that produce essential oils. These are genuine natural sources of bioactive compounds used in food, the cosmetics and chemical industries, aromatherapy and above all in pharmaceutical products (Lagouri et *al*., 1995, El Ouadi *et al*., 2017; Ruberto et *al*., 2000). Once used solely for their pleasant fragrances and curative properties, aromatic plants are increasingly used in cosmetics for the development of beauty products, relaxants, and stimulants. They are also used in therapy as antioxidants (El Ouariachi et *al*., 2014), antimicrobials, and antivirals (Selles et *al*., 2013; Goly et *al*., 2015; Goly et *al*., 2017; Haddou *et al*., 2023). they can be used as an alternative for toxic chemical inhibitors in acidization and acid pickling of mild steel

(Salhi et *al*., 2016; Hmamou et *al*., 2012). For example, *Hyptis suaveolens* (Lamiaceae), widespread in tropical areas of America, Asia and Africa, is used in more than twenty-two countries for its medicinal properties (Tang *et al*., 2018). All parts of the plant are used in traditional medicine in the treatment of many conditions such as respiratory conditions, gastrointestinal infections, antirheumatic, antisuporifics, colic, colds, indigestion, fever, abdominal pain, burns, sores, cramps and multiple skin complications (Mahesh *et al*., 2001,Oliveira *et al*., 2005). However, the leaves are the most used, followed by the grains and the whole plant (Tang *et al*., 2018). Several phytochemical studies have revealed that the leaves of *Hyptis suaveolens* contain essential oil (less than 5% of the mass of dry leaves) which contains menthol, limonene and sesquiterpenes (Tia *et al*., 2011) which have numerous pharmacological and biological (Kumar *et al*., 2015). Leaf extracts revealed the presence of alkaloids, simple sugars, steroids, terpenoids, tannins, flavonoids, anthraquinones and phenols which represent more than 13% of the dry leaf mass (Soumahoro *et al*., 2020). Furthermore, hydroethanolic extracts and their dichloromethane fraction have shown antioxidant (Soumahoro *et al*., 2023) and antibacterial properties (Goly *et al*., 2015). However, few identified molecular structures of the dichloromethane fractions of the plant leaves would justify these biological activities.

Thus, the present study aims to determine the structures of some known compounds present in the leaves of *Hyptis suaveolens* before extraction of the essential oil by the application of HPLC-ESI-Q-TOF-MS/MS analysis.

2. Material and methods

2.1 Material

2.1. 1. Plant material

The leaves of *Hyptis suaveolens* (**figure 1**) were collected in July 2017 in Yamoussoukro (6°47'18.762" North and 5°15'25.9992" West) in the center of Côte d'Ivoire and identified by Mr. Amani N'Guessan, botanist at the Institut National Polytechnique Felix HOUPHOUËT-BOIGNY (INP-HB) of Yamoussoukro.

Figure 1: Photo of *Hyptis suaveolens* (photo soumahoro, 17/07/2023 Yamoussoukro)

A specimen of *Hyptis suaveolens* is listed in the CSRS herbarium under the number: Coll n°: 18027 / bdcsrs: 65599. The leaves were dried in the shade at ambient laboratory temperature (26 to 30°C) for 7 days before being crushed using an IKA M20 brand electric grinder (France). The powder obtained was sifted using a 0.5 mm mesh sieve. The resulting powder was stored in a colored jar at 4°C until further use.

2.1. 2. Experimental equipment

For the 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. The analyzes were carried out 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 11000 (at m/z 922).

2.2. Methods

2.2.1. Sample preparation

The preparation of the total hydroalcoholic extract was carried out according to the method described by Soumahoro and collaborators (Soumahoro *et al.*, 2020). A mass of 100 g of ground sample was macerated in 1 L of an ethanol/water mixture (70/30: v/v) under a magnetic stirrer for 24 hours. After decantation, the mixture was successively filtered through hydrophilic cotton and Watman No. 2 paper. The operation was repeated three (3) times until the ground material was exhausted. The filtrate obtained was concentrated at reduced pressure at a temperature of 40°C using a BUCHI 461 type rotary evaporator then freeze-dried to give the total hydroalcoholic extract (EHA1). The total hydroalcoholic extract obtained was successively fractionated using solvents of increasing polarities (hexane, dichloromethane, ethyl acetate, ethanol, and water) following the method reported by Bouamama and collaborators (Bouamama *et al.*, 2006). The hydroalcoholic extract (10 g) was dissolved in 100 mL of water and partitioned successively with hexane (3x 100 mL), dichloromethane (3x 100 mL) and ethyl acetate (3x 100 mL). The different organic phases obtained were separately dried over anhydrous sodium sulphate. After filtration and elimination of solvents under reduced pressure, the fractions with hexane (FHEX), dichloromethane (FDCM) and ethyl acetate (FAE) before extraction of the essential oil were obtained. Then, 5 mg of dichloromethane fraction are dissolved in 1 mL of analytical methanol, then 1 mL of this solution is taken up using a syringe in 1 mL of methanol. This extract is filtered again using a 0.5 µm filter syringe. Finally, 300 µL are taken to be stored in a case before HPLC-QTOF-MS/MS analysis.

2.2.2. Comparative HPLC-ESI-Q-TOF-MS/MS analysis of the dichloromethane fraction by the dereplicative method

Dereplicative analysis is a new method that allows the rapid identification of known molecules contained in a complex mixture (Jongmin et *al* 2017).. It is based on the use of the coupling of High Performance Liquid Chromatography (HPLC) and Tandem Mass Spectrometry (MS/MS or MS2)/Q-TOF [11]. 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), Route B (5-100% ACN) for 30 minutes. ESI conditions were set 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 11000 (at m/z 922). The injection volume of the samples was set at 5 µL.

An analysis of the dichloromethane fraction from the hydroalcoholic extract of *Hyptis suaveolens* leaves before extraction of the essential oil was carried out by the HPLC-ESI-Q-TOF-MS/MS method. Then an automated integration of the chromatogram obtained, using the MassHunter® (Agilent) Qualitative Analysis B.07.00 software, made it possible to obtain the peaks of the different main compounds from this fraction (**figure 2**).

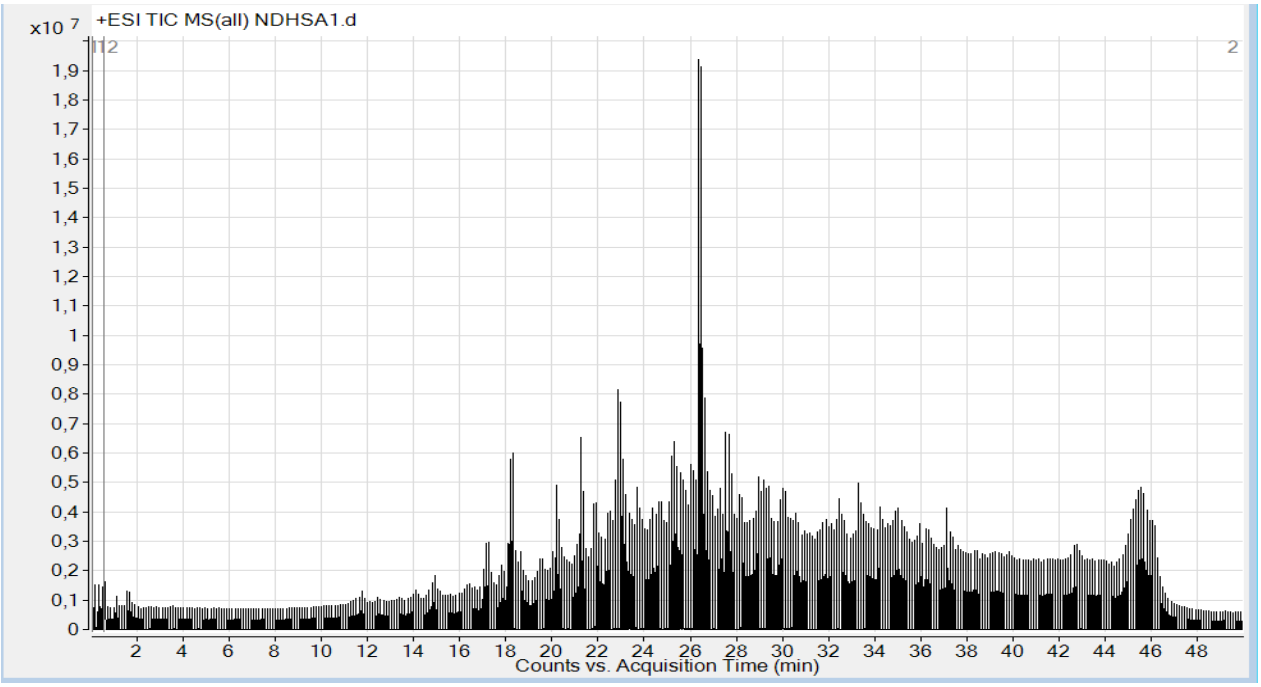


Figure 2. Total ESI/MS chromatographic profile

By clicking on a given peak, the software generates a set of formulas corresponding to the single molecular ion $[M+H]^+$ (**figure 3**).

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Score (MFG)	Mass (MFG)	DBE
●	MFG	C20 H30 O2	(M+H)+	303.2318	98,33	-0,24	98,33	302,2246	6
○	MFG	C18 H28 N3 O	(M+H)+	303.2318	89,9	-4,92	89,9	302,2232	6,5
○	MFG	C13 H30 N6 S	(M+H)+	303.2318	83,53	0,58	83,53	302,2253	2
○	MFG	C15 H32 N3 O S	(M+H)+	303.2318	78,37	5,35	78,37	302,2266	1,5
○	MFG	C16 H26 N6	(M+H)+	303.2318	72,65	-9,64	72,65	302,2219	7
○	MFG	C17 H34 O2 S	(M+H)+	303.2318	65,35	10,08	65,35	302,228	1
○	MFG	C19 Cl3 O9 S4	(M+2H)+2	303.3823	46,84	-1,57	46,84	604,7491	18,5
○	MFG	C14 H2 Cl3 N3 O8 S5	(M+2H)+2	303.3823	46,61	1,79	46,61	604,7511	14
○	MFG	C15 Cl3 O14 S3	(M+2H)+2	303.3823	45,56	2,57	45,56	604,7516	14,5
○	MFG	C14 H Cl2 N O12 S5	(M+2H)+2	303.3823	43,98	-3,45	43,98	604,7479	14
○	MFG	C16 H4 Cl3 O9 S5	(M+2H)+2	303.3823	42,77	4,01	42,77	604,7524	13,5
○	MFG	C23 Cl3 O4 S5	(M+2H)+2	303.3823	38,31	-5,7	38,31	604,7466	22,5
○	MFG	C17 Cl3 N4 O5 S5	(M+2H)+2	303.3823	36,77	6,22	36,77	604,7538	18,5

Figure 3. Raw formulas suggested by MassHunter software

3. Results and discussion

3.1. Dereplicative HPLC-MS/Q-TOF analysis of the dichloromethane fraction

The HPLC-MS/Q-TOF chromatographic profile of the dichloromethane fraction from the hydroalcoholic extracts of *Hyptis suaveolens* leaves before the extraction of the essential oil is shown

in **Figure 4. Figure 4** shows the presence of numerous compounds of different polarities with retention times varying between 1.093 min and 45.56 min. The analyses carried out in positive mode made it possible to determine the molecular masses and the crude formulas of the compounds revealed by chromatography (**Table 1**).

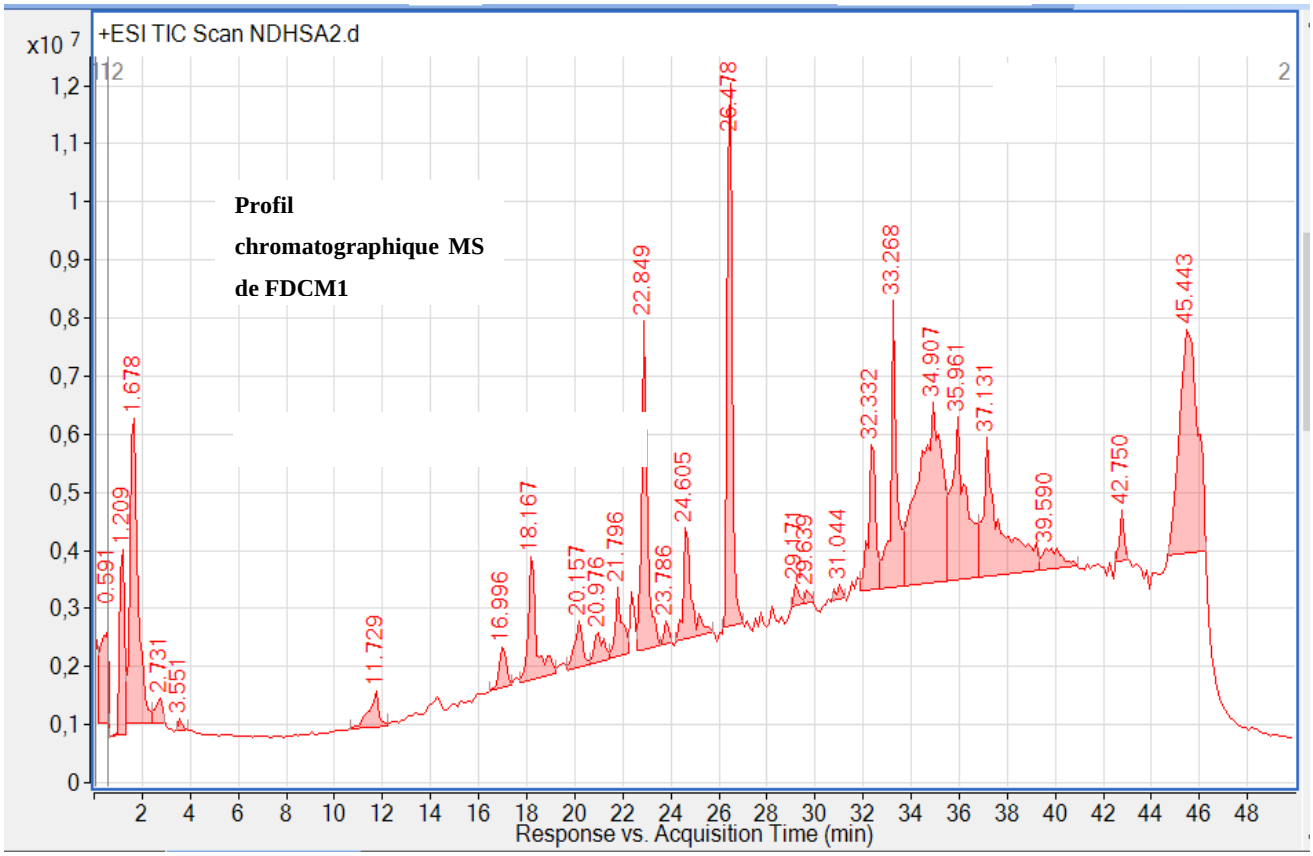


Figure 4. ESI/MS chromatographic profile of the majority compounds of the dichloromethane fraction before extraction of the essential oil

It appears from the analysis of the dichloromethane fraction from the hydroalcoholic extract of the leaves of *Hyptis suaveolens* before extraction of the essential oil that the plant is rich in molecules (32) (**Table 1**). Among the compounds detected in the dichloromethane fraction, ten (10) have already been isolated from the genus Hyptis (Mukherjee *et al.*, 1984, Tang *et al.*, 2018, Ekow *et al.*, 2018) (**Table 2**).

3.2. Confirmation of the structures of known compounds (10) from the dichloromethane fraction

The structures of the compounds were found from the interpretation of data provided by HPLC-ESI-MS/Q-TOF analysis of the dichloromethane fraction of the plant leaves. HPLC-ESI-MS/Q-TOF analysis of the dichloromethane fraction of *Hyptis suaveolens* leaves made it possible to obtain the mass and fragmentation spectra as well as the crude formulas of several major compounds. Among the raw formulas obtained, the NIST, ChemSpider and PubChem databases provided structures. We were interested in the structures corresponding to those of certain compounds already isolated from the Hyptis genus.

Table 1. Compounds detected in the dichloromethane fraction (FDCM1)

PIC number	Retention time (min)	Brute formula	Molecular mass (g/mol)	Score (%)
1	1.209	$C_5H_{13}NO$	103	97.97
2	1.678	$C_9H_{19}N_5O_3S$	277	75.56
3	2.731	$C_6H_{10}N_6O_4$	230	94.21
4	3.551	$C_{12}H_{23}NO_7$	293	99.64
5	11.729	$C_9H_6O_4$	178	87.16
6	16.996	$C_{14}H_{22}O$	206	84.35
7	18.167	$C_8H_{12}N_6O$	208	94.18
8	20.157	$C_8H_{10}O$	122	96.37
9	20.859	*$C_{21}H_{28}O_7$	392	65.20
10	20.976	$C_{17}H_{24}N_6O_5$	392	95.61
11	21.652	*$C_{15}H_{10}O_7$	302	92.89
12	21.796	$C_{13}H_{22}O_2$	210	87.03
13	21.842	*$C_{21}H_{20}O_{12}$	464	85.84
14	22.03	*$C_{10}H_{12}O_2$	164	84.98
15	22.849	$C_9H_6O_3$	162	99.82
16	22.872	*$C_{18}H_{16}O_8$	360	95.01
17	23.786	$C_9H_{18}N_2S$	186	99.74
18	24.605	* $C_{30}H_{40}O_{11}$	576	80.12
19	26.478	$C_8H_4O_3$	148	99.76
20	29.171	$C_{18}H_{28}O_4$	308	95.18
21	29.45	*$C_8H_8O_3$	152	75.46
22	31.044	$C_{17}H_{14}N_2O_3S$	326	89.20
23	32.332	$C_5H_8O_3S$	148	66.25
24	33.034	*$C_{19}H_{28}O$	272	56.21
25	33.268	$C_{12}H_{22}O_2$	198	94.84
26	34.556	*$C_{20}H_{34}O_2$	306	73.87
27	34.907	$C_{21}H_{45}N_{11}O$	467	96.9
28	35.961	$C_{18}H_{35}NO$	281	98.68
29	37.131	$C_{24}H_{38}O_4$	390	99.47
30	39.59	$C_4H_9N_7O$	171	93.0
31	40.643	*$C_{20}H_{32}O$	288	84.45
32	42.75	$C_{44}H_{58}N_2O_3$	662	98.75

*** : Compounds already identified in the genus Hyptis**

Table 2. Compounds detected in the dichloromethane fraction of *Hyptis suaveolens* leaves before extraction of the essential oil

Known compounds detected in the dichloromethane fraction			Before extracting the essential oil	
Name	Brute formula	Molecular mass (g/mol)	Retention time (ms)	Score (%)
2-methyl-3-methylene-2-(4-methylpent-3-en-1-yl) oxirane	$C_{10}H_{16}O$	152.24	18.405	75.46
Propanoate de 1-(5-(hydroxy(2-oxotetrahydro-2H-pyran-3-yl) methyl) tetrahydrofuran-2-yl)ethyl-3-(4-hydroxyphenyl)	$C_{21}H_{28}O_7$	392.45	20.859	65.20
Quercetine	$C_{15}H_{10}O_7$	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)-en-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
(2R,4aS,4bS,10aR)-2,4b,8,8,10a-pentamethyldecahydro-2H-2,4a-methanophenanthren-1(4bH)-one	$C_{20}H_{32}O$	288.48	40.643	84.45
Rosamarinic Acid	$C_{18}H_{16}O_8$	360.32	37.322	95.01

Structure of compound 1

Compound 1 which appears at the retention time equal to 18.40 min (**figure 5**) gives the peak of the molecular ion [M+H]+ at m/z: 153.127 corresponding to the molecular molar mass 152.120 g/mol. The most probable molecular formula is $C_{10}H_{16}O$ (cal. 152.24).

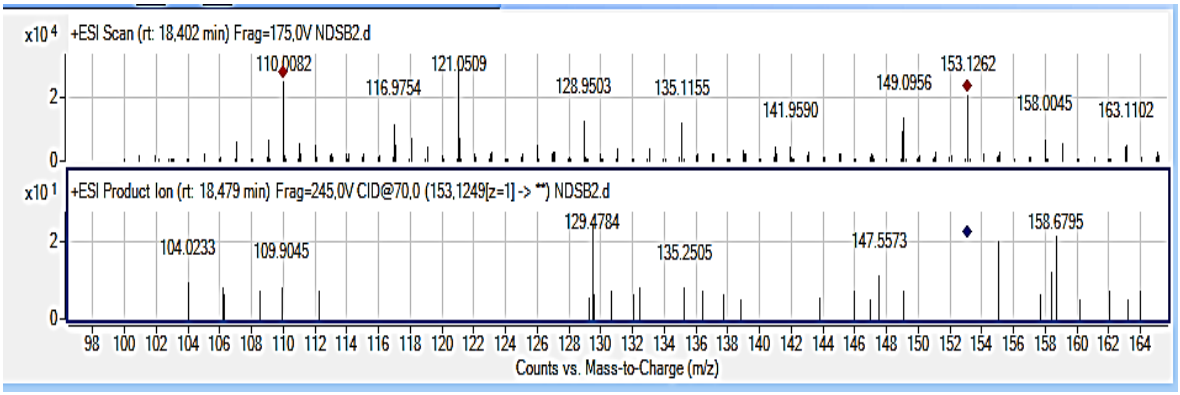
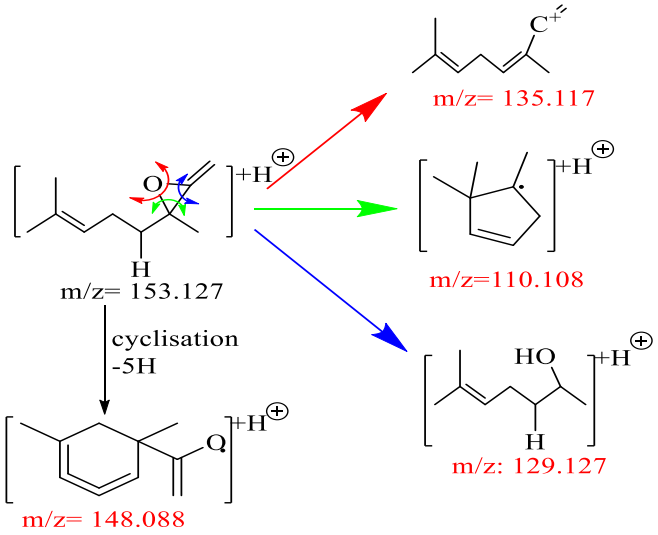


Figure 5. LC-ESI/MS mass spectrum and ESI/MS fragmentation spectrum of compound 1

Analysis of the fragmentation spectrum of compound 1 (**Figure 5**) 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 (pair) would be due to a rearrangement (cyclization) with loss of hydrogen atoms. The fragment at m/z: 110 with an even mass would come from a cyclization followed by the elimination of an ethoxy group (CH_3CO-). The fragments at m/z 135 and 129 result respectively from the loss of a water molecule and a C_2 group. Compound 1 **(Figure 6)** 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 agrees with that of the literature (Joseph *et al.*, 2016).

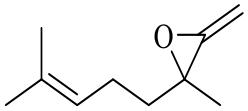


Figure 6. Structure of compound 1

Structure of compound 2

Compound 2 which appears at the retention time equal to 20.859 min corresponds to the molecular ion $[M+H]^+$ m/z: 393.1879 with a molecular molar mass of 392.1835 g/mol. The most probable molecular formula is $C_{21}H_{28}O_7$ (cal. 392.45) (**Figure 7**).

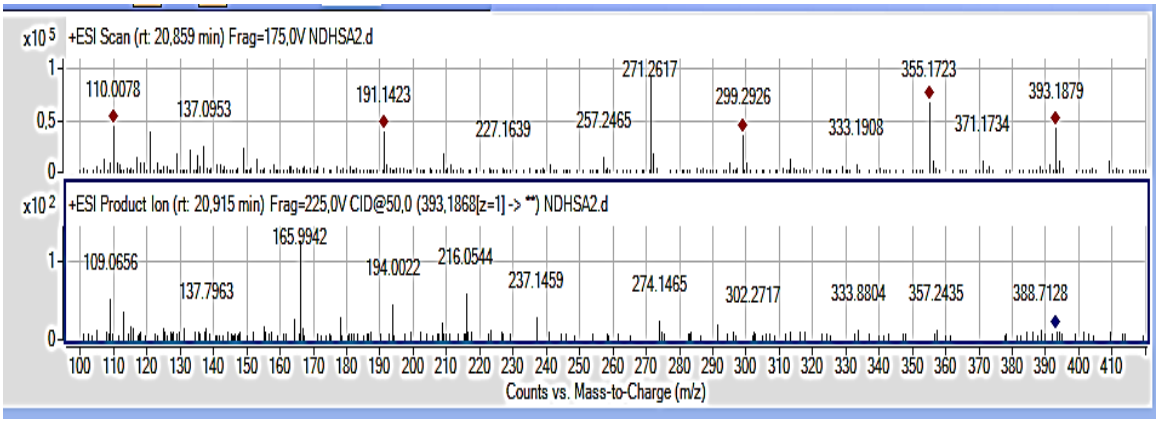
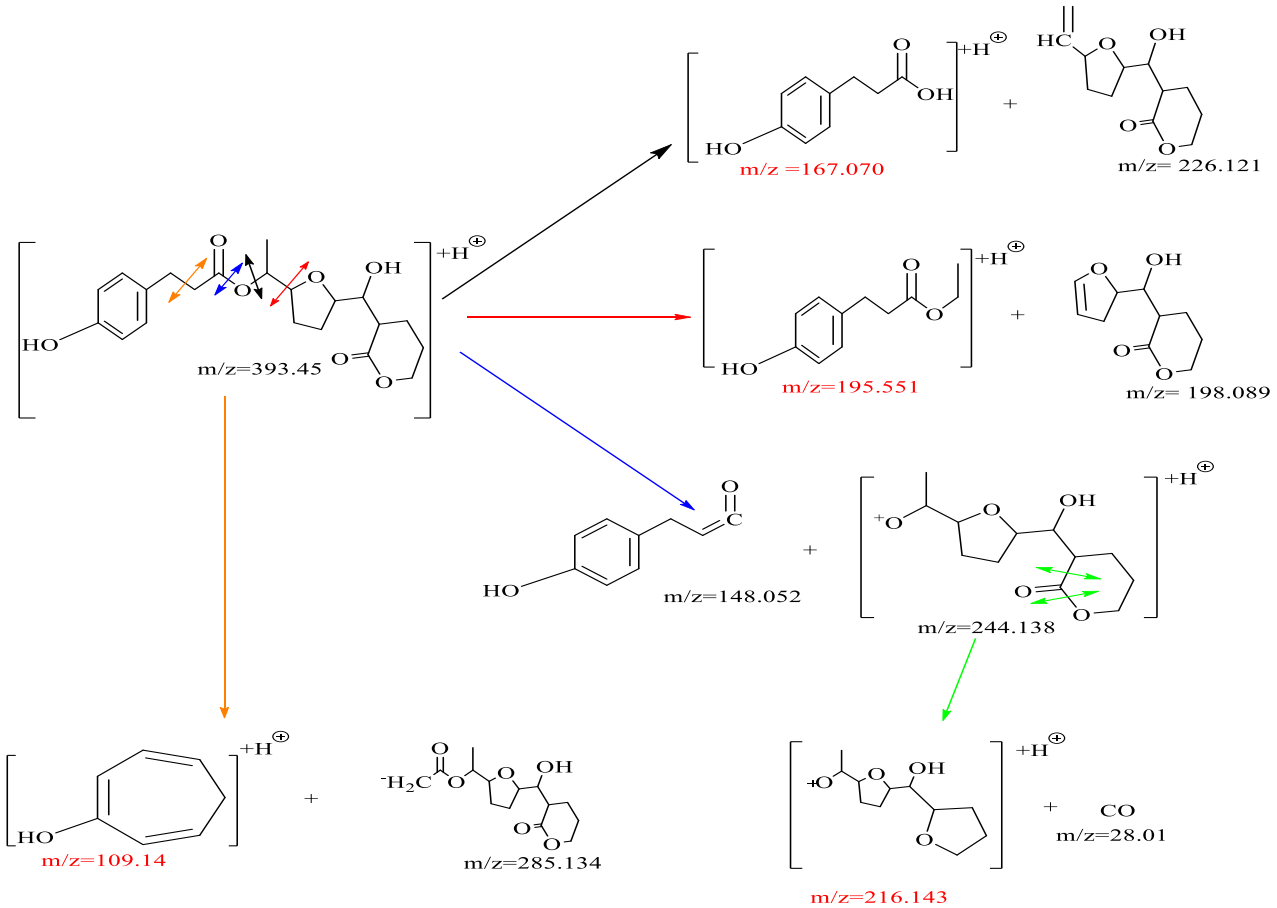


Figure 7. LC-ESI/MS mass spectrum and ESI/MS fragmentation spectrum of compound 2

Analysis of the fragmentation spectrum of compound 2 (**Figure 7**) 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) propanoate -hydroxyphenyl) gives a mode of fragmentation similar to that of the desired compound (**Scheme 2**).



Scheme 2. Proposed fragmentation of compound 2

The basic peak of molecular mass m/z: 167 (166 on the spectrum following a rearrangement according to the nitrogen rule), would be due to a Mc Lafferty type rearrangement with cutting of the carbon-oxygen bond in α (Fragmentation α). The fragment m/z:195 (194 on the spectrum after a rearrangement, nitrogen rule), would come from the splitting of the carbon-carbon bond into α of the methyl group. The m/z:109 fragment would result from the cleavage of the carbon-carbon bond in β of the aromatic ring followed by the formation of the tropylium ion. Compound 2 (**Figure 8**) is therefore 1-(5-(hydroxy(2-oxotetrahydro-2H-pyran-3-yl) methyl) tetrahydrofuran-2-yl) ethyl-3-(4-hydroxyphenyl) propanoate and it belongs to the family of phenolic compounds. Leaves of Hyptis brevipes from the same family as the study plant were also identified (Suárez-Ortiz *et al.*, 2017).

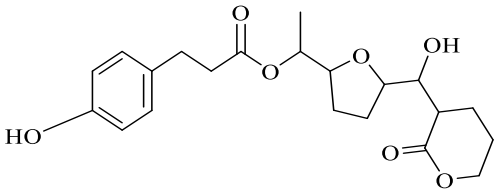


Figure 8. Structure of compound 2

Structure of compound 3

Compound 3 with a retention time equal to 21.814 min corresponds to the molecular ion [M+H]+ at m/z: 303.0494. Its molecular molar mass is 302.0427g/mol. The most probable molecular formula is $C_{15}H_{10}O_7$ (cal. 302.238) (**Figure 9**).

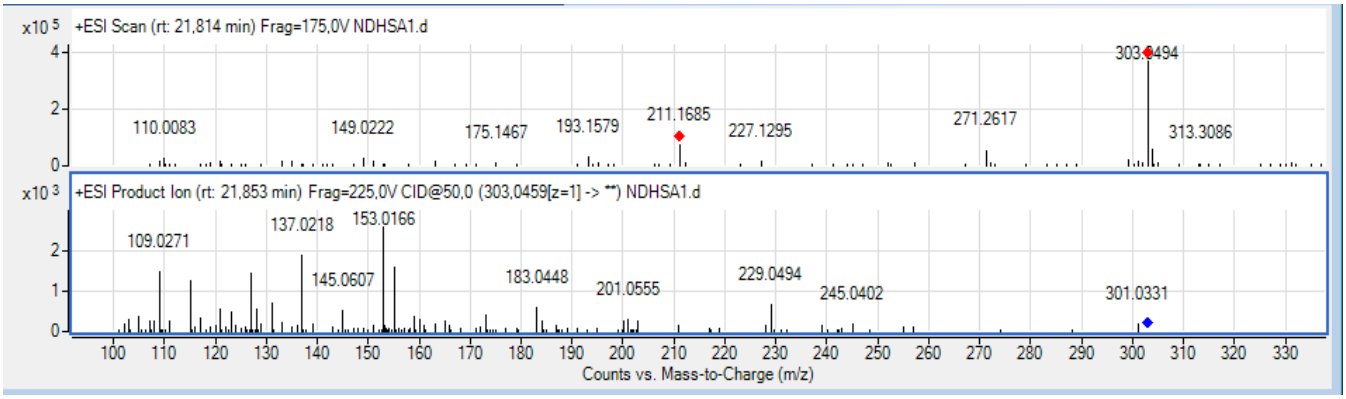
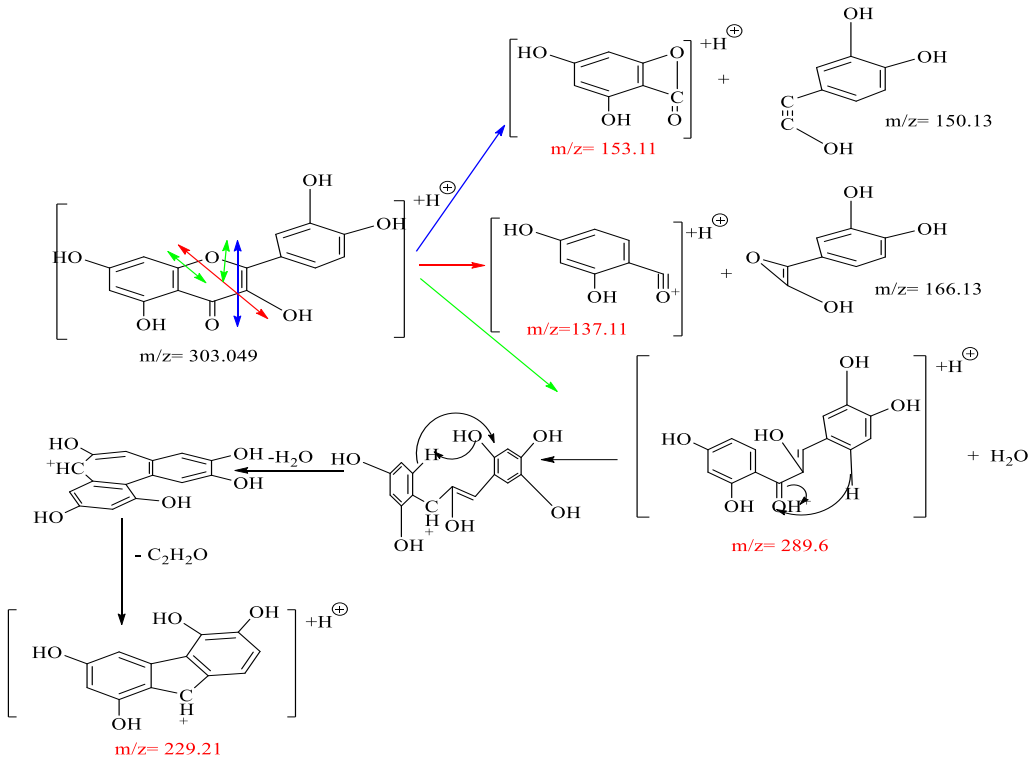


Figure 9. LC-ESI/MS mass spectrum and ESI/MS fragmentation spectrum of compound 3

Analysis of the fragmentation spectrum of compound 3 (**Figure 9**) 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 mode of fragmentation similar to that of the desired compound (**Scheme 3**).

The base peak at m/z: 153 comes from a double cut 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 split on the intermediate C ring of the carbon-oxygen and carbon-carbon bonds respectively in position α of the aromatic ring and β of its hydroxyl group. As for the fragment at m/z: 229, it comes from the elimination of the oxo group, followed by cyclization after the loss of a water molecule and an ethoxy group on the intermediate C ring (**Scheme 3**). Compound 3 (**Figure 10**) is therefore quercetin, and it is from the flavonoid family. This compound is present in the leaves of *H. suaveolens* (Tang *et al.*, 2018).



Scheme 3. Fragmentation of compound 3

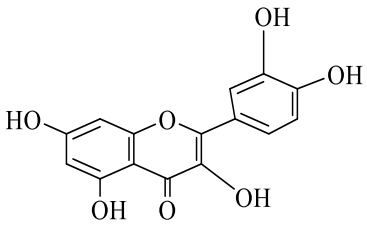


Figure 10. Structure of compound 3 (quercetin)

Structure of compound 4

Compound 4 with a retention time equal to 21.814 min corresponds to the molecular ion [M+H]+ at m/z: 465.1028 with a molecular molar mass of 464.0955g/mol. Thus, the most likely crude formula is $C_{21}H_{20}O_{12}$ (cal. 464.3790) (**Figure 11**).

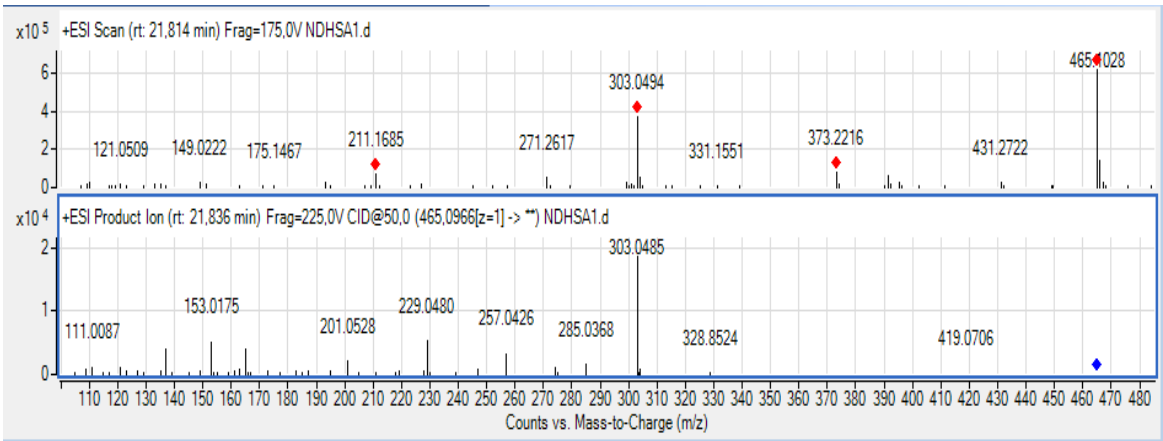
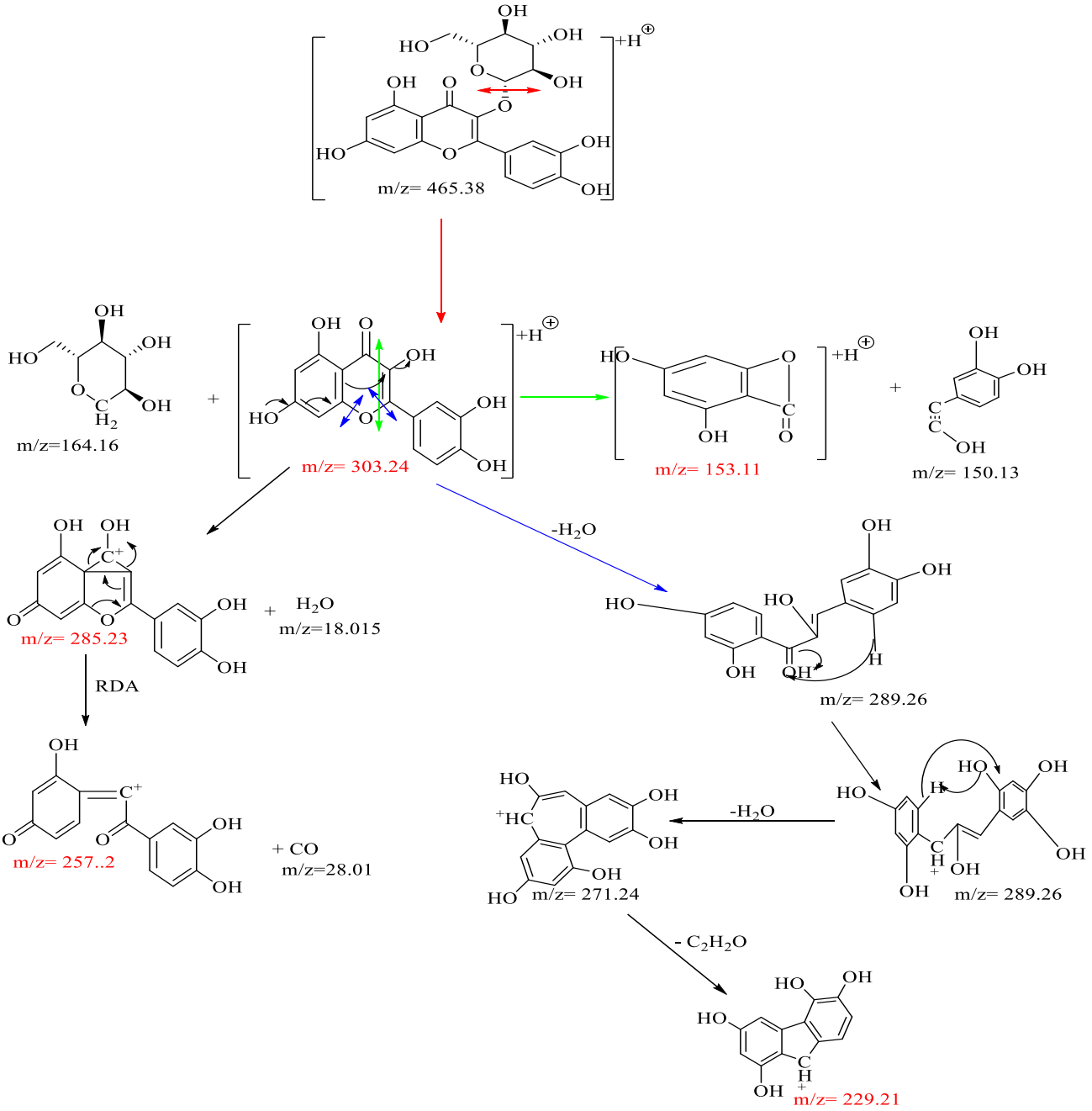


Figure 11. LC-ESI/MS mass spectrum and ESI/MS fragmentation spectrum of compound 4

Analysis of the fragmentation spectrum of compound 4 (**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 mode of fragmentation similar to that of the desired compound (**Scheme 4**). The base peak at m/z: 303 comes from the loss of the glucosyl group. The fragment at m/z: 153 results from the base peak after a double cleavage on the intermediate C ring of the carbon-oxygen and carbon-carbon bonds respectively at β and at α of the hydroxyl group of this cycle. Likewise, the fragment at m/z: 285 comes from the base peak by loss of a water molecule on the intermediate C ring. As for the fragment at m/z: 257, it arises from a Retro Diels-Alder (RDA) mechanism on the intermediate C ring with loss of the carbonyl group (**Scheme 4**). Compound 4 (**Figure 12**) is therefore quercetin 3-O-β-D-glucopyranoside from the flavonoid family. It has already been identified in the plant studied (Ekow *et al.*, 2018).



Scheme 4. Fragmentation of compound 4

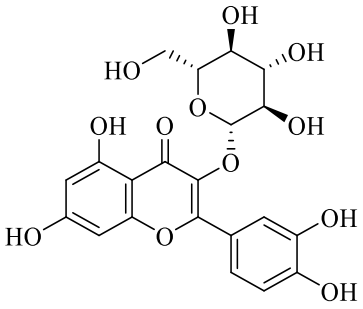


Figure 12. Structure of compound 4

Structure of compound 5

Compound 5 with a retention time equal to 22.165 min corresponds to the molecular ion [M+H]+ at m/z: 165.0901 with a molecular molar mass of 164.0837g/mol. The most probable molecular formula is $C_{10}H_{12}O_2$ (cal. 164.20) (**figure 13**).

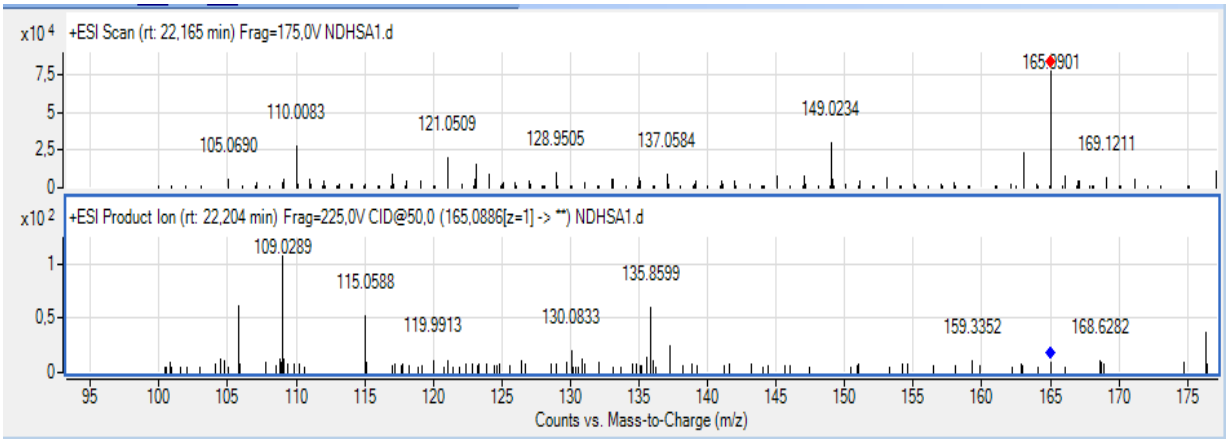
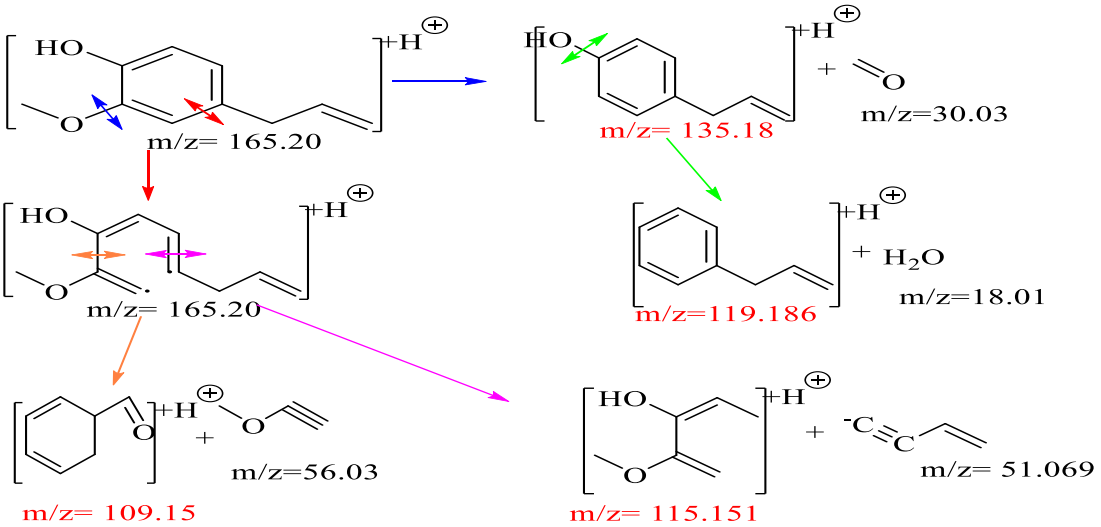


Figure 13. LC-ESI/MS mass spectrum and ESI/MS fragmentation spectrum of compound 5

Analysis of the fragmentation spectrum of compound 5 (**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 mode of fragmentation similar to that of the desired compound (Scheme 5).



Scheme 5. Proposed fragmentation of compound 5

The fragment at m/z: 135 would come from the loss of the methoxyl group in formaldehyde form. The fragment at m/z: 119 would have come from the previous one after dehydration. The majority peak at m/z: 109 would result from a double cleavage on the aromatic ring of the carbon-carbon single bonds in the β and γ position of the methoxyl group. Similarly, the fragment at m/z: 115 would be due to a double scission on the cycle of the single and double α bonds of the propenyl group (**Scheme 5**). Compound 5 (**Figure 14**) is therefore 4-allyl-2-methoxyphenol from the family of phenolic compounds. It was identified in the essential oil of H. suaveolens leaves collected in Tanzania (Malele et al., 2003).

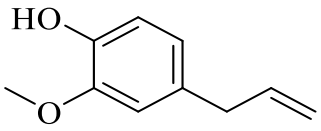


Figure 14. Structure of compound 5

Structure of compound 6

Compound 6 with a retention time equal to 24.605 min corresponds to the molecular ion [M+H]+ at m/z: 577.262 with a molecular molar mass of 576.259 g/mol, as can be seen in Figure 14. The most probable crude formula is $C_{16}H_{12}O_6$ (cal. 576.859) (**figure 15**).

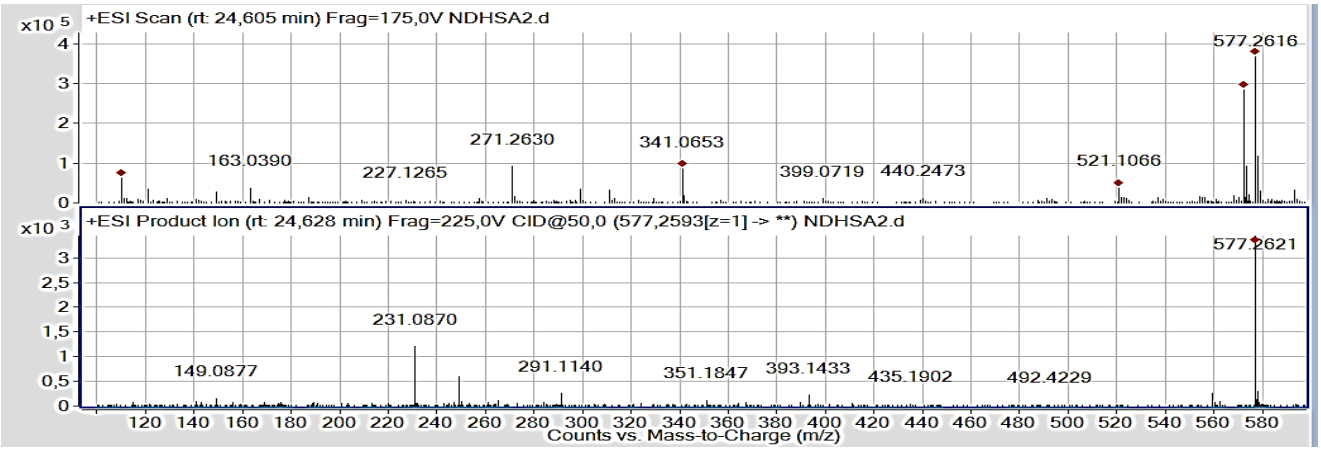
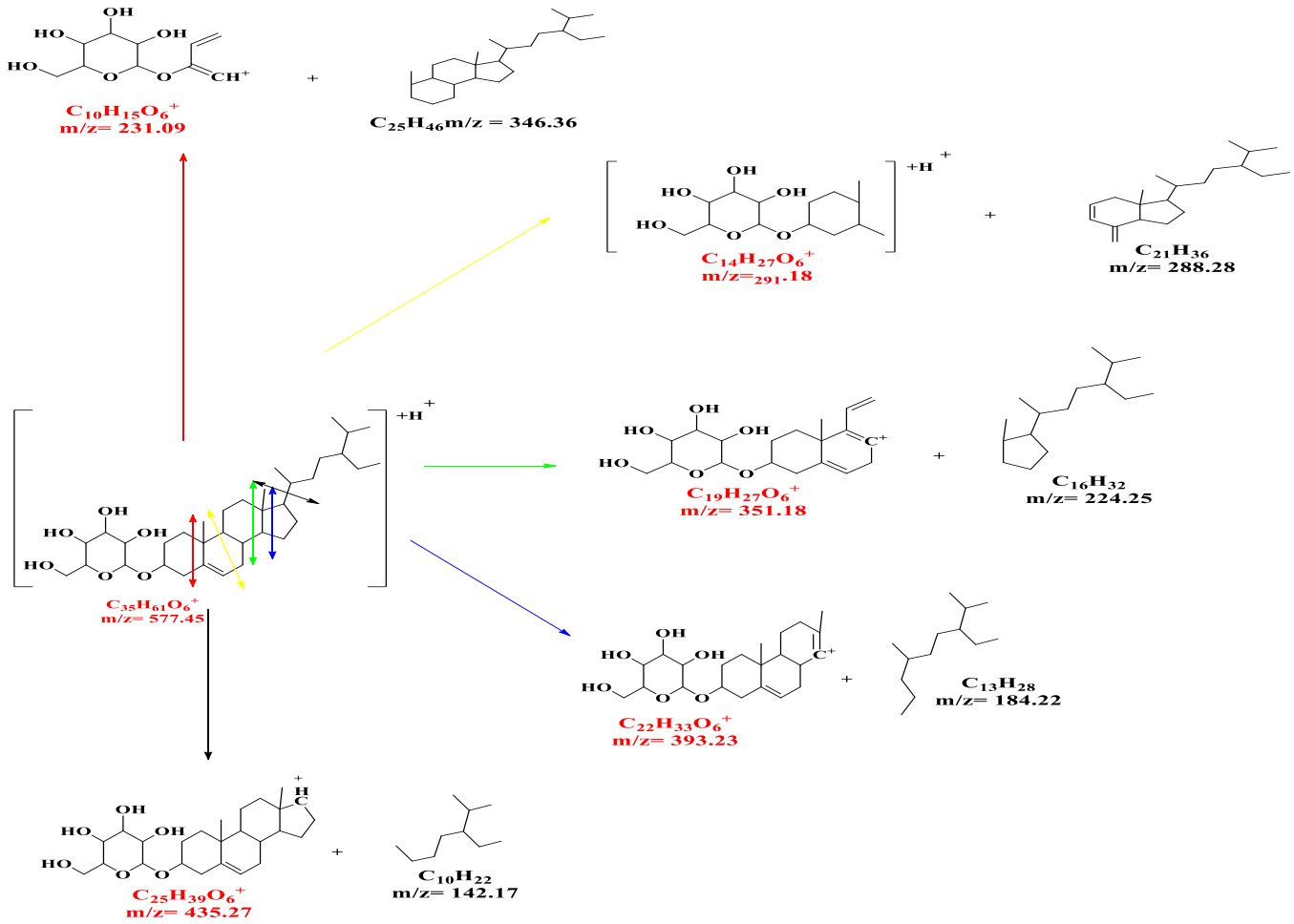


Figure 15. LC-ESI/MS mass spectrum and ESI/MS fragmentation spectrum of compound 6

Analysis of the fragmentation spectrum of compound 6 (**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]. Among the structures proposed by the ChemSpider and PubChem databases, only β-sitosterol glucose gives a fragmentation mode like that of the desired compound (**Scheme 6**).

The molecular peak is the base peak, which denotes the stability of the molecule. The fragment at m/z: 231 would come from the double cleavage on the ring contiguous to the glucosyl group of the two carbon-carbon bonds at α of the following cycle. The fragment at m/z: 291 would also result from a double split on the unsaturated cycle of carbon-carbon bonds between the previous cycle and the following cycle 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 ring in C6 of the carbon-carbon bonds contiguous to the ring in (C_5). The fragment at m/z: 393 would come from the cutting of the 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 1-methyl,4-isopropylhexane ($C_{10}H_{21}$) substituent (**Scheme 6**). Compound 6 (**Figure 16**) is therefore β-sitosterol glucose which belongs to the class of sterols and terpenes. It's present in the plant studied (Ziegler *et al.*, 2002).



Scheme 6. Proposed fragmentation of compound 6

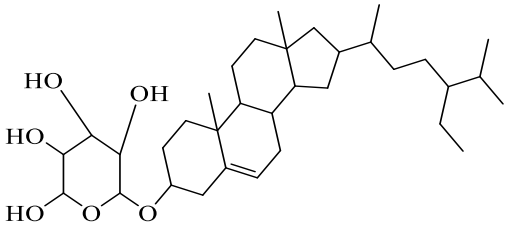


Figure 16. Structure of compound 6

Structure of compound 7

Compound 7 with a retention time equal to 33.106 min corresponds to the molecular ion [M+H]+ at m/z: 273.2210 with a molecular molar mass of 272.2140 g/mol. The most likely crude formula is $C_{19}H_{28}O$ (cal. 272.43). **Figure 17** shows the spectrum of the compound. The analysis of the compound fragmentation spectrum 7 (**Figure 17**) shows the presence of major fragments to M/Z: 159 [M+H-114] (Basic 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 databases at Chemspider and Pubchem, only 5α-Androst-9 (11) -en-12-One gives a mode of fragmentation like that of the compound sought (**Scheme 7**).

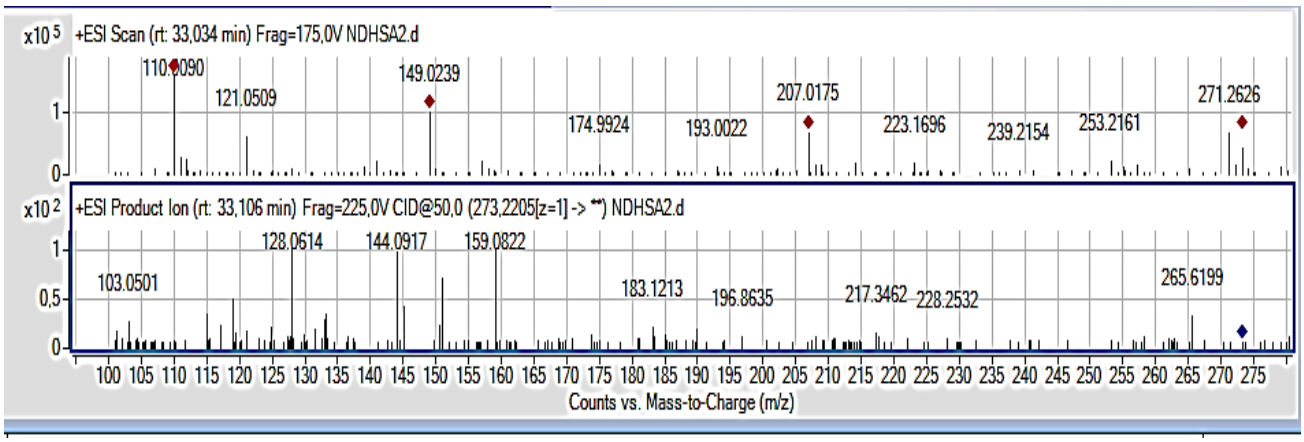
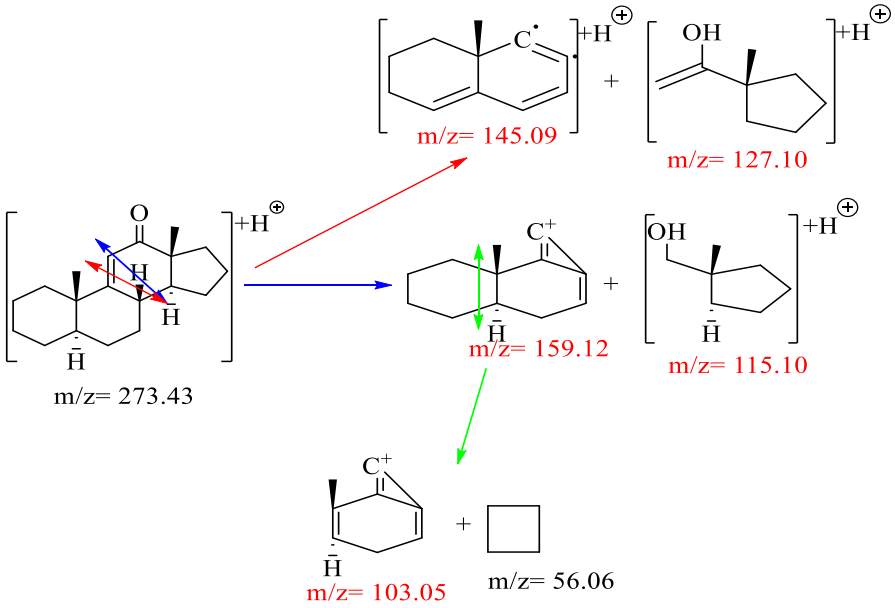


Figure 17. Spectre de masse LC-ESI/MS et spectre de fragmentation ESI/MS du compose 7



Scheme 7. Proposed fragmentation of compound 7

The base peak at m/z: 159 would derive 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 would come from the base peak by loss of a cyclobutane following the cut on the saturated ring of the carbon-carbon bonds contiguous to the second cycle at (C_6). The fragments at m/z: 145 (144 on the spectrum) and m/z: 127 (128 on the spectrum) would come from the breakage on the unsaturated ring of the carbon-carbon bonds contiguous to the intermediate ring at (C_6) (**Scheme 7**). Compound 7 (**Figure 18**) would therefore be 5α-androst-9(11)-en-12-one from the terpene and sterol family. It has previously been identified in the leaves of the plant (Edeoga et al., 2006).

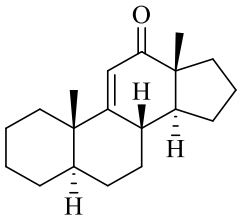


Figure 18. Structure of compound 7

Structure of compound 8

Compound 8 with a retention time equal to 34.691 min corresponds to the molecular ion [M+H]+ at m/z: 307.262 with a molecular molar mass of 306.256 g/mol. The most probable molecular formula is $C_{20}H_{34}O_2$ (cal. 306.49) (**figure 19**).

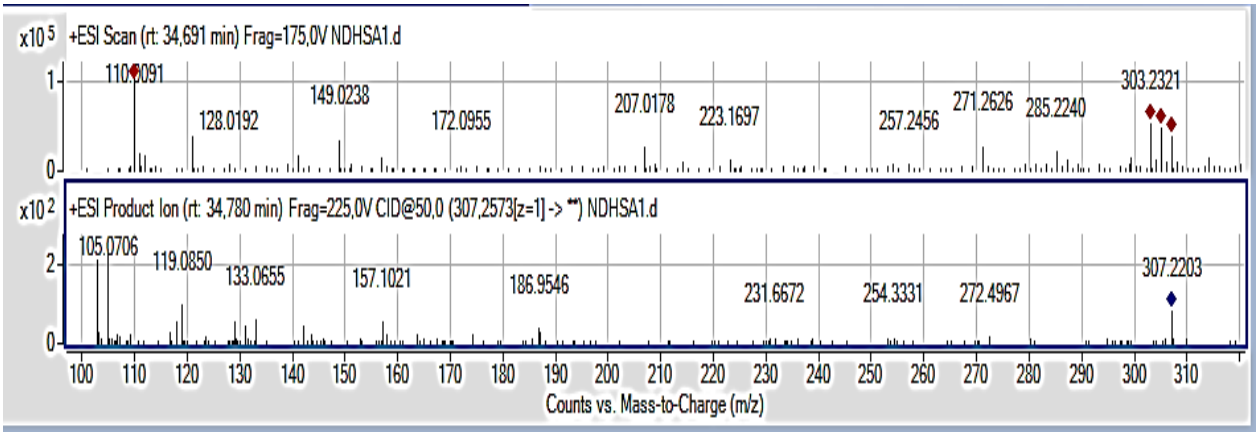
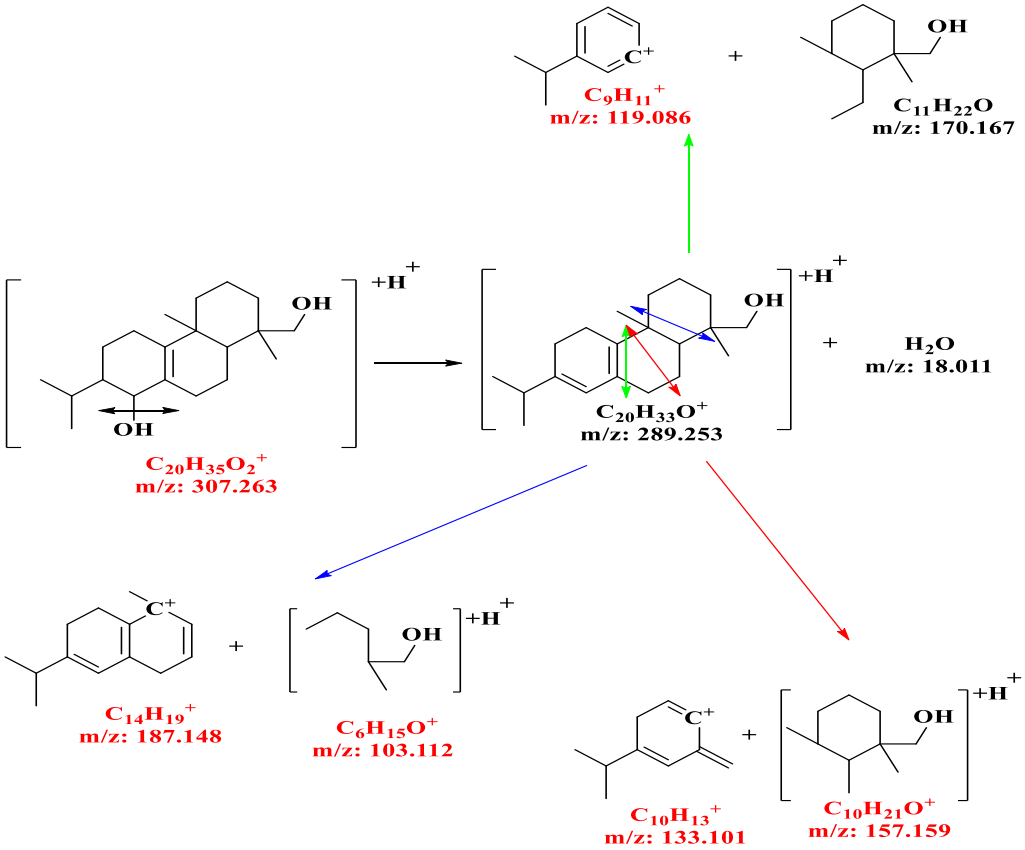


Figure 19. LC-ESI/MS mass spectrum and ESI/MS fragmentation spectrum of compound 8

Analysis of the fragmentation spectrum of compound 8 (**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 gives a mode of fragmentation like that of the desired compound (**scheme 8**).



Scheme 8. Fragmentation of compound 8

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 at the level of the unsaturated ring. The majority fragment at m/z: 103 (105 on the spectrum) and that at m/z: 187, come from cleavage on the saturated ring of the carbon-carbon bonds at α of the adjacent ring. The fragment at m/z: 119 is due to cleavage on the intermediate ring of the carbon-carbon bonds at α of unsaturation. Concerning the fragments at m/z: 157 and at m/z: 133, they result from the double scission on the intermediate cycle of the carbon-carbon bonds in position α and β of the two other cycles (**Scheme 8**). Compound 8 (**figure 20**) is therefore Suaveolol which is from the terpene and sterol family. It has already been reported in the literature (Manchand et al., 1974).

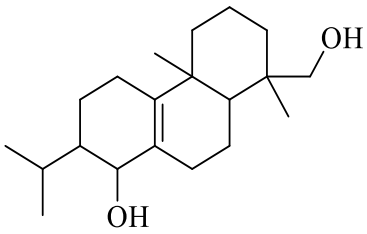


Figure 20. Structure of compound 8

Structure of compound 9

Compound 9 with a retention time equal to 36.330 min corresponds to the molecular ion [M+H]+ at m/z: 289.252 with a molecular molar mass of 288.245 g/mol. The most probable molecular formula is $C_{20}H_{32}O$ (cal. 288.48) (**Figure 21**).

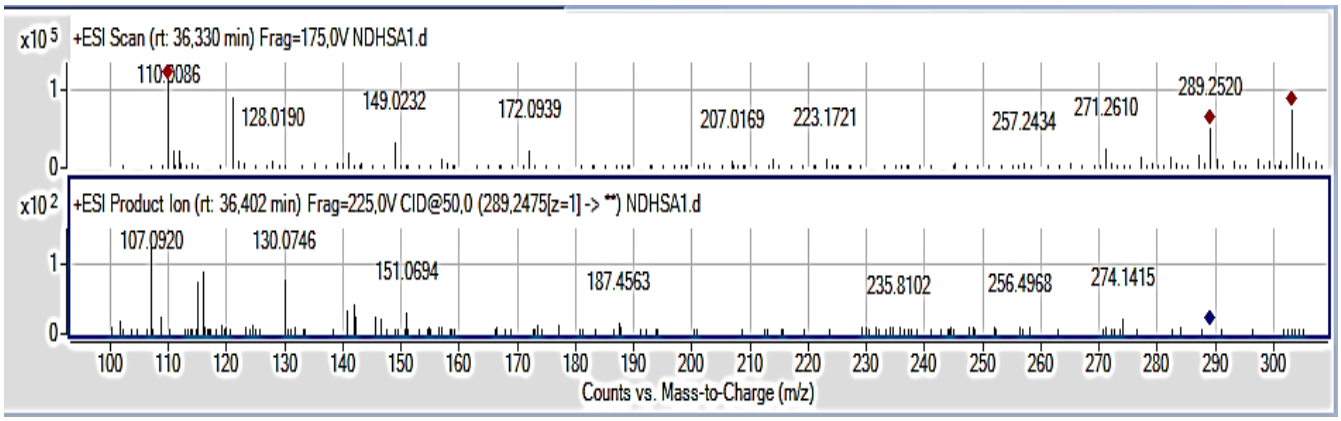
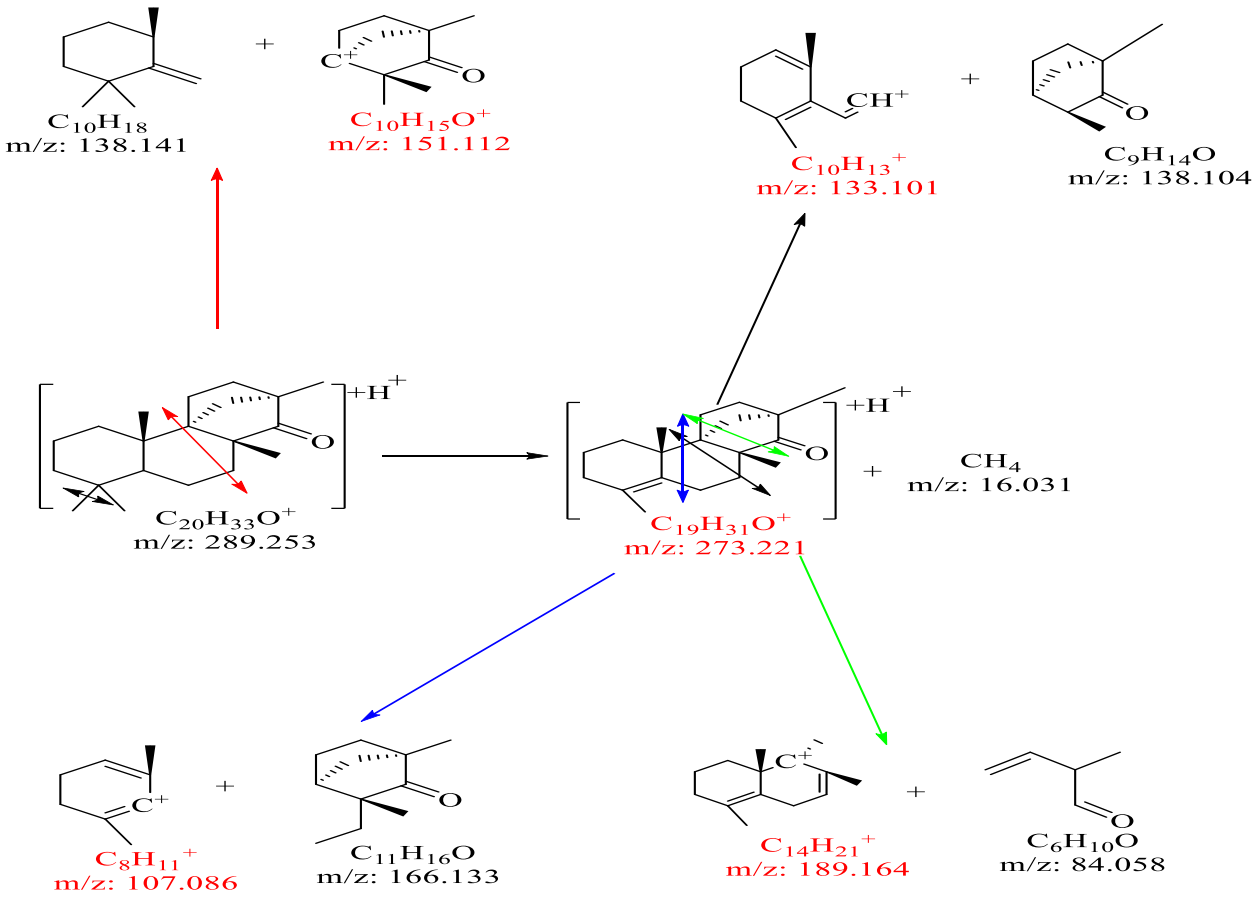


Figure 21. LC-ESI/MS mass spectrum and ESI/MS fragmentation spectrum of compound 9

Analysis of the fragmentation spectrum of compound 9 (**Figure 21**) shows the presence of major fragments at m/z: 274[M+H-15], m/z: 151[M+H-138], m/z : 130[M+H-159], m/z: 107[M+H-172] (base peak). Among the structures proposed by the ChemSpider and PubChem databases, only (2R,4aS,4bS,10aR)-2,4b,8,8,10a-pentamethyldecahydro-2H-2,4a-methanophenanthren-1(4bH)- one gives a mode of fragmentation like that of the desired compound (**Scheme 9**). The fragment at m/z: 151 would be due to the splitting on the intermediate cycle of the carbon-carbon bonds into α and β of two extreme cycles. The fragment at m/z: 273 (274 on the spectrum) would come from the loss of one of the two methyl groups specific to peripheral cyclohexane. The other majority fragments would come from the fragment at m/z: 273. The most abundant fragment at m/z: 107 would result from the cutting on the intermediate cycle of the bonds contiguous to the unsaturated cycle.



Scheme 9. Proposed fragmentation of compound 9

The fragment at m/z: 133 would derive from cleavage on the intermediate ring of the carbon-carbon bonds at α of the carbonyl ring. As for the fragment at m/z: 189, it would result from the breakage on the carbonyl ring of the carbon-carbon bonds in α of the intermediate cycle (**Scheme 9**). Compound 9 (**Figure 22**) is therefore (2R,4aS,4bS,10aR)-2,4b,8,8,10a-pentamethyldecahydro-2H-2,4a-methanophenanthren-1(4bH)-one of the family of sterols and terpenes. This compound has been reported in the literature (Chukwujekwu et al., 2005).

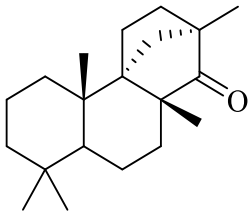


Figure 22. Structure of compound 9

Structure of compound 10

Compound 10 with a retention time equal to 37.322 min corresponds to the molecular ion [M+H]+ at m/z: 361.091 with a molecular molar mass of 360.085 g/mol. The most probable molecular formula is $C_{18}H1_6O_8$ (cal. 360.32) (**Figure 23**). Analysis of the fragmentation spectrum of compound 10 (**Figure 23**) reveals the presence of major fragments at m/z: 277[M+H-84], m/z: 179[M+H-182], m/z : 151[M+H-210], m/z: 123[M+H-238] (base peak). Among the structures proposed by the ChemSpider and PubChem databases, only rosamarinic acid gives a mode of fragmentation like that of the desired compound (**Scheme 10**).

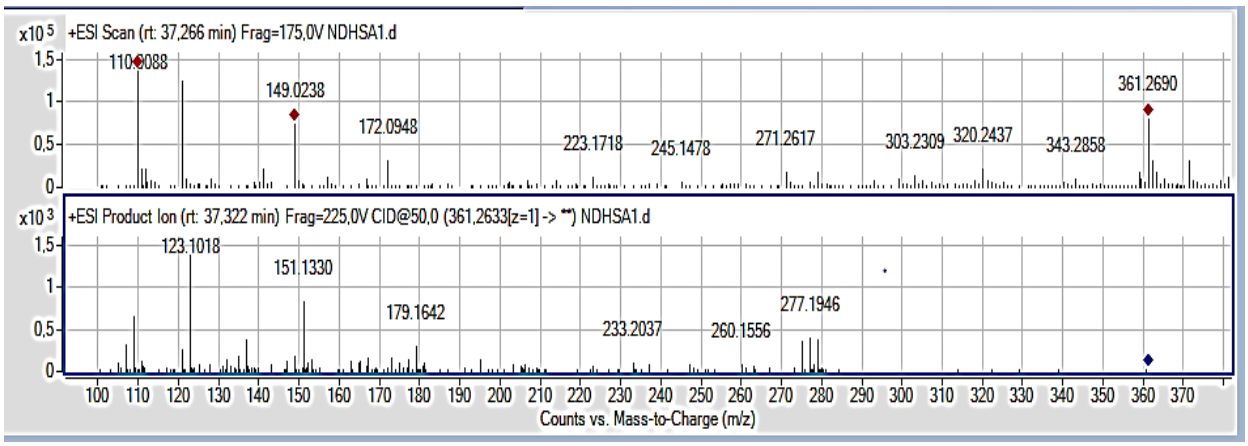
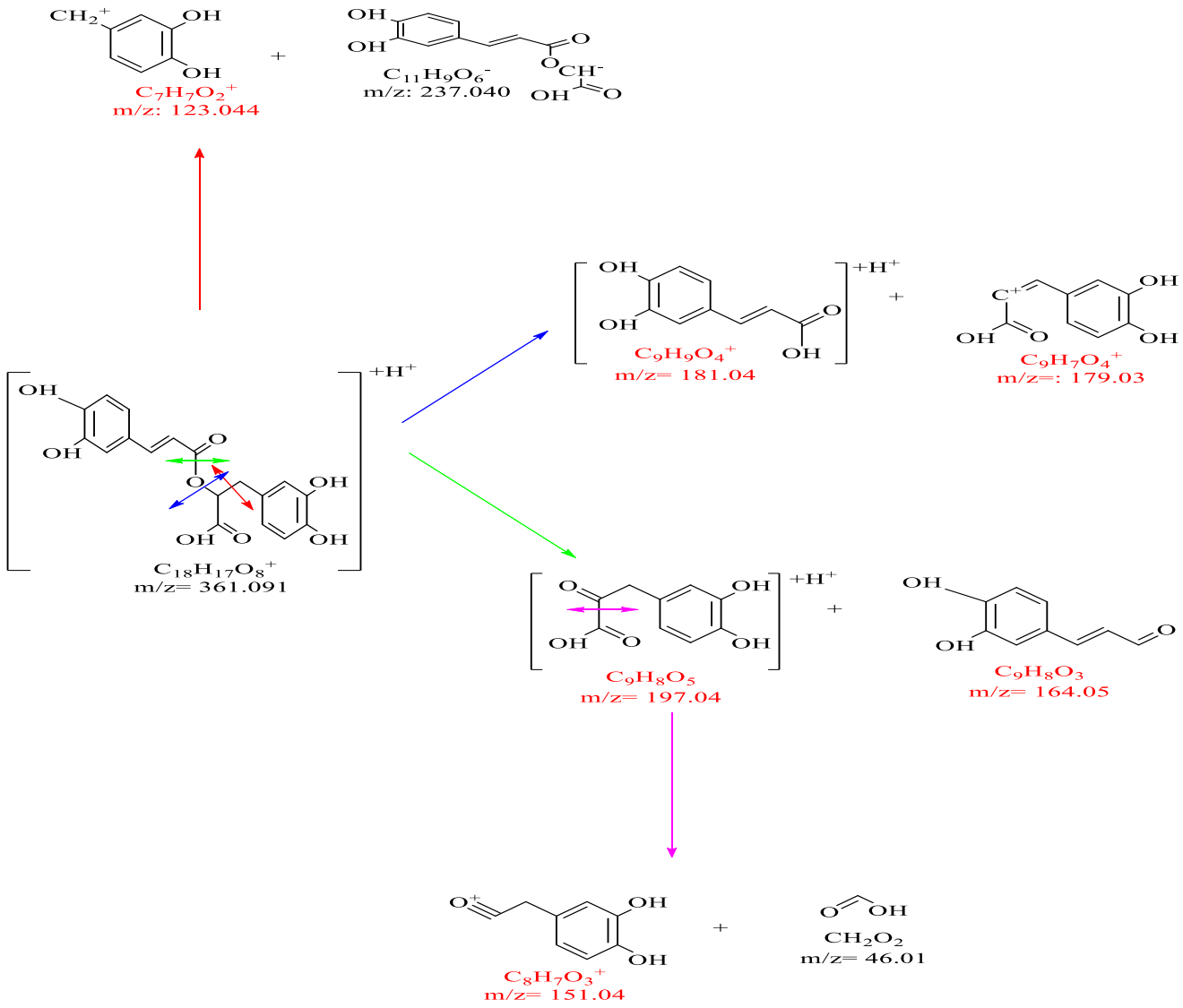


Figure 22. LC-ESI/MS mass spectrum and ESI/MS fragmentation spectrum of compound 10



Scheme 10. Proposed fragmentation of compound 10

The majority peak at m/z: 123 would come from the cleavage of the carbon-carbon bond in the β position of the carboxylic group. The fragment at m/z: 179 would result from the breakage of the carbon-oxygen bond at β of the carboxylic group. The fragment at m/z: 163 would be linked to the

scission of the carbon-oxygen bond at γ of the carboxylic group (Fragmentation α). As for the fragment at m/z: 151, it would result from a double cut at the level of the carbon-carbon and carbon-oxygen bonds respectively at α and γ of the carboxylic group (**Scheme 10**). Compound 10 (**figure 24**) is therefore rosamarinic acid which belongs to the polyphenol family. This compound has been reported in the literature (Prawatsri et al., 2013, Lautie et al., 2008).

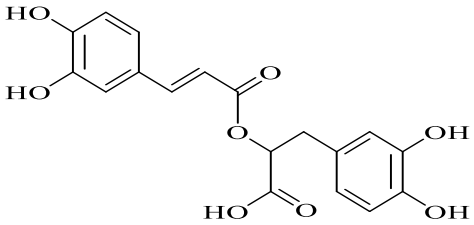


Figure 24. Structure of compound 10

Conclusion

It emerges from this analysis that the dichloromethane fraction resulting from the hydroethanolic extract of the leaves of *Hyptis suaveolens* contains several compounds (approximately 33). Among the molecular formulas proposed, many are those which do not correspond to molecules already identified in the Hyptis genus. On the other hand, ten (10) correspond to structures already isolated from the genre. Of the 10 already known compounds detected in the leaves of the plant during this study, five (05) had not yet been identified in the leaves of *Hyptis suaveolens*. Furthermore, five (05) of the 10 compounds identified are phenolic compounds and the five (05) others are terpenoids. The identification of these families of molecules corroborates the use of this plant in traditional medicine.

Acknowledgement: The technical inputs of Mr Dibi Jacques Konan of organic chemistry and natural substances (LCOSN), UFR-SSMT, University of Felix Houphouët-Boigny are acknowledged.

Conflict of Interest: The authors declare that there are no conflicts of interest.

Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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