Identification of Compounds by HPLC-ESI- Q-TOF -MS/ MS Analysis of the Ethyl Acetate fraction of *Hyptis suaveolens* leaves before Essential Oil extraction

Abstract : *Hyptis suaveolens* is a plant widespread in tropical areas of America, Asia and Africa. Preliminary studies have shown that the plant's leaves possess multiple medicinal properties, certainly due to the presence of sterols and terpenes, saponins and phenolic compounds (**Brahima Soumahoro et al., 2020**). However, few molecular structures of the plant's leaves have been elucidated. The structures of fifteen (15) known compounds were therefore identified by dereplication of the ethyl acetate fraction from the hydroalcoholic extract (70%) of the plant's leaves before extraction of the essential oil. Of these 15 compounds, nine (09) are phenolic compounds and the other six (6) are terpenoids. Of these molecules already identified in the Hyptis genus, five (05) had not yet been elucidated in the leaves of *Hyptis suaveolens*.

Key words : *Hyptis suaveolens*, dereplication, before extraction of the essential oil, molecular structures

1. Introduction

Hyptis suaveolens is a plant used in more than twenty-two countries for these medicinal properties (**Tang et al., 2018**). The plant is used in the treatment of numerous illnesses such as respiratory ailments, gastrointestinal infections, antirheumatics, antisuporifics, colic, colds, indigestion, fever, abdominal pain, burns, wounds, cramps and multiple skin complications (**Kiritikar & Bassu, 1999**), (**Mahesh et al., 2001**), (**Asekun et al., 1999**), (**Oliveira and al**., **2005**). Preliminary analyses have shown that *Hyptis suaveolens* leaves contain alkaloids, steroids, terpenoids, tannins, flavonoids, anthraquinones and phenols representing more than 13% of the dry leaf mass (**Kumar and al., 2015**) (**Goly and al., 2015**). In addition, the hydroethanol extract and ethyl acetate fraction of the plant's leaves have shown very good antibacterial (**Goly et al., 2015**) and antioxidant properties prior to extraction of the essential oil (**Soumahoro et al., 2023**). Recent studies on dichloromethane fractions from hydroalcoholic extracts before and after extraction of the essential oil have elucidated the structures of eleven (11) molecules, including five (05) phenolic compounds and six (06) terpene compounds (**B Soumahoro et al., 2023**) (**Brahima Soumahoro et al., 2024**). However, the molecular structures of the ethyl acetate fractions have not been studied. The aim of this study was to determine the structures of a number of known compounds present in the ethyl acetate fraction obtained from the hydroethanol extract of *Hyptis suaveolens* leaves prior to extraction of the essential oil.

2. Materials 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 central Côte d'Ivoire and identified by Mr Amani N'Guessan, botanist at the Institut National Polytechnique Félix HOUPHOUËT-BOIGNY (INP-HB) in Yamoussoukro. A specimen of *Hyptis suaveolens* is listed in the CSRS herbarium under number : n° Coll : 18027 / bdcsrs : 65599. The leaves were dried in the shade at room temperature in the laboratory (26 to 30 °C) for 7 days before being ground using an electric grinder of the brand IKA M20 (France) and the powder obtained was sieved using a sieve 0.5 mm mesh. The powder obtained was stored in coloured jar at 4°C until further use.ss

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. Analyses were performed in positive mode. A Sunfire® C18 analytical column (150×2.1 mm ; 3.5 µm, Waters) was used. In 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 total hydroalcoholic extract was prepared according to the method described by Soumahoro et al (**Brahima Soumahoro and al., 2023**). A 100g mass of sample crushed material was macerated in 1L of an ethanol/water mixture (70/30 : v/v) under magnetic stirrer for 24 hours. After settling, the mixture was successively filtered through cotton wool 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 and then freeze-dried to give the total hydroalcoholic extract before (EHA1) 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) using the method reported by Bouamama and al. (**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 various organic phases obtained were dried separately over anhydrous sodium sulphate. After filtration and removal of the solvents under reduced pressure, the hexane (F_{HEX}), dichloromethane (F_{DCM}) and ethyl acetate (F_{AE}) fractions were obtained before extraction of the essential oil. Next, 5 mg of ethyl acetate fraction is dissolved in 1 mL of analytical methanol, then 1mL of this solution is taken up using a syringe in 1mL of methanol. This extract is filtered again using a 0.5 µm filter syringe. Finally, 300 µL were taken to be stored in a case prior to HPLC-QTOF-MS/MS analysis.

2.2.2. HPLC- ESI- Q-TOF -MS/ MS analysis of the ethyl acetate fraction using the dereplicative method

Dereplicative analysis is a new method that enables the rapid identification of known molecules contained in a complex mixture (**Jongmin A, Hee-Sung C, Young-Won C, 2017**). It is based on the coupling of High Performance Liquid Chromatography (HPLC) and Tandem Mass Spectrometry (MS/MS or MS2) /Q-TOF (McFarland K, Mulholland DA, 2004). A Sunfire® C18 analytical column (150×2.1 mm ; 3.5 µm, Waters) was used with a flow rate of 250 µL/min and a two-way linear gradient : Lane A (95-0% H_2O plus 0.1% formic acid), Lane B (5-100% ACN) for 30 minutes. ESI conditions were set with a temperature at 320°C, a source voltage at 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 lock masses. Full scans were acquired at a resolution of 11000 (at m/z 922). The sample injection volume was set at 5 µL.

An analysis of the ethyl acetate fraction from the hydroalcoholic extract of *Hyptis suaveolens* leaves prior to 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 from this fraction (**Figure 1**).

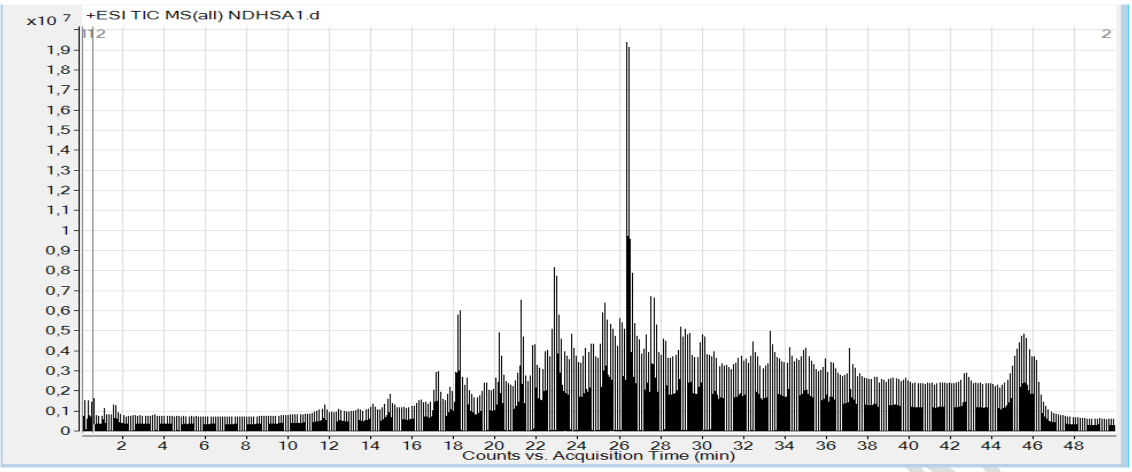
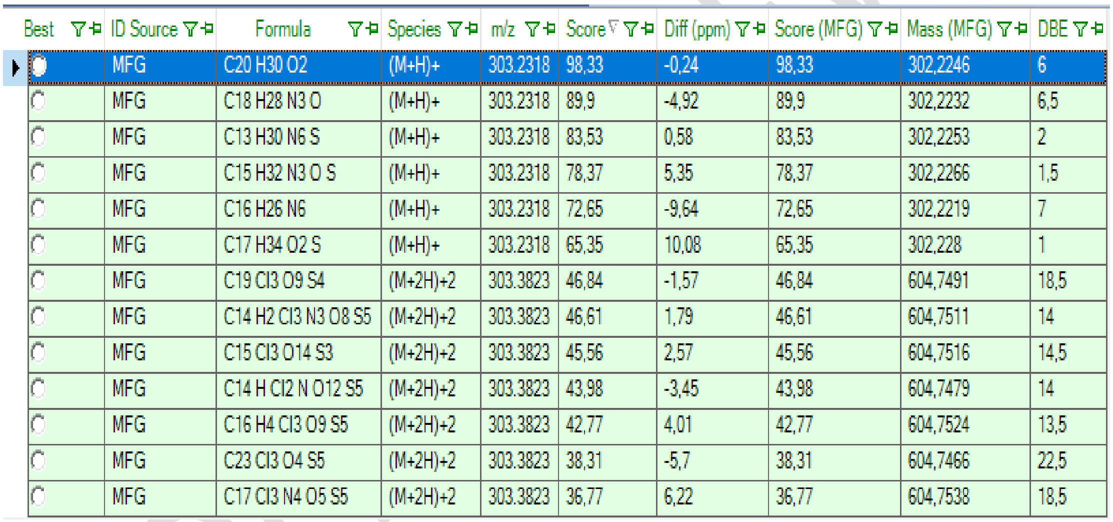


Figure 1 : Total ESI/MS chromatography profile

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

Table 1 : Raw formulas suggested by MassHunter software



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

3. Results and Discussion

3.1. HPLC-MS/Q-TOF analysis of the ethyl acetate fraction

A dereplicative analysis of the ethyl acetate fraction from the hydroalcoholic extract of *Hyptis suaveolens* leaves prior to extraction of the essential oil was carried out using the HPLC-ESI-Q-TOF-MS/MS method. The aim was to determine the structures of some of the compounds present in the leaves of the plant studied.

The HPLC-MS/Q-TOF chromatographic profiles of the ethyl acetate fraction from the hydroethanol extract of *Hyptis suaveolens* leaves before extraction of the essential oil (E.O.) are shown in Figure 2.

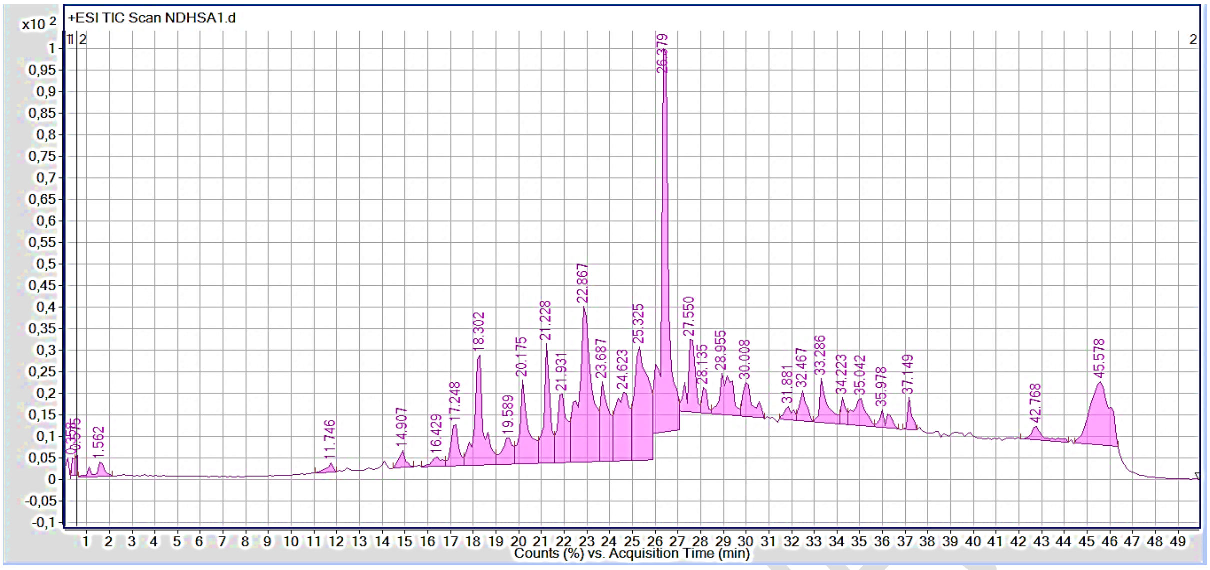


Figure 2 : ESI/MS chromatographic profile of the majority compounds in the Ethyl Acetate Fraction prior to Extraction of H.E.

Figure 2 shows that the secondary metabolites leave the column in the time interval 1.56-45.58 min.

The analyses carried out in positive mode enabled the molecular masses and gross formulae of the compounds revealed by the chromatography to be determined. Table 2 gives the crude formula and corresponding molecular weights with the different scores for each of the main compounds detected.

Table 2 : Compounds detected in the ethyl acetate fraction (FAE1)

	Before Extraction of the Essential Oil			
PIC number	**Retention time (min)**	**Gross formula**	**Molecular mass (g/mol)**	**Score (%)**
1	1,562	$C_{25}H_{34}N_4$	390	98,51
2	11,746	$C_{24}H_{38}O_4$	390	97,15
3	14,907	$C_7H_{14}O_6$	194	86,17
4	16,429	$C_9H_6O_4$	178	86,31
5	17,248	$C_9H_6O_3$	162	98,03
6	17,951	*$C_{11}H_{12}O_2$	176	47,48
7	18,302	$C_{14}H_{12}N_2$	208	87,19
8	19,589	$C_{24}H_{38}O_4$	390	96,28
9	20,175	$C_7H_6O_2$	122	89,6
10	20,994	*$C_{21}H_{28}O_7$	392	50,54
11	21,228	$C_{10}H_{10}O_4$	194	98,83
12	21,814	*$C_{15}H_{10}O_7$	302	98,7
13	21,931	*$C_{21}H_{20}O_{12}$	464	99,49
14	22,165	*$C_9H_8O_3$	164	82,67
15	22,867	$C_9H_6O_3$	162	97,28
16	23,101	*$C_{15}H_{10}O_6$	286	98,49
17	23,687	$C_{30}H_{37}N_{11}O_{10}$	711	97,76
18	24,623	$C_6H_{10}O_3S$	162	83,64
19	25,325	*$C_{16}H_{12}O_6$	300	99,12
20	26,379	$C_8H_4O_3$	148	99,69
21	27,316	*$C_{15}H_{10}O_5$	270	80,07

22	27,55	$C_{13}H_{23}N_{11}$	333	92,08
23	28,135	$C_{20}H_{37}N_{11}O_3$	479	95,52
24	28,955	$C_{18}H_{32}O_2$	280	97,34
25	29,884	*$C_{19}H_{28}O$	272	61,63
26	30,008	$C_{28}H_{24}O_{11}$	536	99,09
27	30,360	*$C_{16}H_{12}O_5$	284	98,53
28	31,881	$C_{23}H_{44}N_6OS$	452	88,24
29	32,467	$C_5H_8O_3S$	148	67,03
30	33,286	$C_9H_{10}O_5$	198	99,59
31	33,520	*$C_{30}H_{46}O_4$	470	91,22
32	34,223	$C_{27}H_{49}NO_9$	531	97,25
33	34.691	*$C_{20}H_{34}O_2$	306	88,25
34	35,042	$C_{24}H_{45}NS$	379	86,93
35	35,978	$C_{18}H_{35}NO$	281	98,31
36	36.330	*$C_{20}H_{32}O$	288	82,67
37	36.40	*$C_{20}H_{30}O$	286	87,94
38	37,149	$C_{24}H_{38}O_4$	390	99,25
39	37,383	*$C_{18}H_{16}O_8$	360	96,53
40	42,768	$C_{44}H_{58}N_2O_3$	662	96,66
41	45,578	NON IDENTIFIE	109	
42				

* Compounds already identified in Hyptis species

Analysis of Table 2 shows that fifteen (15) of the compounds detected in the ethyl acetate fraction have already been isolated from the genus Hyptis (**Tang et al., 2018**) (**Suárez-Ortiz and al., 2017**) (**Mukherjee and al., 1984**).

Table 3 : Compounds already isolated and detected in the ethyl acetate fraction

Compounds detected in ethyl acetate fractions			**Before extraction of the essential oil**	
Name	Gross Formula	Molecular mass (g/mol)	Retention time (ms)	Score (%)
(2E)-1-(2-hydroxyphenyl)-pent-2-en-1-one*	$C_{11}H_{12}O_2$	176,22	17,951	48,47
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	20,994	50,54
Quercetin	$C_{15}H_{10}O_7$	302,24	21,814	98.7
Quercetin 3-O-β-D-glucopyranoside	$C_{21}H_{20}O_{12}$	464,38	21,931	99.49
p-Coumaric acid *	$C_9H_8O_3$	164,20	22,165	82.67
Kaempférol *	$C_{15}H_{10}O_6$	286,46	23,101	98,49
Methyl Wogonine *	$C_{16}H_{12}O_6$	300,27	25,325	99,12
Apigenine *	$C_{15}H_{10}O_5$	270,24	27.316	80.07
Genkwanine *	$C_{16}H_{12}O_5$	284,27	30,360	98.53
5α-androst-9(11)-en-12-one	$C_{19}H_{28}O$	272,43	33.106	61,63
1,19α-dihydroxyl-uros-2(3),12-dien-28-oic acid*	$C_{30}H_{46}O_4$	470,69	33,520	91.22
Suaveolol	$C_{20}H_{34}O_2$	306,49	34,691	88.25
(2R,4aS,4bS,10aR)-2,4b,8,8,10a-pentaméthyldecahydro-2H-2,4a-méthanophénanthren-1(4bH)-one	$C_{20}H_{32}O$	288,48	36.330	82.67
Dehydroabietinol*	$C_{20}H_{30}O$	286,46	36.40	87.94

rosamarinic acid	$C_{18}H_{16}O_8$	360,32	37,383	96.53

* Molecules not identified (08) during our previous studies in the dichloromethane fractions before and after extraction of the essential oil from the leaves of *Hyptis suaveolens*.

3.2 Confirmation of the Structures of known Compounds (15) From the Ethyl Acetate Fraction of the Hydroethanol Extract

The structures of the compounds were determined by interpreting the data provided by HPLC-ESI-MS/Q-TOF analyses of the ethyl acetate fraction of the plant's leaves.

HPLC-ESI-MS/Q-TOF analysis of the ethyl acetate fraction of *Hyptis suaveolens* leaves prior to extraction of the EO provided mass and fragmentation spectra and crude formulae for several major compound structures were obtained from the NIST, ChemSpider and PubChem databases. We were interested in the structures corresponding to those of certain compounds already isolated from the Hyptis genus.

Structure of compound 1

Compound 1, with a retention time of 17.95 min, corresponds to the molecular ion $[M+H]^+$ at m/z: 177.0545 (**Figure 3**). Its molecular molar mass is therefore 176.084 g/mol and the most probable molecular formula (with the highest score) is $C_{11}H_{12}O_2$ (cal. 176.215). The fragmentation spectrum of compound 1 gives the chromatographic profile SM with a molecular ion at m/z : 177 and the collision-induced dissociation fragments of the molecular ion (**Figure 3**)

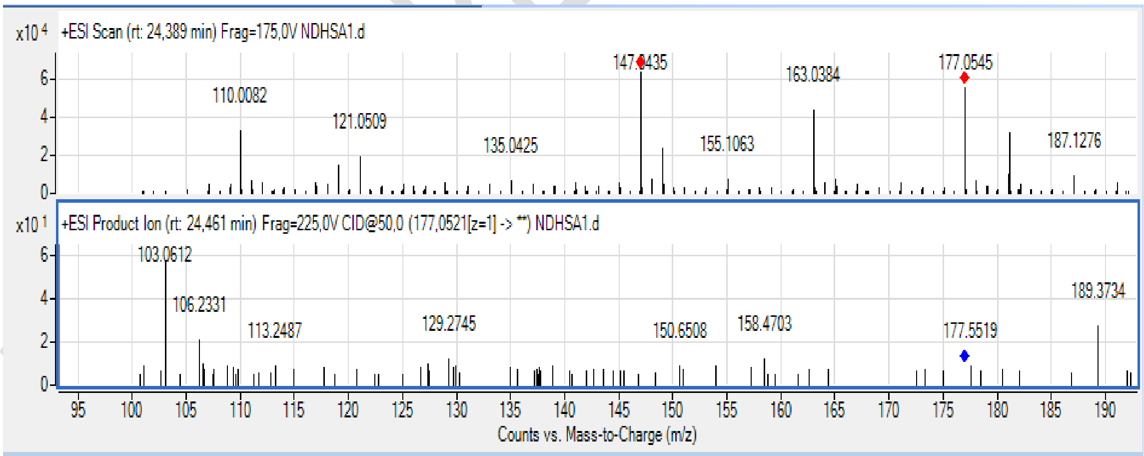


Figure 3 : LC-ESI/MS mass spectrum and ESI/MS fragmentation spectrum of compound 1

Analysis of this spectrum shows major fragment ions at m/z : 158 [M+H-19], m/z : 151 [M+H-26], m/z : 129 [M+H-19-29], m/z : 113 [M+H-19-45], m/z : 106 [M+H-19-52], m/z : 103 [M+H-19-55] (base peak).

Among the structures proposed by the ChemSpider and PubChem databases, only (2E)-1-(2-hydroxyphenyl)-pent-2-en-1-one corresponds to the crude formula giving the following structure.

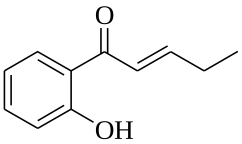
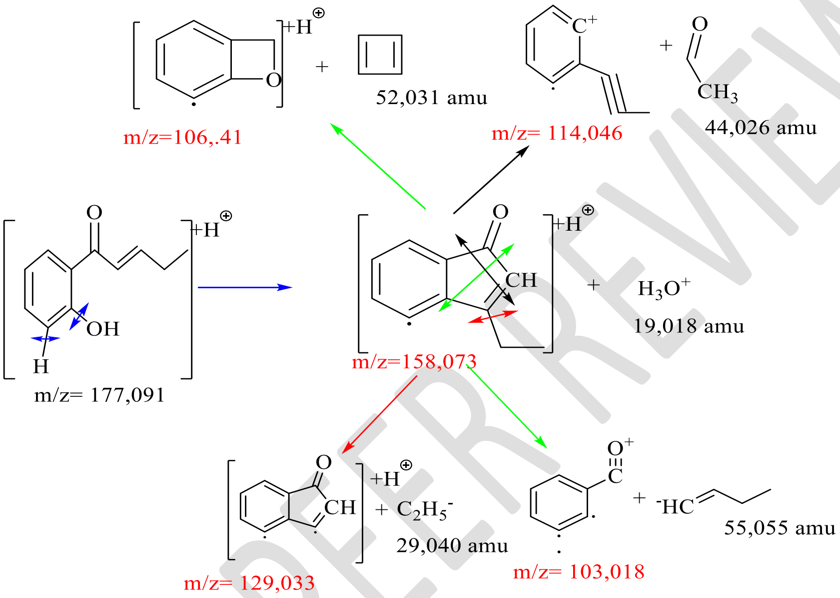


Figure 4 : Structure of compound 1

The fragmentation mode, similar to that of the compound identified, is as follows (**Scheme 1**)



Scheme 1 : Proposal fragmentation of compound 1

hydronium ion (H_3O^+) on the benzene ring followed by a rearrangement (cyclisation) in accordance with the nitrogen rule (the molar mass of the molecular ion being odd, the molar mass of the fragment resulting from the collusion should also be odd). The fragments at m/z :106 and m/z :103 would be derived from the previous fragment by the removal of a cyclobutadiene molecule and a butenyl group respectively following cleavage of the α and γ oxo groups on the (C5) ring. The fragment at m/z : 129 is thought to be due to the departure of the ethyl group at the α position of the ethylenic double bond on the (C5) ring. Compound 1 would therefore be (2E) -1-(2-hydroxyphenyl)-pent-2-en-1-one and belongs to the family of phenolic compounds. This compound has already been identified in the leaves of *Hyptis suaveolens* (**Mukherjee et al., 1984**).

Structure of compound 2

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

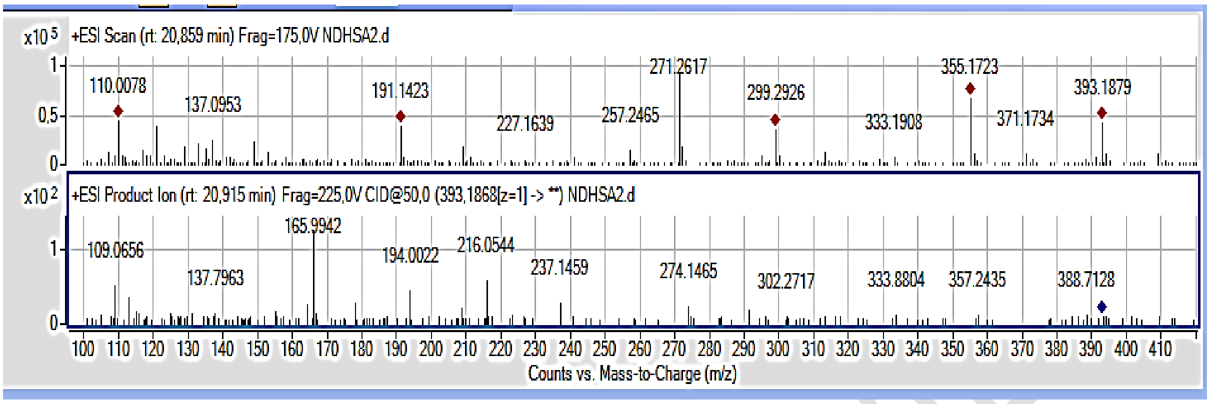


Figure 5: LC-ESI/MS mass spectrum and ESI/MS fragmentation spectrum of compound 2

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 corresponds to the crude formula giving the following structure.

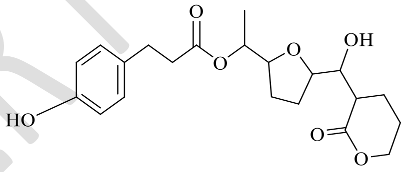
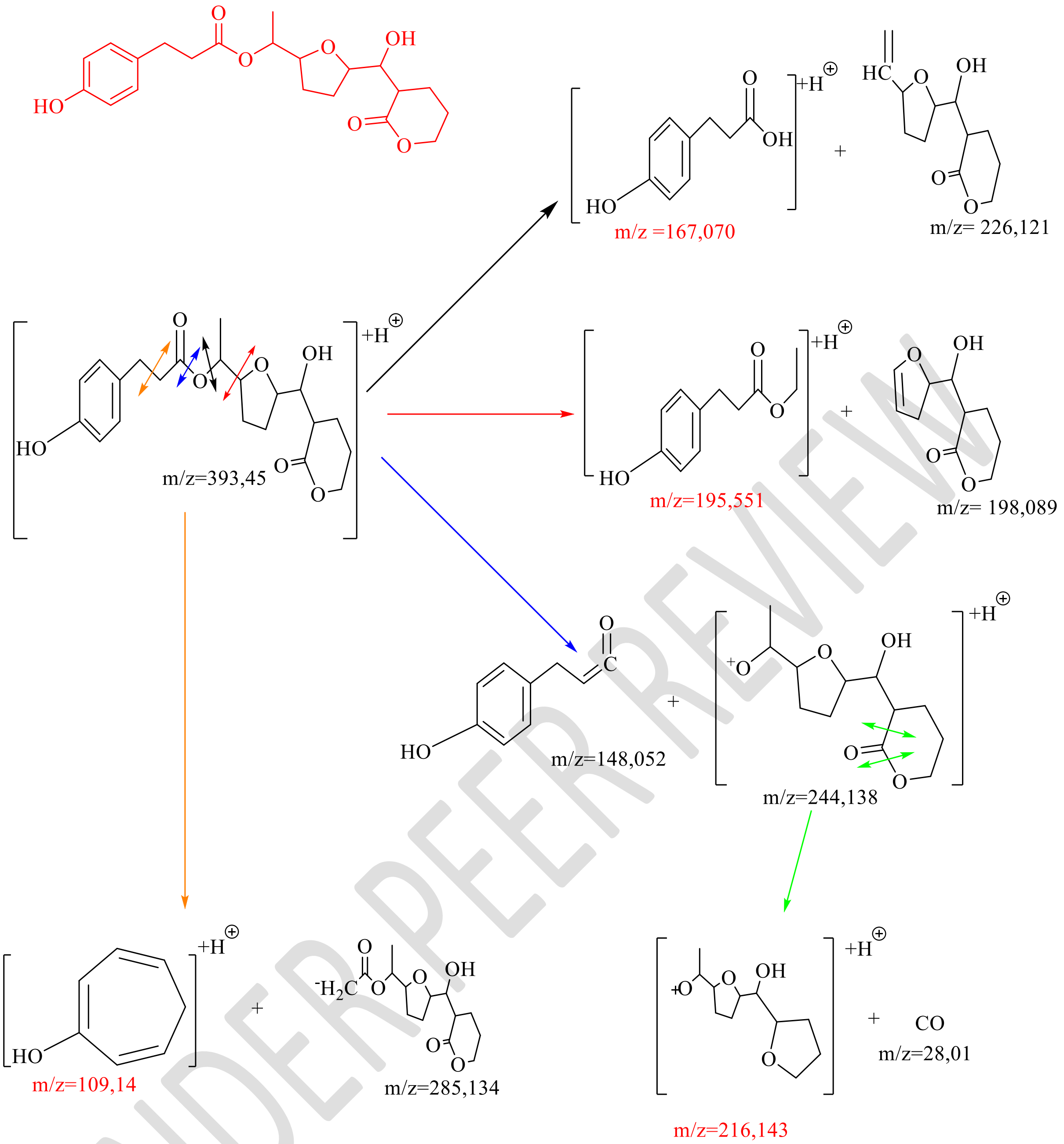


Figure 6 : Structure of compound 2

The fragmentation mode, similar to that of the compound identified, is as follows (**Scheme 2**)



Scheme 2 : Proposal fragmentation of compound 2

The basic molecular mass peak m/z :167 (166 on the spectrum following rearrangement according to the nitrogen rule), would be due to a Mc Lafferty type rearrangement with cleavage of the carbon-oxygen bond at α (α Fragmentation). The fragment m/z :195 (194 on the spectrum after 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 is therefore 1-(5-(hydroxy(2-oxotetrahydro-2H-pyran-3-yl)methyl)tetrahydrofuran-2-yl)ethyl-3-(4-hydroxyphenyl)propanoate and belongs to the family of phenolic compounds. It has also been identified in the leaves of Hyptis brevipes, from the same family as the study plant (Suárez-Ortiz et al., 2017).

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 weight is 302.0427g/mol. The most likely molecular formula is $C_{15}H_{10}O_7$ (cal. 302.238) (**Figure 7**).

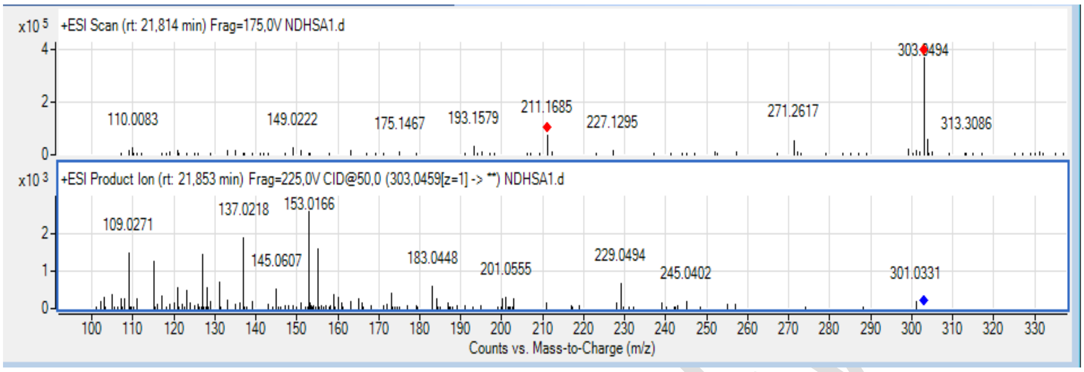


Figure 7 : LC-ESI/MS mass spectrum and ESI/MS spectre de fragmentation spectrm of compund 3

Analysis of the fragmentation spectrum of compound 3 (**Figure 7**) 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 corresponds to the crude formula giving the following structure.

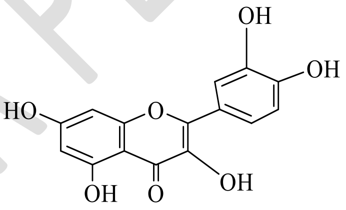
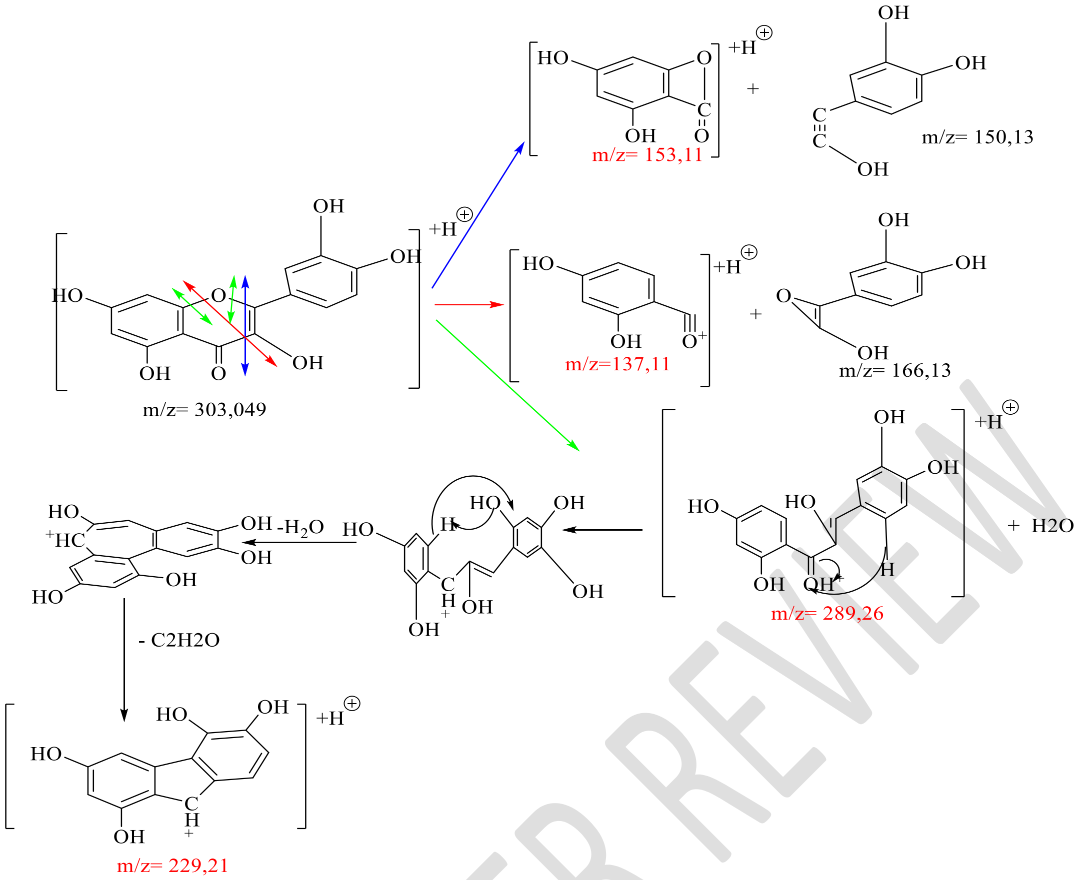


Figure 8 : Structure of compound 3 (quercetin)

The mode of fragmentation, similar to that of the desired compound, is as follows (**Scheme 3**).



Scheme 3 : Proposal of fragmentation of compound 3

The base peak at m/z : 153 arises 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 in the α position of the aromatic ring and β position of its hydroxyl group respectively. As for the fragment at m/z : 229, it is derived from the elimination of the oxo group, followed by cyclisation after the loss of a water molecule and an ethanone group on the intermediate C ring (**Scheme 3**). Compound 3 is therefore quercetin and belongs to the flavonoid family. This compound is actually present in the leaves of H. suaveolens (**Tang et al., 2018**).

Structure of compound 4

Compound 4 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 likely molecular formula is therefore $C_{21}H_{20}O_{12}$ (cal. 464.3790) (**Figure 9**).

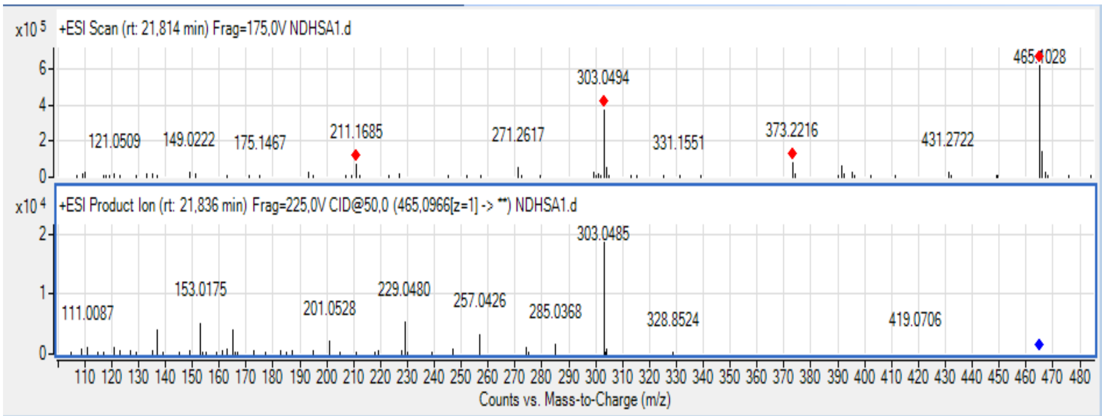


Figure 9 : LC-ESI/MS mass spectrum and ESI/MS spectrum of compound 4

Analysis of the fragmentation spectrum of compound 4 (**Figure 9**) 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 corresponds to the crude formula giving the following structure.

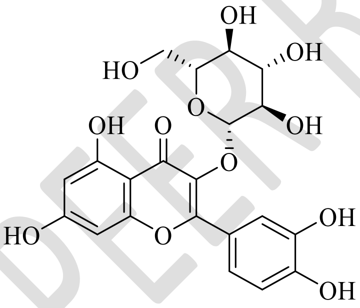
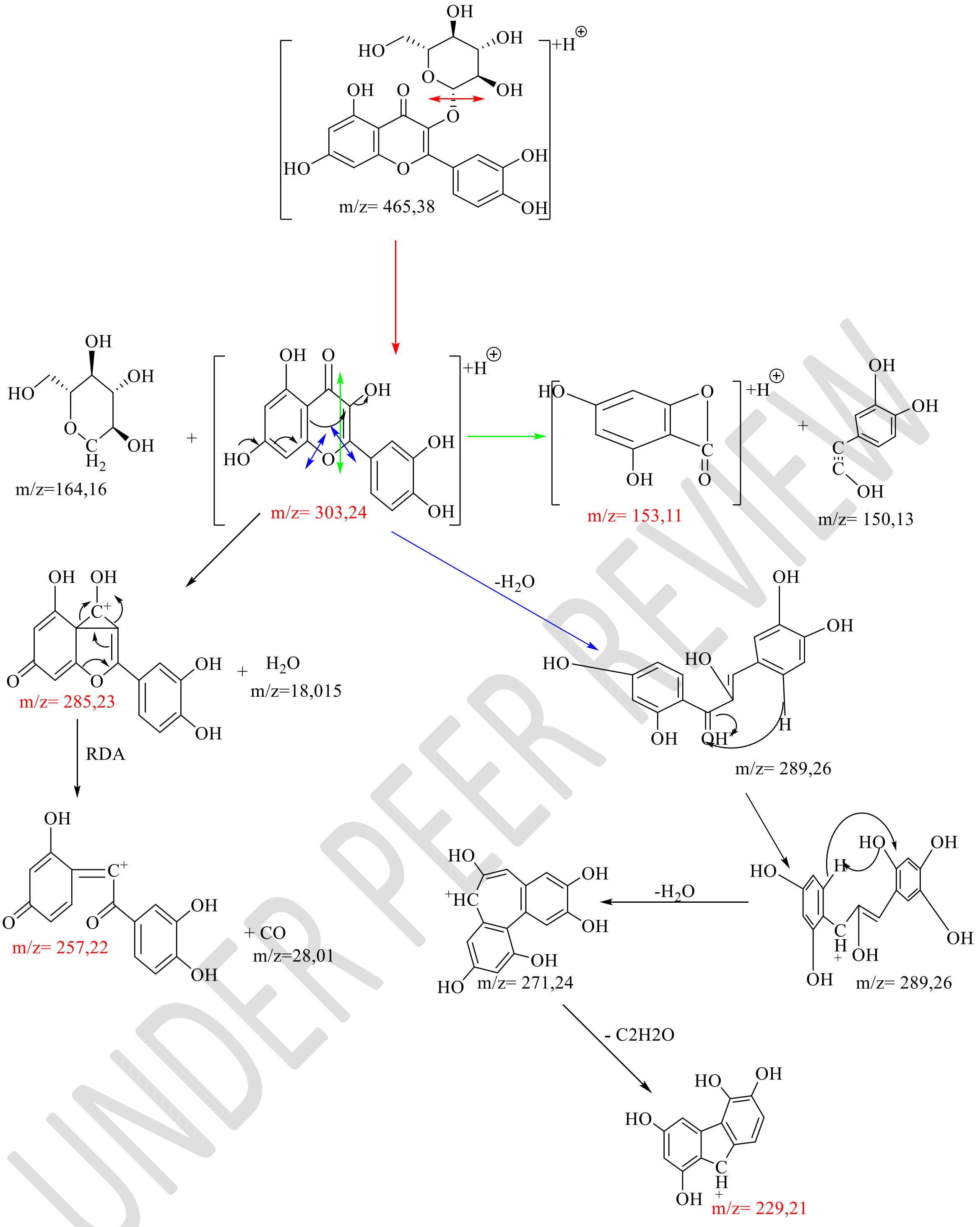


Figure 10: Structure of compound 4

The fragmentation mode, similar to that of the desired compound, is therefore as follows (**Scheme 4**).



Scheme 4 : Proposal fragmentaion of compound 4

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 cleavage 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 4**). Compound 4 is therefore quercetin 3-O-β-D-glucopyranoside from the flavonoid family. It is present in the study plant (**Ekow et al., 2018**).

Structure of compound 5

Compound 5 which appears at 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 11**).

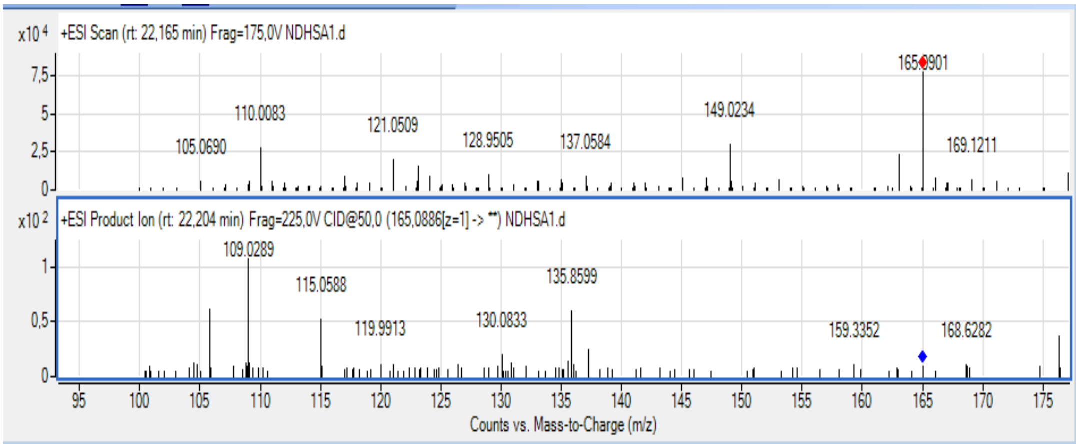


Figure 11 : LC-ESI/MS mass spectrum and ESI/MS spectrum of compound 5

Analysis of the fragmentation spectrum of compound 5 (**Figure 11**) 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 corresponds to the crude formula giving the following structure.

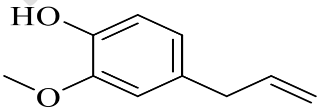
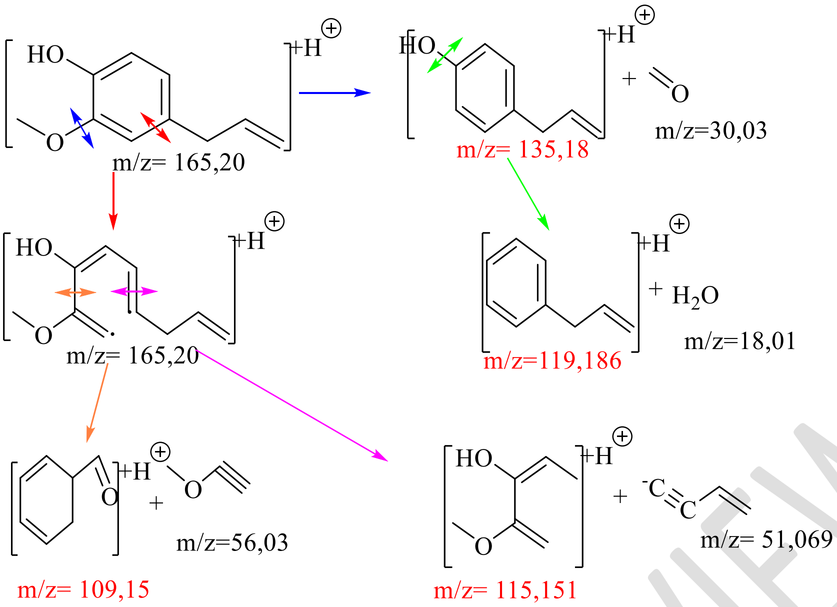


Figure 12 : Structure of compound 5

The fragmentation mode is similar to that of the desired compound (**Scheme 5**).



Scheme 5 : Proposal fragmentation of compound 5

The fragment at m/z : 135 is thought to result from the loss of the methoxyl group in formaldehyde form. The fragment at m/z : 119 is thought to have arisen from the previous fragment after dehydration. The majority peak at m/z : 109 would result 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 α-single and double bonds of the propenyl group (**Scheme 5**). Compound 5 is therefore 4-allyl-2-methoxyphenol from the family of phenolic compounds. It has been identified in the essential oil of H. suaveolens leaves harvested in Tanzania (**Malele et al., 2003**).

Structure of compound 6

Compound 6 with a retention time of 23.101 min corresponds to the molecular ion [M+H]+ at m/z: 287.0544 with a molecular mass of 286.0477g/mol. The most probable molecular formula is $C_{15}H_{10}O_6$ (cal. 286.46) (**Figure 13**).

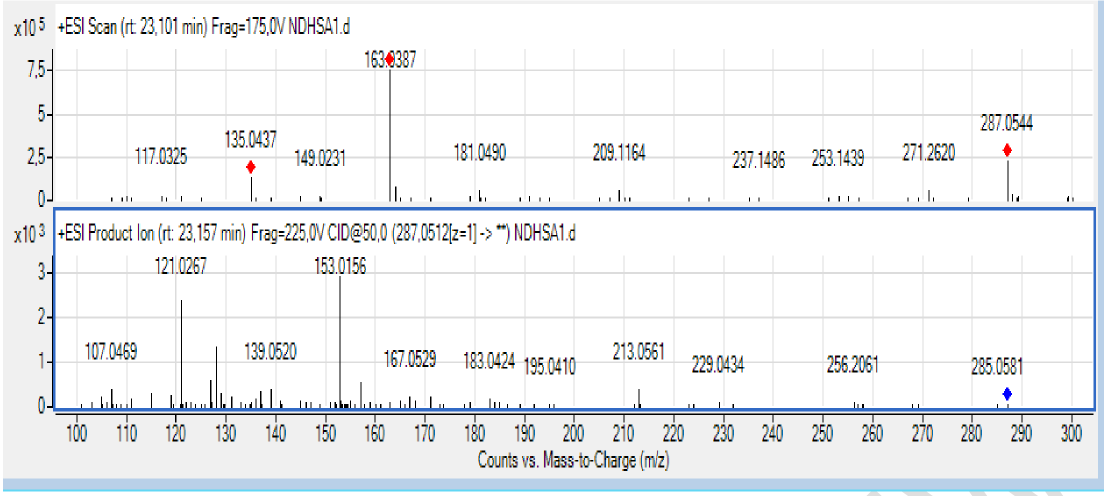


Figure 13 : LC-ESI/MS mass spectrum and ESI/MS spectrum of compound 6

Analysis of the fragmentation spectrum of compound 6 (**Figure 13**) indicates the presence of major fragments at m/z : 213[M+H-74], m/z : 167[M+H-120], m/z : 153[M+H-134] (base peak), m/z : 139[M+H-148], m/z : 121[M+H-166]. Among the structures proposed by the ChemSpider and PubChem databases, only Kaempferol corresponds to the crude formula giving the following structure.

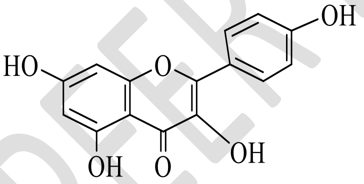
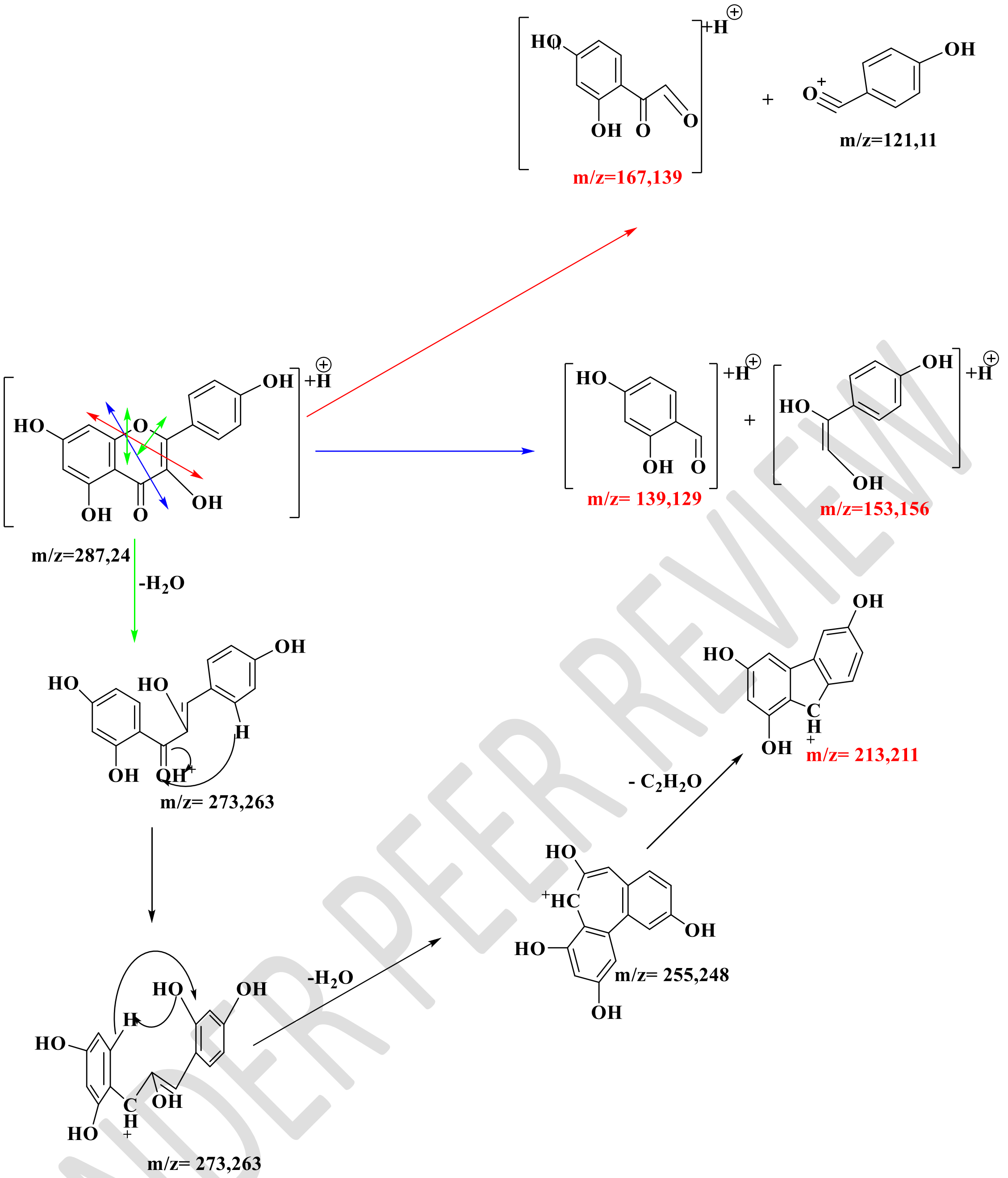


Figure 14 : Structure of compound 6

The fragmentation mode is similar to that of the desired compound (**Scheme 6**).



Scheme 6 : Proposal fragmentation of compound 6

The base peak at m/z : 153 and the fragment at m/z : 139 would result from the double cleavage on the intermediate ring of the carbon -carbon and carbon-oxygen bonds respectively at β and α of the ring adjacent to the intermediate ring (**Scheme 6**). Compound 6 is therefore Kaempferol, a flavonoid. It has already been identified in the leaves of the plant studied (**Ekow et al., 2018**).

Structure of compound 7

Compound 7 with a retention time of 25.325 min corresponds to the molecular ion $[M+H]^+$ at m/z: 301.0706 with a molecular weight of 300.0634 g/mol. The most probable molecular formula is $C_{16}H_{12}O_6$ (cal. 300.2660) (**Figure 15**).

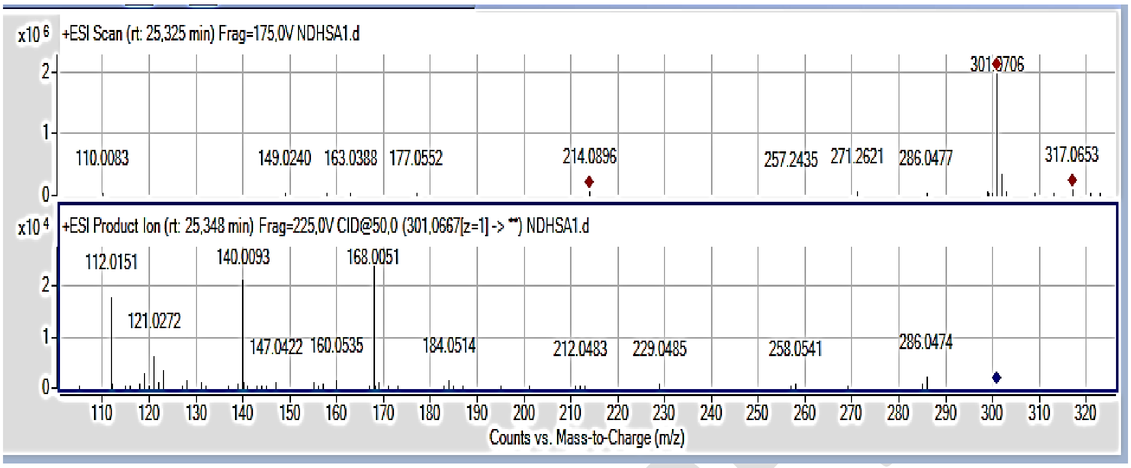


Figure 15 : LC-ESI/MS mass spectrum and ESI/MS spectrum of compound 7

Analysis of the fragmentation spectrum of compound 7 (**Figure 15**) shows the presence of major fragments at m/z : 286 [M+H-15], m/z : 168 [M+H-15-118] (base peak), m/z : 140 [M+H-15-118-28], m/z : 121 [M+H-180], m/z : 112 [M+H-189]. Among the structures proposed by the ChemSpider and PubChem databases, only the methyl Wogonine corresponds to the crude formula giving the following structure.

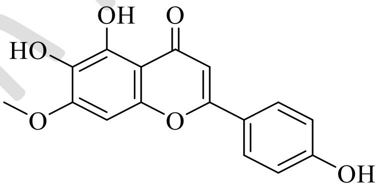
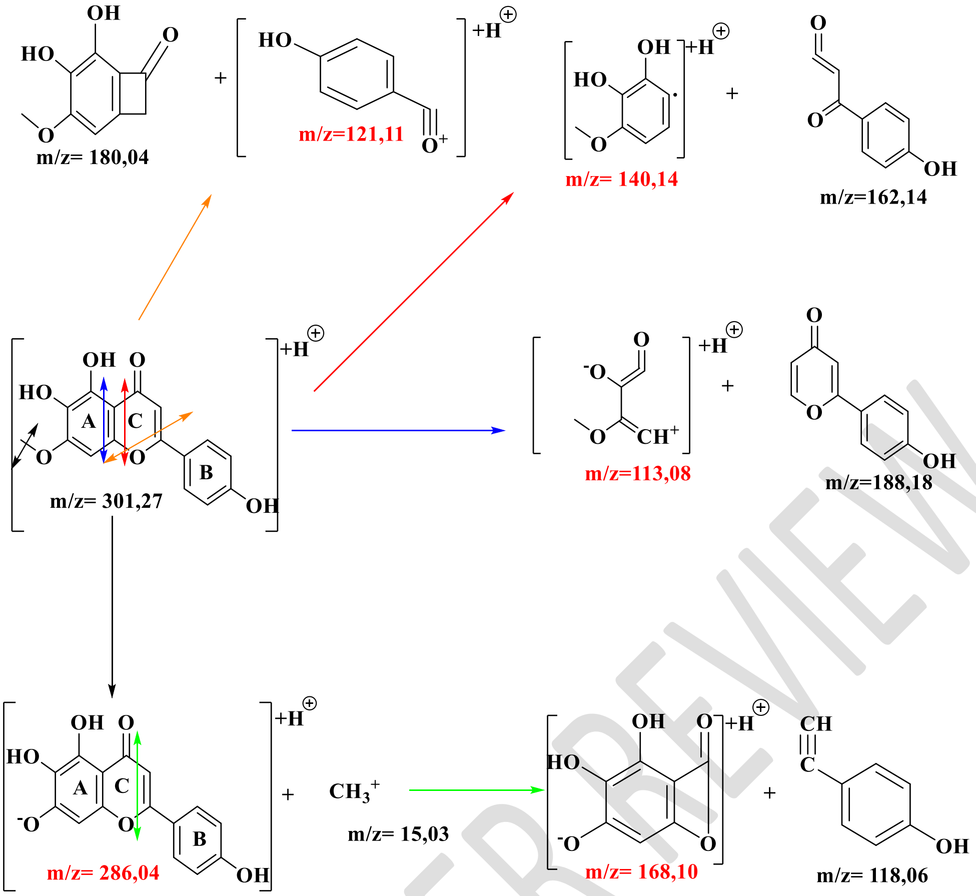


Figure 16 : Structure of compound 7

The fragmentation mode is similar to that of the desired compound (**Scheme 7**).



Scheme 7 : Propoal fragmentation of compound 7

The fragment at m/z : 286 arises from the loss of the methyl group. The base peak at m/z : 168 is derived from the preceding fragment following the cleavage of the carbon-carbon and carbon-oxygen bonds on the intermediate C ring in the β position of the contiguous ring (A). The fragment at m/z : 112 results from the double cleavage on ring (A) of the carbon-carbon bonds at α of the intermediate C ring. The fragment at m/z : 140 results from (RDA)-type cleavage of the carbon-carbon and carbon-oxygen bonds on the intermediate C ring in the α position of the contiguous ring (A). As for the fragment at m/z : 121, it is due to the cleavage of the carbon-carbon and carbon-oxygen bonds respectively at the γ and β positions of the adjacent ring (**Scheme 7**). Compound 7 is therefore the methyl Wogonin of the flavonoid family and has already been described from the plant (**Tang et al., 2018**).

Structure of compound 8

Compound 8 with a retention time of 27.316 min corresponds to the molecular ion $[M+H]^+$ at m/z : 271.0627 with a molecular mass of 270.0528 g/mol. The most probable molecular formula is $C_{15}H_{10}O_5$ (cal. 270.24) (**Figure 17**).

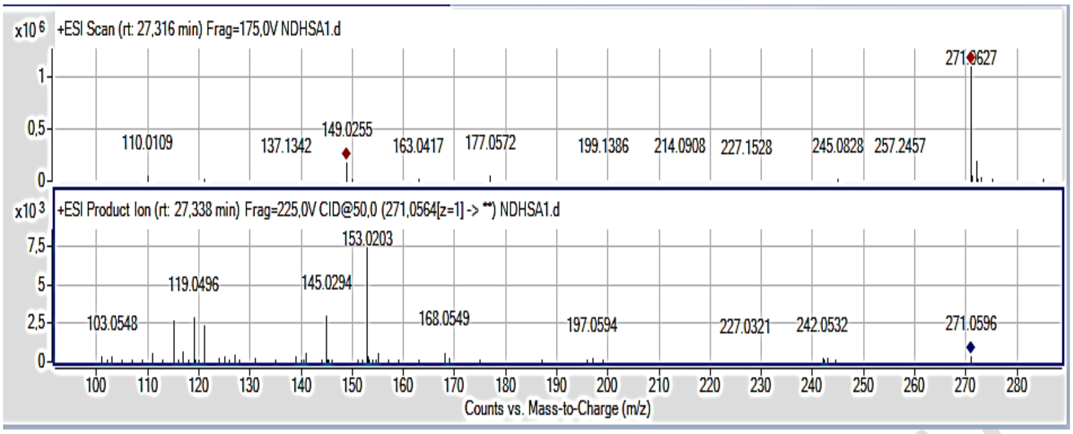


Figure 17 : LC-ESI/MS mass spectrum and ESI/MS spectrum of compound 8

Analysis of the fragmentation spectrum of compound 8 (figure 17) indicates the presence of major fragments at m/z : 153[M+H-118] (base peak), m/z : 145[M+H-126], m/z : 119[M+H-152]. Among the structures proposed by the ChemSpider and PubChem databases, only Apigenin corresponds to the crude formula giving the following structure.

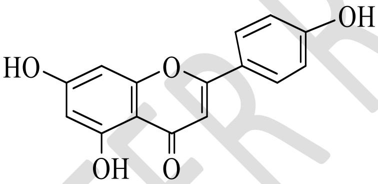
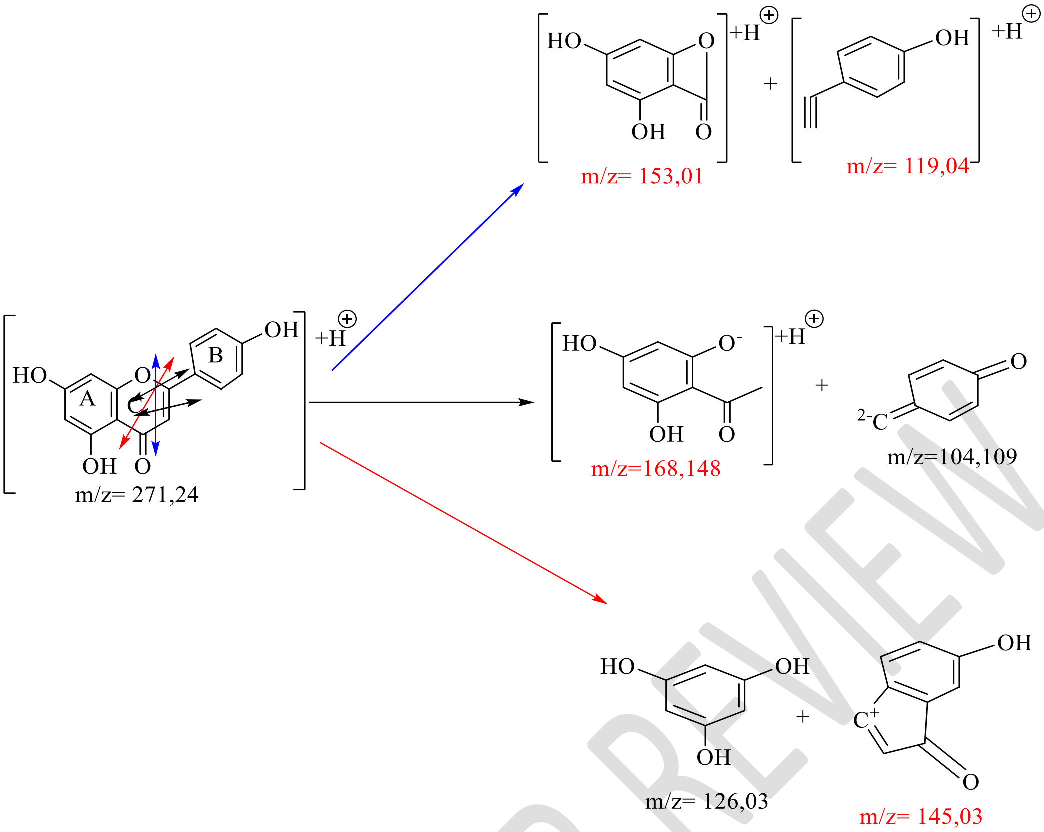


Figure 18 : Structure of compound 8

The fragmentation mode is similar to that of the desired compound (**Scheme 8**).



Scheme 8 : Proposal fragmentation of compound 8

The base peak at m/z : 153 results from the cleavage on the intermediate C ring of the carbon-carbon and carbon-oxygen bonds in β of the contiguous ring (A). The fragment at m/z : 168 results from the cleavage on the intermediate C ring of the carbon-carbon and carbon-oxygen bonds respectively at γ and β of the contiguous ring (A). As for the fragment at m/z : 145, it is due to the cleavage of the carbon-carbon and carbon-oxygen bonds respectively at α and β of the contiguous ring (A) (**Scheme 8**). Compound 8 is therefore Apigenin, which belongs to the flavonoid family. It has been identified in the leaves of the plant (**Ekow et al., 2018**).

Structure of compound 9

Compound 9 with a retention time of 30.360 min corresponds to the molecular ion $[M+H]^+$ at m/z: 285.0763 with a molecular weight of 284.0685 g/mol. The most likely molecular formula is C16H12O5 (cal. 284.2700) (**Figure 19**).

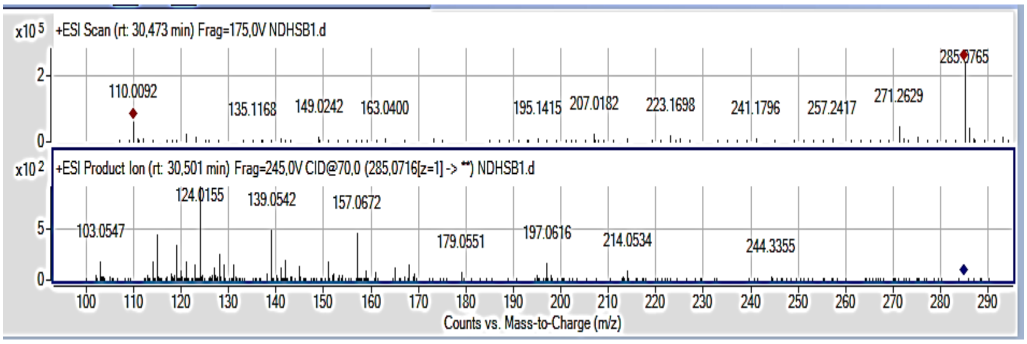


Figure 19 : LC-ESI/MS mass spectrum and ESI/MS spectrum of compound 9

Analysis of the fragmentation spectrum of compound 9 (Figure 19) shows the presence of major fragments at m/z : 197 [M+H-88], at m/z : 157 [M+H-128], at m/z : 139 [M+H-146], at m/z : 124 [M+H-161] (base peak). Among the structures proposed by the ChemSpider and PubChem databases, only Genkwanine corresponds to the crude formula giving the following structure.

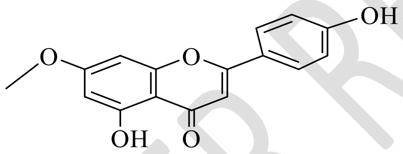
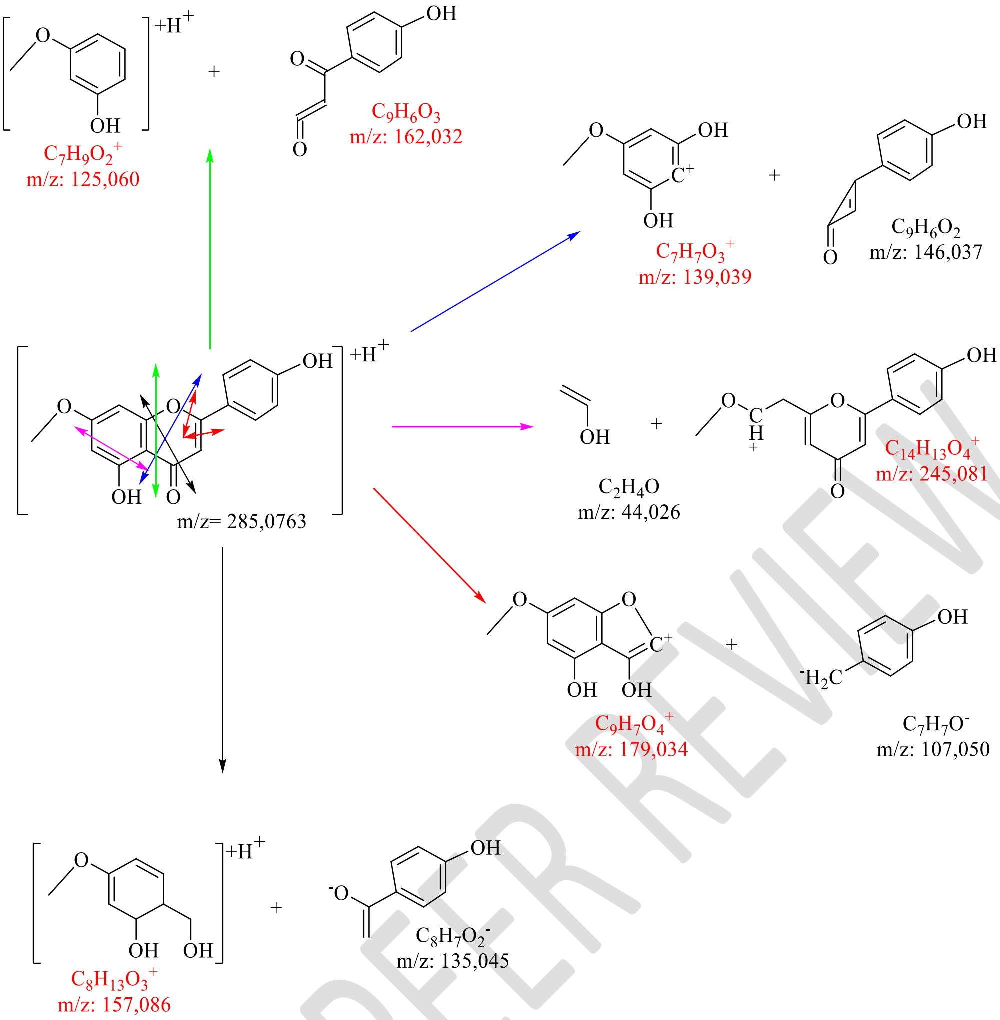


Figure 20 : Structure of compound 9

The fragmentation mode is similar to that of the desired compound (**Scheme 8**).



Scheme 9 : Proposal fragmentation of compound 9

The majority peak at m/z : 125 (124 on the spectrum, difference due to rearrangement according to the nitrogen rule), arises from the cleavage of carbon-carbon and carbon-oxygen bonds on the intermediate C ring at α of the contiguous ring (A). The fragment at m/z : 139 is due to the breaking of carbon-carbon and carbon-oxygen bonds on the intermediate C ring at α and β of the contiguous ring (A) respectively. The fragment at m/z : 245 results from the breakage on the aromatic ring (bearing the methoxyl group) of single carbon-carbon bonds at α of the hydroxyl group and at β of the methoxyl group. The fragment at m/z : 179 results from cleavage of the carbon-carbon and carbon-oxygen bonds on the intermediate C ring in β of the monosubstituted ring. Similarly, the fragment at m/z : results from cleavage on the intermediate C ring of the carbon-carbon and carbon-oxygen bonds at β and α respectively of the methoxylated ring (**Scheme 9**). Compound 9 is therefore Genkwanine, a flavonoid. It has been identified in the leaves of H. suaveolens (**Ekow et al., 2018**).

Structure of compound 10

Compound 10 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 21**).

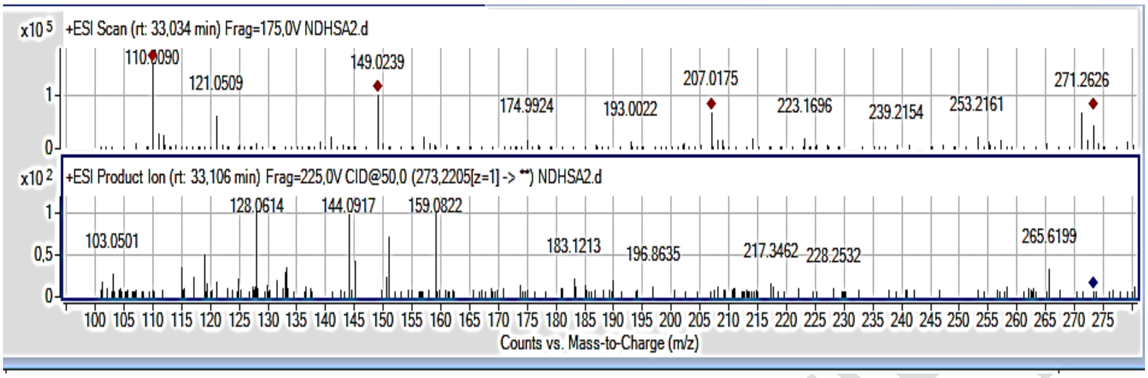


Figure 21 : LC-ESI/MS mass spectrum and ESI/MS fragmentation spectrum of compound 10

Analysis of the fragmentation spectrum of compound 10 (**Figure 21**) 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 corresponds to the crude formula giving the following structure.

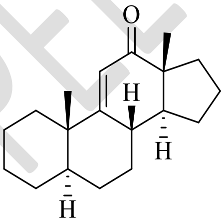
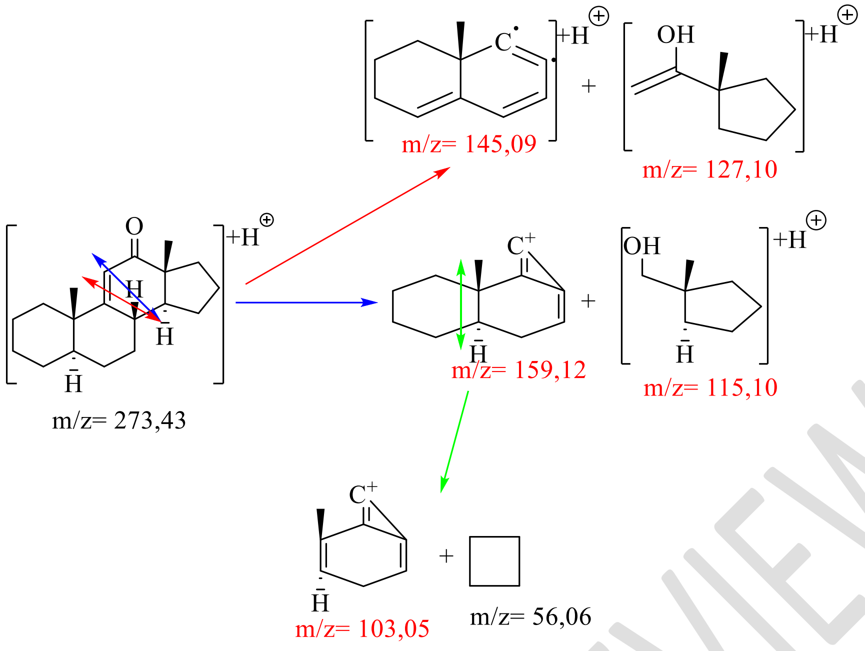


Figure 22: Structure of compound 10

The fragmentation mode is similar to that of the desired compound (**Scheme 10**).



Scheme 10 : Fragmentation of compound 10

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 (C5) ring and the saturated (C6) ring. The fragment at m/z : 103 is thought to have arisen from the base peak as a result of the loss of a cyclobutane following the cleavage of the carbon-carbon bonds adjacent to the second ring at (C6) on the saturated ring. The fragments at m/z : 145 (144 on the spectrum) and m/z : (128 on the spectrum) would result from breakage on the unsaturated ring of the carbon-carbon bonds contiguous to the intermediate ring at (C6) (**Scheme 10**). Compound 10 is therefore 5α-androst-9(11)-en-12-one, a member of the terpene and sterol family. It has been identified in plant leaves (**Edeoga et al., 2006**).

Structure of compound 11

Compound 11 with a retention time of 33.520 min corresponds to the molecular ion $[M+H]^+$ at m/z: 471.346 with a molecular weight of 470.340 g/mol. The most probable molecular formula is $C_{30}H_{46}O_4$ (cal. 470.69) (**Figure 23**).

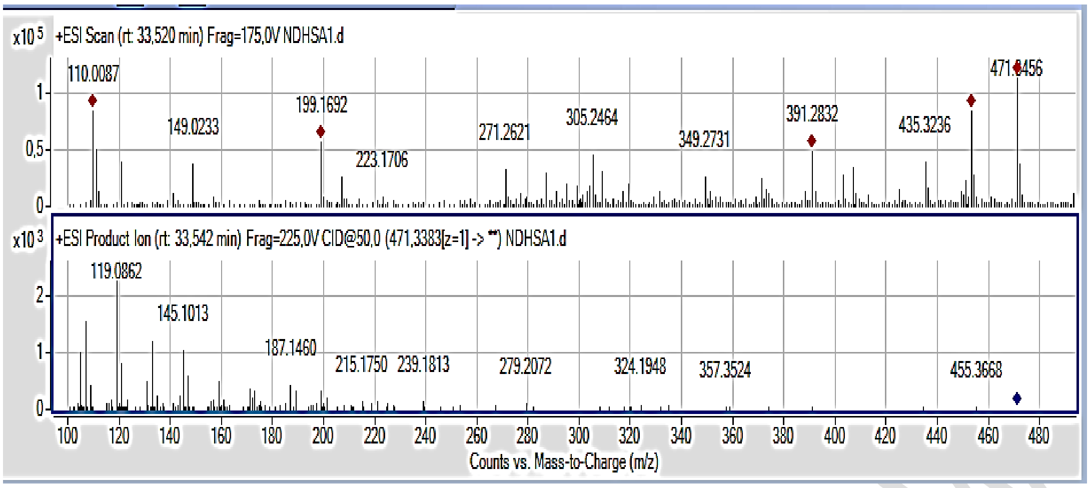


Figure 23 : LC-ESI/MS mass spectrum and ESI/MS fragmentation spectrum of compound 11

Analysis of the fragmentation spectrum of compound 11 (Figure 23) shows the presence of major fragments at m/z : 455[M+H-16], m/z : 357[M+H-16-98], m/z : 187[M+H-16-268], m/z : 157[M+H-16-298], m/z : 145[M+H-16-310], m/z : 119[M+H-16-336]. Among the structures proposed by the ChemSpider and PubChem databases, only 1,19α-dihydroxyl-uros-2(3),12-dien-28-oic acid corresponds to the crude formula giving the following structure.

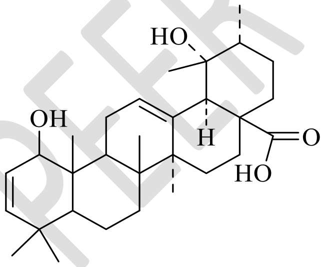
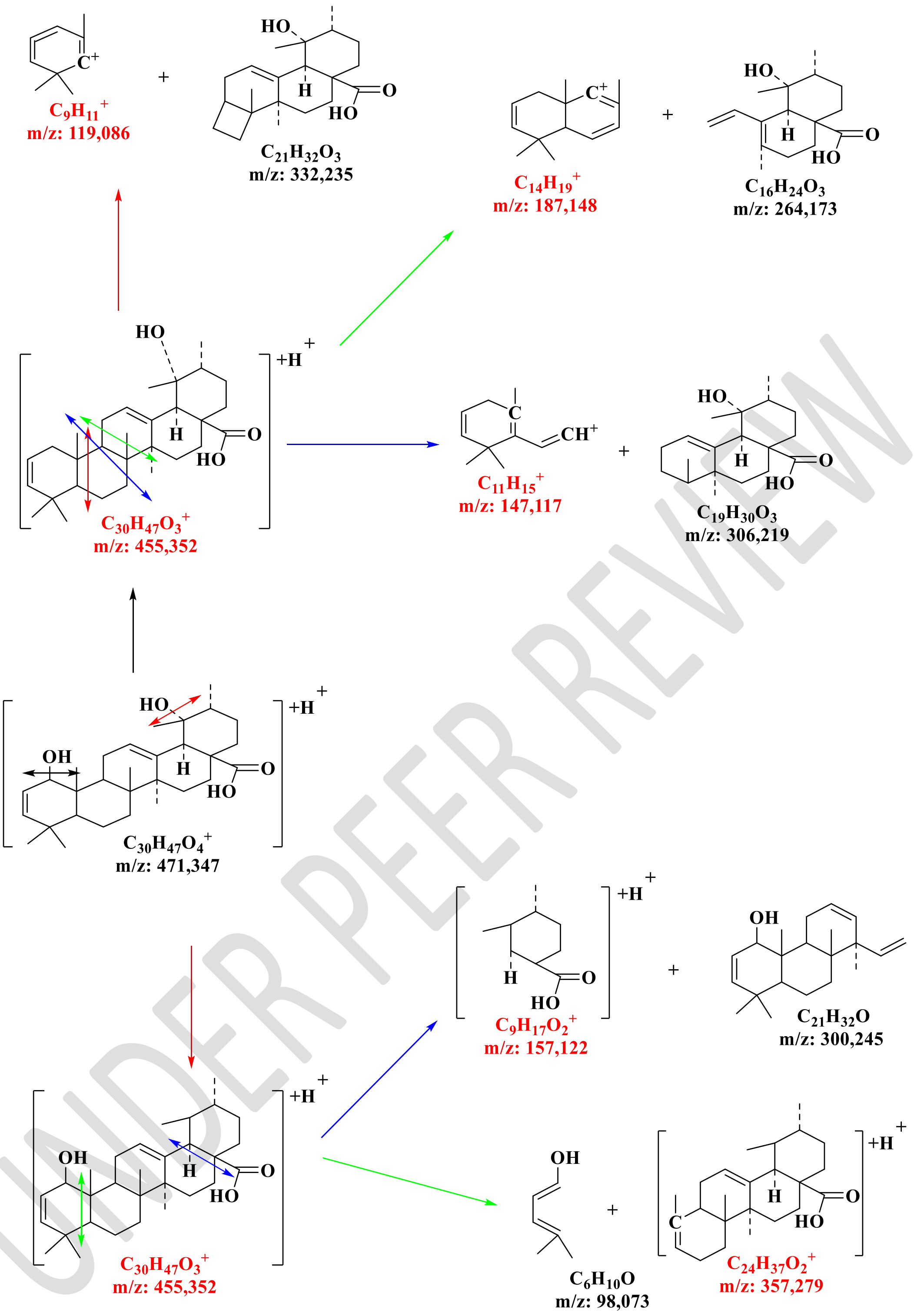


Figure 24 : Structure of compound 11

The fragmentation mode is similar to that of the desired compound (**Scheme 11**).



Scheme 11 : **P**roposal fragmentation of compound 11

The fragment at m/z : 455 is obtained after dehydroxylation on one of the peripheral rings. All other fragments are derived from the preceding fragment. The majority peak at m/z : 119 and the fragments at m/z : 187 and m/z : 147, are thought to have resulted from dehydroxylation on the peripheral unsaturated ring followed by a double cleavage of the carbon-carbon bonds respectively contiguous to the unsaturated ring, between the two unsaturated rings and in γ of the extreme unsaturated ring and finally the bonds contiguous to the saturated ring intermediate to the unsaturated rings. As for the fragments at m/z : 357 and m/z : 157, they result from dehydration on the peripheral saturated ring followed by ~~a~~ double scission of carbon-carbon bonds respectively on the peripheral α-unsaturated ring of the adjacent ring and on the α-saturated ring of the extreme saturated ring (**Scheme 11**). Compound 11 would therefore be 1,19α-dihydroxyl-uros-2(3),12-dien-28-oic acid, a member of the terpene and sterol family. This compound has been identified in the leaves of H. suaveolens (**Raja et al., 1990**) (**Aspinall et al., 1991**).

Structure of compound 12

Compound 12 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 25**).

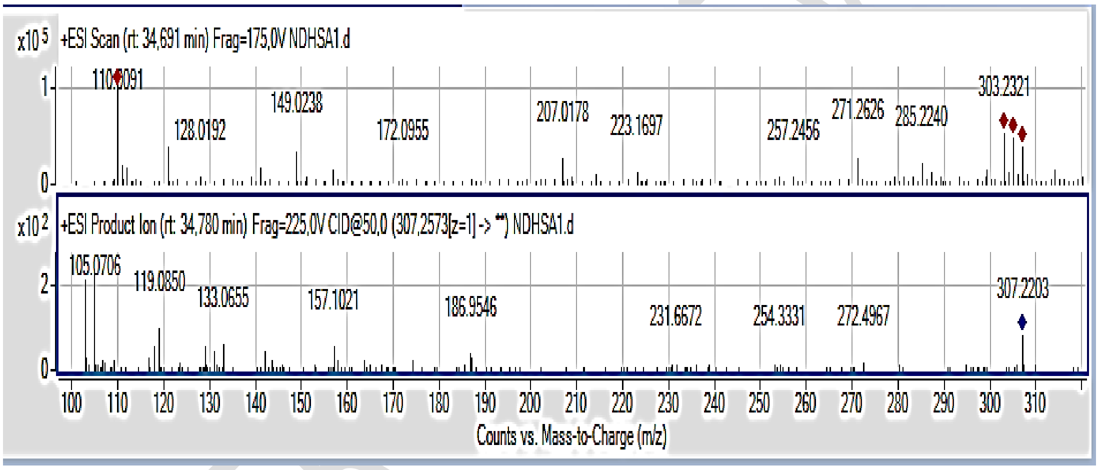


Figure 25 : LC-ESI/MS mass spectrum and ESI/MS fragmentation spectrum of compound 12

Analysis of the fragmentation spectrum of compound 12 (**Figure 25**) 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 corresponds to the crude formula giving the following structure.

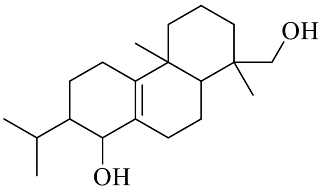
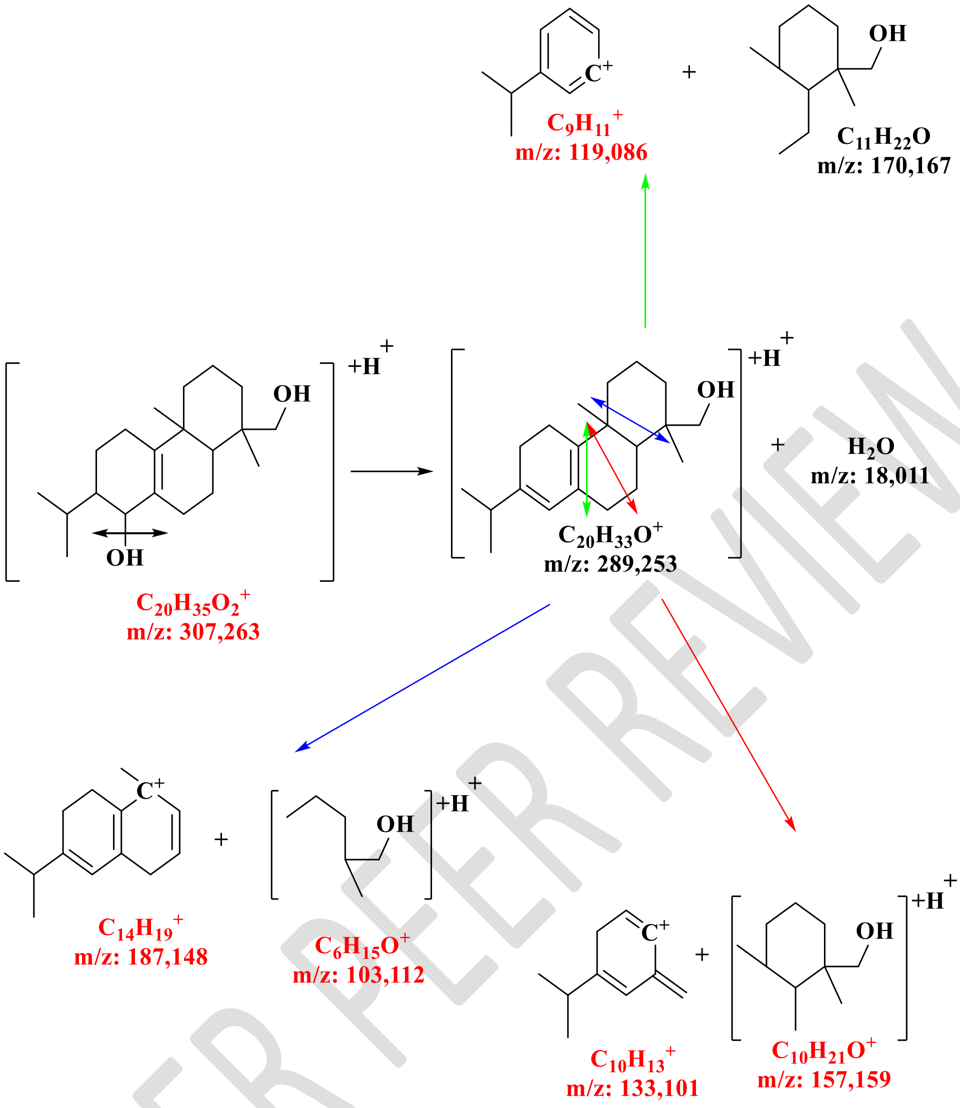


Figure 26 : Structure of compound 12

The fragmentation mode is similar to that of the desired compound (**Scheme 12**).



Scheme 12 : Proposal fragmentation of compound 12

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. As for the fragments at m/z : 157 and m/z : 133, they result from the double cleavage on the intermediate ring of the carbon-carbon bonds in the α and β positions of the other two rings (**Scheme 12**). Compound 12 is therefore Suaveolol, which belongs to the terpene and sterol family. It has already been reported in the literature (**Manchand et al., 1974**).

Structure of compound 13

Compound 13 with a retention time of 36.092 min corresponds to the molecular ion $[M+H]^+$ at m/z: 287.237 with a molecular weight of 286.230 g/mol. The most probable molecular formula is $C_{20}H_{30}O$ (cal. 286.46) (**Figure 27**).

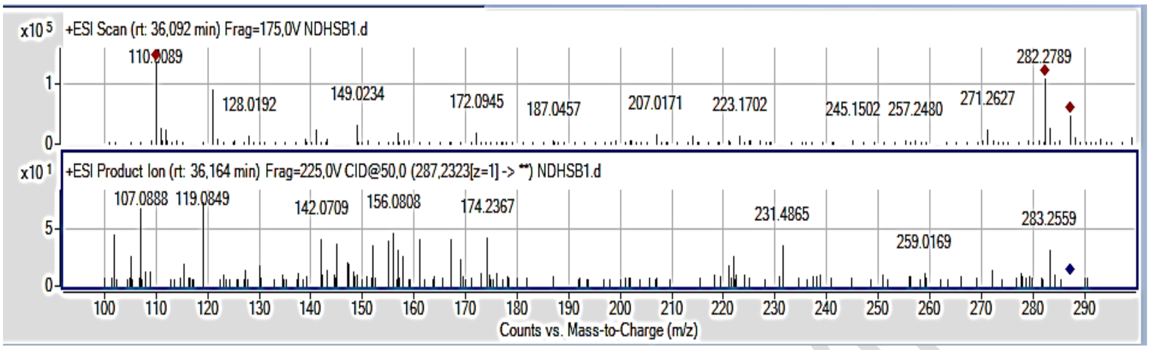


Figure 27 : LC-ESI/MS mass spectrum and ESI/MS fragmentation spectrum of compound 13

Analysis of the fragmentation spectrum of compound 13 (**Figure 27**) indicates the presence of major fragments at m/z : 231[M+H-56], m/z : 174[M+H-113], m/z : 156[M+H-131], m/z : 142[M+H-145], m/z : 119[M+H-168] (base peak), m/z : 107[M+H-180]. Among the structures proposed by the ChemSpider and PubChem databases, only dehydroabietinol corresponds to the crude formula giving the following structure.

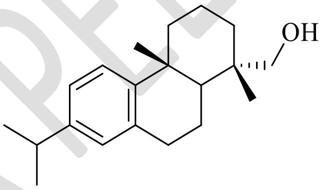
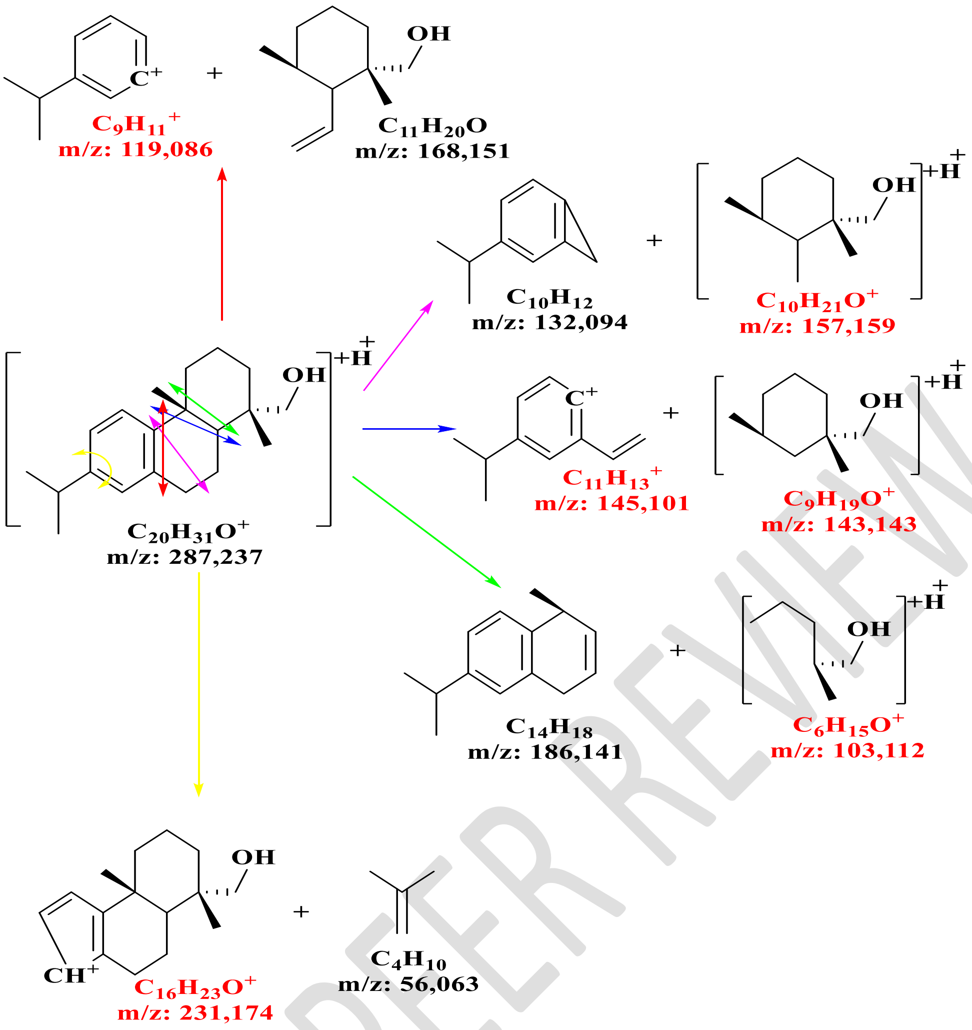


Figure 28 : Structure of compound 13

The fragmentation mode is similar to that of the desired compound (**Scheme 13**).



Scheme 13 : Proposal fragmentation of compound 13

The majority fragment at m/z : 119 would be due to cleavage on the intermediate ring of the carbon-carbon bonds adjacent to the aromatic ring. The fragment at m/z : 103 would result from cleavage on the saturated ring of the carbon-carbon bonds in α of the adjacent ring. As for the fragment at m/z : 157, it would result from the double cleavage on the intermediate ring of the carbon-carbon bonds in the α and β positions of the other two rings. The fragment at m/z : 143 (142 on the spectrum, difference due to a rearrangement), would derive from the cleavage on the intermediate ring of the carbon-carbon bonds in α position of the adjacent saturated ring. The fragment at m/z : 231 would derive from the break on the aromatic ring of the β-carbon bonds of the isopropyl group (**Scheme 13**). Compound 13 would therefore be dehydroabietinol from the terpenes and sterols family. This compound has been reported in the literature (**Misra and al., 1983**).

Structure of compound 14

Compound 14 with a retention time of 36.330 min corresponds to the molecular ion $[M+H]^+$ at m/z : 289.252 with a molecular weight of 288.245 g/mol. The most probable molecular formula is $C_{20}H_{32}O$ (cal. 288.48) (**Figure 29**).

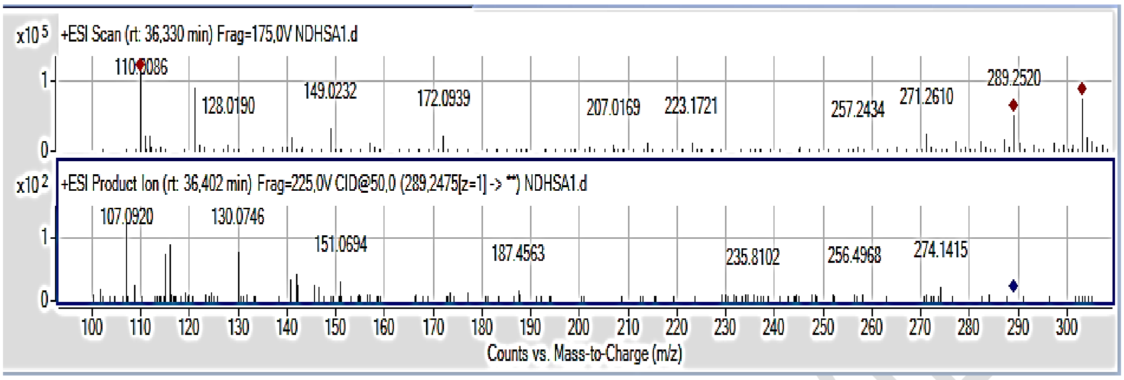


Figure 29 : LC-ESI/MS mass spectrum and ESI/MS fragmentation spectrum of compound 14

Analysis of the fragmentation spectrum of compound 14 (Figure 29) 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-methanophenanthrene-1(4bH)-one corresponds to the crude formula giving the following structure.

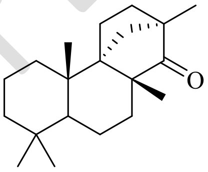
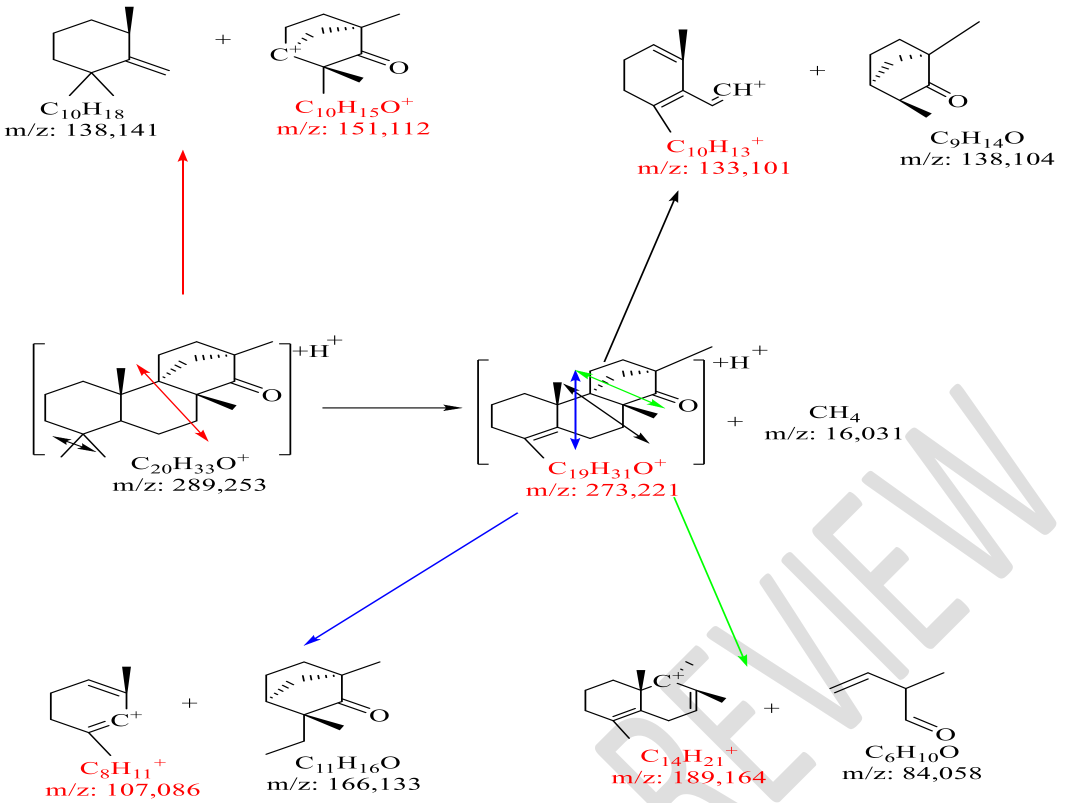


Figure 30 : Structure of Compound 14

The fragmentation mode is similar to that of the desired compound (**Scheme 14**).



Scheme 14 : Proposal fragmentation of compound 14

The fragment at m/z : 151 would be due to the scission on the intermediate ring of the carbon-carbon bonds in α and β of two extreme rings. The fragment at m/z : 273 (274 on the spectrum), would result from the loss of one of the two methyl groups specific to the peripheral cyclohexane. The other major fragments are thought to have originated from the fragment at m/z : 273. The most abundant fragment at m/z : 107 would result from the cleavage of the intermediate ring bonds adjacent to the unsaturated ring. 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 derive from the cleavage on the carbonyl ring of the α-carbon bonds of the intermediate ring (**Scheme 14**). Compound 14 is therefore (2R,4aS,4bS,10aR)-2,4b,8,8,10a-pentamethyldecahydro-2H-2,4a-methanophenanthrene-1(4bH)-one of the sterol and terpene family. This compound has been reported in the literature (**Chukwujekwu et al., 2005**).

Structure of compound 15

Compound 15 with a retention time of 37.322 min corresponds to the molecular ion $[M+H]^+$ at m/z: 361.091 with a molecular weight of 360.085 g/mol. The most probable molecular formula is $C_{18}H_{16}O_8$ (cal. 360.32) (**Figure 31**).

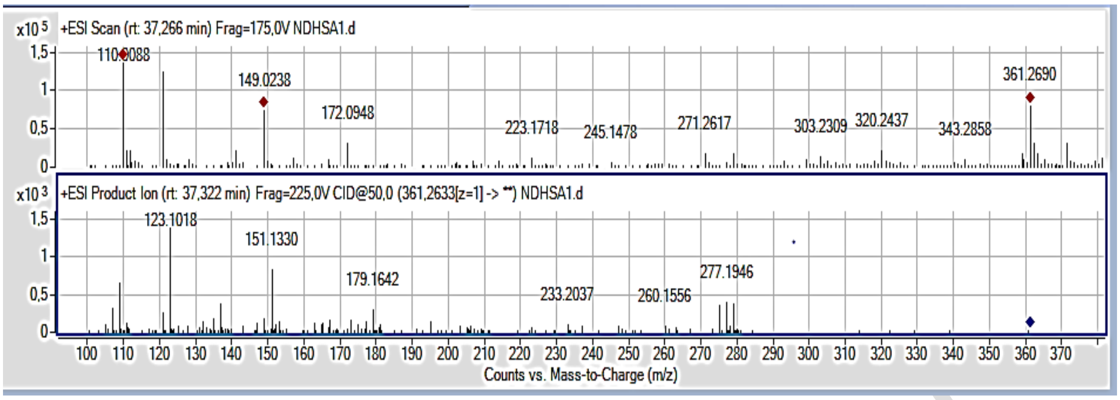


Figure 31 : LC-ESI/MS mass spectrum and ESI/MS fragmentation spectrum of compound 15

Analysis of the fragmentation spectrum of compound 17 (Figure 31) 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 corresponds to the crude formula giving the following structure.

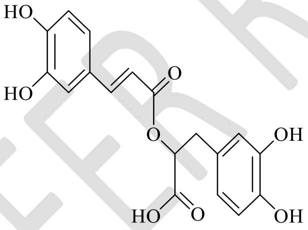
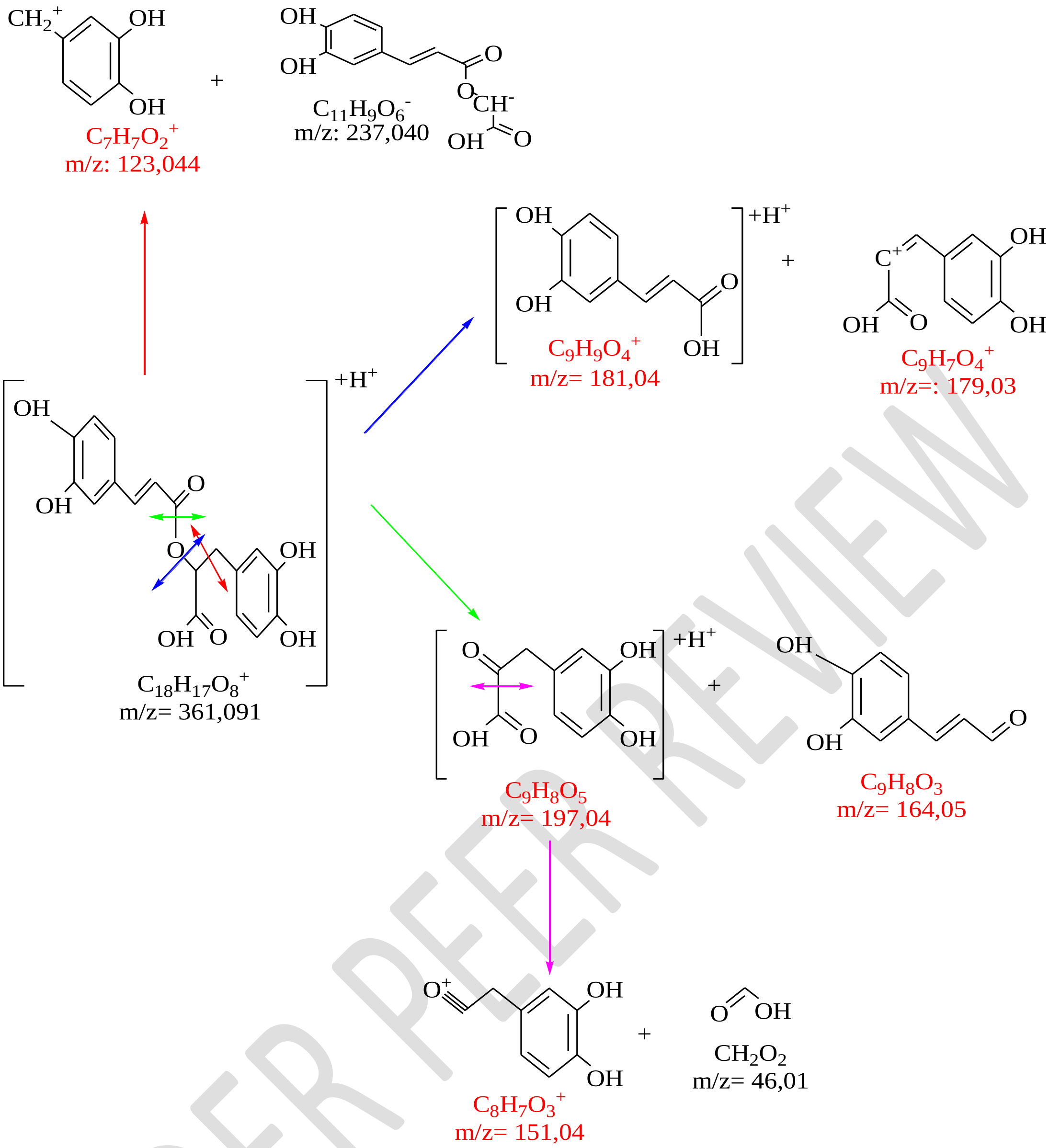


Figure 32: Structure of Compound 15

The fragmentation mode is similar to that of the desired compound (**Scheme 15**).



Scheme 15 : Proposal fragmentation of compound 15

The majority peak at m/z : 123 would result from cleavage of the carbon-carbon bond at the β-position of the carboxyl group. The fragment at m/z : 179 would result from cleavage of the carbon-oxygen bond at the β-position of the carboxyl group. The fragment at m/z : 163 would be linked to the cleavage 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 cleavage at the level of the carbon-carbon and carbon-oxygen bonds respectively at α and γ of the carboxylic group (**Scheme 15**). Compound 15 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**).

4. Conclusion

The dereplicative analysis of the ethyl acetate fraction from the hydroethanol extract of *Hyptis suaveolens* leaves revealed several compound (around 40). Many of the molecular formulas proposed do not correspond to molecules already identified in the Hyptis genus. On the other hand, ten (10) correspond to structures already isolated from the genus. Of the 15 known compounds detected in the ethyl acetate fraction of plant leaves during this study, eight (08) had not been identified in the dichloromethane fractions during our previous studies. Moreover, these eight (08) new molecules are phenolic compounds. In total, of the 15 fifteen elucidated structures, nine (09) are phenolics and the remaining six (6) are terpenoids. The presence of all these bioactive molecules in *Hyptis suaveolens* leaves makes this plant an alternative for combating many diseases caused by oxidative stress.

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