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Characterization of flavonoids in the ethyl acetate extract from *Euphorbia hirta* L. by liquid chromatography and tandem mass spectrometry

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***Abstract*:**

Flavonoids are one of the largest groups of secondary metabolites and are present in plants as aglycones linked to glycosides. LC-MSMS experiments are used in this work to establish the flavonoid content of *Euphorbia hirta L.* Based on the identification of aglycone ions produced upon collision-induced dissociation (CID), the structural characterization of each flavonoid ion is finally achieved based on the MSMS data. Fourteen flavonoids presenting five different aglycones: quercetin, myricetin, kaempferol, luteolin, and apigenin, are identified in the ethyl acetate extract of the plant. Among all the observed flavonoids, myricetin-3-O-glucoside, myricetin-7-O-glucoside, kaempferol-3-O-glucoside, vitexin, and isovitexin are detected for the first time in the plant extract. The distinction between vitexin and isovitexin is achieved using ion mobility mass spectrometry. Quantitative evaluation shows that *Euphorbia hirta* extract contains, 136 ± 2, 64 ± 2 and 6.9 ± 0.3 µg of quercitrin, myricitrin and quercetin per mg of ethyl acetate extract, respectively.

***Keywords*:** *Euphorbia hirta* L.; Flavonoids; Ion mobility; Vitexin; Isovitexin.

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1. Introduction

Plant specific metabolites have been the focus of many studies due to their biological properties. These specific metabolites mostly participate in the self-defence mechanisms in plants [1, 2]. These properties can be directly transferred to applications with high added values in medicine, alimentation, phytomedecine or cosmetics. As for a typical example, flavonoids are ubiquitous plant secondary metabolites and they are for instance efficient antioxidants [3]. Several reports showed their anti-inflammatory, anti-hypertensive, anti-allergic, anti-thrombic, antifungal, anti-microbial and anti-carcinogenic properties [4-7]. The growing interest in these molecules among the scientific community is exemplified by the great number of publications presenting the properties as well as the identification and structural characterization of the different classes of secondary metabolites [7, 8]. Flavonoids represent a family of secondary metabolites widely distributed in plants. They belong to the polyphenols family. Qualitative analysis of these flavonoids however remains challenging due to their extreme structural diversity [9, 10].

Nevertheless, all flavonoids share the same structural pattern based on an aglycone residue (Figure 1). In plants, flavonoids are mainly found in their glycosylated form by association of oligosaccharide chain(s) to an aglycone skeleton. The aglycone can be identified depending on the carbon atom of the C ring on which the B ring is attached and the degree of unsaturation and oxidation of the C ring. Among them, the congener with the B ring linked in position 2 of the C ring in a phenylchromane skeleton can be further classified into several subgroups on the basis of the structural features of the C ring. These subgroups of flavonoids are: flavones, isoflavones, flavonols, flavanones, flavanols or catechins, and anthocyanins.

The hydroxylation at different positions of the aglycone skeleton further increases the structural diversity and affects the biological activity. In addition, the glycosylation leads to an unlimited number of derivatives. Hydroxyl groups in positions 3 and/or 7 are often the sites of O-glycosylation with various mono- or diglycosidic substituents.

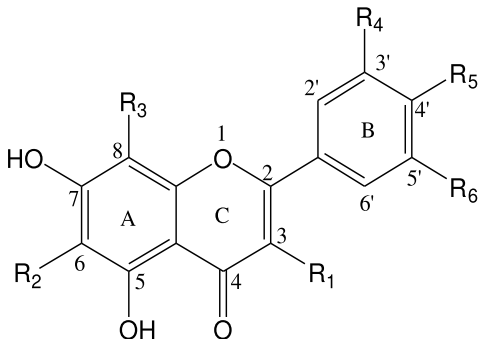

Fig. 1. Flavonoid skeleton with R=H, OH or saccharide.

Several other structures are also known as for instance, C-glycosides, which present their oligosaccharidic chains, appended on a carbon of the aglycone [11-13].

In mass spectrometry analysis by specifically targeting the aglycone ions, the

different aglycones in plant extracts can be successfully identified [14]. The method consists in conducting tandem mass spectrometry (MSMS) experiments, to analyse the plant extracts by monitoring the aglycone fragment ions [15]. Indeed, during the collision-induced dissociation experiments, O-glycoside flavonoid ions undergo complete losses of the saccharidic chains producing thus the aglycone ions of the flavonoid signature [16]. The methodology is also based on accurate mass measurements to establish the compositions of the flavonoid ions. Afterwards, a complete analysis of the MSMS data allow to get the structural characterization of the entire flavonoid ions.

In the present paper, using this targeted aglycone approach, we intend to characterize the flavonoids extracted from *Euphorbia hirta* L. (Euphorbiaceae), a pesticidal and medicinal plant used in Burkina Faso [3]. After flavonoid identification, the quantification of the major compounds will be achieved using the external standard method through LC-MS analysis.

2. Materials and experimental

2.1. Chemicals

Isoquercetin, myricitrin, myricetin, kaempferol, rutin (≥ 99%) and quercitrin (≥ 98.5%) standards are purchased from Extrasynthese (Lyon, France). Quercetin and luteolin (≥ 98%) standards are obtained from Sigma-Aldrich (Paris, France). HPLC grade acetonitrile (CH_3CN), methanol (MeOH), formic acid (HCOOH) are used without any further purification.

2.2. Plant material

The whole plant (leaves, stems, roots and flowers) of *Euphorbia hirta* L. was collected, in mid-February corresponding to dry season in a field in Ouagadougou, Burkina Faso. The plant identification was achieved by Professor Jeanne Millogo, botanist at the Laboratory of Plant Biology and Ecology (LABEV) of the Joseph KI-ZERBO University.

2.3. Extraction of phenolic compounds

The whole plant samples were washed carefully with milli-Q water, dried at room temperature (35 °C) during 96 hours and powdered. The powder (100 g) was defatted with hexane (3×250 ml). The residual plant material was separated by filtration and dried at room temperature. 50 g of the dried residue were macerated in 500 ml of 80% aqueous ethanol during 20 hours with occasional stirring. The extract was filtered on paper Wattman N°1, and concentrated on rotary evaporator at 50 °C until the volume reached 150 ml. The remaining aqueous phase was then freeze-dried to obtain 4 g of crude extract named EB. The EB extract was further dissolved in 150 ml of milli-Q water and extracted successively with 3×50 ml of dichloromethane (DCM), followed by 3×50 ml of ethyl acetate (AcOEt). AcOEt extract was evaporated to dryness on a rotary evaporator at 50 °C and then kept in fridge for further analysis.

2.4. Preparation of standards, calibration solutions and *E. hirta* extract solution

One mg of each standard was dissolved in 400 µl of CH_3CN and then completed with 600 µl of H_2O acidified with HCOOH (1%) to prepare 1 mg mL^{-1} of stock solutions. For the establishment of the calibration curve, the stock solutions were diluted with CH_3CN/H_2O 4/6 (v/v) to obtain different solutions in the concentration range of 0.39 to 50 µg mL^{-1} for quercetin, 0.78 to 150 µg mL^{-1} for quercitrin, 1.56 to 100 µg mL^{-1} for isoquercetin, 6.25 to 75 µg mL^{-1} for myricetin, 3.12 to 100 µg mL^{-1} for myricitrin, 0.39 to 25 µg mL^{-1} for luteolin, 0.39 to 50 µg mL^{-1} for kaempferol and 0.78 to 50 µg mL^{-1} for rutin.

A aliquot (1 mg) of the dried AcOEt extract was dissolved in 400 µl of CH_3CN and 600 µl of H_2O acidified with HCOOH (1%) to prepare 1 mg mL^{-1} solution.

2.5. Mass spectrometry analysis

Separation of flavonoids compounds from *E. hirta* extract was performed on a Waters Alliance 2695 liquid chromatography apparatus. The LC device was coupled to a Waters QTOF Premier or a Waters Synapt G2-Si mass spectrometer (ESI+ and ESI- mode) and consisted of vacuum degasser, a quaternary pump and an auto sampler. A sample volume of 20 µl and 2 µl were injected in all cases respectively for the QTOF Premier and Synapt G2-Si analysis. Chromatography separation was performed on a non-polar column (phenomenex C_{18}, 150×2.1 mm, 5 µm column, kinetex) at 30 °C. The mobile phase (300 µL min^{-1}) was non-linear gradient programmed from acidified water (0.1% HCOOH) (eluent A) and CH_3CN (eluent B). The gradient was programmed as follow: 0 min, 0% B; 20 min, 20% B; 30 min, 30% B; 35 min, 40% B; 40 min, 0% B, and finally, the initial conditions were held for 5 min as a re-equilibration step. The mobile phase flow (300 µL min^{-1}) was split prior to injection in the Electrospray ionization source (5 µL min^{-1}). For the ion mobility experiments, the Waters Synapt G2-Si mass spectrometer was used with a wave velocity of 1000 m/s and wave height of 40 V. Data were processed using Mass Lynx version 4.1 software (Micromass, Manchester, UK) and DriftScope V2.8.

For quantitative studies in LC-MS experiments, the mass-to-charge (*m/z*) ratios of the ions corresponding to the protonated $[M+H]^+$ and the sodiated $[M+Na]^+$ flavonoids were extracted from the total ion current (TIC) chromatogram with a mass error of 0.01 Da. These measurements were realized on standard solutions and on *E. hirta* extract solutions. The area of both $[M+H]^+$ and $[M+Na]^+$ peak signals were then summed and used for the quantitative study. Parameters of analysis were set up in the positive and negative ion mode, with spectra acquired over a mass range from *m/z* 100 to 1000. The optimum values of the ESI-MS parameters were: capillary voltage, 3.1 kV; cone voltage, 20 V; source temperature, 100 °C;

desolvation temperature, 300 °C. Dry nitrogen was used as the ESI gas.

The identification of flavonoid derivatives in *E. hirta* extract was based on the comparison of their retention times with the ones of corresponding standards and the MSMS fragmentations.

3. Results and discussion

3.1. Identification of the aglycones

To build the aglycone targeted approach, the aglycones of the flavonoids present in the *E. hirta* AcOEt extract must first be identified and this is achieved by generating them as fragments in the ion source (increase of the cone voltage, 60 V). The experiments are conducted on a Waters Synapt G2-Si mass spectrometer in both the positive and negative ion modes to secure the aglycone identification. A full scan MS experiment is used to record all the aglycone ions based on their specific *m/z* ratio. The obtained results reveal that, in the AcOEt extract of *E. hirta*, five common aglycones, namely quercetin, myricetin, luteolin, kaempferol and apigenin are present as summarized in table 1.

The confirmation of the elemental composition of these aglycones is achieved using accurate mass measurements (HRMS) and the identification of all the aglycones is further established based on the comparison of their retention times in LC-MS with standard compounds (see Table 2 for complete set of data). The standard compounds are carefully selected based on the information obtained from the detected aglycones and the full scan MS experiments.

Table 1
Main flavonoid aglycones detected in *E. hirta* AcOEt extract

Aglycone	R_1	R_2	R_3	R_4	R_5	R_6	*m/z* $[M+H]^+$
Luteolin	H	H	H	OH	OH	H	287
Kaempferol	OH	H	H	H	OH	H	287
Quercetin	OH	H	H	OH	OH	H	303
Myricetin	OH	H	H	OH	OH	OH	319
Apigenin	H	H	H	H	OH	H	271

3.2. Targeted aglycone approach in LC-MSMS analysis

The data LC-MSMS (Waters Synapt G2-Si) of the AcOEt extract of *E. hirta* have been analysed with a special attention paid on the fragment ions by targeting the aglycone fragment ions through their specific *m/z* ratios. The obtained data are gathered in table 2 that also includes the main ion fragments observed in the LC-MSMS experiments. As shown in table 2, fourteen compounds have been detected in the AcOEt extract of *E. hirta*.

Compounds 1, 4, 7 and 10 produced aglycone fragment ions at *m/z* 319 and 317, respectively from the positively and negatively charged precursor ions (Table 2). When these fragment ions are further submitted to CID experiments, characteristic fragment ions are specifically observed in the negative ion mode at *m/z* 278, 271, 179 and 151 confirming the

presence of the myricetin aglycone, as reported in table 2 [15].

Compounds 6, 9, 12 are characterized by aglycone fragment ions at *m/z* 303 in positive mode and *m/z* 301 in negative mode, as shown in table 2. When these fragments are further submitted to CID experiments in negative mode the characteristic ions at *m/z* 271, 255, 179 and 151 are observed confirming the presence of quercetin [14]. Using the same strategy, compound 13 is readily assigned to luteolin and compound 5 as one of its derivatives. In addition, compound 14 is kaempferol, whereas compounds 8 and 11 are identified as kaempferol derivatives. Finally, compounds 2 and 3 possess the same aglycone identified as apigenin leading to the similar fragmentation pattern with fragment ions detected at *m/z* 341 and at *m/z* 311 (Figure 5).

In summary, the fourteen compounds identified in *E. hirta* extract include 3 quercetin derivatives, 4 myricetin derivatives, 3 kaempferol derivatives, 2 luteolin derivatives and 2 apigenin derivatives.

3.3. Identification of flavonoids

Based on the analytical strategy targeting the aglycones, each flavonoid can further be fully identified in the *E. hirta* extract starting from the corresponding aglycone.

As an example for identification, the following data are obtained for the quercetin derivatives, namely compound 12 and its two derivatives, 6 and 9. Beside compound 12 that is readily identified as quercetin [14] based on HRMS, CID data and retention time (Table 2), compounds 6 and 9 can be identified based on the data gathered in table 2. Compound 6 is detected at *m/z* 465 and *m/z* 463 in the positive and negative ion modes, respectively. The 2 u difference between the positive and negative ions allows to assign them as $[M+H]^+$ and $[M-H]^-$, respectively. Moreover, upon MSMS experiments on the Waters Synat G2-Si, the $[M+H]^+$ and $[M-H]^-$ ions undergo a 162 u loss generating fragment ions detected at *m/z* 303 and *m/z* 301, corresponding to the aglycone ions. By comparison with the literature data [15], the presence of these fragment ions is explained by the loss of an hexose residue [17, 18]. It is also proposed in the literature that a 3-O-glycosylation is often detected by comparing the relative intensities of the $[M-H-162-H]^{-\bullet}$ and $[M-H-162]^-$ fragment ions in the CID spectrum of the $[M-H]^-$ precursor ions [15]. The highest abundance of the $[M-H-162-H]^{-\bullet}$ ions thus reveals that the hexose is attached to the aglycone in 3-OH position [15]. Compound 6 is thus proposed to be quercetin-3-O-glycoside (isoquercetin). For compound 9, the $[M+H]^+$ ions detected at *m/z* 449 in the positive ion mode are characterized by intense *m/z* 303 $[M+H-146]^+$ fragment ions. In the negative mode, when subjected to MSMS experiments, the $[M-H]^-$ precursor ions at *m/z* 447 lead to two abundant fragment ions at *m/z* 301 and *m/z* 300 for respectively $[M-H-146]^-$ and $[M-H-146-H]^{-\bullet}$, corresponding to the loss of a rhamnose unit.

Furthermore, the lowest intensity of the $[M-H-146]^-$ signal compared to $[M-H-146-H]^{-\bullet}$ signal indicates that the glycosylation site is 3-OH according to the literature. Compound 9 is therefore identified as quercetin-3-O rhamnoside (quercitrin) [15].

As far as the myricetin derivatives (1, 4, 7 and 10) are concerned, compound 10 is unambiguously assigned as myricetin based on the data in table 1 [15]. Compounds 1 and 7 are both detected as protonated and deprotonated molecules at *m/z* 481 and 479, respectively in the positive and negative ion modes. These two molecules are isomers with the difference being associated with the position of the monoglycoside residue on the aglycone. In the MSMS spectrum of the $[M-H]^-$ precursor ions from compound 7, the predominance of the $[M-H-162]^-$ fragment ions when compared to the $[M-H-162-H]^{-\bullet}$ ions points to a 7-OH attachment, whereas for compound 1, the reversed situation is observed pointing to the 3-OH position of the hexose. Compound 1 is thus tentatively identified as myricetin-3-O-glucoside and compound 7 as myricetin-7-O-glucoside. Compound 4 is detected as $[M+H]^+$ ions at *m/z* 465 and $[M-H]^-$ at *m/z* 463. The CID spectrum of the $[M+H]^+$ precursor ions features two characteristic fragments at *m/z* 319 for $[M+H-146]^+$ ions and at *m/z* 147 for $[M+H-318]^+$ ions respectively attributed to either a rhamnose loss or the myricetin aglycone loss. Again, the predominance of the $[M-H-146-H]^{-\bullet}$ fragment ions compared to the $[M-H-146]^-$ ions confirms the 3-OH attachment of the rhamnose on the myricetin aglycone [14], meaning that the compound 4 is myricetin-3-O-rhamnoside (myricetrin). This result is in agreement with the literature data [19, 20], that indicate the presence of myricitrin in *E. hirta* extracts.

This analytical strategy fails to distinguish the isomeric compounds 2 and 3 that belong to the apigenin derivatives. Indeed, the CID spectra of the $[M-H]^-$ precursor ions (*m/z* 431) are found nearly superimposable as featured in figure 4.

Nevertheless, several pieces of information can be derived from the analysis of these CID spectra. First, according to the literature data [11, 21], the detection of the fragment ions at *m/z* 341 - $[M-H-90]^-$, and at *m/z* 311 - $[M-H-120]^-$ points to a C-glycosylation in both cases [11, 21]. As candidate aglycones, the isomeric vitexin and isovitexin (Figure 5) can be envisaged [11, 21].

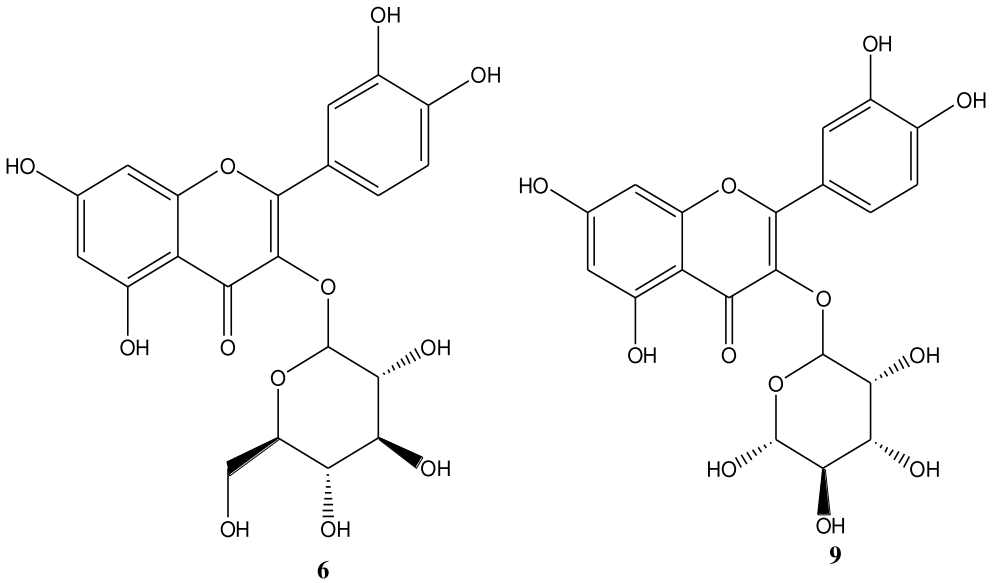

Fig. 2. Chemical structure of compound 6 (quercetin-3-O-glucoside) and compound 9 (quercetin-3-O-rhamnoside).

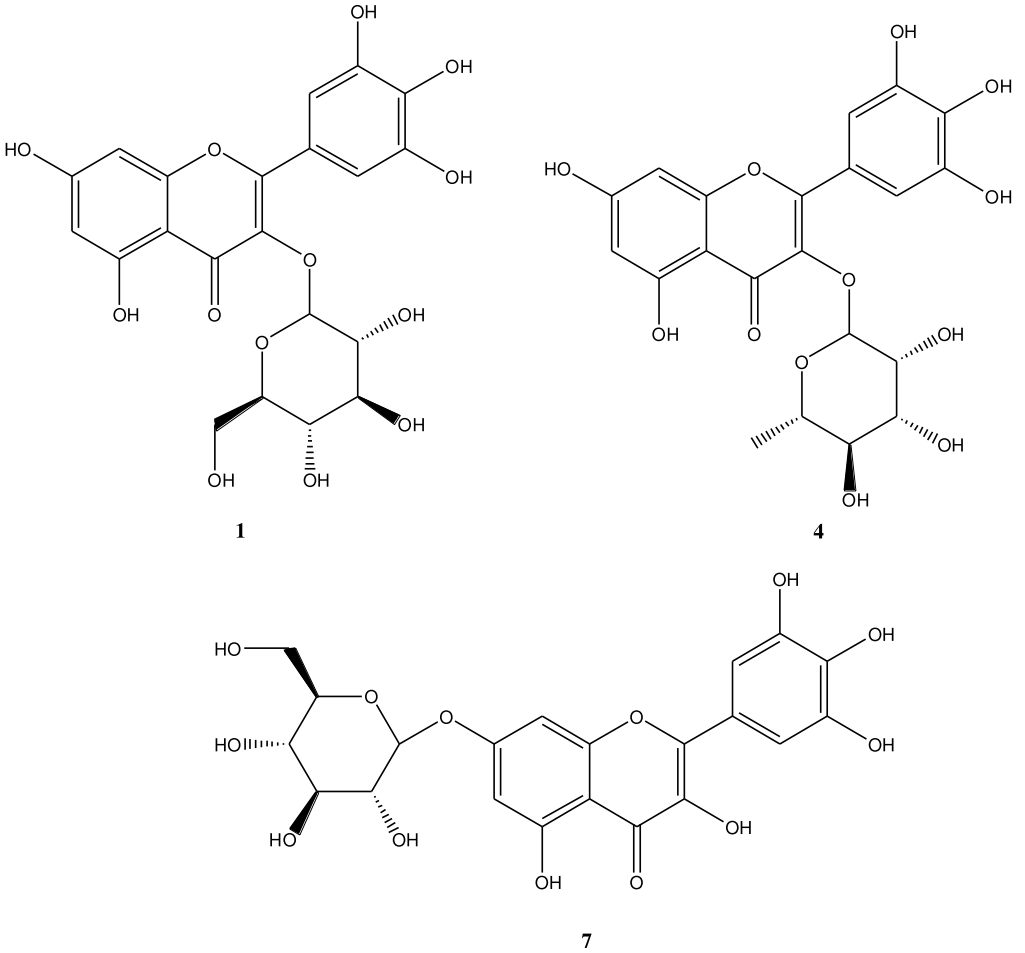

Fig. 3. Chemical structure of compound 1 (myricetin-3-O-glucoside), compound 4 (myricetin-3-O-rhamnoside) and compound 7 (myricetin-7-O-glucoside).

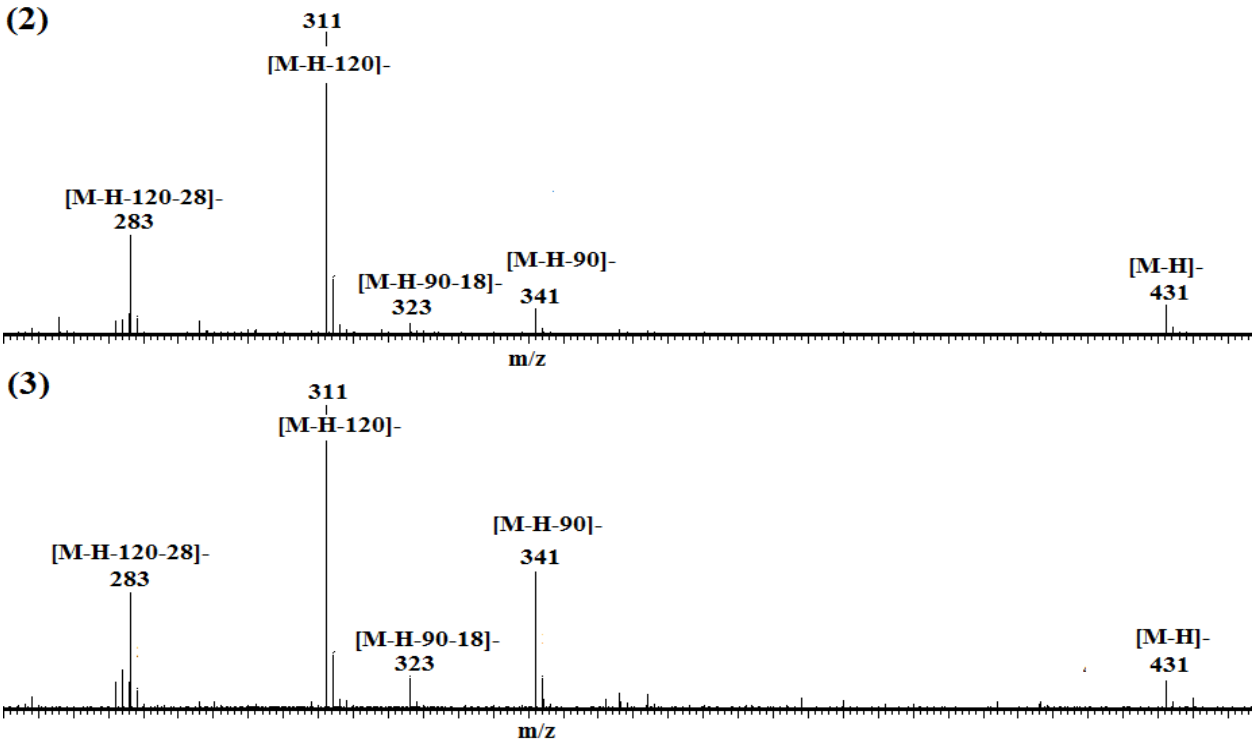

Fig. 4. LC-MSMS analyses (negative mode) of the *E. hirta* extract: CID spectra of the m/z 431 precursor ions corresponding to compounds 2 and 3.

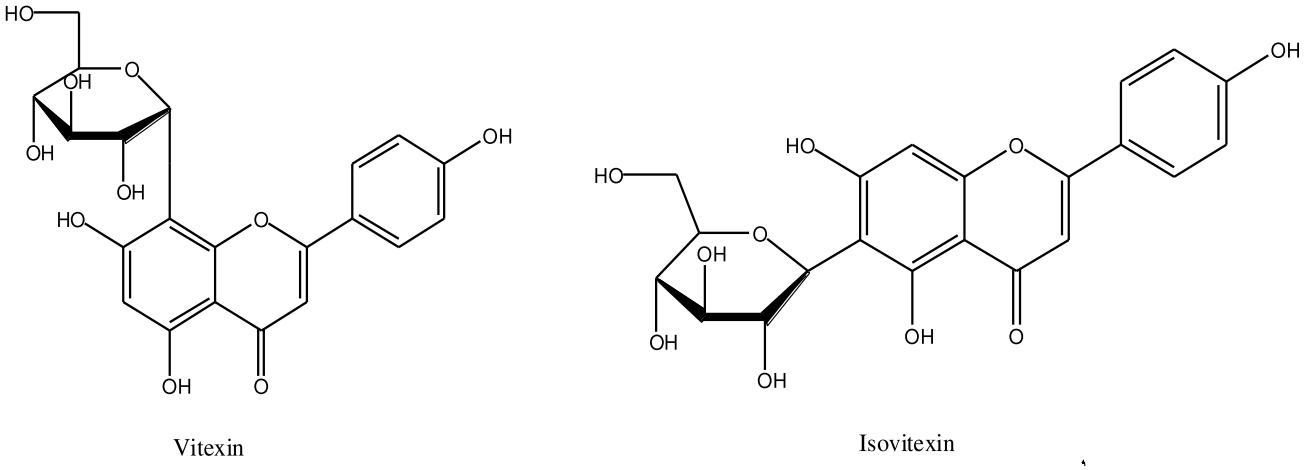

Fig. 5. Chemical structure of Vitexin and Isovitexin.

In the recent literature, ion mobility spectrometry (IMS) experiments are more and more implemented in the MS toolbox for the characterization of natural products. For instance, we recently introduced IMS for the successful characterization of saponins, plant and marine animal secondary metabolites that are challenging to characterize [22, 23]. IMS has also already be used for flavonoid characterization [12]. This methodology allows for the temporal separation of ions based on their mobility in a cell filled with a buffer gas under the influence of an electric field. The drift time (t_D) of the ions across the mobility cell is directly proportional to their collisional cross section (CCS) which reflects the three-dimensional shape of the ions in the gas phase [24]. In order to distinguish the vitexin and isovitexin isomers, the corresponding $[M+H]^+$ ions are subjected to IMS experiments and the arrival time distributions (ATD) are recorded and presented in figure 6. The drift time (apex of the ATD signal) of the $[M+H]^+$ ions from compound 2 is found weaker, 6.46 ms, than the t_D of the $[M+H]^+$ of compound 3, 6.84 ms, confirming the presence of isomeric aglycone. These ions have already been studied using IMS and the direct comparison of the arrival times with the literature data [12] allows proposing that compounds 2 and 3 are respectively vitexin and isovitexin.

Among the 14 detected flavonoids, 5 flavonoids, namely myricetin-3-O-glucoside (1), myricetin-7-O-glucoside (7), kaempferol-3-O-glucoside (8), vitexin (2), and isovitexin (3) are reported for the first time in *E. hirta*.

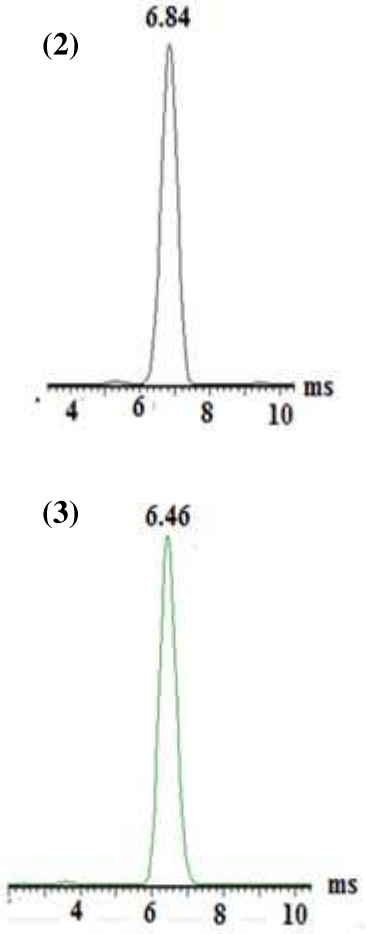

Fig. 6. LC-IMS-MS analysis (negative mode) of the *E. hirta* extract: ATD of the $[M-H]^-$ ions (*m/z* 431) of compounds 2 and 3.

Table 2
LC-MSMS analysis of *E. hirta* AcOEt extract: analytical characteristics of the detected flavonoids in positive and negative ion modes. t_R and RI correspond to the retention time (min) in LC-MS and the fragment ion relative intensities (%) in the CID mass spectra.

Peak N°	t_R (min)	Formula	$[M+H]^+$	$[M-H]^-$	Δm (ppm)	CID analysis - $[M+H]^+$ Fragment ions *m/z* (RI - %)	CID analysis – $[M-H]^-$ Fragment ions *m/z* (RI - %)	Identified flavonoids
1	18.34	$C_{21}H_{20}O_{13}$	481.0843	479.0826	-1.6	481(15), 319(100), 269(3), 167(2)	479(6), 318(4), 317(20), 316(100), 287(3), 271(4), 259(2), 179(0,4), 151(0,4), 137(0,1)	Myricetin-3-O-glucoside
2	19.49	$C_{21}H_{20}O_{10}$	433.1332	431.0978	-1.6	433(49), 343(13), 313(100), 283(21)	431(9), 341(8), 323(3), 312(18), 311(100), 283(32)	Vitexin
3	19.92	$C_{21}H_{20}O_{10}$	433.1332	431.0978	-1.6	433(1), 313(93), 283(100)	431(9), 379(3), 341(45), 323(10), 312(17), 311(100), 283(38), 269(4)	Isovitexin
4	19.93	$C_{21}H_{20}O_{12}$	465.1316	463.0877	-1.5	319(100), 244(7), 171(4), 147(26), 129(17)	463(0,1), 318(9), 317(45), 316(100), 287(11), 271(4), 259(9), 179(0,6), 151(0,1)	Myricitrin
5	20.58	$C_{21}H_{20}O_{11}$	-	447.0927	1.6	-	447(59), 286(10), 285(61), 284(100), 275(2), 227(0,1), 179(2), 175(0,1), 151(6), 133(0,1)	Luteolin-7-O-glucoside
6	20.64	$C_{21}H_{20}O_{12}$	465.1316	463.0877	-1.5	303(100), 163(3), 145(4)	463(0,1),302(9), 301(59), 300(100), 272(5), 271(0,1), 255(6), 243(6), 227(0,1)	Isoquercetin
7	20.86	$C_{21}H_{20}O_{13}$	481.1225	479.0826	-0.8	319(100)	479(65), 318(22), 317(100), 316(88), 301(18), 299(7), 287(5), 275(7), 243(8), 205(9), 179(8), 169(12), 151(9), 137(4)	Myricetin-7-O-glucoside
8	21.96	$C_{21}H_{20}O_{11}$	-	447.0927	1.6	-	447(22), 285(35), 284(100), 255(17), 229(0,1), 227(26), 161, 151(1), 109	Kaempferol-3-O-glucoside
9	22.54	$C_{21}H_{20}O_{11}$	449.1332	447.0927	1.6	449(5), 303(100), 287(11), 147(25), 129(19)	447(10), 302(16), 301(65), 300(100), 272(30), 271(16), 255(14), 243(14), 227(2), 179(1), 163(2), 151(3)	Quercitrin
10	23.35	$C_{15}H_{10}O_8$	319.0634	317.0297	1.6	319(100), 237(25), 173(15)	317(33), 287(3), 271(13), 227(3), 217(1), 179(26), 165(47), 151(100), 137(87), 125(4), 109(36)	Myricetin
11	24.93	$C_{21}H_{20}O_{10}$	433.1332	431.0978	-1.6	433(7), 287(100), 147(24), 129(18)	431(9), 286(9), 285(64), 284(100), 257(5), 255(37), 228(8), 227(36), 151(1),	Afzelin
12	27.46	$C_{15}H_{10}O_7$	303.0644	301.0348	-1.0	303(100), 257(15), 137(15)	301(26), 273(20), 271(1), 255(6), 245(19), 229(6), 227(6), 179(16), 161(8), 151(100), 149(33), 139(9), 121(48), 109(4)	Quercetin
13	27.82	$C_{15}H_{10}O_6$	287.0698	285.0399	0.7	287(100)	285(66), 257(1), 217(1), 201(2), 199(2), 175(4), 171(1), 151(13), 149(4), 133(100)	Luteolin
14	31.50	$C_{15}H_{10}O_6$	287.0698	285.0399	0.7	287(100), 269(12), 225(36), 224(38), 194(37)	285(100), 257(2), 255(2), 243(1), 239(2), 229(4), 227(3), 211(3), 187(4), 185(4), 171(2), 159(3), 157(1), 151(1)	Kaempferol

3.4. Quantitative analysis of flavonoids

Figure 7 presents the results of the quantitative analysis of the major flavonoids present in the *E. hirta* AcOEt extract. The quantification has been achieved in LC-MS on a Waters QToF Premier mass spectrometer using commercially available flavonoids as external standards. The results reported in figure 7 are presented in microgram of analyte per gram of dry extract (µg/g).

As shown in figure 7, the AcOEt contains three major constituents, namely quercitrin (9), myricitrin (4), and isoquercetin (6) at 136.0 ± 1.8, 63.9 ± 2.1 and 30.9 ± 1.39 µg/g of extract, respectively. The data can be combined by merging all the flavonoids sharing the same aglycone. This quantitative evaluation gives 6.9 ± 0.3 µg/g of extract for the quercetin derivatives, 1.25 µg/g for myricetin and 1.1 ± 0.1 µg/g for kaempferol and 0.2 µg/g of extract for luteolin.

Our data are in agreement with the literature data, since quercetin was previously detected as the major aglycone for flavonoid derivatives in *E. hirta* [18]. The AcOEt fraction of the whole plant of *E. hirta* was also previously found to content kaempferol (0.0487%), quercetin (0.0789%), and rutin (0.0184%) [25].

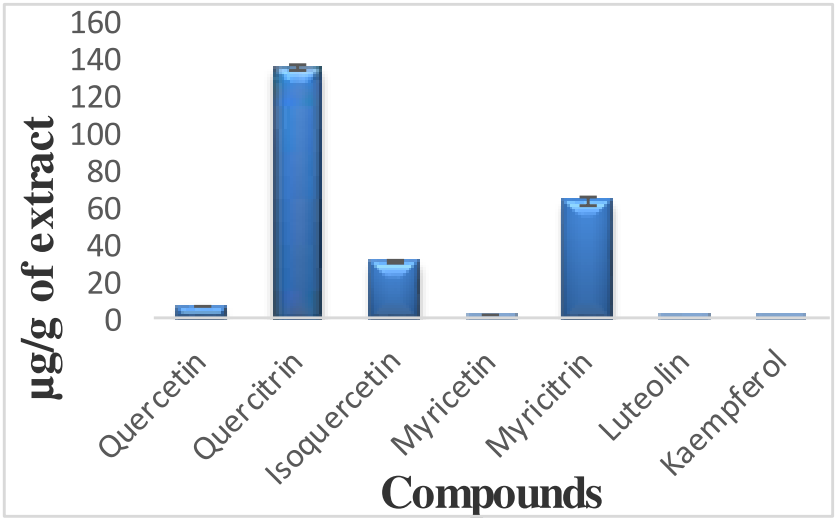

Fig. 7. LC-MS quantitative analysis of the major flavonoids present in the AcOEt extract of *E hirta.*

4. Conclusion

The present investigation is based on a LC-MSMS method and intends to identify the flavonoids contained in the AcOEt extract of *E. hirta*. Based on accurate mass measurements and MSMS experiments, 14 flavonoids have been identified. Among these flavonoids, myricetin-3-O-glucoside, myricetin-7-O-glucoside, kaempferol-3-O-glucoside, vitexin and isovitexin are here reported for the first time in *E. hirta*. In addition to the diversity of flavonoids in the AcOEt extract, the quantitative analysis clearly showed that *E. hirta* contains a high content of flavonoids with the major compounds being quercitrin, myricitrin and other quercetin derivatives.

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