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Identification and characterization of major flavonoids and caffeoylquinic acids in three *Compositae* plants by LC/DAD-APCI/MS

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Abstract

In this study, a liquid chromatography/diode array detector-atmospheric pressure chemical ionization/mass spectrometry (LC/DAD-APCI/MS) was successfully developed to identify and characterize the main flavonoids and caffeoylquinic acids (CQAs) of three common *Compositae* plants (*Chrysanthemum morifolium* Raman, *Artemisia annua*, and *Chrysanthemum coronarium*) which have been used as herbal medicine. Identifications were performed by comparing the retention time, UV and mass spectra of samples with standards or/and earlier publications. The crude methanolic extracts of these plants were assayed directly using LC/MS without any further pretreatment. The proposed method is rapid and reproducible and is useful for characterization and evaluation of different plant flavonoids and CQAs. A total of 41 different flavonoids and 6 CQAs were identified and confirmed by APCI-MS. The main components of three *Compositae* plants were also compared. Although there exist some similarities in the flavonoidic content of the leaf and flower of *C. morifolium*, significant variations in their varities and concentrations were observed. *Artemisia annua* processes substantial amount of alkylated derivatives of flavones and *Chrysanthemum coronarium* contains only CQAs. These findings suggest that although all the plants studied are from the same *Compositae* family, their flavonoids and phenolic compositions are markedly different. The proposed method is useful for further chromatographic fingerprinting of plant flavonoids.

Keywords: LC/DAD-APCI/MS; Flavonoids; Caffeoylquinic acids; *Compositae* plant; Chromatographic fingerprinting

1. Introduction

The *Compositae* family has been cultivated for more than 3000 years in human history. For instance, the flower of *Chrysanthemum morifolium* (*C. morifolium*) has been used for centuries as beverage and folk medicine in many Asian countries such as China, Japan, South Korea and Thailand. Other important plants of this family include *Artemisia annua* (*A. annua*) which is used mainly as anti-parasitic medicine, and *Chrysanthemum coronarium* (*C. coronarium*) which is an edible plant and is commonly consumed as a vegetable. It has been reported that the flower and leaf of *Compositae* plant possesses anti-bacterial, anti-fungal, anti-viral and anti-inflammatory activities [1], although some of the bioactive components are yet to be identified. *Chrysanthemum* species have also been demonstrated to produce a wide variety of flavonoids, phenols and phenolic acids [1–3]. Some of these compounds, especially the flavonoids have been shown to exhibit anti-inflammatory and anti-cancer properties. For example, apigenin and acacetin derivatives isolated from *C. morifolium* were found to possess an anti-human immunodeficiency virus type 1 (HIV-1) action [2,3]. Acacetin and related glycoside compounds such as linarin and linarin acetate isolated from *C. morifolium* can inhibit cell growth and induce apoptosis in human prostate cancer cell [4]. Our recent findings have suggested that luteolin from the flower of *C. morifolium* possesses strong anti-cancer property, especially when used together with other chemotherapeutic agents [5,6]. Thus, development of a method for comprehensive and accurate identification and determination of flavonoids and phenols in *Compositae* plants would be useful for the study of other functional aspects of this group of

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compounds. For qualitative identification of flavonoids, liquid chromatography/mass spectrometry (LC/MS) was considered as the most powerful approach [7–9]. LC/MS has been widely used to identify all kinds of flavonoids in different plant samples such as cocoa, fresh herbs, cauliflower, mango and kale [9–13]. It is ascribed that LC/MS not only provides the molecular mass of the different constituents but also able to identify unstable compounds in solution, such as acylated flavonoids [7]. Moreover, LC/MS^n could also be used to differentiate *O*-glycosides, *C*-glycosides and *O*, *C*-glycosides [7,8,14] as well as the position of some functional groups such as hydroxyl and carboxylic groups in flavonoids [15–17]. As far as we know, there has yet to be a study which will provide more comprehensive information of the flavonoids and caffeoylquinic acid (CQAs) in the *Compositate* plants. On the other hand, the chromatographic fingerprints have become a pivotal tool in the quality control of complex herbal plants and vegetables [18]. In this study we attempted to develop a liquid chromatography-diode array detector coupled with atmospheric pressure chemical ionization/mass spectrometry (LC/DAD-APCI/MS) method for characterizing and chromatographic fingerprinting of various flavonoids and CQAs in three different *Compositae* plants. Our findings suggest that there are over 40 different flavonoids occur in these *Compositae* plants. The data obtained from this study will provide some useful information for further study of *Compositae* plants for different applications.

2. Experimental

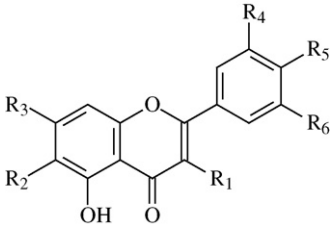
2.1. Reagents and chemicals

Chlorogenic acid (CGA), caffeic acid (CA), quinic acid (QA), gallic acid, luteolin, apigenin, acacetin, quercetin, kaempferol and kaempferide (chemical structures were listed in Table 1) were purchased from Sigma–Aldrich GmbH (Steinheim, Germany). Methanol and acetonitrile (HPLC-grade) were purchased from Tedia (Fairfield, OH, USA). Absolute alcohol, formic acid, ammonium hydroxide and trifluoroacetic acid (TFA) were purchased from Merck (Darmstadt, Germany). All the stock solutions were prepared individually with methanol at a concentration of 1 mg/mL and were stored at 4 °C. All stock solutions are stable within three months. Working standard solutions with various concentrations were diluted by methanol with stock solutions. Ultra-pure water was produced by Millipore system (Saint-Quentin, France) and used throughout this study.

2.2. Sample preparation and extraction

Flower and leaf of *C. morifolium* Raman were obtained directly from the grower in Hangzhou, the biggest production area of *C. morifolium* plants in China. *A. annua* was a gift from Yunnan Herbarium, China. *C. coronarium* was purchased

Table 1
Chemical structures of some common flavonoid aglycones



Compounds number	R1	R2	R3	R4	R5	R6	MW	Compounds name
1	H	H	OH	H	H	H	254.2	Chrysin
2	H	H	OH	H	OH	H	270.2	Apigenin
3	H	H	OH	H	OMe	H	284.2	Acacetin
4	H	H	OH	OH	OH	H	286.2	Luteolin
5	H	H	OMe	OH	OMe	H	314.3	Pilloin
6	H	OMe	OMe	OH	OH	H	330.2	Cirsiliol
7	H	OMe	OMe	OMe	OH	H	344.2	Cirsilineol
8	OH	H	OH	H	OH	H	286.2	Kaempferol
9	OH	H	OH	H	OMe	H	300.2	Kaempferide
10	OH	H	OH	OH	OH	H	302.3	Quercetin
11	OH	H	OH	OH	OMe	H	316.3	Tamarixetin
12	OH	H	OH	OH	OH	OH	318.3	Myricetin
13	OH	H	OH	OH	OH	OMe	332.3	Laricitin
14	OH	H	OH	OH	OMe	OH	332.3	Mearnsetin
15	OH	H	OH	OMe	OH	H	316.2	Isorhamnetin
16	OH	H	OH	OMe	OH	OMe	346.2	Syringetin
17	OH	H	OMe	OH	OH	H	316.3	Rhamnetin
18	OMe	OMe	OMe	H	OH	H	344.3	Chrysosplenol
19	OMe	OH	OMe	OMe	OH	H	360.3	Chrysosplenol C
20	OMe	OME	OMe	OH	OH	H	360.3	Chrysosplenol D
21	OMe	OMe	OMe	OMe	OH	H	374.3	Chrysosplentin

from a local supermarket in Singapore. The plant samples were freeze-dried with an LABCONCO® FreeZone 2.5 plus dryer (Missouri, USA) for 24–48 h depending on their moisture content. Under dim lighting environment, the lyophilized plant was ground with pestle and mortar to a fine powder and stored at −70 °C until analysis. Twenty milligrams of powdered sample was weighed and added in a 2-mL microcentrifuge tube. For the investigation of the extraction efficiency of different solvent, 1 mL of different concentration of methanol (60%, 80% and 100%), 99.7% ethanol and pure acetonitrile as well as 5.0 μg of CGA, quercetin, luteolin, apigenin, kaempferide and acacetin standards was added to the tube, respectively. For final sample analysis, 1 mL methanol was added to the tube. The tube was then placed on a shaker (Vortemp™, UniEquip, Germany) with a shaking speed of 900 rpm at 28 °C for 30 min. The mixture was then centrifuged at 16,000 *g* for 5 min and the supernatant was filtered with a VectaSpin Micro filter (Whatman, England). Finally, the filtrate was transferred into a 2-mL vial for LC/MS/MS analysis.

2.3. LC/DAD-APCI/MS analysis

The LC system comprises of a series of Agilent Technologies 1100 diode array detector (DAD), autosampler, binary pump and on-line vacuum degasser (Santa Clara, CA, USA). A Zorbax SB-C_{18} (150 mm × 2.1 mm, i.d. 3.5 μm, Ringoes, NJ, USA) was used as LC/MS analysis column. The mobile phase A was made up of acetonitrile while B was made of 0.1% formic acid (pH 4.0, adjusted with ammonium hydroxide) aqueous solution. The gradient was performed at 0.2 mL/min with an initial condition of 12% of mobile phase A and 88% of mobile phase B for 10 min. The mobile phase A was increased to 25% at 60 min and linearly increased to 60% at 80 min and then increased to 100% at 85 min (hold 5 min) to clean up the hydrophobic residues on the column. The system was subsequently returned to the initial condition and equilibrium for 10 min before the next injection. For *C. coronarium* sample, a shorter gradient elution program (within 25 min) was used due to its simple composition. UV spectra were scanned from 200 to 450 nm. Peaks were simultaneously determined at 335, 280 and 220 nm. Mass spectrometric analyses were performed on a Finnigan LCQ™ ion trapping mass spectrometer (San Jose, CA, USA) fitted with electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) interface. The instrumental parameters of APCI were vaporizer temperature, 450 °C; discharge current, 7.0 μA; capillary voltage, 47.0 V; capillary temperature, 140 °C; sheath gas flow rate, 60 arbitrary unit; auxiliary gas flow rate, 10 arbitrary unit. The instrument was operated in both positive and negative mode. The full mass scanning range was from *m/z* 50 to 800. To avoid the interference from background, the selection ion monitoring (SIM) mode was also used for some samples. The chromatographic and mass spectrometric analyses were controlled by the instrument built-in LCQ™ software. The full identifications were performed by comparing the retention time, UV and mass spectra of samples with standards or earlier publications.

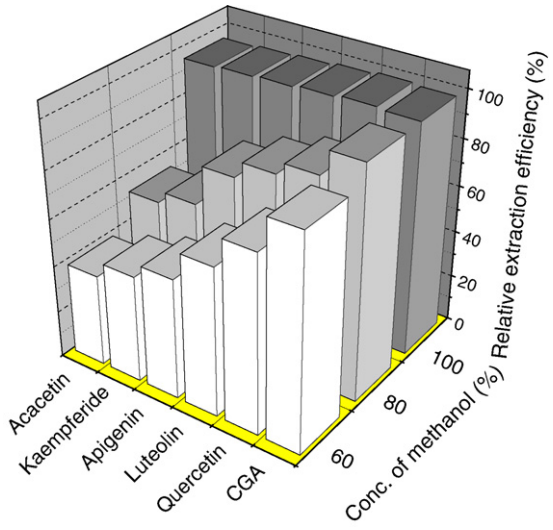


Fig. 1. The relative extraction efficiency of some flavonoids using different extraction solvents.

3. Results and discussion

3.1. Selection of extraction solvent and mobile phase

For the extraction of flavonoids in plants, some researchers used 62.5% aqueous methanol as extraction solvent [10,19]. In order to investigate the most efficient medium for extraction, 5 μg of CGA, quercetin, luteolin, apigenin, kaempferide and acacetin were added to 20 mg of *C. morifolium* leave samples for the recovery assay. Methanol of 60%, 80%, 100%, 99.7% ethanol and 100% acetonitrile were used for the extraction. The relative peak area was used to calculate the extraction efficiency. It can be seen from Fig. 1 that methanol at 60% and 80% were not effective for more hydrophobic compounds such as acacetin and kaempferide, with a recovery of below 60%, although it is efficient for more hydrophilic components, such as CGA and quercetin. Conversely, all of the above mentioned components could be effectively extracted using 100% methanol. Ethanol and acetonitrile were found to be less efficient for the flavonoid extraction (data not shown). Therefore, 100% methanol was employed for extraction in all the subsequent studies.

Indeed, the crude methanolic extracts of the three *Compositae* plants studied contain a wide range of flavonoids and CQAs; ranging from water-soluble to lipid-soluble components, especially the *A. annua* extract. It is well established that for the separation of flavonoids, choice of column, mobile phase and an appropriate gradient elution sequence are critical. Some researchers preferred to use methanol-water as mobile phase with a C_{18} column [10,19]. This procedure is generally sufficient for samples which contain few components. For more complex sample such as *A. annua*, an acetonitrile–water gradient elution system was found to be more effective, especially for the glycoside flavonoids separation when C_{18} column was used. Fig. 2 shows that a satisfactory separation for all flavonoids of interest could be obtained under the proposed conditions.

3.2. Identification of flavonoids and CQAs in Chrysanthemum morifolium flower

As can be seen from Fig. 2a and Table 2 (peak **1–17**), there are more than 17 flavonoids and CQAs found in the crude methanolic extract of *C. morifolium* flower. It can be concluded from Table 2 that, except for CGA, most of these compounds are flavonoids which occur as aglycones or/and their glucoside (Glu), glucuronide (GluA), acetyl glucoside (AGlu), neopesperidoside (Neo) and rutinoside (Rut). These flavonoid glucosides were protonated under acidic condition and easily lose their glycan which giving rise to the corresponding protonated aglycones even without any collision energy. Most of the aglycone-related ions of these flavonoid glucosides could be observed in MS^1 spectra. This is ascribed that a charge-remote neutral molecule loses a glycosyl residue through a rearrangement reaction following protonation at the C-3 carbonyl group [20]. Thus, the fragmental mass ions of the flavonoid aglycones were evidently observed in total ion chromatography (TIC). The main aglycones mass ionic fragments include quercetin (*m/z* 303.1), luteolin (*m/z* 287.2), apigenin (*m/z* 271.2), acacetin (*m/z* 285.2), kaempferol (*m/z* 287.2) and kaempferide (*m/z* 301.2) (Table 2). Based on these fragmental ions, most of the components in the flower could be confirmed by MS^1 and UV spectra. Among them, kaempferol-GluA, apigenin-AGlu, kaempferol-AGlu and chrysosplenol were detected for the first time in *C. morifolium* flower. It is noted that luteolin and kaempferol have the same molecular weight, but their retention times vary owing to the difference in position of the hydroxyl group. Our investigation suggested that luteolin is eluted faster than kaempferol under the

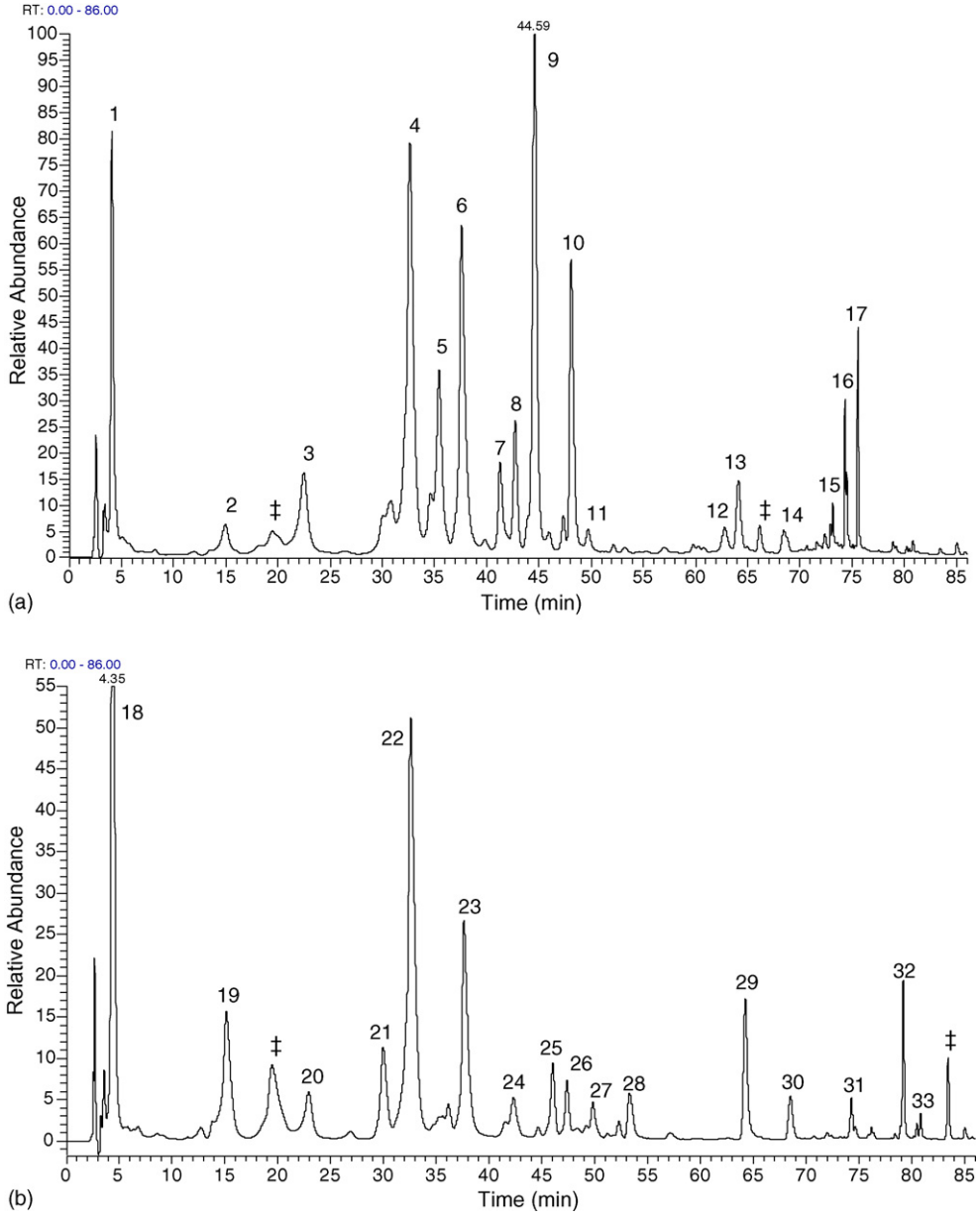


Fig. 2. Chromatograms of (a) *C. morifolium* flower; (b) *C. morifolium* leaf; (c) *A. annua* leaf; and (d) *C. coronarium* leaf (chromatographic conditions: refer to Section 2.3) (*note*: (‡) no mass signal).

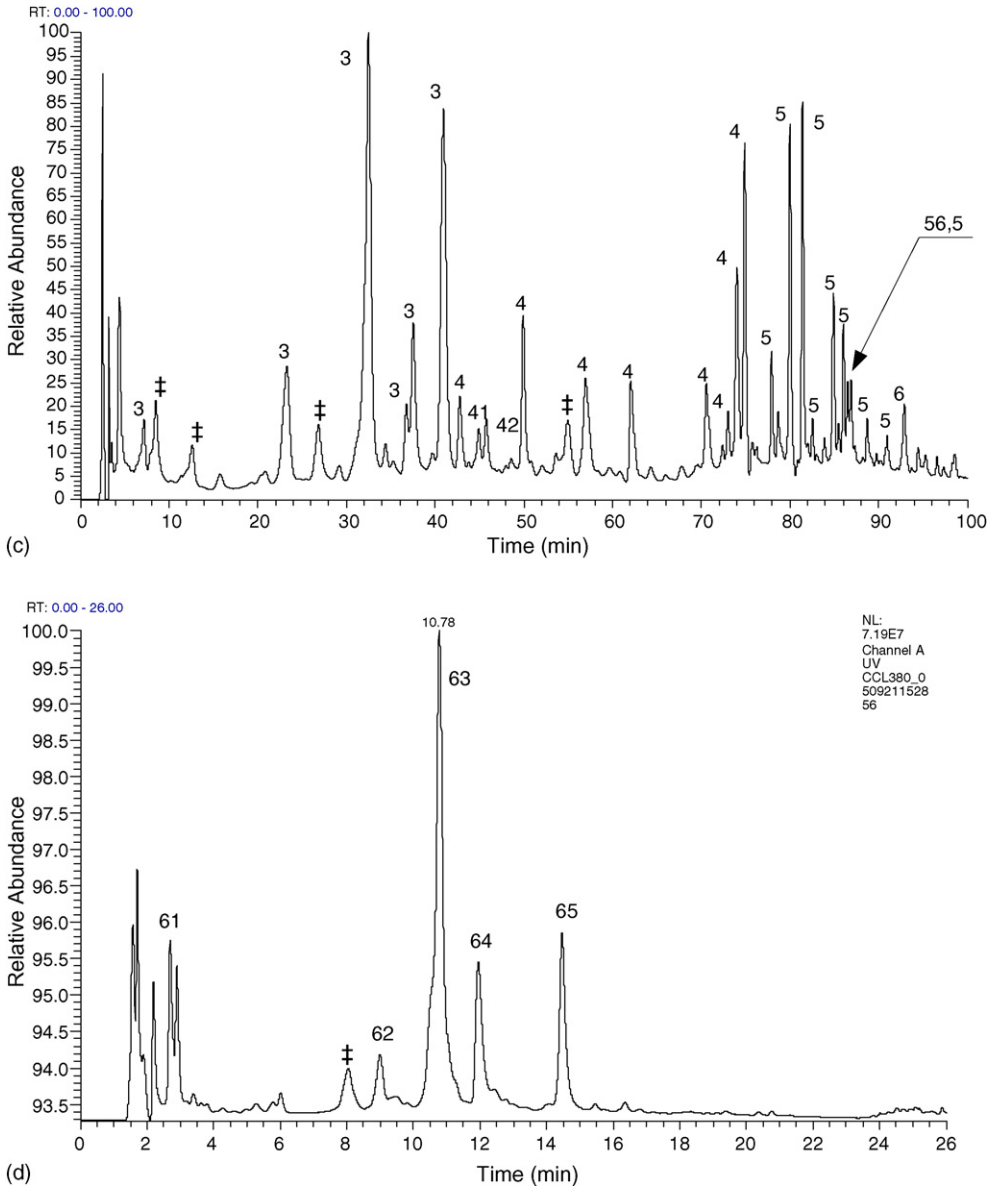


Fig. 2. (*Continued*).

proposed chromatographic condition. This is due to the fact that luteolin is more polar than kaempferol owing to the different position of hydroxyl. For kaempferol, the charge of hydroxyl group on C3 (Table 1, R1) could be easily dispersed to A and B rings when conjugation occurred. As a result, the polarity of hydroxyl on C3 in kaempferol is weakened. On the other hand, the polarity of hydroxyl group on C3′ (Table 1, R4) in luteolin is likely to be only slightly weakened. Their corresponding glycosyl compounds appeared with the same elution order. Thus it is possible to differentiate those molecules which posses the same molecular weights but different position of functional groups. The main components of *C. morifolium* flower are chlorogenic acid (CGA), quercetin-Glu, luetolin-AGlu, apigenin-Glu and apigenin-AGlu. The typical mass identification and proposed chemical structures of peak **5** and **9** are shown in Fig. 3. Incidentally, CGA was recently reported to be able to inhibit the proliferation of human cancer cells *in vitro*. It was also able to increase the enzymatic activities of various phase 2 metabolic enzymes, including tathione *S*-transferases (GST) and NAD (P) H: quinone oxidoreductase [21].

3.3. Identification of flavonoids and CQAs in Chrysanthemum morifolium leaf

The *C. morifolium* leaf was also analyzed using the same conditions as that for the flower and the results are shown in Fig. 2b and Table 2 (peak **18**–**33**). It is interesting to note that there are numerous components such as CGA, luteolin-AGlu, luetolin-GluA, kaempferol-GluA, apigenin-Neo, linarin, acacetin-AGlu and chrysosplenol that could be found in both flower and leaf of *C. morifolium*, except for the difference in concentrations. This finding suggests that the flavonoids in different parts of the plant of *C. morifolium* are rather similar. The distinct differences are peak **4** (Fig. 2a) and **22** (Fig. 2b) which have the same retention

Table 2
Flavonoids and CQAs of the three *Composiate* plants identified by mass spectra and UV-DAD

Peak no.	Mass spectra (molecular ion/fragmental ions)	UV (λ_{max})* (nm)	References	Proposed compounds
Chrysanthemum morifolium flower				
1	354.9/193.1, 163.1	194, 218, 326		Chlorogenic acid
2	463.2/287.2	198, 284, 326	[10]	Luteolin-GluA**
3	463.2/287.2	216, 236, 328		Kaempferol-GluA
4	465.2/303.1	194, 218, 326	[10]	Quercetin-Glu
5	449.1/287.2	204, 266, 348	[3,10,23]	Luteolin-Glu
6	491.1/287.2	218, 242, 326	[10]	Luteolin-AGlu
7	491.1/287.2	206, 266, 348		Kaempferol-AGlu
8	579.1/433.1, 271.2	200, 266, 338	[10]	Apigenin-Neo
9	433.0/271.2	198, 268, 338	[1,2]	Apigenin-Glu
10	475.1/271.2	198, 268, 338		Apigenin-AGlu
11	463.2/301.1, 287.2	206, 268, 344		Kaempferide-Glu
12	287.2	212, 266, 346	[2,23]	Luteolin
13	593.1/447.0, 285.2	208, 268, 334	[1,2,4]	Linarin
14	489.0/447.0, 285.2	236, 268, 334	[2]	Acacetin-AGlu
15	271.2	238, 268, 330	[10,23]	Apigenin
16	361.2/331.2, 301.2	232, 298, 310		Chrysosplenol C
17	361.2/331.2, 301.2	230, 296, 310		Chrysosplenol D
Chrysanthemum morifolium leaf				
18	354.9/163.1	194, 218, 326		Chlorogenic acid
19	463.1/287.2	200, 284, 328	[10]	Luteolin-GluA
20	463.1/287.2	216, 234, 328		Kaemperol-GluA
21	445.2/271.2	198, 268, 336	[3,10]	Apigenin-GluA
22	516.8/449.0, 354.9, 163.0	218, 240, 326	[3,17,27]	3,5-DCQA
23	491.1/287.2	218, 242, 326		Luteolin-AGlu
24	579.0/432.9, 271.2	200, 288, 328	[10]	Apigenin-Neo
25	611.0/464.9, 431.0, 303.0	200, 284, 328	[10]	Quercetin-Neo
26	609.0/463.0, 301.2	204, 268, 346		Kaempferide-Neo
27	463.0/301.0	204, 254, 346		Kaempferide-Glu
28	505.0/301.1	206, 268, 332		Kaempferide-AGlu
29	593.0/447.0, 285.1	198, 268, 334	[1,2,4]	Linarin
30	489.0/447.0, 285.2	236, 268, 334	[2]	Acacetin-AGlu
31	361.2/331.2, 316.2	216, 274, 346		Chrysosplenol C
32	345.2/330.2	216, 274, 344		Chrysosplenol
33	375.1/360.1, 317.2	223, 278, 350	[18,24]	Chrysosplentin
Artemisia annua leaf				
34	354.9/193.1	226, 288, 336		Chlorogenic acid
35	193.1/178.1	204, 228, 344		Quinic acid
36	494.9/433.1,303.2	216, 244, 328	[23]	Quercetin-Glu
37	494.9/333.2,318.2	210, 258, 348		Mearnsetin-Glu
38	494.9/333.2,303.2	220, 242, 326		Laricitin-Glu
39	207.1/191.2	230, 294, 344		Flaviolin
40	448.9/303.2, 287.1	206, 256, 344		Quercetin-Rhad
41	479.0/317.2	236, 298, 328		Isorhamnetin-Glu
42	479.0/317.2	236, 298, 324		Rhamnetin-Glu
43	677/357, 339, 265, 177	238, 328	[19]	GMC
44	303.2	236, 296	[2,13,19,23]	Quercetin
45	287.2	244, 298, 328	[2,19,23]	Luteolin
46	317.2/262.1	232, 290, 330	[21]	Isorhamnetin
47	271.2	238, 298, 334	[10,19,23]	Apigenin
48	301.1/287.2	244, 288, 314		Kaemfperide
49	317.1/302.2, 286.1	196, 232, 292	[10,22]	Rhamnetin
50	361.2/345.2, 303.1	236, 258, 350		Chrysosplenol C
51	361.2/346.2, 303.1	230, 260, 345	[22]	Chrysosplenol D
52	315.1/282.0, 254.1	230, 276, 334		Pilloin
53	345.2/331.2, 315.1	228, 280, 348		Chrysosplenol
54	375.2/359.2, 342.1, 317.2	225, 278, 350	[22]	Chrysosplentin
55	301.2/285.2, 259.2	238, 298, 319,		Kaempferide
56	301.2/285.2	236, 296, 325		Isokaempferide
57	331.2/316.2, 301.2, 271.1	239, 296, 335		Cirsiliol
58	361.2/331.2, 303.2	236, 265, 348		Isochrysosplenol C
59	389.2/374.2, 356.2, 331.2	241, 286, 327		Unknown
60	361.1/301.2	237, 290, 332		Isochrysosplenol D

Table 2 (*Continued*)

Peak no.	Mass spectra (molecular ion/fragmental ions)	UV (λ_{max})* (nm)	References	Proposed compounds
Chrysanthemum coronarium leaf				
61	354.9/163.1	194, 218, 326		Chlorogenic acid
62	516.9/449.0, 163.1	220, 242, 328	[16,17]	1,5-DCQA
63	516.9/449.0, 163.1	218, 242, 326	[16,17]	1,3-DCQA
64	516.9/449.0, 163.1	220, 242, 326	[15,17]	3,5-DCQA
65	616.8, 499.1, 437.0, 163.1	220, 242, 330	[25]	4-Succinyl-3,5-DCQA

Note: (*) All data from DAD; (**) GluA: glucuronide; Glu: glucoside; AGlu: acetyl glucoside; Neo: neohesperidoside; GMC: 7-*O*-Glu-[5′-*O*-Glu-(2″-*O*-GluA)]-4-methylcoumarine; DCQA: dicaffeoylquinic acid; Rha: rhamnoside.

time but with different mass spectra. The mass spectrum of peak **4** suggested that it is quercetin-Glu which possesses both molecular ion *m/z* 465.2 and fragmental ion *m/z* 303.1 while peak **22** is DCQA which produces the notable molecular ion *m/z* 516.8 and fragmental ions *m/z* 449.0, 354.9 and 163.0 in positive mode. In addition, it is also interesting to note that the main components in *C. morifolium* flower, apigenin-Glu (peak **9**) and apigrnin-AGlu (peak **10**), could not be detected in *C. morifolium* leaf. In contrast, quercetin-Neo (peak **25**), kaempferide-Neo (peak **26**), kaempferide-AGlu (peak **28**), chrysosplenol (peak **32**) and chrysosplentin (peak **33**) were found in the leaf but could not be detected in flower. The main constituents of *C. morifolium* leaf are CGA (**18**), 3,5-DCQA (**22**), luteolin-AGlu (**23**), linarin (**29**) and chrysosplenol (**32**). The typical mass spectra of peak **18** and **29** in *C. morifolium* leaf are shown in Fig. 4. The results suggest that peak **18** has the molecular ion *m/z* 354.9 and the

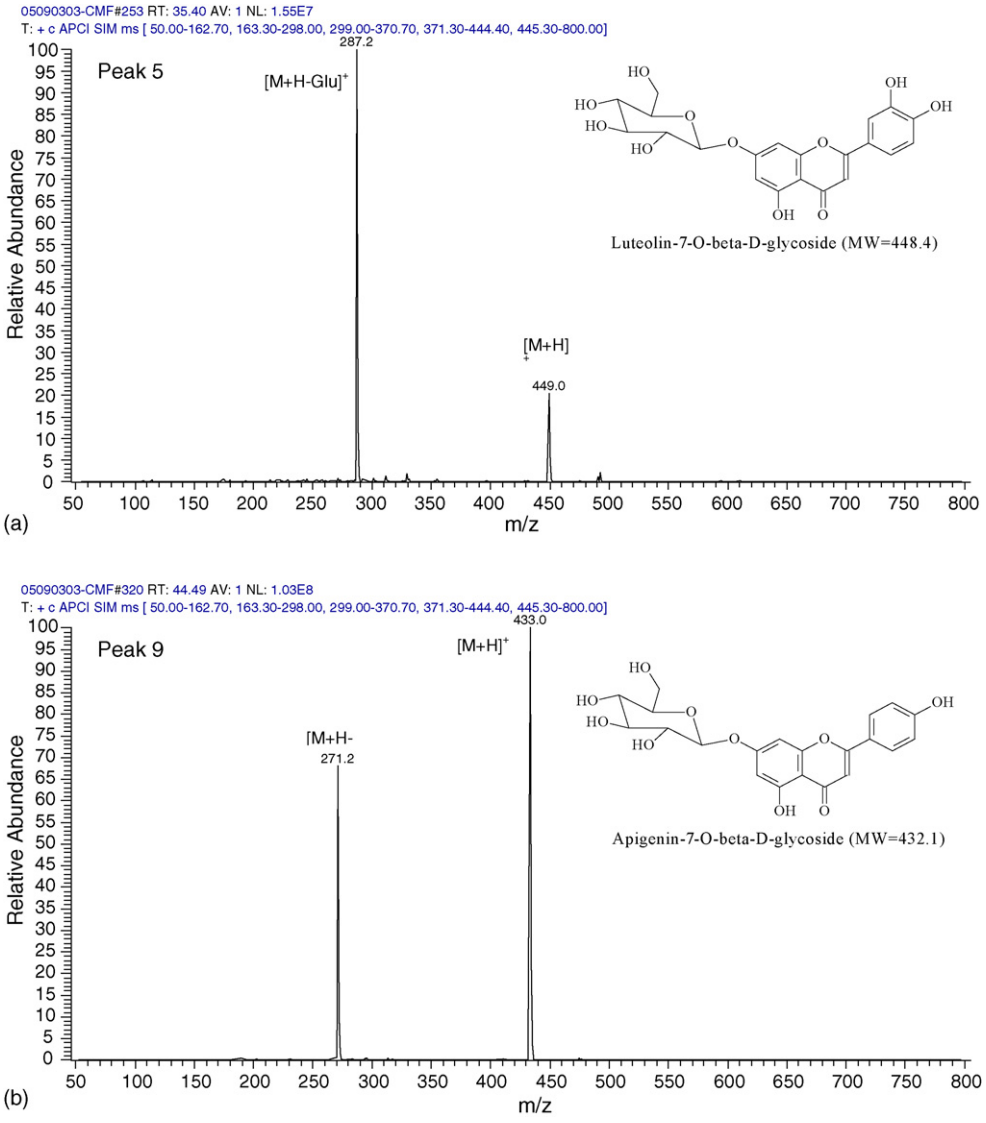


Fig. 3. The mass spectra and proposed chemical structures of peak **5** and **9** in *C. morifolium* flower.

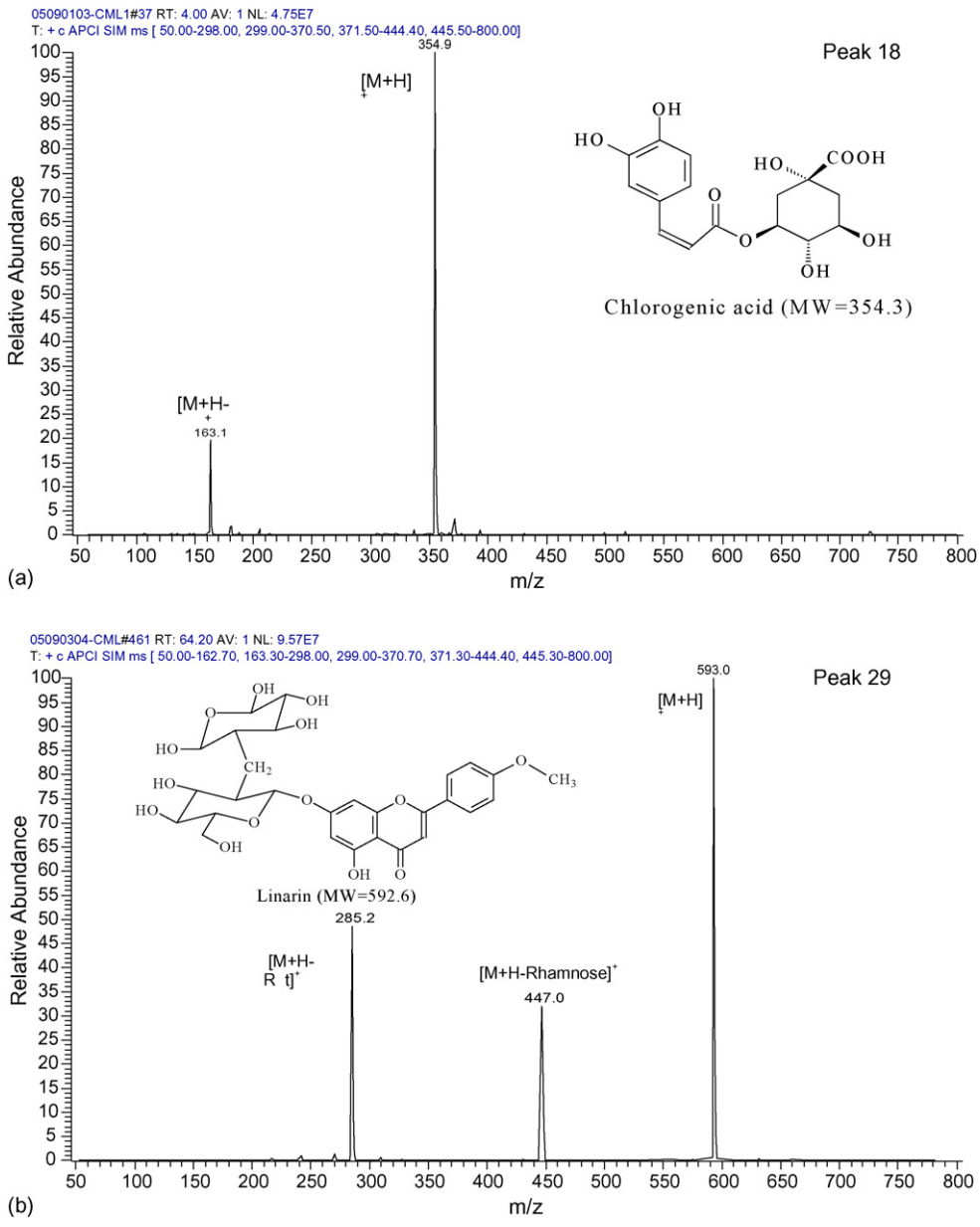


Fig. 4. The mass spectra and proposed chemical structures of peak **18** (a) and peak **29** (b) in *C. morifolium* leaf.

fragmental ion *m/z* 163.1 (Fig. 4a) in positive mode. The typical mass characters evidently prove that peak 18 is CGA. Peak **29**, which molecular ion *m/z* 593.0 and fragmental ion *m/z* 447.0 and 285.2 in positive mode, was identified as linarin (Fig. 4b) [1,2,4]. The mass spectra, UV and references of other components in *C. morifolium* leaf are listed in Table 2 (peak **18**–**33**).

3.4. Identification of flavonoids and CQAs in Artemisia annua

A. annua has been used as a traditional medicine to treat malaria and dysentery for centuries [22]. Certain methoxylated flavones isolated from *A. annua* have been identified [23–25], although their specific functions have not been verified. Our LC-UV chromatogram (Fig. 2c) suggests that there are more than 40 components when detected at 335 nm, and more than 27 of them could be identified by MS^1 (peak **34**–**60**). The main components are quercetin-Glu (**36**), flaviolin (**39**), rhamnetin (**49**), chrysosplenol D (**51**) and pilloin (**52**). These flavonoids are very different from those found in the leaf or flower of *C. morifolium*. The UV and mass spectra data demonstrate that there are more methylated flavones (Fig. 2c and Table 2, peak **46**, **48**–**60**) existed in *A. annua* than in *C. morifolium*. It is also noted that the component in *A. annu* methanolic extraction is generally more complex. The bioactivities of some of these components are worthy to be further investigated. The mass spectra and their proposed chemical structures of peak **39** and **52** in *A. annu* are indicated in Fig. 5. It is noted that peak **52** (Fig. 5b) produces *m/z* 315.2, 300.1, 282.0 and 254.1 which correspond to the $[M+H]^+$, $[M+H–CH_3^{\bullet}]^+$, $[M+H–CH_3^{\bullet}–H_2O]^+$ and $[M+H–2\ OCH_3^{\bullet}]^+$ (chrysin) frag-

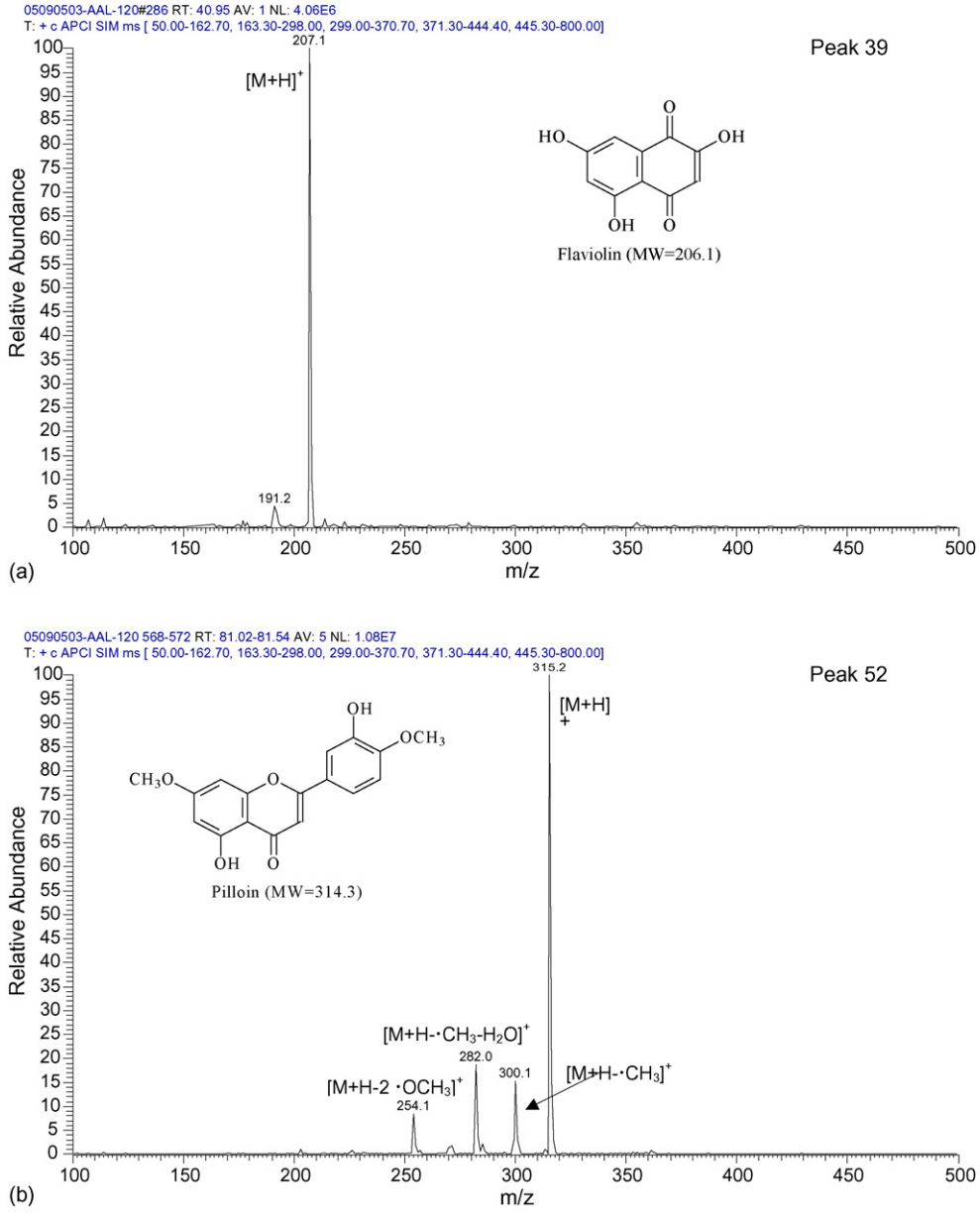


Fig. 5. The mass spectra and proposed chemical structures of peak **39** (a) and peak **52** (b) in *A. annua* leaf.

ments. Therefore, this peak was identified as pillion, first time that has been identified in this plant. It is reported that these methylated flavonoids exhibit specific fragmentation with the loss of •CH3 radical from the parent molecules [9,10]. Our study also suggested that these methylated flavonoids could easily lose their methyl radicals even without induced collision energy to produce fragmental ions, which are favorable of the structure identification. The other components of *A. annu* are listed in Table 2 (peak **34**–**60**).

3.5. Identification of CQAs in Chrysanthemum coronarium

It has been reported earlier that 3,5-DCQA and 4-succinyl-3,5-DCQA could be found in *C. coronarium* [26]. DCQA and dicaffeoyltartaric acids have also been shown to be potent inhibitors of HIV-1 integrase and viral replication [27,28]. Our results further confirmed that these two DCQAs are present in *C. coronarium*. Furthermore, the present results showed that CGA and other two DCQAs could also be detected in this *Compositae* plant (Fig. 2d). Our investigation suggested that peak **61**, **62** and **63** (Table 2) produced the same molecular ions *m/z* 517.0 in positive mode and *m/z* 515.2 in negative mode. It has been reported that there are six positional isomers of DCQAs, including 1,3-, 1,4-, 1,5-, 3,4-, 3,5- and 4,5-DCQA. However, they are able to be differentiated by MS^1–MS^4 fragments [17]. Comparing our mass spectra with the literature, peak **61**, **62** and **63** were identified as 1,5-, 1,3- and 3,5-DCQA, respectively. The identifications and chemical structures of peak **63** and **65** are shown in Fig. 6.

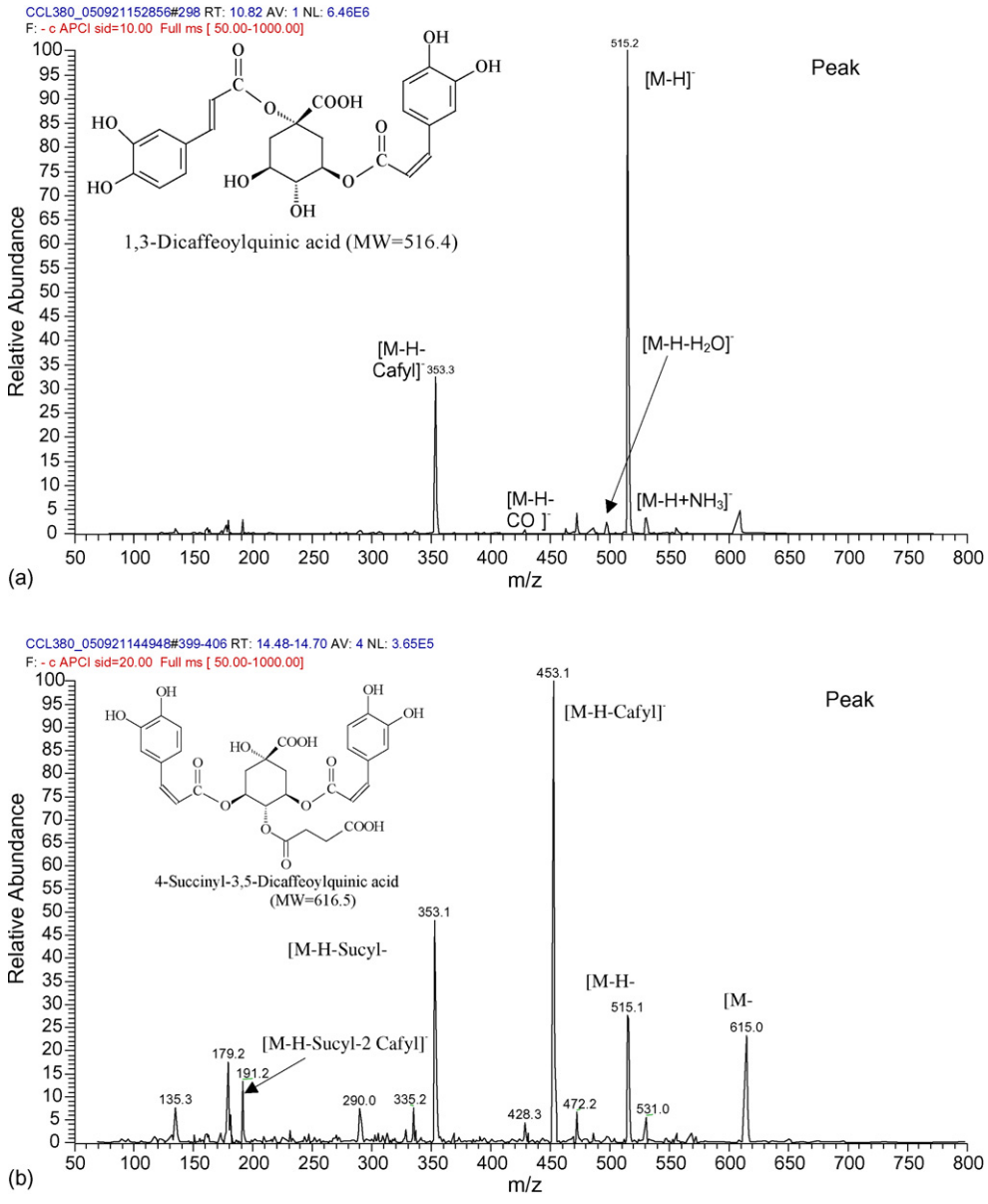


Fig. 6. The mass spectra and proposed chemical structures of peak **63** (a) and peak **65** (b) in *C. coronarium* leaf.

4. Conclusion

In this study, we attempt to develop a liquid chromatography-diode array detector-atmospheric pressure chemical ionization/mass spectrometry (LC/DAD-APCI/MS) method for comprehensive identification and characterization of the flavonoids and CQAs in *Compositae* plants. The identification and evaluation were performed by comparing the retention time, UV and mass spectra of samples with standards or/and earlier publications. The proposed method is rapid and reproducible for identification and characterization of various flavonoids in *Compositae* plants. The proposed method and flavonoids obtained are also useful for further chromatographic fingerprinting of plant flavonoids. The overall findings of this study suggest that although all three plants studied are from the same *Compositae* family their bioactive components and concentrations varied significantly. It is worthwhile to further examine the bioactivity and the specific medicinal property of the major flavonoids that have been identified in these plants. This investigation is currently being carried out in our laboratory.

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