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Full Length Research Paper

Liquid chromatography–mass spectrometry-electrospray ionisation analysis of *Centella asiatica* l., *Curcuma longa* L. and *Strobilanthes crispus* L. methanol extracts

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All the three plants were analysed in liquid chromatography-mass spectrometry-electrospray ionisation to identify plant active principles. Chromatographic separation of the compound of interest was achieved on C-18 column with detection in positive ion mode. The test showed that the most active principles found in *Curcuma longa* L. were curcuminoids mainly curcumin, demethoxycurcumin, bisdemethoxycurcumin, and dihydrocurcumin. In *Centella asiatica* L. madecassic acid was found at ion/mz 490. While the active principle in *Strobilanthes crispus* L. is verbascoside, which was detected at ion/mz 625. As plant active principles were excellently determined, the plant bioactivities such as anti-cancer, antiviral and/or antibacterial could be explored and developed.

Key words: *Centella asiatica* L., *Curcuma longa* L., *Strobilanthes crispus* L., liquid chromatography–mass spectrometry-electrospray ionisation (LC-ESI- MS), curcuminoids, madecassic acid, verbascosid.

INTRODUCTION

Chromatography is one of the most powerful, versatile and well established analytical separation techniques to modern chemist, due to its capacity to determine a single component present in a mixture in one analytical procedure. In addition, chromatography is also able to handle various forms of samples which are gaseous, liquid or solid and can range in complexity containing widely different chemical species. In addition, the versatility of this separation technique comes from its competency to carry out an analysis on a simple, inexpensive thin layer plate (Derksen et al., 2002; Herbert and Robert, 2003). Thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC) are applied for phytochemical preparations of medicinal plant analysis. However, identification problems arise when reference standards are not available, and this is often the case for plant extracts. Screening with HPLC with on-line ultraviolet detection, semi-preparative isolation of unknown compound followed by hydrolysis and liquid or gas chromatography are time consuming and laborious. On the contrary, liquid chromatography-mass spectrometry (LC-MS) represents a fast, favorable and reliable method to analyze non-volatile compound (Pietta et al., 1994; Zhang et al., 2012).

The LC-MS is described by the mobile phase as liquid and the analytes will travel through the stationary phase

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in the column. Unlike other kinds of spectrometry, the mass spectrometry takes a brute-force approach to structure determination. The mass spectrometry (MS) detector revealed the amount of compounds and the molecular weight (Lehman, 2002). Furthermore, due to its high power of mass separation, very good selectivity can be obtained, a factor of utmost importance in trace analysis (Hostettmann and Wolfender, 1995). It has become an important method for the efficient detection and rapid identification of natural products in complex biological matrices such as plant extracts. For on-line MS detection, thermospray (TSP) and electrospray ionisation (ESI) interfaces have been used by different authors for the analysis of flavonoids and terpenes in plant extracts. Between them, ESI-MS allows a softer ionization, thus, it allows structural information to be obtained. Besides, ESI-MS can discriminate between various flavonoids classes, and due to the low level of fragmentation, the technique is suitable to characterize complex mixtures, like those herbal extracts (Mauri and Pietta, 2000).

Studies had shown that medicinal benefits of *Centella asiatica* L., *Curcuma longa* L., and *Strobilanthes crispus* L. (Somchit et al., 2004, 2005; Wang et al., 2010) highlighted the urgent need for analytical techniques to determine and identify their active compounds. The *C. asiatica* L. is an ayurvedic medicine and different uses are claimed for the plant. These properties have been ascribed to their active principles: asiatic acid, asiaticoside, medecassic acid, and madecassoside (Somchit et al., 2004). One of the earliest uses of chromatography is to separate and quantify curcumin as described by Srinivasan (1953). The method involved separation of curcumin using a liquid chromatographic procedure and spectrophotometric detection (Heath et al., 2003). Whilst in *S. crispus* L., other than caffeic acid, verbascoside has been reported to respond as anti-oxidant, antibacterial and antiviral agents (Benassi et al., 2008). Latest methods and techniques for identification and determination of active constituents in *C. asiatica* L., *C. longa* L., and *S. crispus* L. are available for electrospray mass spectrometric, HPLC, ultraviolet (UV), infrared, and nuclear magnetic resonance (NMR) analysis (Mauri and Pietta, 2000; Heath et al., 2003; Benassi et al., 2008; He et al., 1998).

A combination of LC and MS, using ESI has proven to be a useful on-line system to identify the component in botanical extracts (Pietta et al., 1994). Among the active principles present in medicinal plants, flavonoids, triterpenoids, terpenes, and caffeic acid derivatives attracted a great interest. An increasing number of publications have been reported on the chemistry of flavonoids especially due to their medicinal properties (antibacterial, antiviral, and anticancer) (Harborne et al., 1975), as well as triterpernoid, is generally among the major effective constituents of numerous medicinal plants for wide bioactivities, e.g., antitumor, antiviral, and anti-inflammatory activities. However, because of high polarity, thermal lability and low contents in plants may result in component losses during the processing steps (Li et al., 2005). There were numbers of studies using LC-mass spectrometry to identify the presence of flavonoids, triterpernoid, saponins, naphtalenes, naphthodianthrones and their glycosides (Derksen et al., 2002). The LC-MS-ESI has demonstrated its great advantages for structural analysis with high sensitivity, short time and low consumption of the samples (Li et al., 2005). Thus, the purpose of this study is to use LC-MS-ESI for the separation and determination of *C. asiatica* L., *C. longa* L., and *S. crispus* L. active constituents.

MATERIALS AND METHODS

Plant materials

The rhizomes of *C. longa* L. was obtained from Sungai Buloh, Selangor. While the two other plants, the leaves of *C. asiatica* L. and S. *crispus* L., were obtained from a farm in Batu Pahat, Johore. The botanical identification of collected plants was done by a taxonomist of plant genetics unit, Institute of Bioscience, Universiti Putra Malaysia. Voucher specimens (SK 571/05 for *C. longa* L., SK 271/02 for *C. asiatica* L., and SK 473/04 for S. *crispus* L.) are conserved at the Phytomedicinal Herbarium of Institute of Bioscience. All of them were washed thoroughly before air-dried for 24 h. Then, they were cut into smaller pieces and grounded into powder. The powder of all three plants (1000 g) was soaked in methanol in ratio 1:10 for 24 h. The procedure was repeated three times (Salleh et al., 2002). Then, the extracts were sieved by a Buchner filter and evaporated by rotary evaporator at 40°C for approximately 3 h. The filtrate then was again diluted with methanol, and the extract concentrations were in the range of 0.5 to 5 mg/ml prior to LC-MS separation (Mauri and Pietta, 2002).

Chemicals

Methanol (LC grade) obtained from Merck (Dramstadt, Germany) and deionized water (< 8 MΩ cm resistivity) from MiliQ Water purification system (Millipore, BedFord, MA, USA). Formic acid was obtained from Acros (Geel, Belgium). All LC solvents were degassed by vacuum filter over 0.45 µm membrane filter (Type RC, Scleicher and Schuell), prior to use.

Liquid chromatography with mass spectrometry

LC-MS separation was performed by using Esquire 3000 plus, connected with a HP 1100 Agilent HPLC (Agilent Technologies, Palo Alto, CA, USA). The column effluent directly introduced into the quadruple mass spectrometer operates in the positive ESI mode. MS data were recorded in the full scan mode (m/z 50 to 1000). A quantitative analysis was carried out by the monitoring of each protonated molecular ion ($[M+H]^+$) in the positive ion mode of ESI-MS. Detection and integration of chromatographic peaks were performed by the Agilent Chemstation data analysis system (Agilent Technology, Palo Alto, CA, USA).

LC separation was carried out at room temperature on Alltima – C_{18} column (5 µm particle size, 150 × 4.6 mm i.d). The following gradient system was used with a mobile phase A: 0.1% (v) formic acid (pH 2.6) in water, and a mobile phase B: methanol with 0.1% (v) formic acid (pH 2.6) delivered at 0.4 ml/min, A:B are 100:0 (0 min), 80:20 (7.5 min), 30:70 (12.5 min), 20:80 (15 min), and 80:20 (20 min) for a total run time of 20 min. The detector was set at 450 nm. The sample of 20 µl of diluting extract was suspended in 1 ml

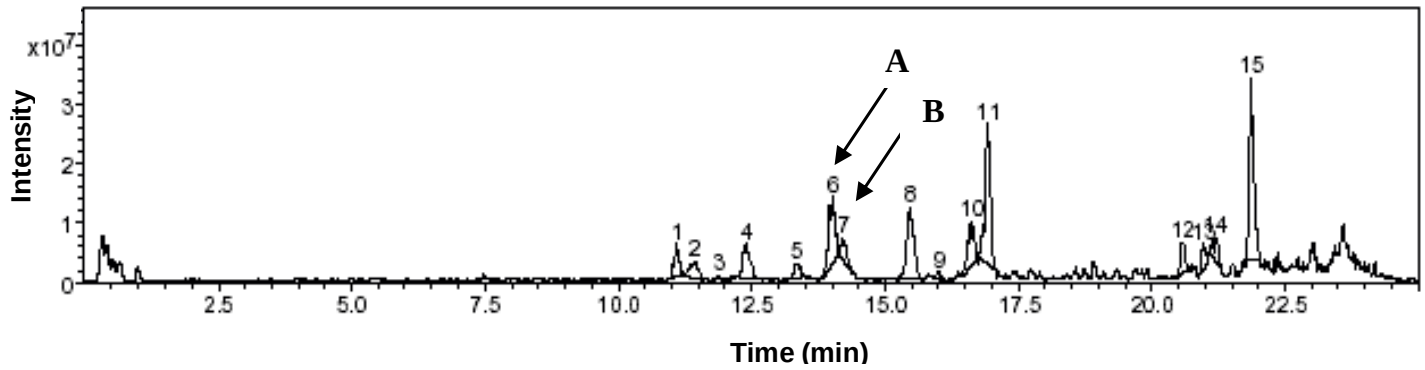


Figure 1. Typical positive mass spectrum of *C. asiatica* L. extract. Peaks 6 and 7 are madecassic acid (A and B). Chromatographic conditions: Column Alltima C_{18} (5 µm particle size, 150 × 4.6 mm i.d); linear gradient of 0.1% (v) formic acid (pH 2.6) in water (Solvent A), methanol with 0.1% (v) formic acid (pH 2.6) (Solvent B), A:B 100:0 (0 min), 80:20 (7.5 min), 30:70 (12.5 min), 20:80 (15 min), and 80:20 (20 min), at a flow rate of 0.4 ml/min. A mass spectrum was obtained by PI-ESI.

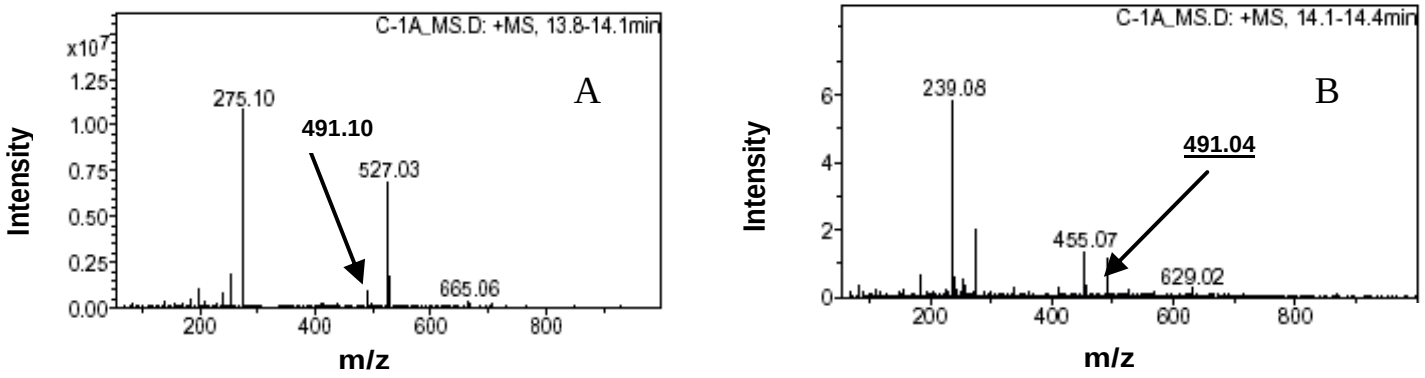


Figure 2. Mass spectra of the chromatographic peaks for individual sapogenins madecassic acid (at m/z 491.1 A and 491.04 B showed by arrow). Chromatographic conditions: Column Alltima C_{18} (5 µm particle size, 150 × 4.6 mm i.d); linear gradient of 0.1% (v) formic acid (pH 2.6) in water (Solvent A), methanol with 0.1% (v) formic acid (pH 2.6) (Solvent B), A:B 100:0 (0 min), 80:20 (7.5 min), 30:70 (12.5 min), 20:80 (15 min), and 80:20 (20 min), at a flow rate of 0.4 ml/min. Mass spectra were obtained with PI-ESI.

of water and filtered over a 0.45 µm membrane filter (Type RC, Scleicher and Schuell). This is to discard any other impurities that can hinder the actual active compound of the plants. The injection volume was 3 µl.

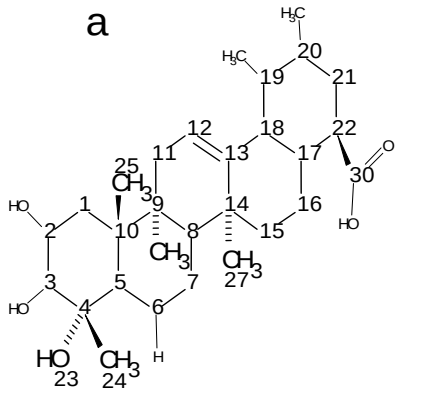
Nitrogen gas was used as nebulization and was delivered at a flow rate 12 L/min at 350°C. The nebulizer pressure was 50 psig, vaporizer temperature was 350°C, and capillary voltage was 3500 V.

RESULTS AND DISCUSSION

LC-ESI-MS analysis of *C. asiatica* L.

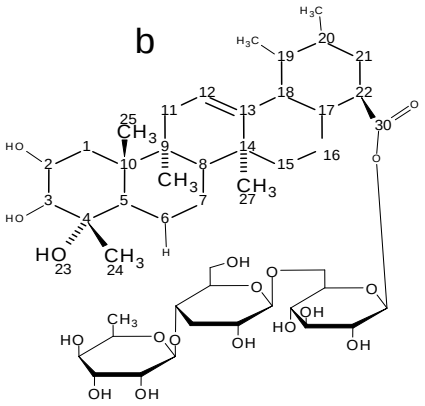
Triterpene glycosides (asiaticoside and madecassoside) and their sapogenins (asiatic acid and madecassic acid) are regarded as the active principles in *C. asiatica* L. (Castellani et al., 1981; Newall et al., 1996). The main ions m/z 474 and 490 correspond to asiatic acid and madecassic acid, respectively. While the ions m/z for asiaticoside is 929 and madecassoside is 945. Under optimum LC-ESI-MS conditions, however only madecassic acid was successfully screened at 13.8 to 14.4 min. Peaks 6 and 7 showed similar UV spectra (from typical positive mass spectrum) (Figure 1), which are characteristic of madecassic acid (Figure 2A and B).The chemical structures of these compounds obtained by ESI-MS are shown in Figure 3a, b, c and d.

Four active principles were screened out, there was only one compound screened which was sapogenin; madecassic acid. In contrast to results reported by some researchers, all four active principles in methanol extract of *C. asiatica* L. have been identified by using a reverse-phase gradient HPLC and ESI-MS, respectively (Mauri and Pietta, 2000; Inamdar et al., 1996). This discrimination could be due to different ions m/z reported for each of the active constituents, by which ions m/z 488 and 504 correspond to asiatic acid and madecassic acid, respectively (Mauri and Pietta, 2000). While the ions m/z for asiaticoside is 957 and madecassoside is 974.



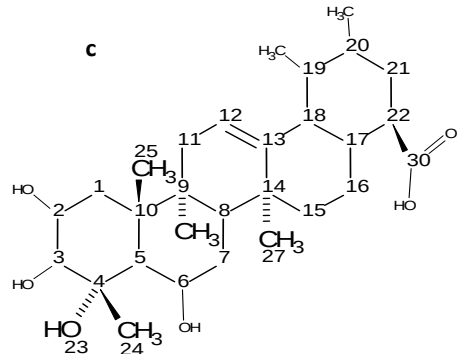
a

Molecular Formula = $C_{29}H_{46}O_5$
Formula Weight = 474.673
Composition = C(73.38%) H(9.77%) O(16.85%)
Molar Refractivity = 131.88 ± 0.4 cm^3
Molar Volume = 395.5 ± 5.0 cm^3
Parachor = 1068.2 ± 6.0 cm^3
Index of Refraction = 1.581 ± 0.03
Surface Tension = 53.2 ± 5.0 dyne/cm
Density = 1.20 ± 0.1 g/cm^3
Dielectric Constant = Not available
Polarizability = 52.28 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass = 474.334525 Da
Nominal Mass = 474 Da
Average Mass = 474.683489 Da



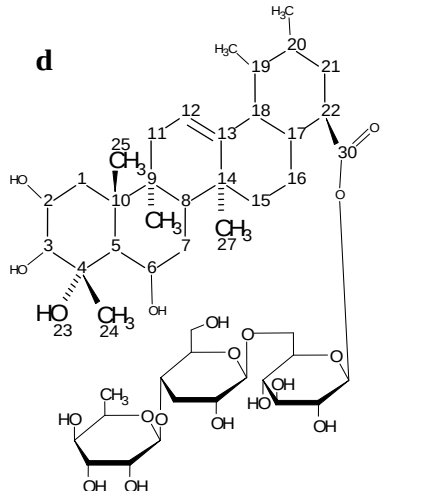
b

Molecular Formula = $C_{47}H_{76}O_{18}$
Formula Weight = 929.096
Composition = C(60.76%) H(8.24%) O(31.00%)
Molar Refractivity = 230.00 ± 0.4 cm^3
Molar Volume = 651.3 ± 5.0 cm^3
Parachor = 1913.7 ± 6.0 cm^3
Index of Refraction = 1.624 ± 0.03
Surface Tension = 74.5 ± 5.0 dyne/cm
Density = 1.42 ± 0.1 g/cm^3
Dielectric Constant = Not available
Polarizability = 91.17 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass = 928.50317 Da
Nominal Mass = 928 Da
Average Mass = 929.112398 Da



c

Molecular Formula = $C_{29}H_{46}O_6$
Formula Weight = 490.672
Composition = C(70.99%) H(9.45%) O(19.56%)
Molar Refractivity = 133.39 ± 0.4 cm^3
Molar Volume = 393.3 ± 5.0 cm^3
Parachor = 1083.2 ± 6.0 cm^3
Index of Refraction = 1.593 ± 0.03
Surface Tension = 57.4 ± 5.0 dyne/cm
Density = 1.24 ± 0.1 g/cm^3
Dielectric Constant = Not available
Polarizability = 52.88 ± 0.5 10^{-24} cm
Monoisotopic Mass = 490.32944 Da
Nominal Mass = 490 Da
Average Mass = 490.682794 Da



d

Molecular Formula = $C_{17}H_{76}O_{19}$
Formula Weight = 945.095
Composition = C(59.73%) H(8.11%) O(32.16%)
Molar Refractivity = 231.51 ± 0.4 cm^3
Molar Volume = 649.0 ± 5.0 cm^3
Parachor = 1928.7 ± 6.0 cm^3
Index of Refraction = 1.632 ± 0.03
Surface Tension = 77.9 ± 5.0 dyne/cm
Density = 1.45 ± 0.1 g/cm^3
Dielectric Constant = Not available
Polarizability = 91.77 ± 0.5 10^{-24} cm^3
Nominal Mass = 944 Da
Average Mass = 945.111703 Da
Monoisotopic Mass = 944.498085 Da

Figure 3. a: chemical structure of asiatic acid; b: chemical structure of asiaticoside; c: chemical structure of madecassic acid; d: chemical structure of madecassoside.

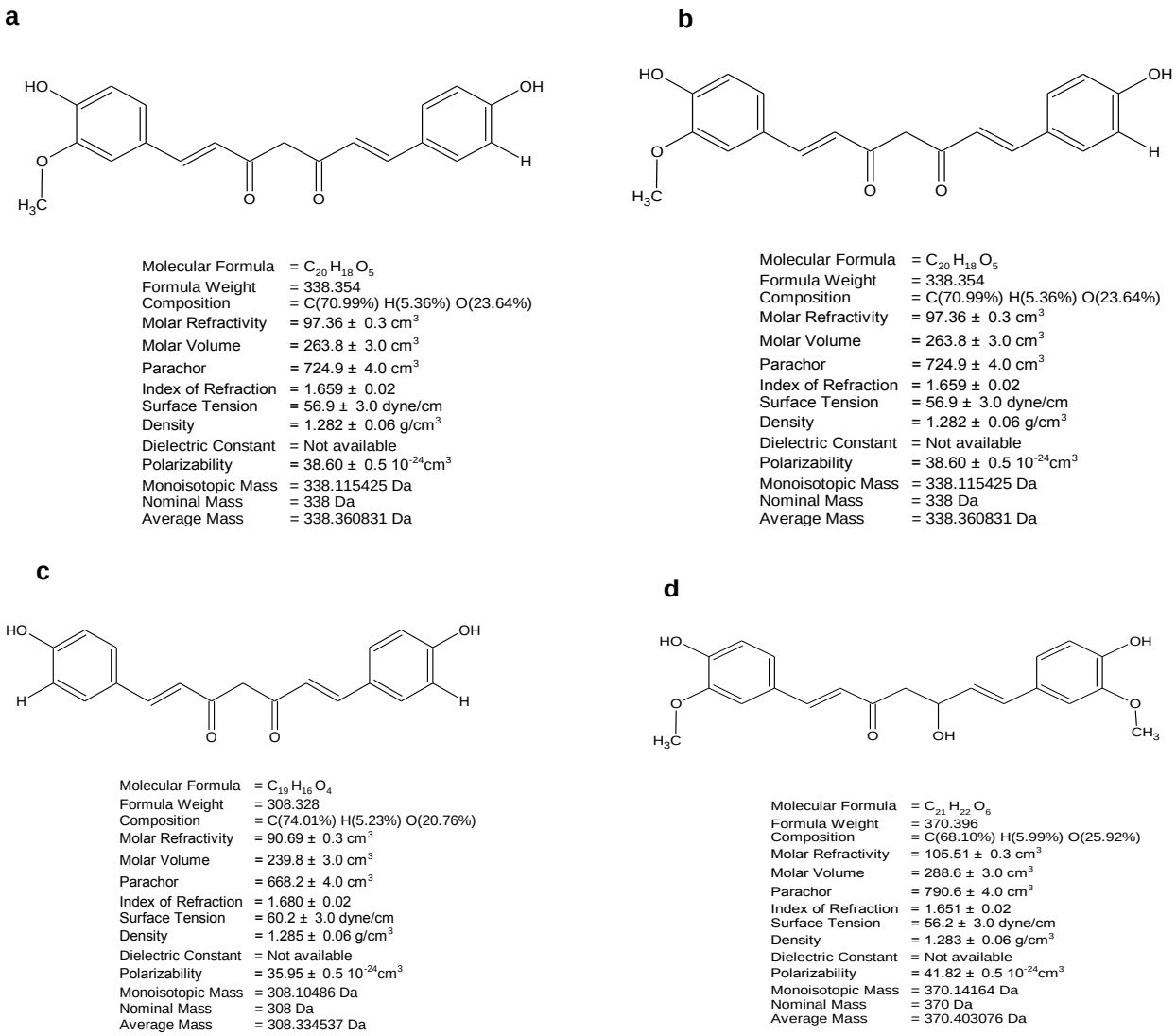


Figure 4. Chemical structures of: a: curcumin; b: demethoxycurcumin; c: bisdemethoxycurcumin; and d: dihydrocurcumin.

Besides, alternative explanation could be due to positive-ion (PI) mode of ESI used in this experiment which is less sensitive and producing unlimited fragmentation, though it can yield much more structurally significant information of the compound (Li et al., 2005), and characterised the unknown compounds (Rezanka and Dembitsky, 2003). It was also reported that all the four active constituents in *C. asiatica* L. were detected by NI-ESI (Mauri and Pietta, 2000).

A study has concluded that flavonoids analysis is best done in negative-ion (NI) mode because of its better sensitivity and limited fragmentation (Praisin et al., 2004). In addition, it was reported that NI could provide immediate information, including the acyl groups on aglycone and the type of esterification on glucuronyl residue (Li et al., 2005), that is, for the detection of saponins in extract of *Panax natoginseng.* Besides, previous study had reported that, madecassic acid was detected at 21 min by reverse-phase gradient HPLC (Inamdar et al., 1996) instead of 13.8 to 14.4 min by LC-ESI-MS in the present study. This inconsistent result indicates that madecassic acid in the samples could not be resolved under different chromatographic conditions and methods (Lu et al., 2004).

LC-ESI-MS analysis of *C. longa* L.

Three major yellow pigments, generally called curcuminoids are regarded as active principles in *C. longa* L. These diarylheptane derivatives also give *C. longa* L. its distinctive color. They are curcumin, demethoxycurcumin (DMC), bisdemethoxycurcumin (BDMC), and asymmetrical derivative, dihydrocurcumin (He et al., 1998). The chemical structures of these compounds are shown in Figure 4a, b, c and d.

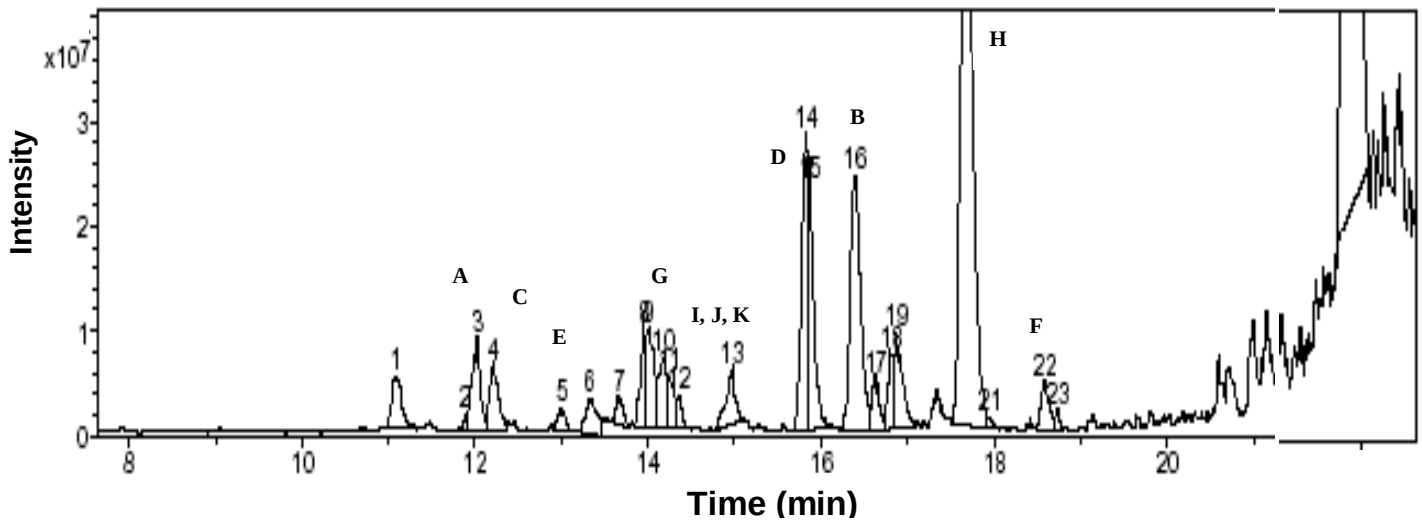


Figure 5. Typical positive mass spectrum of *C. longa* L. extract. Individual chromatographic peaks are alphabetically labeled. Chromatographic conditions: Column Alltima C_{18} (5 µm particle size, 150 × 4.6 mm i.d); linear gradient of 0.1% (v) formic acid (pH 2.6) in water (Solvent A), methanol with 0.1% (v) formic acid (pH 2.6) (Solvent B), A:B 100:0 (0 min), 80:20 (7.5 min), 30:70 (12.5 min), 20:80 (15 min), and 80:20 (20 min), at a flow rate of 0.4 ml/min. Mass spectra were obtained with PI-ESI.

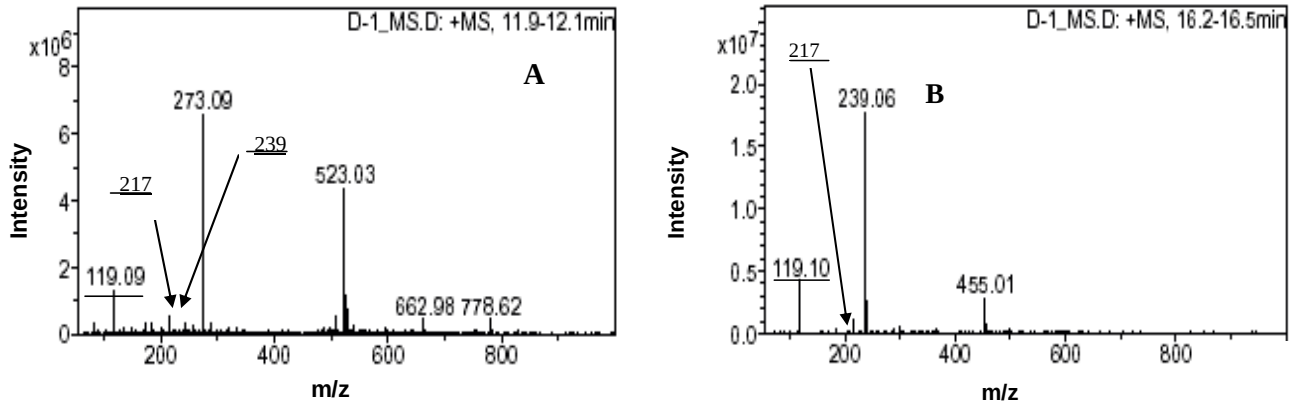


Figure 6. Mass spectra of the chromatographic peaks for individual *C. longa* L. essential oil ar-turmerone at m/z 217 and 239 and α cleavage to the aromatic ring at m/z 119 (underlined) (A and B). Chromatographic conditions: Column Alltima C_{18} (5 µm particle size, 150 × 4.6 mm i.d); linear gradient of 0.1% (v) formic acid (pH 2.6) in water (Solvent A), methanol with 0.1% (v) formic acid (pH 2.6) (Solvent B), A:B 100:0 (0 min), 80:20 (7.5 min), 30:70 (12.5 min), 20:80 (15 min), and 80:20 (20 min), at a flow rate of 0.4 ml/min. Mass spectra were obtained with PI-ESI.

The mass spectra for individual chromatographic peaks (alphabetically labeled) of *C. longa* L. extract are depicted in Figure 5. Peaks 3 and 16 showed a characteristic of ar-turmerone. The spectrum showed the molecular ion at m/z 217 (intense protonated molecule $[M + H]^+$), adduct ion $[M+Na]^+$ at m/z 239, and α cleavage to the aromatic ring at m/z 119 (Figure 6A and B). Peaks 4 and 15 were attributed to curcumenone, curcumenol, procurcumenol, and dehydrocurdione. All compounds showed intense protonated molecules $[M + H]^+$ at m/z 234 and less intense adducts ions $[M+Na]^+$ at m/z 257 (Figure 7C and D). Peaks 5 and 21 showed an intense protonated molecule $[M + H]^+$ at m/z 219 and adduct ion $[M+Na]^+$ at m/z 241. These two peaks can be attributed to curlone, α-turmerone, β- turmerone, and bisacumol which have the same molecular mass of 218 (Figure 8E and F). Peaks 8 and 20 were tentatively identified as germacrone-13-al and zingiberene, respectively based on their mass data (Figure 9G and H). Peak 13 showed the three major constituents of *C. longa* L., which are curcumin (I), DMC (J), and BDMC (K). Compounds I, J and K all showed very intense protonated molecules $[M + H]^+$ at m/z 369, 339, and 309; less intense adducts ions $[M+Na]^+$ at m/z 391, 361, and 331; and sodiated dimmer ions $[2M + Na]^+$

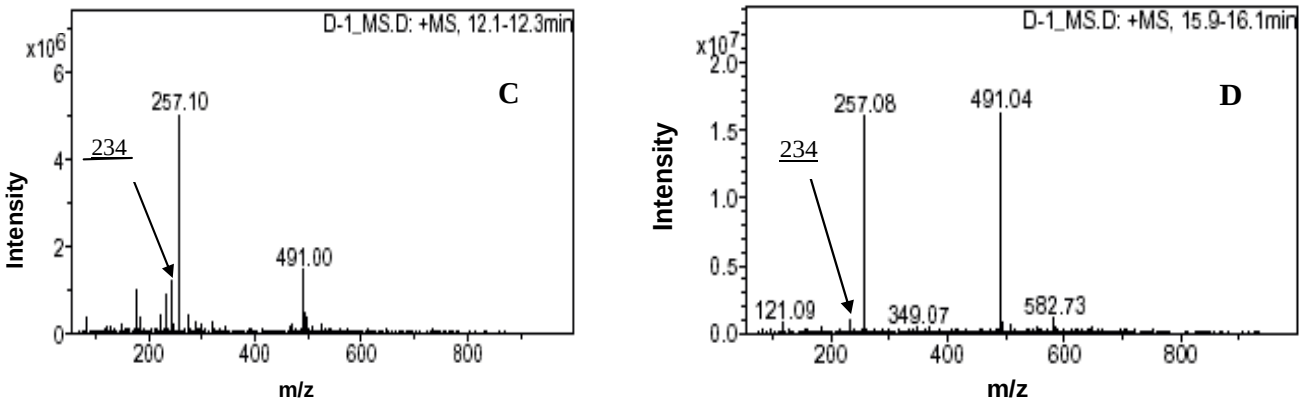


Figure 7. Mass spectra of the chromatographic peaks for individual *C. longa* L. essential oil curcumenone at m/z 234 and 257 (C and D). Chromatographic conditions: Column Alltima C_{18} (5 µm particle size, 150 × 4.6 mm i.d); linear gradient of 0.1% (v) formic acid (pH 2.6) in water (Solvent A), methanol with 0.1% (v) formic acid (pH 2.6) (Solvent B), A:B 100:0 (0 min), 80:20 (7.5 min), 30:70 (12.5 min), and 20:80 (15 min)- 80:20 (20 min), at a flow rate of 0.4 ml/min. Mass spectra were obtained with PI-ESI.

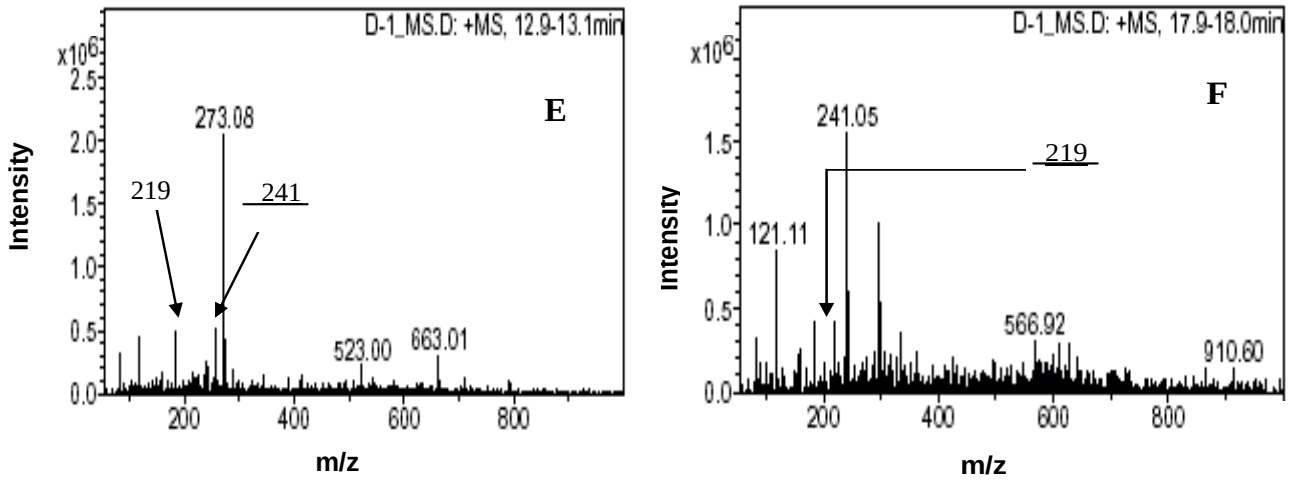


Figure 8. Mass spectra of the chromatographic peaks for individual *Curcuma longa* L. essential oil curlone, α-turmerone, β-turmerone and bisacumol at m/z 219 and 241 (E and F). Chromatographic conditions: Column Alltima C_{18} (5 µm particle size, 150 mm × 4.6 mm i.d); linear gradient of 0.1% (v) formic acid (pH 2.6) in water (Solvent A), methanol with 0.1% (v) formic acid (pH 2.6) (Solvent B), A:B 100:0 (0 min)-80:20 (7.5 min)-30:70 (12.5 min)- 20:80 (15 min)- 80:20 (20 min), at a flow rate of 0.4 ml/min. Mass spectra were obtained with PI-ESI.

at m/z 759, 699 and 639, respectively (Figure 10). Peak assignments and MS data for analysis of *C. longa* L. extract are shown in Table 1.

Chromatographic conditions

Column Alltima C_{18} (5 µm particle size, 150 × 4.6 mm i.d); linear gradient of 0.1% (v) formic acid (pH 2.6) in water (Solvent A), methanol with 0.1% (v) formic acid (pH 2.6) (Solvent B), A:B 100:0 (0 min), 80:20 (7.5 min), 30:70 (12.5 min), 20:80 (15 min), and 80:20 (20 min), at a flow rate of 0.4 ml/min. Mass spectra were obtained by PI-ESI.

In *C. longa* L. rhizome compound identification, LC-ESI-MS identified most of the active principles in the plant. It also showed that the mass spectra with hydrogen $[M + H]^+$ and sodium $[M + Na]^+$ adducts were observed in the chromatogram. This course is in agreement with the theory that the molecules need a functional group, which may donate a lone pair of electrons, to form stable hydrogen and sodium adducts (Blasco et al., 2004), and MS response signals of PI-ESI mode should come with the adducts (Luo et al., 2003).

The molecular weight (MW) detection of plant compounds by LC-ESI-MS was consistent with many researchers (25, 14). Comparison with GC-MS also found that, MW detected was similar (25). However, they were

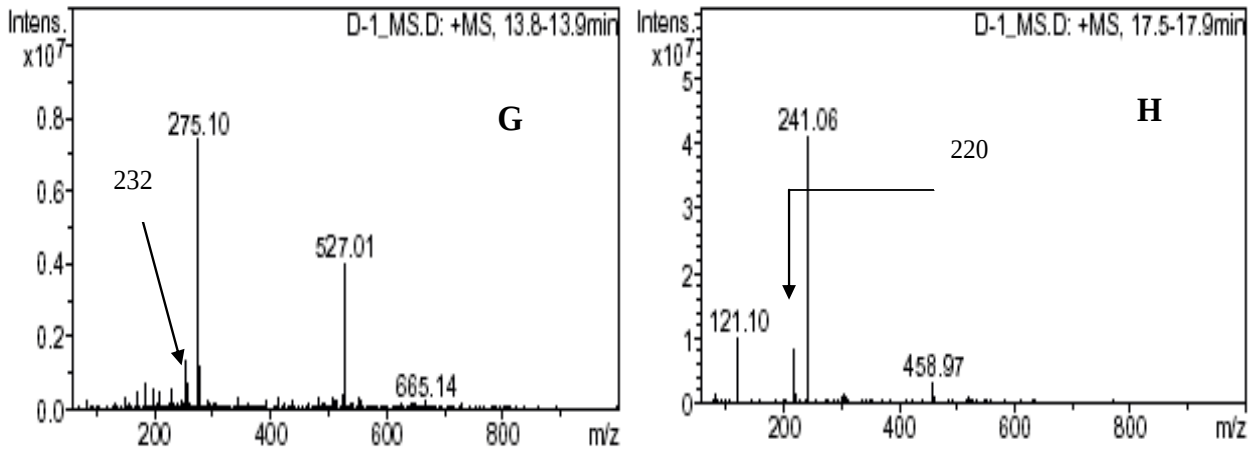


Figure 9. Mass spectra of the chromatographic peaks for individual *Curcuma longa* L. essential oil germacrone-13-al at m/z 232 (G), zingiberene at m/z 220 (H). Chromatographic conditions: Column Alltima C_{18} (5 µm particle size, 150 mm × 4.6 mm i.d); linear gradient of 0.1% (v) formic acid (pH 2.6) in water (Solvent A), methanol with 0.1% (v) formic acid (pH 2.6) (Solvent B), A:B 100:0 (0 min)-80:20 (7.5 min)-30:70 (12.5 min)- 20:80 (15 min)- 80:20 (20 min), at a flow rate of 0.4 ml/min. Mass spectra were obtained with PI-ESI.

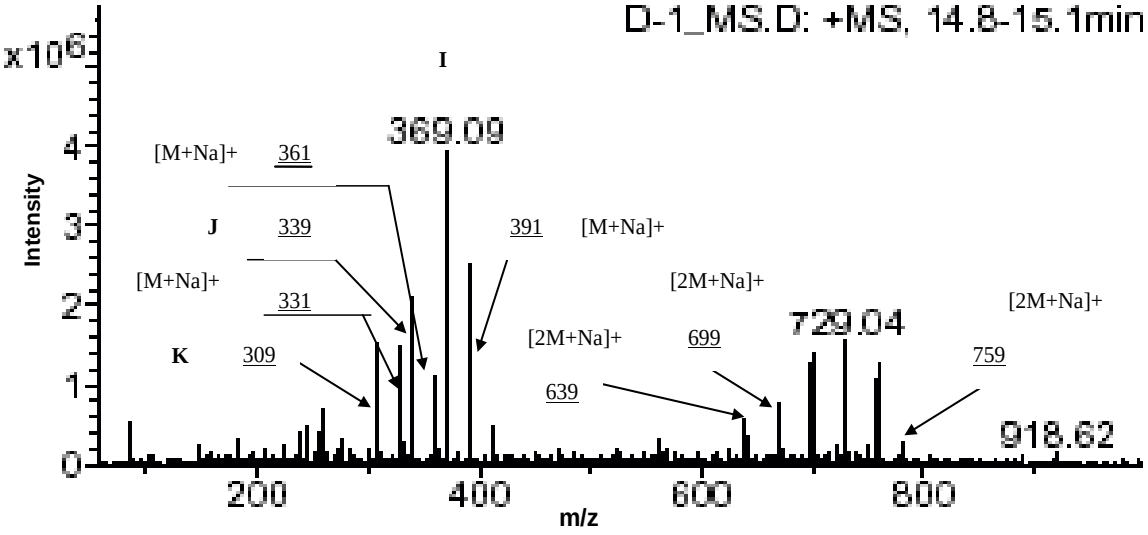


Figure 10. Mass spectra of the chromatographic peaks for individual *C. longa* L. curcuminoids; curcumin (I), DMC (J) and BDMC (K) at m/z 369, 339 and 309, respectively. Protonated molecules and sodium adducts ion are labeled. Chromatographic conditions: Column Alltima C_{18} (5 µm particle size, 150 × 4.6 mm i.d); linear gradient of 0.1% (v) formic acid (pH 2.6) in water (Solvent A), methanol with 0.1% (v) formic acid (pH 2.6) (Solvent B), A:B 100:0 (0 min), 80:20 (7.5 min), 30:70 (12.5 min), 20:80 (15 min), and 80:20 (20 min), at a flow rate of 0.4 ml/min. Mass spectra were obtained with PI-ESI.

detected at different retention time. There were numbers of studies reported regarding retention time of curcuminoids and sesquiterpenoids in *C. longa* L. In present analysis, Curcumin, BDMC and DMC were detected at 14.8 to 15.1 min. The result was inconsistent with many reports before, where curcuminoids were detected at 13 min and below, and this could be due to different chromatographic methods and conditions applied (Hiserodt et al., 1996; Tonnesen and Karlsen, 1983; Zhao et al., 2005; Peret-Almeida et al., 2005). Besides curcuminoids, sesquiterpenoids were also identified in *C. longa* L. at different retention time (Hiserodt et al., 1996). It has also been reported that curcuminoids and sesquiterpenoids in *C. longa* L. identification applied PI mode in ESI interface (He et al., 1998).

Table 1. t_R, MS data and peak assignments for the analysis of *C. longa* L. extract.

Peak number/compound number	t_R (min)	$[M + H]^+$ m/z	$[M + Na]^+$ m/z	$[2M + Na]^+$ m/z
3	12.0	217	239	-
4	12.2	234	257	-
5	13.0	219	241	-
8	14.0	232	-	-
	14.8	309	331	639
13	15.0	339	361	699
	15.3	369	391	759
15	15.9	234	257	-
16	16.4	217	239	-
20	17.7	220	-	-
21	17.9	219	241	-

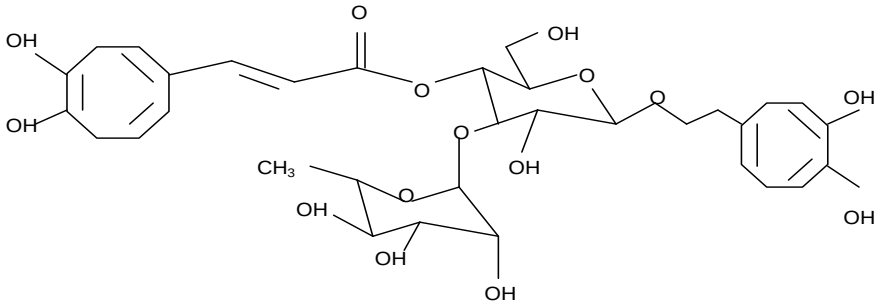


Figure 11. Chemical structure of verbascoside, active constituent of *S.crispus* L.

LC-ESI-MS analysis of *S. crispus* L.

Verbascoside was isolated from methanol extract of *S. crispus* L. leaves extract. It is a disaccharide ester containing a glucose unit linked to rhamnose and forming a glycoside. This compound is regarded as the active principle of the plant and has been claimed for many medicinal properties such as antioxidant and anticancer. The molecular formula of verbascoside is $C_{29}H_{36}O_{15}$ and the molecular weight is 624.594 (Benassi et al., 2008). The chemical structure of the active constituent is shown in Figure 11. Peak 3 showed a characteristic of verbascoside. It was detected at 7.9 min (Figure 12). A mass spectrum for verbascoside was showed in Figure 13.

In *S. crispus* L. LC-ESI-MS plant analysis, verbascoside which is considered as plant active constituent was successfully detected at 8 min. The verbascoside has a very complicated structure due to the present of glucose and rhamnose, and it caused overlapping of some spectrum. Besides, due to the complicated structure, the compound was not purely isolated. There are little impurities attached to sugar units, and impurities peaks were observed in the spectrum (Benassi et al., 2008). There is no report found for *S. crispus* L. plant analysis by using LC-ESI-MS or any other equivalent chromatographic separation. Thus, this study could be foundation for future plant compound identification.

Conclusion

Methanol was found to be the most suitable and chosen solvent in plant extraction method for plant compound analysis, than any other solvents as it extracts out most of the plant compounds. However, analysis of plant compound in *C. asiatica* L. and *C. longa* L. was reported to be most efficient in methanol extracts. Other solvents were also used to extract the plant compound, that is, ethanol. These differences, depends on the characteristics of the plant compound of interest that is to be isolated.

Many studies concluded that plant compound analysis is best done in NI-ESI mode. However, there were also experiments reported that, plant compound determination was done by both PI and NI-ESI to obtain more detailed

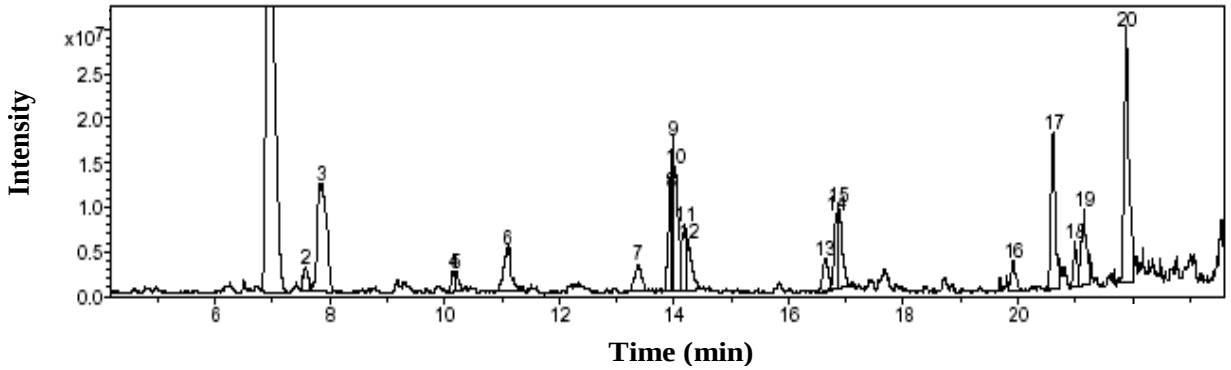


Figure 12. Typical positive mass spectrum of *S. crispus* L. extract. Peak 3 (arrowed) a characteristic of Verbascoside. Chromatographic conditions: Column Alltima C_{18} (5 µm particle size, 150 × 4.6 mm i.d); linear gradient of 0.1% (v) formic acid (pH 2.6) in water (Solvent A), methanol with 0.1% (v) formic acid (pH 2.6) (Solvent B), A:B 100:0 (0 min), 80:20 (7.5 min), 30:70 (12.5 min), 20:80 (15 min), and 80:20 (20 min), at a flow rate of 0.4 ml/min. Mass spectra were obtained with PI-ESI.

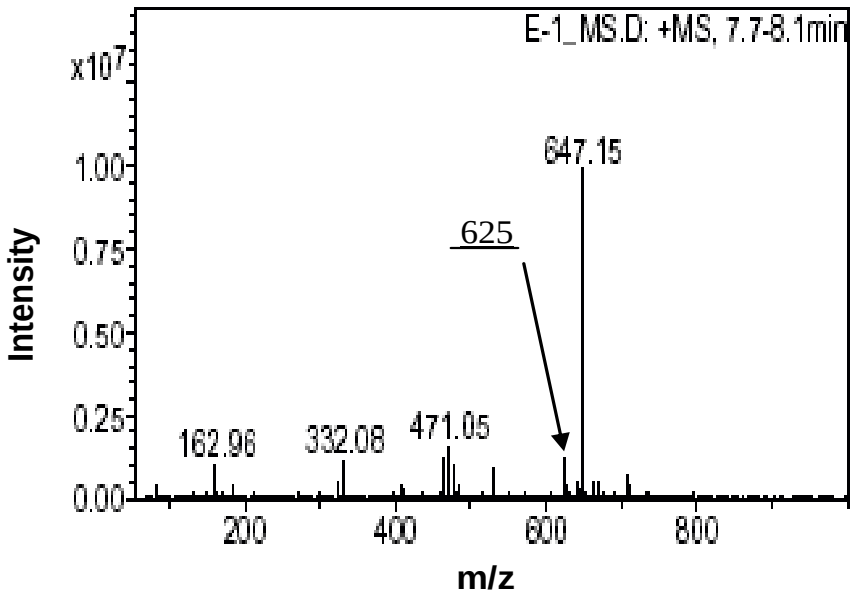


Figure 13. Mass spectra of the chromatographic peak for individual verbascoside at m/z 625 (arrowed). Chromatographic conditions*:* Column Alltima C_{18} (5 µm particle size, 150 × 4.6 mm i.d); linear gradient of 0.1% (v) formic acid (pH 2.6) in water (Solvent A), methanol with 0.1% (v) formic acid (pH 2.6) (Solvent B), A:B 100:0 (0 min), 80:20 (7.5 min), 30:70 (12.5 min), 20:80 (15 min), and 80:20 (20 min), at a flow rate of 0.4 ml/min. Mass spectra were obtained with PI-ESI.

structural information of the related constituents. ESI was employed because it produces a soft ionization and, thus, intense molecular ion species are generated. Chromatographic conditions, that is, the type of organic solvents and additives used have significant influence on ionisation efficiency in the ESI ion source. In this study, methanol was used as organic solvent with 0.1% formic acid, and almost all the compounds of interest from the plant were identified.

Furthermore, by using LC-ESI-MS techniques, active principles in the *C. asiatica* L., *C. longa* L., and *S. crispus* L. extracts identification can be done without, time consuming pre-purification step or optimization of chromatographic procedures. It is also, often unnecessary to use any extraction techniques that is capable to determine both free and conjugated forms of flavonoids. The techniques have showed good performances both in terms of sensitivity and specificity, and provided two independent parameters, that is, retention time and mass information. The ESI interface which is relatively simple has showed to be a robust instrument. A wide range of flavonoids and other

polyphenols can be analysed directly without derivatization, and ionisation technique requires very little sample work up. The coupling of LCMS with ESI has been demonstrated to be a very powerful tool for the identification of natural product in plant extracts owing to their soft ionisation, which favors the analysis of polar, non-volatile, and thermally labile kinds of compounds. Therefore, the LC-ESI-MS provide comparable types of spectroscopic information to those recorded for pure constituents, and their sensitivity does not compromise the HPLC separation.

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REFERENCES

Benassi R, Ferrari E, Lazzari S, Spagnolo F, Saladini M (2008). Theoretical study on Curcumin: A comparison of calculated spectroscopic properties with NMR, UV–vis and IR experimental data. J. Mol. Struct., 892: 168-176

Blasco C, Font G, Pico Y. (2004). Solid-phase microextraction-liquid chromatography-mass spectrometry applied to the analysis of insecticides in honey. J. Chromatogr. (A), 1028: 267-273.

Castellani C, Marai A, Vacchi P (1981). The *Centella asiatica*. Bollet. *chimica farmacia,* 1(20): 570-605.

Derksen GCH, Niederlander HAG, van Beek TA (2002). Analysis of anthraquinones in Rubia tinctorum L. by liquid chromatography coupled with diode-array UV and mass spectrometric detection. J. Chromatogr. (A), 978: 119-127.

Harborne JB, Mabry JJ, Mabry H (1975). Flavonoids. Chapman and Hall, London. p. 39.

He XG, Lin LZ, Lian LZ, Lindenmaier M (1998). Liquid chromatography–electrospray mass spectrometric analysis of curcuminoids and sesquiterpenoids in turmeric (*Curcuma longa*). J. Chromatogr. (B), 818: 127-132.

Heath DD, Pruitt MA, Brenner DE, Rock CL (2003). Curcumin in plasma and urine: quantitation by high-performance liquid chromatography. J. Chromatogr. (B), 783: 287-295.

Herbert CG, Robert AW (2003). Mass Spectometry Basics. CRC Press, Boca Raton, p. 245.

Hiserodt T, Hartman TG, Ho CT, Rosen RT (1996). Characterization of powdered turmeric by liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry. J. Chromatogr. (A), 740: 51-63.

Hostettmann K, Wolfender JL (1995). Phytochemistry of Medicinal Plants. Arnason JT, Mata R, Romeo JT. (Eds). Plenum Press, New York and London, p. 190.

Inamdar PK, Yeole RD, Ghogare AB, de Souza NJ (1996). Determination of biologically active constituents in *Centella asiatica*. J. Chromatogr. (A), 742: 127.

Lehman JW (2002). Multiscale Operational Organic Chemistry. A problem solving approach to the laboratory course. Upper Saddle River, NJ, Prentice Hall, p. 492.

Li B, Abliz Z, Tang M, Fu G, Yu S (2005). Rapid structural characterization of triterpenoid saponins in crude extract from *Symplocos chinensis* using liquid chromatography combined with electrospray ionization tandem mass spectrometry. J. Chromatogr. (A), 1101: 53-62.

Lu GH, Chan K, Chan CL, Leung K, Jiang ZH, Zhao ZZ (2004). Quantification of ligustilides in the roots of *Angelica sinensis* and related umbelliferous medicinal plants by high-performance liquid chromatography and liquid chromatography-mass spectrometry. J. Chromatogr. (A), 1046: 101-107.

Luo XB, Chen B, You SZ, Zeng JG (2003). Simultaneous analysis of caffeic acid derivatives and alkamides in roots and extracts of *Echinacea purpurea* by HPLC-photodiode array detection-electrospray mass spectrometry. J. Chromatogr. (A), 986: 73-81.

Mauri P, Pietta P (2000). Electrospray characterization of selected medicinal plant extracts. J. Pharma. Biomed. Anal., 23: 61.

Newall C, Anderson CA, Philipson JD (1996). Herbal Medicine- A Guide for Health Care Professionals. Pharmaceutical Press, London, p.170.

Peret-Almeida L, Cherubino APF, Alves RJ, Dufosse L, Gloria MBA (2005). Separation and determination of the physicochemical characteristics of curcumin, demethoxycurcumin and bisdemethoxycurcumin. Food Res. Int., 38: 1039-1044.

Pietta P, Facino RM, Carini M, Mauri P (1994). Thermospray liquid chromatography-mass spectrometry of flavonol glycosides from medicinal plants J. Chromatogr. (A), 661: 121-126.

Praisin JK, Wang CC, Barnes S (2004). Mass Spectrometric Methods for the Determination of Flavonoids in Biological samples. Free Rad. Biol. Med., 37: 1324-1350.

Rezanka T, Dembitsky VM (2003). Identification of acylated xanthone glycosides by liquid chromatography-atmospheric pressure chemical ionization mass spectrometry in positive and negative modes from the lichen *Umbilicaria proboscidea*. J. Chromatogr. (A), 995: 109-118.

Salleh MN, Runnie I, Roach PD, Mohamed S, Abeywardena MY. (2002). Antioxidant Activity of the Main Phenolic Compounds Isolated from Hot Pepper Fruit (*Capsicum annuum* L.) J. Agric. Food Chem., 50: 3693-3697.

Somchit MN, Sulaiman MR, Zuraini A, Samsuddin L, Somchit N, Israf DA, Moin S (2004). Antinociceptive and antiinflammatory effects of *Centella asiatica*. Indian J. Pharmacol., 36: 377-380.

Somchit MN, Zuraini A, Ahmad Bustamam A, Somchit N, Sulaiman MR, Noratunlina R (2005). Protective activity of turmeric (*Curcuma longa*) in paracetamol-induced hepatotoxicity in rats. Int. J. Pharmacol., 1: 252-256.

Tonnesen HH, Karlsen J (1983). High-performance liquid chromatography of curcumin and related compounds. J. Chromatogr., 259: 367-371.

Wang L, Zhang RM, Liu GY, Wei BL, Wang Y, Cai HY, Li FS, Xu YL, Zheng SP, Wang G (2010). Chinese herbs in treatment of influenza: A randomized, double-blind, placebo-controlled trial. Respirat. Med., 104: 1362-1369.

Zhao L, Huang C, Shan Z, Xiang B, Mei L (2005). Fingerprint analysis of *Psoralea corylifolia* L. by HPLC and LC-MS. J. Chromatogr. B, 821: 67-74.

Zhang AH, Sun H, Wang P, Ying Han Y, Wang X (2012). Modern analytical techniques in metabolomics analysis. Anal., 137: 293-300.