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Mass fragmentation study of the trimethylsilyl derivatives of arctiin, matairesinoside, arctigenin, phylligenin, matairesinol, pinoresinol and methylarctigenin: Their gas and liquid chromatographic analysis in plant extracts

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ABSTRACT

The mass fragmentation patterns and the characteristic behavior of the trimethylsilyl (TMS) derivatives of the dibenzylbutyrolactone-type (arctiin, arctigenin, methylarctigenin, matairesinoside, matairesinol) and those of the diphenylperhydrofurotetrahydrofurane-type (phylligenin, pinoresinol) lignans, obtained by gas chromatography–mass spectrometry (GC–MS), were presented. It was shown that upon acidic hydrolysis the dibenzylbutyrolactone-type lignans are stable while the diphenylperhydrofurotetrahydrofurane-type ones decompose. As a novelty to the field we confirmed that the fragment species of the derivatized lignan glycosides, in the presence of excess hexamethyldisilazane, leaded to their in situ derivatization. Quantification of the selective fragment ions of the TMS derivatives by GC–MS, in respect of the ions found one by one, and concerning the selective fragment ions {SFI(s)} in total, provided acceptable reproducibilities, suitable for quantitation purposes: varying between 1.20% and 6.6% relative standard deviation percentages (RSD%). For characterization of the behavior of various type of lignans, analyses were performed with the untreated and with the trifluoroacetic acid hydrolyzed plant extracts, from the same sample, in parallel, both by GC–MS and by high performance liquid chromatography–mass spectrometry, working in the positive electron ionization mode (HPLC–ESPI-MS). The analysis of lignans in fruit and leaf extracts (obtained from the *Arctium*, *Centaurea* and *Forsythia* plants) was confirmed both by GC–MS and by HPLC–ESPI-MS. Our multicomponent system (including the identification and quantification of sugars, sugar alcohols, and several members of various homologous series of acids, anthraquinones and flavonoids) has been extended to the analysis of lignan glycosides and to the free lignans. Reproducibilities in the quantitation of lignans in plant matrices, as averages on GC and HPLC basis, varied between 0.9% and 11% (RSD). The distribution of the lignan constituents was presented for 5 *Arctium*, for 8 *Centaurea* and for 4 *Forsythia* plant extracts: the total of lignan contents varied between 0.42 and 87.9 mg/g, respectively.

1. Introduction

The relevancy of lignans (being regarded as versatile, beneficial impact furnishing natural compounds) is associated with their invaluable physiological/biological/medicinal characteristics.

Lignans, present in selected species of *Arctium* (*A.*) [1,2], *Centaurea* (*C.*) [3–7] and *Forsythia* (*F.*) [8,9] genera proved to be biologically active compounds. The extracts of *Arctium lappa* [1,2] and various *Centaurea* species, like *C. macrocephala* [3], *C. americana* [4] and *C. dealbata* [6] manifested antioxidant and antitumor [1] activity. The neuroprotective effect of arctigenin [4] is under confirmation even in our laboratories. Others, like *C. montana* [4,5], *C. schischkinii* [7] have exhibited anticarcinogenic, while *F. koreana* [8,9] has been used in herbal medicine for its anti-inflammatory, antidiuretic and/or antibacterial activities.

Chromatographic analysis of lignans has been reviewed in 2004 [10] and in 2006 [11], focusing, in general on the extraction/separation, identification and quantification of several plant, biological and food samples [10,11], with special attention on the analysis of tree lignans, determined by gas chromatography isotope dilution mass spectrometry [11].

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On the basis of a detailed literature overview it turned out that concerning the online chromatographic identification and quantification of the lignans extractable from *Arctium* [1,2,12–16], *Centaurea* [3–7,17–26] and *Forsythia* [8,9,27–32] species no [2,3,5–27,30,31] or, few attention was paid [1,16,28,31,32]. This trend is understandable, since the considerable part of proposals preferred the preparation, hydrolysis, enrichment/clean-up of pure lignans: suitable to confirm their different biological activities.

In the fruit, leaf and root extracts of *A. lappa*, by means of LC–MS/MS, arctiin, matairesinol and arctigenin, were identified by Ferracane et al. [1]: quantitative data were not given.

The methanol extract of *A. lappa*'s fruits was analyzed by microemulsion electrokinetic chromatography (MEEKC) with UV detection [16]. The lignans extracted from fruits, leaves and stems of *F. coreana* have been analyzed by LC–MS/MS, applying electrospray ionization in the negative mode (ESNI) [28]: 10 constituents were identified according to their molecular masses in variously prepared extracts. The simultaneous HPLC–DAD determination of 12 major constituents of *F. suspensa* was described in 33 herbal samples [31].

The arctigenin and phylligenin contents of *F. intermedia* were identified by GC–MS based on their underivatized SFIs, including their molecular masses (m/z $[M]^{+\bullet} = 372$) [32].

The dibenzylbutyrolactone-type matairesinol and the diphenylperhydrofurotetrahydrofurane-type pinoresinol (molecular mass of both: Mw = 358) were identified and quantified by LC–MS [33–35] and by GC–MS [36–40] methods in various biological [33,34,36] and food [35–40] matrices.

Matairesinol was determined by HPLC–ESNI-MS, in human plasma [33], in serum [34] and in flaxseed extract [35], while by GC–MS, as its silyl derivative in urine and food samples [36].

Matairesinol and pinoresinol were identified and quantitated, simultaneously, by GC–MS as their TMS derivatives, without any fragmentation pattern analysis: in flaxseed [35,37], in pumpkin seed [37], in sesame seed [39] and in various fruits [38,40].

As a unified characteristic of all GC–MS studies [32,36–40], it is to be noted that detailed fragmentation pattern analysis were not reported.

This work was undertaken in order (i) to study and to document the characteristic behavior and the mass fragmentation patterns of the lignans of *Arctium*, *Centaurea* and *Forsythia* species, as their TMS derivatives, by GC–MS, (ii) to confirm the identity of these lignans, from the same samples, both by GC–MS and HPLC–MS, as well as (iii) to demonstrate the possibilities of our GC–MS multicomponent analysis system extended to the analysis of the group of lignans.

2. Experimental

2.1. Materials

Materials and reagents were all analytical reagent grade of the highest purity available, except solvents used for the HPLC–DAD-MS: all of them were of HPLC grade chemicals.

Methyl alcohol, pyridine, hydroxylamine hydrochloride, acetonitrile and hexane were from Reanal (Budapest, Hungary), hexamethyldisilazane (HMDS) and trifluoroacetic acid (TFA) from Serva (Heidelberg, Germany). Model lignans, such as arctiin, arctigenin, matairesinol methyl iodide, potassium carbonate and acetone were from Sigma–Aldrich (St. Louis, MO, USA). Matairesinoside, was isolated from *Centaurea scabiosa*, arctiin from *A. lappa*, while pinoresinol and phylligenin from *Forsythia viridissima*. Plant samples (fruits of *Arctium* and *Centaurea* and leaves of *Forsythia* species), were from various sources: collected from different Hungarian (H) areas, or purchased from B and T World Seeds sarl (Paguignan, Aigues Vives, France) (F); Further on, like *A. lappa* grown in different places in Hungary: *A. lappa*H-1–*A. lappa*H-5, or *C. montana*F; (samples' joint designation: plant samples).

2.2. Chromatography

2.2.1. Gas chromatography–mass spectrometry (GC–MS)

The apparatus was the Saturn II GC–MS system of Varian (Walnut Creek, CA, USA), supplied with an ion trap detector (ITD) system, a Varian 8200 autosampler and with a septum-equipped programmable injector (SPI). A SGE BPX5 (30 m, 0.25 mm id, 0.25 μm film thickness) capillary column was used (SGE Incorporated, Austin, TX, USA).

Separation of the trimethylsilyl (oxime) ether/ester derivatives: Injections were made at 150 °C, held at 150 °C for 2 min, heated up to 330 °C within 1 min and hold at 330 °C for 5 min. Elutions under temperature gradient conditions were performed as follows: injections were made at 150 °C, held at 150 °C for 4.5 min, heated up to 330 °C within 22.5 min and hold at 330 °C for 7 min (total elution time 34 min).

2.2.2. MS conditions

Electron energy was 70 eV. The temperatures of the transfer line and that of the manifold were 280 and 80 °C, respectively. Multiplier offset was 250 eV. The actual parameters of the ITD were defined by the automatic set-up mode.

Actual automatic set-up conditions—mass range: 40–650 amu; scan rate was 1 s/scan; acquisition time: 34 min; filament/multiplier delay was 420 s, omitting the acquisition of reagent peaks; peak threshold 100 count; mass defect: 100 mmu/100 u; background mass: 50 u.

2.2.3. Preparation of the trimethylsilyl (oxime) ether/ester derivatives

The mixtures of various amounts of standard (arctiin, arctigenin, matairesinol), or isolated (pinoresinol, phylligenin, arctiin, matairesinoside), or synthesized (methylarctigenin) test substances, as well the corresponding amounts containing stock solutions of plant extracts were evaporated to dryness on a vacuum evaporator, at 30–40 °C, in 2–4 mL screw capped vials. The dry residues were treated with 250 μL hydroxylamine hydrochloride containing pyridine (2.5 g hydroxylamine hydrochloride/100 mL), at 70 °C, for 30 min. Thereafter silylation was performed with 450 μL HMDS + 50 μL TFA, and heated at 100 °C for 60 min. After dilution with HMDS, 1 μL of the diluted solutions was injected into the GC–MS system.

2.2.4. High performance liquid chromatography-photo diode array and mass detections (HPLC–DAD-MS, applying electrospray ionization (ES) in the positive mode (HPLC–ESPI-MS)

The apparatus was the TSQ Quantum AM Triple Quadrupol Mass Spectrometer of Thermo Finnigan (River Oaks Parkway, San Jose, CA, USA), supplied with the Surveyor MS Pump (quaternary), with Surveyor Liquid Chromatography, with Autosampler and with Surveyor PDA5 Photo Diode Array Detector.

For the HPLC–UV and HPLC–ESPI-MS analyses the same column, the same eluents and the same gradient were used.

Column: GraceSmart RP18 (5 μm), 150 × 4.6 mm (Grace Davison Discovery Sciences Lokeren, Belgium);

Eluents: eluent A, ACN: 0.07 M acetic acid 15:85 (v/v), eluent B, ACN: 0.07 M acetic acid 85:15 (v/v).

Gradient: Eluent A was changed for eluent B. Program: 0 min, 15% B; 5 min, 30% B; 12 min, 44% B and 20 min, 60% B. Total elution time: 20 min; flow rate: 1 mL/min, detection at 280 nm; injected volumes 20 μL.

Table 1
Quantitative analysis of the fragmentation of the trimethylsilyl derivatives of lignans.

Lignans (Mw)	Selective fragment ion (SFI), *m/z*	Relative abundance of SFIs, expressed in percentages of TIC			Average[n]	RSD%
		A	*B* = *A* × 4	*C* = *A* × 10		
Arctiin (534)	444	17.8	17.5	17.4	17.6	1.2
	361	10.4	9.7	8.9	9.7	7.8
	209	–	–	0.18	–	–
	151	3.54	3.70	3.52	3.59	2.8
	179	–	0.15	0.21	–	–
SFI in total:		31.7	31.1	30.2	31.0	2.4
Arctigenin (372)	444	16.6	16.4	16.7	16.6	0.9
	209	16.3	16.6	15.7	16.2	2.8
	151	5.1	5.2	4.9	5.1	3.0
	179	3.09	3.30	3.16	3.18	3.4
	193	1.80	1.84	1.78	1.81	1.7
SFI in total:		42.8	43.4	42.3	42.8	1.3
Matairesinoside (520)	502	8.1	8.8	8.2	8.4	4.5
	361	7.3	6.8	7.5	7.2	5.0
	209	0.98	2.04	2.52	–	–
	179	–	0.30	0.45	–	–
SFI in total:		16.4	17.9	18.7	17.7	6.6
Matairesinol (358)	502	17.1	16.6	17.7	17.1	3.2
	209	23.8	23.1	23.6	23.5	1.5
	179	6.3	6.2	6.4	6.3	2.0
	193	1.33	1.24	1.22	1.26	4.6
SFI in total:		48.5	47.1	48.9	48.2	2.0
Phylligenin (372)	444	12.0	11.5	11.8	11.8	2.1
	223	4.30	4.11	4.10	4.20	2.7
	165	3.50	3.45	3.30	3.42	3.0
	151	2.95	3.07	2.76	2.93	5.3
	429	1.87	2.02	1.92	1.94	3.9
	193	1.60	1.69	1.70	1.66	3.3
SFI in total:		26.2	25.8	25.6	25.9	1.2
Pinoresinol (358)	502	14.0	14.7	13.7	14.1	3.6
	223	4.91	4.82	4.70	4.80	2.2
	487	1.70	1.70	1.80	1.73	3.2
	193	0.68	0.70	0.63	0.67	5.4
SFI in total:		21.3	21.9	20.8	21.3	2.6
Methylarctigenin (386)	386	12.4	–	–	–	–
	151	31.8	–	–	–	–
SFI in total:		44.2	–	–	–	–

Indications: TIC = total ion current values; *A* = injected ng, varying from 2.5 to 10 ng; (Mw) = molecular weight; – = not available (in shortage of material); Average[n] = at least of three separate derivatization and two injections of each; detailed fragmentation pattern of the trimethylsilyl derivatives of lignans (SFIs) are shown in Fig. 1.

MS parameters: chamber, ES (electrospray); ion mode: positive; scan range of 140–700 *m/z*; drying gas N_2: 8 L/min (≈1.5 Torr), used at ambient temperature; capillary voltage: 3000 V; fragmentation voltage (collision energy): 80 V; collision gas was Argon, characterized with its pressure.

2.3. Isolation of arctiin, matairesinoside, pinoresinol and phylligenin

For this purpose the analytical HPLC apparatus was connected to a preparative HPLC column: Nucleosil100, C18 (10 μm), 15 cm × 1 cm (Teknokroma, Sant Cugat del Vallès, Barcelona, Spain); eluents were the same as described above, while gradient was altered for: 0 min, 15% B; 10 min, 40% B; and 15 min, 100% B; flow rate: 3 mL/min; detection at 280 nm.

For isolation purposes, 2.0 g of the dried pulverized and homogenized fruits of *A. lappa*, or *C. scabiosa* were defatted at 40 °C, for 2 h, consecutively twice with 20–20 mL n-hexane. The centrifuged residue was dried at room temperature for 12 h. The defatted residues (*F. viridissima* samples without treating with hexane), were extracted, twice, with 25–25 mL 80% (v/v) methyl alcohol, at 60 °C, applying a reflux condenser. The unified extracts were evaporated to dryness on a vacuum evaporator at 30–40 °C. The residue was dissolved in 2.5 mL 80% (v/v) methyl alcohol, using its 100 μL aliquots for separation.

2.4. Synthesis of methylarctigenin

Methylarctigenin was prepared from arctigenin (100 mg), in acetone (4 mL)/potassium carbonate (75 mg) and methyl iodide (50 mg) containing medium, applying reflux condenser (oil bath, 80 °C, 24 h) [41]. The reaction mixture was centrifuged and methylarctigenin was obtained by the vacuum evaporation of the supernatant (40 °C, water bath). The residue was further purified by preparative HPLC.

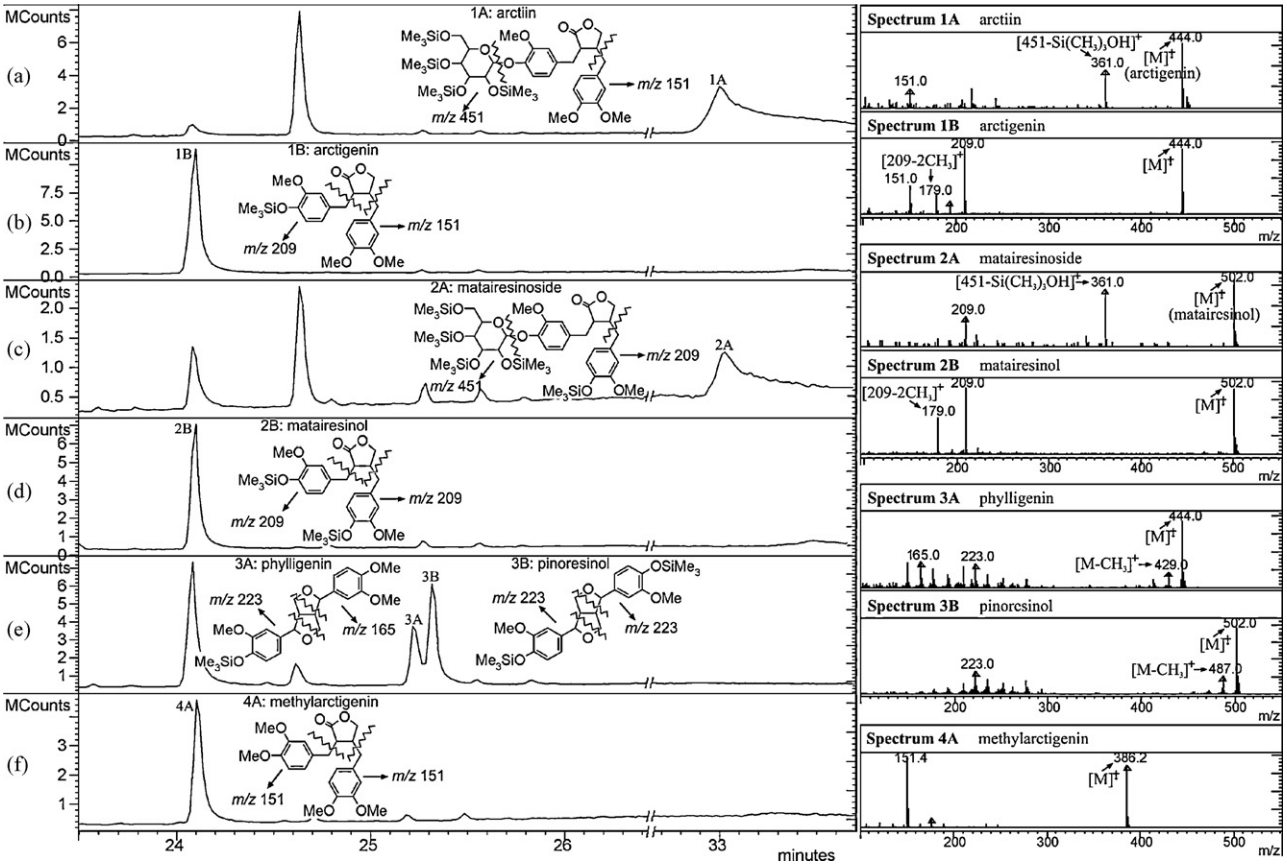

Fig. 1. Peak profile, fragmentation patterns and mass spectra analyzed in the intact and in the hydrolyzed extracts of *A. lappa*H-1 (traces 1a and 1b, spectra 1A and 1B), *C. scabiosa*H-1 (traces 1c and 1d, spectra 2A and 2B), *F. viridissima*H (traces 1e, spectra 3A and 3B) and in the synthesized methylarctigenin (trace 1f, spectrum 4A), as TMS derivatives, by GC–MS. Traces: 1a, 1c, 1e (intact extracts) and 1b, 1d (hydrolyzed extracts).

2.5. Preparation of plant extracts

0.02 g of dried (60 °C, 5 days), pulverized and homogenized samples (weighed with analytical precision) were extracted three times. First, with 2 mL 80% (v/v) methyl alcohol applying a reflux condenser (1 h). Thereafter, the insoluble, centrifuged material was extracted for the second time (2 mL) and for the third time (1 mL), as before. The unified supernatants were completed to 5.0 mL stock solutions.

2.6. Hydrolysis of extracts

0.25–1.00 mL aliquots of the stock solutions (weighed with analytical precision) were evaporated to dryness on a vacuum evaporator at 30–40 °C in 2–4 mL screw capped vials. Hydrolyses were performed with 200 μL 2 M TFA, for 1 h. Samples were dried on a vacuum evaporator, at 30–40 °C. Dried samples were analyzed subsequently to their derivatization by GC–MS, or without derivatization using HPLC–UV and HPLC–ESPI-MS.

3. Results and discussion

3.1. Fragmentation patterns of the trimethylsilyl derivatives of lignans by GC–MS

Based on our earlier experiences, the basic mass fragmentation properties of the TMS (oxime) ether/ester derivatives for sugars [42], for organic acids (members of various homologous series [43,44]) and for amino acids [45] were available for their simultaneous analysis from a single solution, by one injection. The simultaneous analysis principle of organic compounds has been extended recently for the groups of anthraquinones [46], flavonoids [47] and for the glycosidic linkages containing saccharides [48].

In order to complete our analysis principle also with lignans they had to be derivatized, in the frame of a basic study, one by one, in various concentrations.

The fragmentation pattern analysis of lignans (Table 1, Fig. 1) showed both for identification and quantitation purposes relevant, not yet discussed characteristics.

(1) The molecular ions of free lignans were detected for all the studied compounds (Fig. 1): they corresponded to *m*/*z* 444 for the TMS derivatives of arctigenin (spectrum 1B) and phylligenin (spectrum 3A), to *m*/*z* 502 for matairesinol (spectrum 2B) and pinoresinol (spectrum 3B) and to *m*/*z* 386 for the initial form of methylarctigenin (spectrum 4A). The relative abundance percentages both of the molecular ions and the total of selective fragment ions (SFIs) proved to be characteristic to the lignans and proportional to the amount of compounds analyzed (Table 1). Reproducibility of experiments was characterized with the relative standard deviation percentages (RSD%) of analyses. RSD% values varied between 0.90% and 7.8% RSD (average 3.2% RSD), confirming that method is suitable for analytical purposes.

(2) To our knowledge at first (Fig. 1, traces 1a, 1c and spectra 1A, 2A, Table 1), the silylated, free lignans and glucose have been identified in the spectra of their corresponding glycosides (*m*/*z* 444 for arctigenin in arctiin, *m*/*z* 502 for matairesinol in matairesinoside and *m*/*z* 361, in both, for glucose). This

experience might be attributed to the fact that under the fragmentation of glycosides, into the corresponding free lignan and glucose, at 330 °C, in the presence of excess HMDS, the immediate derivatization of the released hydroxyl groups of lignans occurs. As a result of these processes, the spectra of the corresponding silylated, free lignans (Fig. 1, trace 1a, spectrum 1A: *m*/*z* 444; trace 1c, spectrum 2A: *m*/*z* 502, Table 1) and the spectrum of the TMS ether of glucose (Fig. 1, traces 1a and 1c, spectra 1A and 2A: *m*/*z* 361) appear as abundant fragment ions at the expected retention times of the intact lignan-glycoside derivatives (Fig. 1, trace 1a, t_R = 33.00 min for arctiin and Fig. 1, trace 1c, t_R = 33.03 min for matairesinoside).

(3) The SFIs, characteristic to the symmetrical terminal parts of lignans showed up as the most peculiar fragment ions: *m*/*z* 209 for matairesinol (Fig. 1, trace 1d, Table 1: relative abundance 23.5%), *m*/*z* 223 for pinoresinol (Fig. 1, trace 1e, Table 1: relative abundance 4.80%) and *m*/*z* 151 for methylarctigenin (Fig. 1, trace 1f, Table 1: relative abundance 31.8%).

(4) Lignans with unsymmetrical terminal parts do have SFIs specific to their molecular structure: SFIs with *m*/*z* 151 and with *m*/*z* 209 are characteristic to the dibenzylbutyrolactone-type lignans (Fig. 1, traces 1a and 1b, spectra 1A and 1B), while with *m*/*z* 165 and with *m*/*z* 223 to the diphenylperhydrofurotetrahydrofurane-type ones (Fig. 1, trace 1e, spectrum 3A).

3.2. Identification of lignans by HPLC–ESPI-MS

These HPLC–ESPI-MS investigations (Fig. 2) serve for confirming evidence of prepared lignans' identity. Found fragment ions, in all cases investigated, met our expectation: being informative masses. Abundant ions, without exception in all spectra (Fig. 2 spectra 1–7), were the hydrated molecular ions ($[M+H_2O]^+$).

For this study the same extracts were used as for the GC–MS fragmentation pattern analysis (Section 3.1). These HPLC–ESPI-MS investigations (Fig. 2) serve for confirming evidence of prepared lignans' identity.

As seen all spectra (Fig. 2, traces 1–7) manifest the protonated, and/or the hydrated molecular ions ($[M+H]^+$, ($[M+H_2O]^+$), as well as, the cationized molecular ions: by Na ($[M+Na]^+$ and by K ($[M+K]^+$), in the following manner.

1: Matairesinoside (Mw = 520); *m*/*z* [M-glucose+H]$^+$ = 359.2; *m*/*z* $[M+H_2O]^+$ = 538.3; *m*/*z* $[M+Na]^+$ = 543.2; *m*/*z* $[M+K]^+$ = 559.2;

2: Arctiin (Mw = 534), *m*/*z* [M-glucose+H]$^+$ = 373.2; *m*/*z* $[M+H_2O]^+$ = 552.3; *m*/*z* $[M+Na]^+$ = 557.2; *m*/*z* $[M+K]^+$ = 573.2;

3: Pinoresinol (Mw = 358); *m*/*z* $[M+H]^+$ = 359.2; *m*/*z* $[M+H_2O]^+$ = 376.1; *m*/*z* $[M+K]^+$ = 397.2;

4: Matairesinol (Mw = 358); *m*/*z* $[M+H]^+$ = 359.1; *m*/*z* $[M+H_2O]^+$ = 376.1; *m*/*z* $[M+Na]^+$ = 381.1; *m*/*z* $[M+K]^+$ = 397.2;

5: Phylligenin (Mw = 372); *m*/*z* $[M+H]^+$ = 373.1; *m*/*z* $[M+H_2O]^+$ = 390.2; *m*/*z* $[M+K]^+$ = 411.1;

6: Arctigenin (Mw = 372); *m*/*z* $[M+H]^+$ = 373.1; *m*/*z* $[M+H_2O]^+$ = 390.2; *m*/*z* $[M+Na]^+$ = 395.1; *m*/*z* $[M+K]^+$ = 411.2;

7: Methylarctigenin (Mw = 386); *m*/*z* $[M+H]^+$ = 387.2; *m*/*z* $[M+H_2O]^+$ = 404.2; *m*/*z* $[M+Na]^+$ = 409.1; *m*/*z* $[M+K]^+$ = 425.1;

Spectra traces were taken from plant extracts (Fig. 2, traces 1–6) or from a synthesized product (Fig. 2, trace 7). In cases of arctiin, arctigenin and matairesinol also the authentic standards were available (not shown): providing the same spectra, compared to those obtained from the plant extracts.

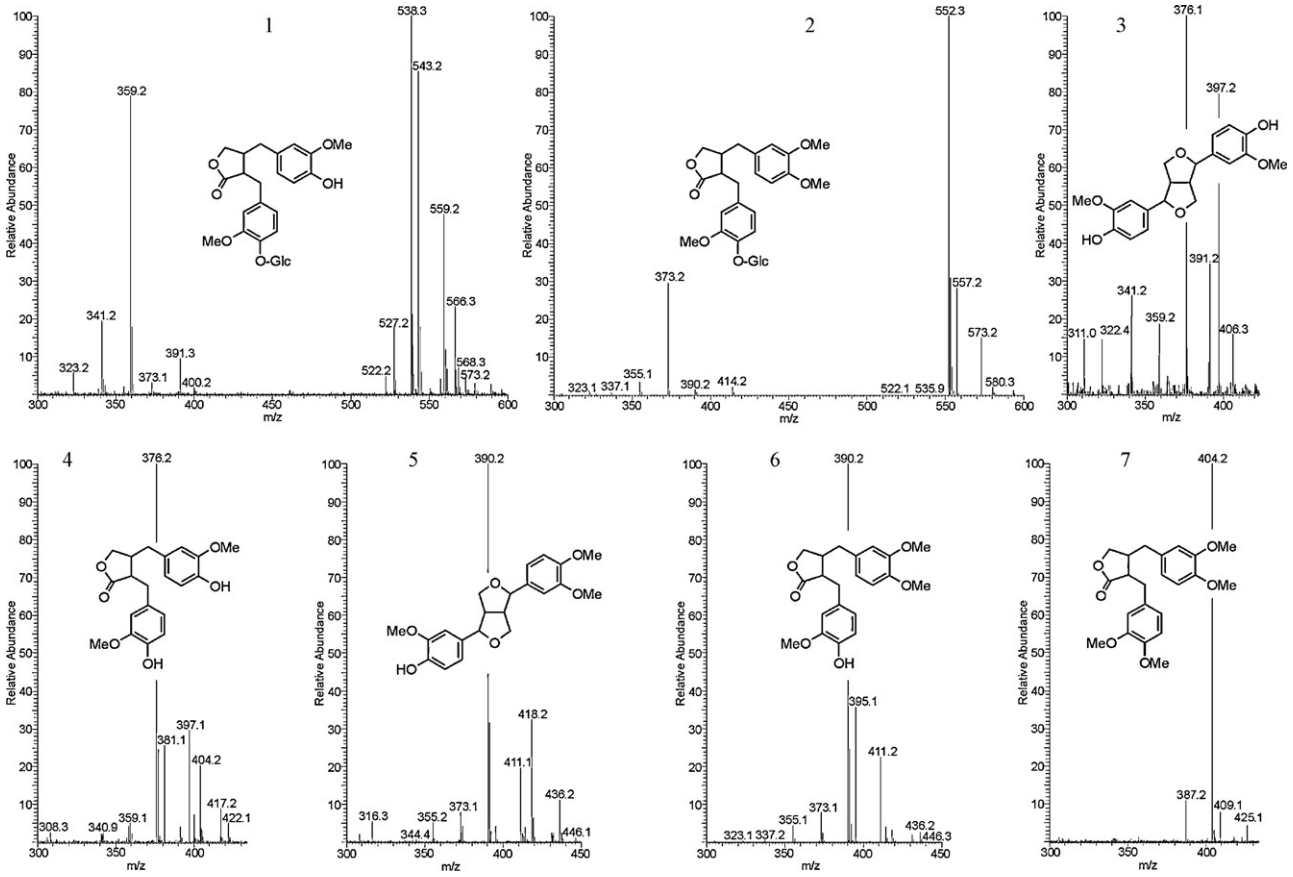

Fig. 2. HPLC–ESPI-MS spectra of lignans, prepared from the fruit extracts of *C. scabiosa*H-1 (traces 1 and 4), from *A. lappa*H-1 (traces 2 and 6), and from the leaf extract of *F. viridissima*H (traces 3 and 5), or synthesized from arctigenin (7). Traces: 1, matairesinoside; 2, arctiin; 3, pinoresinol; 4, matairesinol; 5, phylligenin; 6, arctigenin; 7, methylarctigenin.

Concerning the role and importance of LC–ESPI-MS studies it is to be noted that both in the identification and in the quantification of lignans they proved to be of primary importance (details in Section 3.3): especially in those cases when the gas chromatographic evaluations of the hardly separable pairs could be performed on the basis of selective fragment ions of lignans, only.

3.3. Identification and quantification of the constituents extracted from the fruits of Arctium lappa and Centaurea scabiosa and from the leaves of Forsythia viridissima

3.3.1. Reproducibility in the quantitation of lignans, sugars, sugar alcohols and organic acids from model solutions

Prior to the identification and quantitation of plant extracts reproducibility studies were performed with various amounts of standards obtained both with GC–MS and with HPLC–UV analyses (Table S1, Supplementary material). Model studies have been carried out in the concentration range of ~0.20–100 ng (GC–MS) and in the range of ~40–500 ng (HPLC–UV), calculated, one by one, from various injected amounts of compounds.

The TMS (oxime) ether/ester derivatives of all standard compounds, determined by GC–MS, have been evaluated on the basis of their total ion current (TIC) responses, while lignans in parallel, also on their selective fragment ion (SFI) values (the structure and relative intensities of SFI ions are detailed in Fig. 1 and in Table 1).

Reproducibility of response values (Table S1, Supplementary material), calculated as integrator units/ng compound, have been characterized with their relative standard deviation percentages (RSD). GC–MS (TIC, SFI) and HPLC–UV data, in average, confirmed the reproducibility of 3.4%, RSD: varying between 1.4 and 6.2%, RSD. (In cases of the non-linear responses quantitation was performed by means of calibration curves.)

3.3.2. Analysis of plant extracts

Typical GC–MS chromatograms completed by selected spectra (Fig. S1, Supplementary material) and HPLC–UV chromatograms (Fig. 3), are presented as examples of our working strategy. It means, the principle of our approach can be characterized by two independent chromatographic methodologies. Parallel GC–MS and HPLC–UV separations and quantitations have been performed from the same extracts, both in their initial condition (it means without hydrolysis of the extract) and subsequently to their TFA hydrolyses (Table 2). Thus, the reproducibility of the entire analysis procedure, including sample preparation, hydrolysis and derivatization steps should be in acceptable agreement: should not exceed the 12% RSD, in wide concentration range (~0.1 to ~80 mg/g). According to our working strategy lignans were identified and quantified by both methods (GC–MS, HPLC–UV), while, lignans, simultaneously with sugars, sugar alcohols, and organic acids, as derivatives by GC–MS; partly on the basis of their TIC, partly on their SFI values.

Regarding the analysis of various functional group containing organics simultaneously, from one solution, by a single injection, it has been repeatedly proven that GC–MS is the method of choice (Fig. S1, Supplementary material). In this special case, in the hydrolyzed extractum of *A. lappa*H-1, the TMS esters of caffeic and the various C_{18} fatty acids have been differentiated on their SFIs (Fig. S1, Supplementary material trace b, Spectrum 1A, 2A, 3A, 4A, Table 2).

As to the convincing identification and quantification of lignans in the simultaneous presence of each others, GC–MS and HPLC–UV or HPLC–MS data are important, completing contributions:

The GC–MS identification and quantification of the TMS derivatives of arctigenin/matairesinol and arctiin/matairesinoside (if present in the same sample) are limited to their SFI basis (Table 1 and Fig. 1). Thus, GC–MS is preferred in those cases when either the arctiin/arctigenin, or the matairesinoside/matairesinol pairs, are to

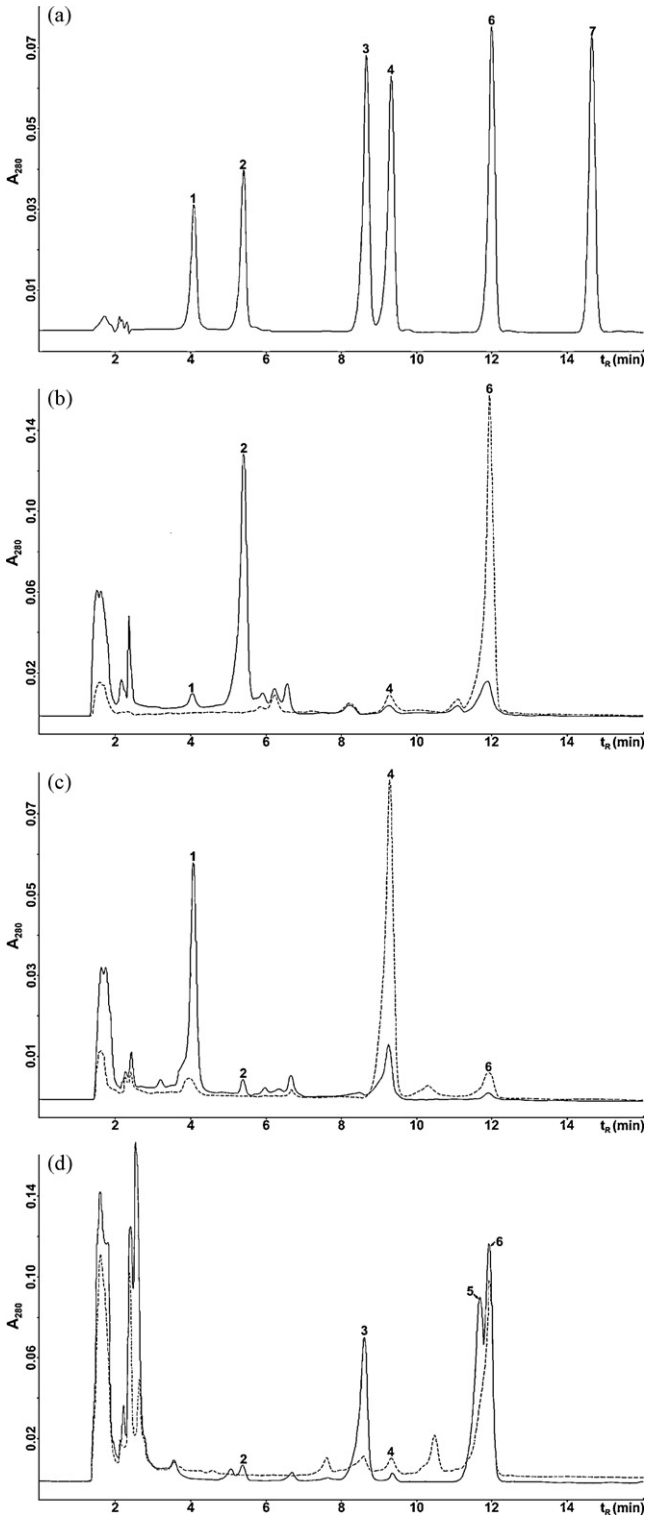

Fig. 3. Peak profile of the lignan constituents obtained by HPLC–UV (280 nm) from model solution (trace 3a) and from the fruit extracts of *A. lappa*H-1 (trace 3b), from *C. scabiosa*H-1 (trace 3c), and from the leaf extracts of *F. viridissima*H (trace 3d). Continuous lines: intact extracts, dashed lines: hydrolyzed extracts. Peak numbers: 1, matairesinoside; 2 arctiin; 3, pinoresinol; 4, matairesinol; 5, phylligenin; 6, arctigenin; 7, methylarctigenin;. compounds 1–6 in Table 2 are indicated with signs 1♠–6♠.

Table 2
Composition of the fruit (*A. lappa*H-1, *C. scabiosa*H-1) and the leaf (*F. viridissima*H) extracts in intact (not hydrolyzed) and in hydrolyzed plant samples, analyzed as trimethylsilyl (oxime) ether/ester derivatives by GC–MS and without derivatization by HPLC–UV (♠).

Compounds			Constituents of extracts: calculated on the dry matter content of samples (mg/g)											
No	Name	t_R (min)	*A. lappa*H-1				*C. scabiosa*H-1				*F. viridissima*H			
			Intact		Hydrolyzed		Intact		Hydrolyzed		Intact		Hydrolyzed	
1	Arabinose 1,2	7.95/8.06	–		0.39		–		0.48		0.19		1.86	
2	Rhamnose	9.03	–		–		–		–		–		28.8	
3	Quinic acid	9.57	0.49		4.08		0.24		0.28		23.9		23.9	
4	Mannitol	10.52	0.58		0.54		0.72		0.70		–		–	
5	Sorbitol	10.63	0.052		0.021		0.049		0.036		–		–	
6	Fructose 1,2	10.70/10.82	0.24		10.6		0.61		6.5		40.4		22.3	
7	Galactose	10.85	0.13		0.25		2.46		2.37		19.6		1.92	
8	Glucose 1,2	11.52/11.75	0.66		84.2		0.65		44.2		160		189	
9	Palmitic acid	12.63	2.20		6.34		1.63		5.1		1.96		3.41	
10	Inositol	12.75	0.69		0.89		0.57		1.35		16.7		18.6	
11	Caffeic acid	13.78	0.11		0.68		–		–		0.047		2.59	
12	Linoleic acid	14.70	1.31		36.4		3.21		26.6		1.76		2.38	
13	Oleic acid	14.75	4.90		16.1		2.39		13.9		2.86		3.51	
14	Stearic acid	15.03	0.59		1.54		0.36		1.33		0.42		0.42	
15	Sucrose	18.57	26.4		0.67		18.9		0.57		8.2		–	
16	Fatty acid[ni]	20.15	0.71		6.2		0.18		5.3		–		–	
19	Raffinose	24.63	27.2		–		7.2		–		4.34		–	
17	Arctigenin[a]	24.08	2.20	**2.47** (9.5)	58.3	**56.4** (4.8)	0.081	**0.077** (8.3)	0.41	**0.44** (8.1)	38.2	**35.4** (11)	38.5	–
6♠		12.03	2.73		54.5		0.072		0.46		32.5		ns	
18	Matairesinol[a]	24.09	0.83	**0.81** (4.4)	1.52	**1.51**(0.90)	6.8	**6.5** (6.5)	37.1	**38.1** (3.5)	0.89	**0.95** (8.2)	1.38	**1.3** (8.7)
4♠		9.29	0.78		1.50		6.2		39.0		1.00		1.22	
20	Phylligenin[a]	25.22	–		–		–		–		19.9	**21.5** (10)	3.18	–
5♠		11.67	–		–		–		–		23.0		ns	
21	Pinoresinol[a]	25.32	–		–		–		–		29.8	**28.7** (5.7)	2.71	**2.93** (10)
3♠		8.58	–		–		–		–		27.5		3.14	
22	Arctiin[a]	33.00	82.0	**78.0** (7.3)	–		0.72	**0.68** (8.3)	–		1.34	**1.32** (2.1)	–	
2♠		5.42	73.9		–		0.64		–		1.30		–	
23	Matairesinoside[a]	33.03	1.30	**1.21** (11)	–		42.9	**41.7** (4.1)	–		–		–	
1♠		4.06	1.11		–		40.5		–		–		–	
Lignans in total (mM/g)		one by one	**0.157**		**0.156**		**0.100**		**0.107**		**0.100**[b] {**0.138**}		**0.107**[b] {**0.017**}	
		Average (RSD%)	0.157 (0.45)				0.104 (4.8)				0.104 (4.8)			
Found in the extracts, in total (%)[c]			66.1		100.8		57.9		98.8		84.2		73.6	

Indications, as in Tables 1 and 2, as well as: ♠ = peak numbers as in Fig. 3; () = indicate the relative standard deviation percentages of averages; additional constituents, including in the total of *F. viridissima*H: its intact extract contains 9.4 mg/g chlorogenic acid, 13 mg/g not identified disaccharide, hydrolyzed for 1.41 mg/g; ns = arctigenin and phylligenin not separable; [ni] = not identified; {} = mM/g contents of the perhydrotetrahydrofurane-type lignans.

[a] Identified and quantified on the basis of their SFIs (selective fragment ions) (details in Fig. 1 and Table 1).

[b] Except the perhydrotetrahydrofurane-type lignans;

[c] Calculated on the dry basis of extracts; bold printed data = averages of the GC–MS and HPLC–UV analyses.

be determined, together with phylligenin/pinoresinol. However, in all cases, which rarely occur, when arctiin, matairesinoside, arctigenin and matairesinol are to be quantified in the same sample matrix in commensurable concentrations, the simultaneous use of HPLC–UV or HPLC–MS is necessary.

The HPLC separation of matairesinoside, arctiin, pinoresinol, matairesinol, arctigenin and methylarctigenin are excellent (Fig. 3, compounds 1–4, 6, 7), there are one hardly separable pair arctigenin and phylligenin (Fig. 3, trace 3d, compounds 5 and 6), for the reliable evaluation of these lignans GC–MS serves as completing evidence.

3.3.3. Practical applicability of the method

In the analysis of the present complex matrix, to follow the dual analysis strategy, GC and HPLC, was of primary importance, and it was inevitable necessary. Since, GC enables the quantification of arctigenin and phylligenin present in extremely different concentrations (Fig. 1, insufficient separation; Table 2: remarks in the last vertical column, ns = not separable (present in not commensurable ratios)). While, HPLC reassures the analysis of arctiin, arctigenin, matairesinoside and matairesinol, evaluable by GC on their selective fragment ion values, only (Table 1 and Fig. 1: spectra 1A, 1B, 2A and 2B).

It is to be noted that all technical parameters expected to improve the resolution and decrease the widening of the coelution partners like arctiin/matairesinoside (Fig. 1, traces 1a/1c) and arctigenin/matairesinol (Fig. 1, traces 1b/1d), have been varied in all possible combinations (temperature program variation of the injector and the gradient programs). The only help, what really proved to be excellent was the evaluation of the not separable pairs, on the basis of their selective fragment ions, obtained by GC–MS, as their TMS ether derivatives (Fig. 1, spectra 1A/2A and 1B/2B, Tables 1 and 2). The completing evidence furnished by the parallel HPLC analyses, – being in good agreement with the GC–MS ones, – were reassured and confirmed the utility of the principle of the dual working strategy.

The usefulness and advantage of our working strategy based on parallel, GC–MS and HPLC–UV analyses of the plant extracts, comparing also the composition of the intact (not hydrolyzed) and hydrolyzed version of the same extract, provided relevant and reliable informations. Thus, it means that a given plant extract was analyzed by both chromatographic methods, in intact and in hydrolyzed conditions, in two separate tests of each (Table 2).

The evaluation of the lignan glycoside and the free lignan contents of extracts is of primary importance: because these constituents could be analyzed by both chromatographic methods. The arctiin and the matairesinoside contents of extracts (bold printed average data from the two chromatographic processes), in order of listing, varied between 0.68 and 78.0 mg/g and between 1.21 and 41.7 mg/g, respectively. Regarding the reproducibilities of lignan glycosides and free lignans (data in parentheses, next to the bold printed average values), obtained with the two methods, varied from 0.90 to 11 RSD%, providing an average of 7.0 RSD%. The reliability and utility of our working strategy can be best characterized by the excellently correlating total amounts of the butyrolactone-type lignans, found both in the intact and hydrolyzed extracts (the lignan content of plant extracts, to be comparable, have been calculated in all extracts on lignan basis, mM/g: values in the last but two horizontal row of Table 2). Analyses revealed that, (1) the dibenzylbutyrolactone-type arctigenin and matairesinol contents in intact and in hydrolyzed extracts, in order of listing, within the experimental error of our analyses proved to be the same (*A. lappa*H-1, 0.157 and 0.156 mM/g, *C. scabiosa*H-1, 0.100 and 0.107 mM/g, *F. viridissima*H, 0.100 and 0.107 mM/g. In contrary, (2) the perhydrofurotetrahydrofurane-type phylligenin and pinoresinol contents of *F. viridisssima* in intact extract was 0.138 mM/g, and decreased upon hydrolysis to 0.017 mM/g.

These data revealed in particular important messages. They confirmed the intrinsic peculiarity of the behavior of lignans upon hydrolysis due to their different molecular structure. This means, the unambiguous stability of the dibenzylbutyrolactone-type arctigenin and matairesinol and the considerable decomposition of the perhydrofurotetrahydrofurane-type phylligenin and pinoresinol.

The main advantage of the GC–MS analyses performed as the TMS (oxime) ether/ester derivatives of constituents is associated with the irreplaceable possibility of this technique: suitable for the identification and quantification of various functional group containing organics from a single solution by on injection. As to the lignan containing extracts, this type of analysis was shown at the first time. Evaluating the total amount of identified constituents (Table 2, last horizontal line, expressed on the dry basis of extracts) revealed a high percentage of identifiable compounds in hydrolyzed extracts of *A. lappa*H-1 and *C. scabiosa*H-1 proved to be 100.8% and 98.8% (due to the stability of the dibenzylbutyrolactone-type lignans). These values, being close to 100%, showed the utility of this technique, proving that the overwhelming part of constituents of these two plant extracts, within the experimental error of our analyses, have been identified and quantified. Because of the decomposition of the perhydrofurotetrahydrofurane-type phylligenin and pinoresinol, and because of the not identified compounds in the extracts of *F. viridissima*H, the total amount of identified constituents was less in hydrolyzed extract (73.6%) compared to its intact one (84.2%). (Note: compounds 1–23, analyzed by GC–MS correspond to those indicated in Fig. S1, Supplementary material, while compounds, indicated by numbers 1♠–6♠, obtained by HPLC, are identical to constituents shown at Fig. 2, by spectra traces 1–6 and at Fig. 3, by compounds 1–6.)

3.3.4. Distribution of lignans of the Arctium, Centaurea and Forsythia genera collected from different area

The knowledge of the total amount of lignans and their distribution, obtained from the intact and hydrolyzed samples, calls attention to the importance of plant selection: in order to find the most suitable area and the species providing lignans in as high concentrations as possible.

For this purpose several plants, collected in Hungary and obtained from France have been analyzed (Fig. S2, Supplementary material). Amount of lignans, expressed in mg/g of dried plants (fruits or leaves) revealed spectacular differences.

The total amount of lignans, calculated on the plants' dry basis varied from 0.42 mg/g (*C. cyanus*F) to 87.9 mg/g (*F. viridissima*H).

As to the initial properties of lignans – being present in free, or in glycosidic form – this is the intrinsic characteristic of the plants. Lignans in *Arctium* and in *Centaurea* species exist overwhelmingly as glycosides, while in *Forsithya* ones they are present, primarily in free forms.

Due to the fact (detailed already in the introductory section of this paper) that the overwhelming part of publications dealt with the preparation, hydrolysis, enrichment/clean-up of lignans, numerical data, characterizing their concentration in different parts of the plants are regarded of marginal importance. This means, lignan contents for the *Centaurea* species have not been found at all. Concerning the arctigenin and arctiin contents of the fruits of *A. lappa* of Chinese origin ([16]: arctiin 55.0 mg/g and arctigenin 2.63 mg/g) proved to be commensurable with our ones, harvested all in Hungary (Fig. S2 Supplementary material, *A. lappa*H-1–*A. lappa*H-5, varying in the rage of 2.7–5.2 mg/g arctigenin and 47.0–73.9 mg/g arctiin contents). According to this experience the lignan contents of the same species are independent on their origin and it is characteristic to the species.

The only numerical data found for the arctigenin content of *Forsythia koreana*'s leaves (0.41 mg/g) [28] collected at the Seoul National University (South Korea) confirms the primary influence of the production site of the globe. The impact of the species, in shortage in our collection of *F. koreana* could not be evaluated. Comparing to the arctigenin content found in the leaves of four *Forsythia* species grown in Hungary (Fig. S2, Supplementary material), due to the difference in the arctigenin contents of four, various Hungarian *Forsythia* species (varying between 17.0 mg/g and 35.4 mg/g; average ≅26 mg/g), seems to be negligible.

Thus, on the basis of our experiences, we are convinced that the preliminary quantitative analysis of extractable lignans, before of plant selection for any purposes, is unavoidable advantageous and necessary.

4. Conclusion

1. Characteristic behavior and fragmentation patterns of the trimethylsilyl derivatives of lignans (arctiin, arctigenin, matairesinoside, matairesinol, phylligenin, pinoresinol, methylarctigenin) were discussed.
2. The identity of lignans separated from plant matrices has been proved both by GC–MS and HPLC–ESPI-MS from the same extracts.
3. As a novelty of the study, it has been confirmed, that under the gas chromatographic mass spectrometric elution condition of the silylated lignan glycosides (at 330 °C, at their expected t_R values, in the presence of excess hexamethyldisilazane), their fragmentation into the corresponding free lignans and glucose occur. Fragmentations are instantaneously followed by derivatization of the released species resulting in the fully silylated arctigenin (m/z 444) or matairesinol (m/z 502) and glucose derivatives (m/z 361), shown by their characteristic spectra.
4. The structure dependent difference concerning the stability of lignans upon trifluoroacetic acid hydrolysis has been described at the first time proving that the benzylbutyrolactone-type lignans are stable, while the perhydrotetrahydrofurane-type ones do decompose.
5. Results of basic studies have been utilized for the identification and quantification of the lignan contents of the fruit and leaf extracts of *Arctium*, *Centaurea* and *Forsythia* species.
6. For identification and quantitation purposes, parallel GC–MS and HPLC–UV separations have been performed from the same extracts both in their initial condition and subsequently to their trifluoroacetic acid hydrolyses. According to this approach (i) lignans were identified and quantified by both methods (GC–MS, HPLC–UV), while, (ii) lignans, simultaneously with sugars, sugar alcohols and organic acids, as trimethylsilyl (oxime) ether/ester derivatives by GC–MS (partly on the basis of their total ion current, partly on their selective fragment ion response values).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.01.019.

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