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LC-PDA-ESI/MS Identification of the Phenolic Components of Three Compositae Spices: Chamomile, Tarragon, and Mexican Arnica

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Abstract

Chamomile (*Matricaria chamomilla* L.), tarragon (*Artemisia dracunculus* L.) and Mexican arnica (*Heterotheca inuoides*) are common compositae spices and herbs found in the US market. They contain flavonoids and hydroxycinnamates that are potentially beneficial to human health. A standardized LC-PDA-ESI/MS profiling method was used to identify 51 flavonoids and 17 hydroxycinnamates. Many of the identifications were confirmed with authentic standards or through references in the literature or the laboratory's database. More than half of the phenol compounds for each spice had not been previously reported. The phenolic profile can be used for plant authentication and to correlate with biological activities.

Keywords

Chamomile; Tarragon; Mexican arnica; Flavonoids; Caffeoylquinic acids; LC-PDA-ESI/MS

Chamomile (*Matricaria chamomilla* L.) flowers and tarragon (*Artemisia dracunculus* L.) leaves are among the common compositae spices and herbs that are used worldwide. They are used as home spices, health foods, and herb teas, and their extracts are used in some pharmaceutical preparations [1–4]. As a traditional medicine, Mexican arnica (*Heterotheca inuloides* Cass) has been taken internally for the treatment of nervous disorders, stomach ailments, and fever [5,6], and the flowers have been used as a spice in Mexico and the USA. Chemically, they contain volatile oils, flavonoids, and hydroxycinnamic acid derivatives [1,6–16]. The flavonoids and hydroxycinnamates are potentially beneficial to human health [17]. These plants have been previously studied using liquid chromatography-mass spectrometry [18–23], but their phenolic components have not been systematically studied.

The systematic identification and quantification of the phenolic compounds in food is necessary in order to determine their impact on human health. Liquid chromatography-photodiode-array-mass spectrometry (LC-PDA-MS) has been shown to be a powerful tool for on-line identification of plant phenolic compounds [24,25]. The only drawback is the inability to identify isomers, e.g. specific sites of attachment of the saccharides. As part of our project of systematic identification of the phenolic compounds in plant derived foods, including spices and herbs, over 200 standards and 400 food samples have been screened using a standardized LC-PDA-ESI/MS method. More than 1000 food phenolic compounds

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have been identified and stored in our food phenolic database. They are used as references to provide reliable identification of the compounds in subsequent analyzed samples [25–27]. In this study, as many as 37 phenolic compounds were identified in chamomile, tarragon and Mexican arnica. More than half are new for these spices.

Identification of flavonoids and caffeoylquinic acids

Chromatograms (350 nm) of the extracts of chamomile, tarragon and Mexican arnica are shown in Figure 1. The retention times (t_R), wavelength of maximum absorbance (λ_{max}), molecular ions ($[M+H]^+/[M-H]^-$), and major fragment ions (PI/NI) are listed in Table 1.

The LC-PDA-ESI/MS instrument offered the UV spectra, retention time, and mass data for each of the phenols in a plant extract in a single run. The molecular ions and their fragments, including the aglycone ions of a flavonoids and the acyls of the cinnamates, were obtained with positive and negative ionization at low (100 V or less) and high (250 V or higher) fragmentation energies. The positive and negative mass data were always used to confirm the mass of each compound in each chromatographic peak. Tentative identification was made based on the UV and MS spectra and retention times. Positive identification was achieved by comparison to data for either authentic standards or positively identified compounds in the reference plant samples. In Table 1, positively and tentatively identified compounds are indicated with superscript “a” and “b”, respectively.

All 17 of the hydroxycinnamates and 27 of the 46 glycosylated flavonoids were positively identified based on standards or reference compounds from previously tested Compositae plants [25–27]. The 19 remaining flavonoids were tentatively identified with reasonable confidence. The positive identification of the aglycones (chromatograms not shown) resulting from hydrolysis of the extracts confirmed the flavonoid glycoside identifications. Some of the compounds in Table 1 have been reported previously in Compositae plants (superscript “c” next to peak number) and were identified by comparison of the LC-MS spectra [1,6–16,18–23].

The main phenolic components of Chamomile flowers were the glycosides of flavones, while hydroxycinnamates were the main phenolic components of tarragon leaves. Mexican arnica flowers contained hydroxycinnamates and the glycosides of flavones and flavonols. All 3 plants can be distinguished easily.

A systematic LC-DAD-ESI/MS plant phenolic component analysis requires a gram or less of material and can be completed in several hours. Use of a standardized approach to compile retention times and UV and MS spectra greatly facilitates compound identification [24–27]. Characterization of the herb chemical component profile is valuable not only for identification and quality control, but will also enhance understanding of their biological activity and their benefit to human health.

Experimental

Plant materials and extraction

Dried chamomile flower, tarragon leaves, and Mexican arnica flowers were purchased from local food stores in Maryland. All were finely powdered and passed through a20-mesh sieve prior to extraction. Dried ground material (100 mg) was extracted with methanol-water (5.0 mL, 60:40, v/v) using a sonicator (Fisher Scientific, Pittsburg, PA, USA) at 40 KHz and 100 W for 60 min. at room temperature. The extract was filtered through a 0.45 μm nylon acrodisk 13 filter (Gelman, Ann Arbor, MI, USA), and a 10 μL of the extract was injected onto the analytical column for analysis [25].

LC-PDA-ESI/MS analysis

The LC-PDA-ESI/MS instrument and operating parameters have been previously described [25]. Briefly, the LC-PDA-ESI/MS consisted of an 1100 HPLC (with a diode array detector) coupled to a mass spectrometer (MSD, SL mode), both from Agilent (Palo Alto, CA). A 250 × 4.6 mm i.d., 5 μm Symmetry C18 column (C18, 5 μm,) (Waters Corp., Milford, MA) and a 20 × 3.9 mm i.d., 5 μm sentry guard column (Symmetry, 3.9 × 20 mm) (Waters Corp., Milford, MA) were used with a flow rate of 1.0 mL/min. Symmetry Shield column (250 × 4.6 mm i.d., 5 μm) was also used further to separate some overlapped peaks. The column oven temperature was set at 25°C. The mobile phase consisted of a combination of A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). The gradient was varied linearly from 10% to 26% B (v/v) in 40 min, to 65% B at 70 min, to 100% B at 71 min, and held at 100% B to 75 min. The PDA was set at 350, 310 and 270 nm to provide real-time records of the peak intensity and UV spectra were recorded from 190–650 nm for plant component identification. Mass spectra were simultaneously acquired using electrospray ionization in the positive and negative ionization (PI and NI) modes at low and high fragmentation voltages (100 V and 250 V) over the range of *m/z* 100–2000. A drying gas flow of 13 L/min, a drying gas temperature of 350°C, a nebulizer pressure of 50 psi, and capillary voltages of 4000 V for PI and 3500 V for NI were used. The LC system was directly coupled to the MSD without stream splitting.

Acid hydrolyzed extracts

The filtered extract (0.50 mL) was mixed with concentrated HCl (37%, 0.10 mL) and heated in a covered tube at 85°C for 2 h. Then, 0.40 mL of methanol was added to the mixture and the solution was sonicated for 10 min. The solution was re-filtered prior to HPLC injection [25].

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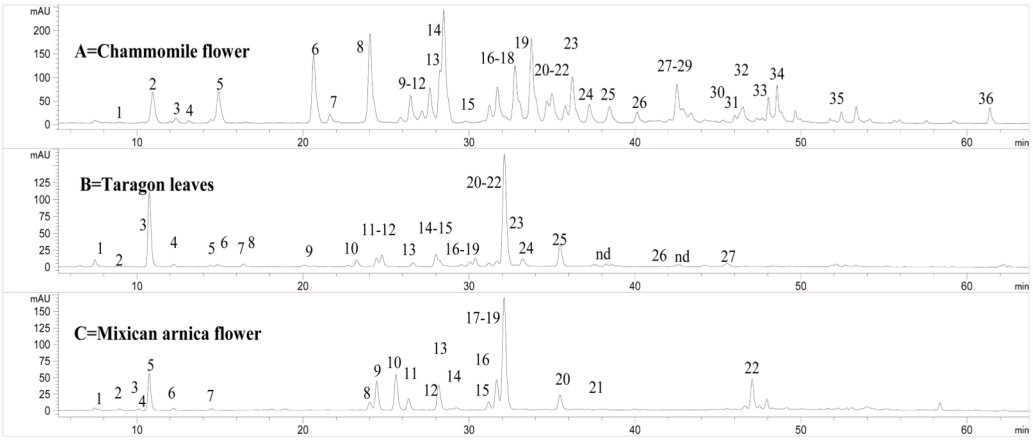

Figure 1.
The LC chromatograms of Chammomile flowers (A), tarragon leaves (B) and Mexican arnica flowers (C).

Table 1a

The identification of the phenolic compounds in chamomile flower (C), tarragon leaf (T) and Mexican arnica flower (MA).

Compound	Peak Number			t_R (min)	[M+H]+ and [M−H]$^-$ *m/z*	Major fragments *m/z*	UV λ_{max} (mn)
	C	T	MA				
Hydroxycinnamates							
1-Caffeyolquinic acid[a]	nd	1	1	6.4	---/353	191, 179, 167, 135	240sh, 300sh, 330
3-Caffeoylquinic acid[a]	1[c]	2	2	7.3	---/353	191, 179, 167, 135	240sh, 300sh, 330
Chlorogenic acid[a]	2[c]	3	5[c]	11.5	---/355	191, 179, 167, 135	240sh, 300sh, 330
4-Caffeoylquinic acid[a]	3[c]	4	6	12.3	---/356	191, 179, 167, 135	240sh, 300sh, 330
Caffeic acid[a]	nd	5	7[c]	14.7	---/179	163	240, 298sh, 326
Caffeoyltartaric acid[a]	nd	7	nd	18.4	---/311	179	nd
5-Feruloylquinic acid[a]	nd	8	nd	18.6	---/367	193, 191	nd
Ferulic acid glucose[a]	5[c]	nd	nd	14.4	---/355	193	nd
Ferulic acid glucose[a]	8[c]	nd	nd	23.2	---/355	193	240, 298sh, 326
1,4-Dicaffeoylquinic acid[a]	nd	17	nd	30	---/515	353,191, 179, 167, 135	nd
3,4-Dicaffeoylquinic acid[a]	16	20	16	31.2	---/515	353,191, 179, 167, 135	240, 300sh, 328
1,5-Dicaffeoylquinic acid[a]	17[c,d]	21	17	31.7	---/517	353,191, 179, 167, 135	240, 300sh, 328
3,5-Dicaffeoylquinic acid[a]	nd	22	18	32.1	---/517	353,191, 179, 167, 135	240, 298sh, 326
4,5-Dicaffeoylquinic acid[a]	24	25	20	35.2	---/519	353,191, 179, 167, 135	240, 300sh, 328
3-Caffeoyl-5-feruloyquinic acid[a]	nd	26	nd	38.3	---/529	367, 353, 191,179,135	240, 326
3,4,5-Tricaffeoylquinic acid[a]	nd	27	nd	48.8	---/667	515,353,515,191,179	nd
Flavone 7-*O*-glycosides							
Hexahydroxyflavone 3-*O*-hexoside[b]	6	nd	nd	20	481/479	319/317	260, 354
Pentahydroxyflavone 7-*O*-hexoside[b]	7	nd	nd	20.9	465/463	303/301	282, 342
Leteolin 7-*O*-rutinoside[a]	9	nd	nd	24.9	595/593	287/285	256, 350?
Pentahydroxyflavone 7-*O*-hexoside[b]	11	nd	nd	26.8	465/463	303/301	268, 338
Luteolin 7-*O*-glucoside[a]	12[c]	nd	nd	27.5	449/447	287/285	256, 266, 348

Compound	Peak Number			t_R (min)	[M+H]+ and [M−H]⁻ *m/z*	Major fragments *m/z*	UV λ_{max} (mn)
	C	T	MA				
Hexahydroxyflavone 3-*O*-dihexoside[b]	15	nd	nd	30.3	643/641	319/317	nd
Apigenin 7-*O*-glucoside[a]	19[c]	nd	nd	33.6	433/431	287/285	256, 266, 348
Apigenin 7-O-acetylglucoside[a]	29[c]	nd	nd	42.3	475/473	271/269	268, 338
Apigenin 7-O-acetylglucoside[a]	32[c]	nd	nd	47.2	475/473	271/269	268, 338
Apigenin 7-*O*-malonylglucoside[a]	28	nd	nd	42.6	519/517	271/269	268, 338
Apigenin 7-*O*-caffeoyllucoside[b]	30	nd	nd	45	595/593	271/269	nd
Apigenin 7-*O*-molonylglucoside[a]	31	nd	nd	45.6	519/517	271/269	268, 338
Apigenin 7-*O*-malonylacetylglucoside[b]	33	nd	nd	47.9	561/559	271/269	268, 338
Apigenin 7-*O*-malonylacetylglucoside[b]	34	nd	nd	48.2	561/559	271/269	268, 338
Apigenin 7-*O*-malonylacetylglucoside[b]	35	nd	nd	49.1	561/559	271/269	268, 338

[a] the identification was confirmed by comparison to standards or positively identified compounds in reference plant samples;

[b] identified tentatively from the LC-MS data;

[c] reported in the plant previously;

[d] previous identification was not corrected; nd: not determined.

Table 1b

The identification of the phenolic compounds in chamomile flower (C), tarragon leaf (T) and Mexican arnica flower (MA).

Compound	Peak Number			t_R	[M+H]+ and [M−H]⁻ *m/z*	Major fragments *m/z*	UV λ_{max} (mn)
	C	T	MA	(min)			
Flavone C-glucoside							
Apigenin 6,8-di-*C*-glucoside[a]	nd	6	nd	15.3	595/593	577, 475, 455	270, 338
Flavone aglycones							
Luteolin[a]	nd	nd	22	47.9	287/285		256, 266, 348
Apigenin[a]	36[c]	nd	nd	51.9	271/269		268, 338
Dihydroxy-tetramethoxyflavone[b]	37	nd	nd	60.2	375/373		256, 272, 352
Flavonol *O*-glycosides							
Quercetin 3-*O*-glucuronide-7-*O*-galactoside[b]	nd	nd	3	9	641/639	/303	256, 266, 354
Quercetin 3-*O*-glucuronide-7-*O*-glucoside[b]	nd	nd	4	10.1	641/639	/303	256, 266, 354
Hexahydroxyflavone 3-*O*-dihexoside[a]	4	nd	nd	13.7	641/639	479, 317/315	256, 266, 356
Quercetin 3-*O*-rhamnosylgalactoside[a]	nd	nd	8[c]	23.1	611/609	303/301	256, 266, 354
Quercetin 3- *O*-rutinoside[a]	nd	nd	9[c]	24.2	611/609	303/301	256, 266, 354
Quercetin 7-*O*-glucoside[a]	nd	10	nd	24.7	465/463	303/301	254, 266, 370
Petuletin 3-*O*-robinobioside[b]	nd	13[c]	nd	24.7	641/639	495, 333/331	258, 266sh, 350
Pentahydroxyflavone 7-*O*-hexoside[a]	10	nd	nd	25.7	465/463	303/301	256, 370
Quercetin 3-*O*-galactoside[a]	nd	nd	10[c]	26.3	463/461	303/301	256, 266sh,352
Petuletin 3-*O*-glucoside[a]	nd	14[c]	nd	26.6	495/493	333/331	258, 266sh, 350
Quercetin 3-*O*-glucoside[a]	nd	nd	11[c]	27.1	463/461	303/301	256, 266sh,352
Pentahydroxymethoxyflavone 7-*O*-glucoside[b]	13	nd	nd	27.3	495/493	333/331	256, 370
Pentahydroxymethoxyflavone glucoside[b]	14	nd	nd	27.6	495/493	333/331	260, 360
Petuletin 3-*O*- manolylrobinobioside[a]	nd	15	nd	28	727/725	495, 333/331	258, 266sh, 350
Quercetin 3-*O*-glucuronide[a]	nd	nd	12	28.2	479/447	303/301	256, 266sh,352
Quercetin 3-arabinoside[a]	nd	nd	14	29	435/433	303/301	256, 266, 354

Compound	Peak Number			t_R	[M+H]+ and [M−H]⁻ *m/z*	Major fragments *m/z*	UV λ_{max} (mn)
	C	T	MA	(min)			
Kaempferol-3-*O*-rutinoside[a]	nd	nd	13[c]	29	595/593	287/285	266, 348
Isorhamonetin-3-*O*-rhamnosylgalactoside[a]	21	16	nd	29.5	625/623	479, 317/315	258, 266sh, 350
Isorhamonetin-3-*O*-rutinoside[a]	nd	18	nd	30.1	625/623	479, 317/315	258, 266sh, 350
Syringenin 3-*O*-rhamnosylhexoside[b]	nd	19	nd	30.4	655/653	509, 347/347	258, 266sh, 350
Kaempferol 3-*O*-glucoside[a]	nd	nd	15[c]	32.1	449/447	287/285	266, 348
Kaempferol-3-*O*-glucuronide[a]	nd	nd	19	32.7	465/463	287/285	nd
Isorhamnetin 3-*O*-glucoside[a]	21	23	nd	32.9	479/477	317/315	256, 266, 350
Petuletin 3-*O*-manolylrhamnosylhexoside[b]	nd	24	nd	33.2	741/739	333/331	256, 266, 350
Isorhamnetin 7-*O*-glucoside[a]	20	nd	nd	33.6	479/477	317/315	254, 372
Petuletin 7-*O*-glucoside[b]	22[c]	nd	nd	34.3	495/493?	333/331	258, 368
Tetrahydroxy-dimethooxyflavone 7-*O*-glucoside[b]	23	nd	nd	34.6	509/507	347/345	258, 368
Pentahydroxymethoxyflavone caffeoylglucoside[b]	25	nd	nd	36.1	657/655	333/331	278, 338
Pentahydroxymethoxyflavone caffeoylglucoside[b]	26	nd	nd	37.4	657/655	333/331	278, 334
Quercetin 3-*O*-caffeoylglucoside[a]	nd	nd	21	37.3	627/625	303/301	256, 266sh, 336

[a] the identification was confirmed by comparison to standards or positively identified compounds in reference plant samples;

[b] identified tentatively from the LC-MS data;

[c] reported in the plant previously;

[d] previous identification was corrected; nd: not determined.