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Comparison of the Phenolic Component Profiles of Skullcap (*Scutellaria lateriflora*) and Germander (*Teucrium canadense* and *T. chamaedrys*), a Potentially Hepatotoxic Adulterant

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Abstract

Introduction—*Scutellaria lateriflora*, commonly known as skullcap, is used as an ingredient in numerous herbal products. Unfortunately, it has occasionally been adulterated with *Teucrium canadense* or *T. chamaedrys*, commonly known as germander, which contains potentially hepatotoxic diterpenes. Chromatographic profiles of the phenolic components provide a means of distinguishing between these plants and enhancing public safety.

Objective—To develop a chromatographic method for the identification of *Scutellaria lateriflora* and two *Teucrium* species and to quantify the latter as adulterants.

Methodology—Samples were extracted with aqueous methanol and the extracts were analysed using a standardised LC-DAD-ESI/MS profiling method to obtain their phenolic profiles.

Results—Skullcap contained primarily flavonoids, while the major phenolic components of the two *Teucrium* species were the phenylethanoids, verbascoside and teucrioside. Using the phenylethanoids as markers, it was possible to clearly distinguish between the two genus and to determine 5% *Teucrium* mixed with *Scutellaria* using either ultraviolet absorption spectrometry or mass spectrometry in the total ion count mode. Using MS in the selective ion monitoring (SIM) mode, 1% *Teucrium* could be measured.

Conclusions—This study showed that chromatographic profiling was able to identify *Scutellaria* and *Teucrium*, separately and when mixed together.

Keywords

Scutellaria lateriflora; two *Teucrium* species; flavonoids; phenylethanoids; diterpenoids; LC-MS phenolic component profiles

Introduction

Skullcap (or mad dog skullcap), *Scutellaria lateriflora* L. (Lamiaceae) is a botanical medicine and supplement that is used as an ingredient in numerous herbal supplements that are available over the counter. *S. lateriflora* has a long history of use in Western herbal

medicine as a nervine, to quiet and support the nervous system and to reduce anxiety, sleeplessness and various types of spasms (Rafinesque, 1830; King, 1866; Mills and Bone, 2000; Sarris, 2007; Wojcikowski *et al.*, 2007). The single clinical trial testing its efficacy for relieving anxiety was based on a non-validated, subjective assessment scale (Wolfson and Hoffmann, 2003). Pre-clinical data have suggested pharmacological mechanisms (e.g. GABA and serotonin receptor modulation) that may be partially responsible for *Scutellaria's* putative effects (Awad *et al.*, 2003; Gafner *et al.*, 2003b; Xu *et al.*, 2006).

S. lateriflora has been subjected to adulteration with various species of germander, *Teucrium canadense* L. and *T. chamaedrys* L. (Lamiaceae), which contain potentially hepatotoxic neoclerodane diterpenes such as teucrin (Fig. 1) (Bedir *et al.*, 2003; Sundaresan *et al.*, 2006; Rader *et al.*, 2007). The hepatotoxicity of *Teucrium* arises from the bioactivation of teucrin A by cytochrome P450 to create reactive metabolites (Kouzi *et al.*, 1994; Haouzi *et al.*, 2000). The data suggest that oxidation of the furan ring is necessary for hepatotoxicity. The neoclerodane diterpenes in skullcap contain a tetrahydrofurofuran ring rather than the furan rings found in *Teucrium* (Bruno *et al.*, 1998, 2002; Rosselli *et al.*, 2004). The main phenolic components of *T. canadense* and *T. chamaedrys* are the phenylethanoid glycosides (Fig. 1; Sticher and Lahloub, 1982; Gafner *et al.*, 2003a; Kadifkova-Panovska *et al.*, 2005; Serrilli *et al.*, 2007).

The purpose of this study was to develop analytical methodology that could be used to compare *S. lateriflora* and *Teucrium* using polyphenols and/or diterpenes. Phenolic profiles were acquired using high performance liquid chromatography with diode array and electrospray ionisation/mass spectrometric detection (LC-DAD-ESI/MS) (Lin and Harnly, 2007). Seventy phenolic components were identified in *S. lateriflora* and *Teucrium* spp. The chromatographic profiles showed clear differences between the two genus and the three species.

Experimental

Standards and chemicals

Luteolin (5,7,3',4'-tetrahydroxyflavone), luteolin 7-*O*-glucoside, apigenin (5,7,4'-trihydroxyflavone), baicalein (5,6,7-trihydroxyflavone) and baicalin (baicalein 7-*O*-glucuronide) were purchased from Sigma Chemical Co. (Saint Louis, MO, USA). Wogonin (5,7-dihydroxy-8-methoxyflavone), scutellarein (5,6,7,4'-tetrahydroxyflavone), scutellarin (scutellarein 7-*O*-glucuronide), verbascoside and isoverbascoside were purchased from Chroma-Dex Inc. (Irvine, CA, USA). Luteolin 7-*O*-rutoside, diosmetin (5,7,3'-trihydroxy-4'-methoxyflavone), diosmin (diosmetin 7-*O*-rutoside) and chrysin (5,7-dihydroxyflavone) were purchased from Extrasynthese (Genay, Cedex, France).

Formic acid, hydrochloric acid (37%) and HPLC-grade solvents (acetonitrile and methanol) were purchased from VWR Scientific (Seattle, WA, USA). HPLC-grade water was prepared from distilled water using a Milli-Q system (Millipore Lab., Bedford, MA, USA).

Plant materials

Nine samples of the aerial parts of *S. lateriflora* (SL), four samples of *Teucrium canadense* (TCA) and seven samples of *T. chamaedrys* (TCH) were obtained from the American Herbal Pharmacopoeia (AHP). All the samples, except one, were dried plant materials. The exception was sample SL P120, which was in the form of a tincture prepared from 1.0 g of the plant materials extracted with 5.0 mL of 65% ethanol water. A collection of botanically authenticated and commercial materials was obtained. Vouchers for the authenticated materials and retention samples were deposited in the herbarium of the AHP.

Extraction

One hundred milligrams of dried ground sample was mixed with 5.0 mL of methanol–water (60:40, v/v) and sonicated for 60 min using an FS30H sonicator (40 kHz, 100 W) (Fisher Scientific, Pittsburgh, PA, USA) at room temperature. The slurry was centrifuged at 2500 rpm for 15 min (IEC Clinical Centrifuge, Damon/IEC Division, Needham, MA, USA). The supernatant was filtered through a 17 mm (0.45 μ m) PVDF syringe filter (VWR Scientific, Seattle, WA, USA) and 50 μ L of the extract was injected onto the LC column (Lin and Harnly, 2007).

The tincture was diluted 1:10 (v/v) with the aqueous methanol extraction solvent described above and filtered prior to injection. To avoid errors arising from unexpected degradation of the phenolic compounds, the LC determinations were completed within 24 h of the extraction.

Acid hydrolysed samples

Filtered sample extracts (0.50 mL) were 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 sonicated for 10 min. The solution was re-filtered prior to HPLC injection (Lin and Harnly, 2007).

LC-MS conditions

All samples were analysed by LC-DAD-ESI/MS. The instrument consisted of an Agilent 1100 HPLC (Agilent, Palo Alto, CA, USA), coupled sequentially with a DAD, and MS (MSD, SL mode). A Waters (Waters Corp., Milford, MA, USA) Symmetry column (C₁₈, 5 μ m, 250 $\times$ 4.6 mm) with a Sentry guard column (Symmetry, C₁₈, 5 μ m, 3.9 $\times$ 20 mm) was used at flow rate of 1.0 mL/min. The column oven temperature was set at 25°C. The mobile phase consisted of A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile) and the gradient increased linearly from 10% B initially to 26% B (v/v) at 40 min, to 65% B at 70 min, and finally to 100% B at 71 min and held at 100% B until 75 min. The DAD was set at 280, 310, 330 and 350 nm to provide real-time traces of the chromatograms. The UV–VIS spectra from 190 to 650 nm were recorded for plant component identification. Mass spectra were simultaneously acquired in the positive and negative ionisation (PI and NI) modes at low and high fragmentation voltages (100 and 250 V) over the range of m/z 100–2000. MS data were available in the total ion count (TIC) and selective ion monitoring (SIM) mode (Lin and Harnly, 2007).

Detection of the diterpenoids of the two *Teucrium* plants (Sundaresan *et al.*, 2006; Fig. 1) was accomplished in the SIM mode by monitoring m/z 329/327 (PI/NI) for teuflin and teucvidin (MW = 328), m/z 345/343 for teucrin A, teuflidin and isoteuflidin (MW = 344), m/z 377/375 for dihydroteugin (MW = 376) and m/z 391/389 for teucrin G (MW = 390). For *Scutellaria lateriflora* diterpenoids (Bruno *et al.*, 1998), m/z 469/467 for scutelcyprol A (MW = 468), m/z 493/491 for scutellaterin A and scuteocyprol A (MW = 492), m/z 535/533 for scutelaterin B (MW = 534), m/z 551/549 for ajugapitin (MW = 550) and m/z 553/551 for scutelaterin C (MW = 552) were monitored.

Results and Discussion

General characteristics of the phenolic components of *Scutellaria* and *Teucrium*

Figures 2–4 show the LC profiles of *S. lateriflora*, *T. canadense* and *T. chamaedrys* recorded at 350 nm. Tables 1–3 summarise the chromatographic data, i.e. retention times (t_R), wavelength of maximum absorbance (λ_{max}), protonated/deprotonated parent molecules ([M

+ H]⁺/[M-H]⁻), and major fragment ions (including aglycones, [A + H]⁺/[A-H]⁻), and the peak identification.

In general, peak identification was made by analysis of the recorded data listed in the tables. Both positive and provisional (isomers not specified) identification are provided for the main flavonoids. Positive identification was possible by comparison of data for specific peaks to data for standards or by comparison of the data in the tables with that reported in the literature (Harborne and Baxter, 1999; Gafner *et al.*, 2003a, b; Bergeron *et al.*, 2005). Confirmation of the identity of the aglycones was obtained by repeating the chromatographic analysis after hydrolysis of the samples.

The primary flavonoids found in *Scutellaria* were flavones and flavanones, which were easily distinguished by their UV spectra. For example, the UV spectra of the flavanones (e.g. dihydroxy-scutellarein 7-*O*-glucuronide, Table 1, peak 4) have a λ_{max} around 286–290 nm (band II) with a shoulder between 340 and 360 nm (band I). This is different from the flavones (e.g. scutellarein 7-*O*-glucuronide, Table 1, peak 5), which have a λ_{max} at 282 and 336 nm.

Glucuronides of the flavones and flavanones were the major flavonoid components of *Scutellaria*. The glucuronides were easily distinguished using in-source collision-induced dissociation with a high fragmentation voltage. The glucuronides contributed 176 *amu* to the molecular weight of the aglycones. In general, they behaved similarly to other glycosides. They were not, however, completely hydrolysed using the conditions normally used for hydrolysis of flavonoid glycosides (Lin and Harnly, 2007).

***S. lateriflora* samples**

Figure 2 shows the chromatographic profiles of five samples of aerial parts of *S. lateriflora* (SL): (A) SL 2322, (B) SL 2492, (C) SL 811, (D) SL 482 and (E) SL P120 (an aqueous ethanol tincture). The first four [Fig. 2(A–D)] represent typical chromatograms of the extracts and agree well with four other samples (SL 1050, SL 1057, SL 2341 and SL 2491) that are not shown.

To date, 10 flavonoids have been positively identified in *S. lateriflora* (Gafner *et al.* 2003a, b). In this study, identification of dihydroxyscutellarein 7-*O*-glucuronide (Table 1, peak 4), scutellarein 7-*O*-glucuronide (scutellarin) (peak 5), ikonnikoside I (5,6,7,2'-tetrahydroxyflavone 7-*O*-glucuronide) (peak 6), baicalin (baicalein 7-*O*-glucuronide) (peak 12), lateriflorin (5,6,7-trihydroxy-2'-methoxyflavone 7-*O*-glucuronide) (peak 13), 5,6,7-trihydroxyflavanone 7-*O*-glucuronide (peak 14), oroxylin A 7-*O*-glucuronide (peak 17), baicalein (peak 23), wogonin (peak 27) and oroxylin (or oroxylin A, 5,7-dihydroxy-6-methoxyflavone) (peak 29) was based on previously reported data. Some of the flavonoids (peaks 3, 5, 12, 23 and 27) were identified by direct comparison with standards as indicated in Table 1. Vitexin (apigenin C8-glucoside, peak 3) has not been previously reported in this plant. Three previously reported flavonoids (scutellarein, 5,6,7,2'-tetrahydroxyflavone, and 5,6,7-trihydroxy-2'-methoxyflavone) were not detected in the extract at this concentration and injection volume.

All of the confirmed *S. lateriflora* samples [Fig. 2(A–D) and the five samples not shown] showed similarly characteristic profiles. In each, baicalin (peak 12) was the dominant flavonoid (greatest peak area) with scutellarein 7-*O*-glucuronide (peak 5), lateriflorin (peak 13), ikonnikoside (peak 6) and 5,6,7-trihydroxyflavanone 7-*O*-glucuronide (peak 14) also providing strong peaks.

Two *Teucrium* species

Figures 3 and 4 show the chromatographic profiles for *T. canadense* and *T. chamaedrys* samples, respectively. The data are summarised and identifications are provided in Table 2. The phenylethanoids, verbascoside and teucrioside, have been previously reported (Sticher and Lahloub, 1982; Gafner *et al.*, 2003a; Serrilli *et al.*, 2007) and were easily identified by their UV and MS data. The presence of verbascoside and its isomer isoverbascoside was confirmed by direct comparison to standards. Other phenylethanoid glycosides, cinnamic acid derivatives and flavonoids were identified as shown in Table 2.

The four *T. canadense* samples (TCA 407, TCA 420, TCA 2312 and TCA 2312a) shown in Fig. 3 have similar phenolic profiles. For each, verbascoside (peak 6) was the largest peak, although the areas varied by as much as a factor of 42 (Table 3). The verbascoside content ranged from 0.23 to 9.66%, by dry weight, with TCA 407 having the highest verbascoside content and TCA 2312a the lowest.

Chromatographic profiles for five (TCH 137, TCH 2272, TCH 2311, TCH 2490 and TCH 2708) of the seven *T. chamaedrys* samples analysed are presented in Fig. 4. The two samples not shown (TCH 138 and TCH 2311b) had profiles identical to that of TCH 137. All contained teucrioside (peak 10) as their major phenolic compound with peak areas varying by a factor of 5. The teucrioside contents of the seven samples ranged from 0.45 to 2.30%, by dry weight (Table 3).

Verbascoside was used as a standard for the quantitative determination of teucrioside with a correction for the difference in the molecular weights since they are structurally identical except for the sugar at the R₁ position. This difference, however, does not influence the absorption coefficient.

The profiles in Fig. 4 show that the same flavonoids appear in all the *T. chamaedrys* samples but the relative ratio of the individual flavonoid and total flavonoid content to that of teucrioside (based on peak area) varies considerably. TCH 2490 had the highest flavonoid content followed by TCH 2272. Taxonomically, however, the phenolic profiles show that these samples are very similar to each other and are distinguishable from *T. canadense* and the *Scutellaria* genus.

MS detection of diterpenoids in *Teucrium* and *Scutellaria lateriflora*

The terpenoids are only detectable by MS. This approach has been used previously for the determination of terpenoids in several plants, including the terpene lactones in *Ginkgo biloba* leaves and products (Lin *et al.*, 2008). The structures of the seven diterpenoids reported in the literature (Sundaresan *et al.*, 2006) for *T. canadense* and *T. chamaedrys* are shown in Fig. 1.

Figure 5 shows the characteristic chromatographic profiles, in the TIC and SIM modes, of two *Teucrium* samples (TCA 420 and TCH 2708) obtained with positive ionisation and a low fragmentation voltage (PI 100 V). The major diterpenoid peaks detected were teuflin (peak 1, *t_R* 60.89 min, [M + H]⁺ = *m/z* 329), teucvidin (peak 2, *t_R* 61.90 min, [M + H]⁺ = *m/z* 329), dihydroteugin (peak 3, *t_R* 48.91 min, [M + H]⁺ = *m/z* 377), teucrin G (peak 4, *t_R* 51.86 min, [M + H]⁺ = *m/z* 391) and teucrin A (peak 5, *t_R* 52.53 min, [M + H]⁺ = *m/z* 345). Teucrin A may have co-eluted with its isomers (isoteuflidin and teuflidin, MW = 344). With MS detection, the isomers could not be differentiated.

Five diterpenoids have been reported in *S. lateriflora* (Bruno *et al.*, 1998, 2002; Rosselli *et al.*, 2004), but none of these compounds were detected in any of the authenticated *S. lateriflora* or *Teucrium* samples using TIC or SIM detection. However, in the SIM mode, a

small peak was observed for ajugapatin (t_R 66.42 min, $[M + H]^+ = m/z$ 551) in the *S. galericulata* sample (SG 2478b) (chromatogram not shown).

Estimation of *Teucrium* adulteration of *Scutellaria lateriflora*

The data presented above suggest that chromatographic profiling could be used for detecting adulteration of *S. lateriflora* by *Teucrium*. The phenylethanoid glycosides specific to *T. canadense* and *T. chamaedrys*, verbascoside (peak 6 in Fig. 3 and P-2 in Fig. 6) and teucrioside (peak 10 in Fig. 4 and P-1 in Fig. 6), respectively, have been previously proposed as possible markers for adulteration (Gafner *et al.*, 2003a). While detection of these peaks in a chromatogram of *S. lateriflora* would definitely indicate adulteration, the level of adulteration would be difficult to determine. In this study, the verbascoside and teucrioside concentrations in the 11 plants tested varied by factors of 42 and 5, respectively (Table 3).

Figure 6 shows chromatographic traces (with UV detection at 350 nm) of *S. lateriflora* sample 2322 spiked with varying amounts of *T. canadense* (5% TCA 2312a, 5% TCA 2312 and 1% TCA 407) and *T. chamaedrys* (5% TCH 2490, 5% TCH 2311 and 1% TCH 2708). The final, diluted concentrations were 0.11, 0.034 and 0.097% (dry weight) for verbascoside and 0.022, 0.045 and 0.023% (dry weight) for teucrioside. In each case, the phenylethanoids were detectable. Similar results were obtained using MS detection in the TIC mode. For both detection systems, the detection limits (see Experimental section) for verbascoside and teucrioside were approximately 0.002%. With MS detection in the SIM mode, the detection limit was approximately 5 times lower, at 0.0004%.

Each of the remaining *Scutellaria* samples (SL 811, SL 842, SL 1050 and SL 2492) were spiked with either 1% (TCA 407, TCA 420, TCH 137, TCH 138, TCH 2272, TCH 2311b and TCH 2708) or 5% (TCA 2312a, TCA 2312, TCH 2490 and TCH 2311) of the *Teucrium* samples, depending on the phenylethanoid concentration. In each case, verbascoside and teucrioside were clearly detected using both UV and MS in the TIC mode (chromatograms not shown).

These preliminary results suggest that low levels of adulteration of *S. lateriflora* by *Teucrium* could be detected using chromatographic profiling and may be relevant for products consisting of powders. However, further analyses with more samples of varying concentrations of compounds and different degrees of admixtures would strengthen these findings and are necessary to establish a validated method.

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Phenylethanoid glycosides

Verbascoside: R=caffeoyl, R₁=rhamnosyl, R₂=H, MW=624

Isoverbascoside: R₂=caffeoyl, R₁=rhamnosyl, R=H, MW=624

Teucrioside: R=caffeoyl, R₁=2''-xylosylrhamnosyl, R₂=H, MW=756

Figure 1.

Structures of the phenylethanoids and diterpenoids of *T. canadense* and *T. chamaedrys*.

Figure 2.
Chromatograms (350 nm) of 5 *S. lateriflora* samples: (A) SL 2322; (B) SL 2492; (C) SL 811; (D) SL 482; and (E) SL P120.

Figure 3. Chromatograms (350 nm) of five *T. canadense* samples: (A) TCA 420; (B) TCA 407; (C) TCA 2312; (D) TCA 2312a.

Figure 4.
Chromatograms (350 nm) of 7 *T. chamaedrys* (TCH) samples: (A) TCH 2272; (B) TCH 2490; (C) TCH2311; (D) TCH 2708; and (E) TCH 137.

Figure 5.

Chromatograms of *T. canadense* and *T. chamaedrys* samples with MS detection: (A) TCA 420, PI TIC; (B) TCA 420, PI SIM; (C) TCH 2708, PI TIC; and (D) TCH 2708, PI SIM. Peaks 1 and 2 = teuflin (M_r 328) and teucvidin (M_r 328), peak 3 = dihydroteugin (M_r 376), peak 4 = teucrin G (M_r 390), peak 5 = teucrin A (M_r 344), isoteuflidin (M_r 344) and teuflidin (M_r 344).

Figure 6.

Chromatograms (350 nm) of extracts of *S. lateriflora* (SL 2322) containing: (A) 5% TCA 2312a; (B) 5% TCA 2312; (C) 1% TCA 407; (D) 5% TCH 2490; (E) 5% TCH 2311; and (F) 1% TCH 2708. Peak P-1 = teucrioside and P-2 = verbascoside, and the numbers for the UV peak area of peaks 1 in (A–C) are 37.28, 113.96 and 319.82, and those for peak 2 in (D–F) are 97.48, 131.10 and 154.82, respectively.

Table 1

Flavonoids of *Scutellaria lateriflora*

Peak. no.	t_R (min)	$[M + H]^+ / [M - H]^-$ (m/z)	PI/NI aglycone, other ion (m/z)	UV* λ_{max} (nm)	Identification
1	23.56	—/251	—/—	314	n.d.
2	23.31	479/477	303/301	282, 336	Pentahydroxyflavone 7-O-glucuronide
3	25.26	433/431	313/311	270, 332	Vitexin ^a
4	28.85	465/463	289/287	286, 360sh	Dihydroxyscutellarein 7-O-glucuronide ^b
5	29.37	463/461	287/285	282, 332	Scutellarein 7-O-glucuronide ^{ba}
6	31.57	463/461	287/285	274, 336	Ikonnikoside ^b
7	33.64	463/461	287/285	n.d.	Tetrahydroxyflavone 7-O-glucuronide
8	34.56	—/293	—/—	314	n.d.
9	36.37	447/445	271/269	270, 338	Apigenin 7-O-glucuronide ^a
10	38.44	477/475	301/299	272, 332	Trihydroxymethoxyflavone O-glucuronide
11	40.58	417/415	271/269	272, 314sh	Baicalin 7-O-rhamnoside
12	42.77	447/445	271/269	278, 316	Baicalin ^{ba}
13	46.03	477/475	301/299	274, 332	Lateriflorin ^b
14	46.71	449/447	273/271	242, 282, 364sh	5,6,7-Trihydroxyflavanone 7-O-glucuronide ^b
15	47.61	447/445	271/269	280, 360sh	Hydroxymethoxyflavanone O-glucuronide
16	47.98	477/475	301/299	284, 350sh	Hydroxyldimethoxyflavanone glucuronide
17	48.51	461/459	285/283	272, 312sh	Oroxylin A 7-O-glucuronide ^b
18	49.73	507/505	331/329	278, 338sh	Hydroxyl-trimethoxyflavanone glucuronide
19	50.28	461/459	285/283	274, 342sh	Dimethoxy or flavanone glucuronide
20	50.83	463/461	287/285	288, 342sh	Dihydrowogonin 7-O-glucuronide
21	51.70	491/489	315/313	272, 336sh	Trimethoxy or flavanone glucuronide
22	52.24	345/343	—/—	282, 346sh	Tetramethoxy or flavanone
23	54.74	271/269	—/—	276, 322	Baicalin ^{ba}
24	55.22	715/713	271/269	n.d.	n.d.
25	56.85	715/713	539/537	280, 318sh	n.d.
26	58.55	287/285	—/—	292, 364sh	Dihydroxymethoxyflavanone
27	61.54	285/283	—/—	268, 338	Wogonin ^{ba}

Peak. no.	t_R (min)	$[M + H]^+ / [M - H]^-$ (m/z)	PI/NI aglycone, other ion (m/z)	UV* λ_{max} (nm)	Identification
28	62.56	315/313	—/—	272, 334	n.d.
29	63.08	285/283	—/—	270, 318	Oroxylin A ^b

^a Identified by comparison with a standard or reference compound.

^b Previously reported in the plant.

n.d., Not determined or unidentified.

Table 2
Phenolic compounds and diterpenoids of *Teucrium canadense* and *T. chamaedrys*

Peak. no.	t_R (min)	$[M + H]^+ / [M - H]^- (m/z)$	PI/NI aglycone, other ion (m/z)	UV* λ_{max} (nm)	Identification
<i>Teucrium canadense</i> phenolic compounds (Fig. 3)					
1	7.63	—/353	—/191,179,161,135	242,298sh,330	3-Caffeoylquinic acid ^a
2	12.62	—/353,451	—/242	298sh,330	n.d.
3	18.52	—/639	—/—	242,298sh,330	Phenylethanoid glycoside
4	19.12	—/639	—/—	242,298sh,330	Phenylethanoid glycoside
5	25.73	—/653	—/—	242,298sh,330	Phenylethanoid glycoside
6	27.45	—/623	—/—	242,298sh,330	Verbascoside ^a
7	29.66	609/607	—/—	252,266,342	Flavone glucuronide
8	30.71	—/623	—/—	242,298sh,330	Isoverbascoside ^a
9	31.78	609/607	301/299	274,334	Trihydroxymethoxyflavone glycoside
10	32.59	639/637	331/329	268,330	Dimethoxytrihydroxyflavone glycoside
11	33.44	609/607	301/299	256,274,344	Trihydroxymethoxyflavone glycoside
12	36.36	447/445	271/269	268,338	Apigenin 7- <i>O</i> -glucuronide ^a
13	37.28	477/475	301/299	252,266,348	Trihydroxymethoxyflavone glucuronide
14	38.46	447/445	271/269	268,338	Trihydroxyflavone 7-glucuronide
15	40.26	637/635	285/283	268, 336	Dihydroxymethoxyflavone di-glucuronide
16	46.15	491/489	315/313	250,268,348	Dihydroxydimethoxyflavone glycoside
17	47.33	n.d.	n.d.	n.d.	n.d.
18	50.16	461/459	285/283	266,344	Dihydroxymethoxyflavone 7-glucuronide
<i>Teucrium canadense</i> diterpenoids (Fig. 5)					
1	60.89	329/—	—/—	—	Teuflin
2	61.90	329/—	—/—	—	Teuvidin
<i>Teucrium chamaedrys</i> phenolic compounds (Fig. 4)					
1	11.23	—/353	—/191,179,161,135	216,298sh,330	5-Caffeoylquinic acid ^a
2	14.92	—/179	—/—	216,298sh,326	Caffeic acid ^a
3	17.76	—/771	—/—	216,298sh,330	Phenylethanoid glycoside
4	18.54	—/771	—/—	216,298sh,330	Phenylethanoid glycoside
5	23.19	611/609	287/285	252,268,342	Luteolin 7- <i>O</i> -diglucoside

Peak no.	t_R (min)	$[M + H]^+ / [M - H]^-$ (m/z)	PI/NI aglycone, other ion (m/z)	UV* λ_{max} (nm)	Identification
6	24.16	787/785	287/285	252,268,342	Luteolin 7- <i>O</i> -glycoside
7	24.48	787/785	287/285	252,268,342	Luteolin 7- <i>O</i> -glycoside
8	25.21	581/579	287/285	252,268,342	Luteolin 7- <i>O</i> -pentosylglucoside
9	25.78	595/593	287/285	252,268,342	Luteolin 7- <i>O</i> -rutinoside
10	26.29	—/755	—/—	242,298sh,330	Teucreoside
11	27.37	—/755	—/—	242,298sh,330	Teucreoside isomer
12	27.69	—/623	—/—	242,298sh,330	Verbascoside ^a
13	29.85	757/755	287/285	252,268,342	Luteolin 7- <i>O</i> -glycoside
14	30.67	653/651	287/285	252,268,346	Luteolin 7- <i>O</i> -glycoside
15	32.20	771/769	301/299	282,330	Diosmetin 7- <i>O</i> -glycoside
16	33.27	609/607	301/299	252,268,342	Diosmetin 7- <i>O</i> -rutinoside
17	34.32	449/447	287/275	268,334	Tetrahydroxyflavone 7- <i>O</i> -glycoside
18	36.59	447/445	271/269	266,336	Apigenin 7- <i>O</i> -glucuronide ^a
19	37.80	653/651	287/285	276,304,328	Tetrahydroxyflavone glycoside
20	38.29	667/665	301/299	252,268,346	Diosmetin 7- <i>O</i> -glycoside
21	45.71	593/591	285/283	268,328	Dihydroxymethoxyflavone glycoside
22	47.43	287/275	—/—	268,334	Luteolin ^a
23	49.19	667/665	301/299	278,306,328	Diosmetin 7- <i>O</i> -glycoside
24	52.50	n.d.	n.d.	266,340	n.d.
25	53.78	331/329	—/—	256,272,346	Diosmetin ^a
<i>Teucrium chamaedrys</i> diterpenoids (Fig. 5)					
3	48.91	377/—	—/—	—	Dihydroteuigin
4	51.86	391/—	—/—	—	Teucin G
5	52.53	345/—	—/—	—	Teucin A, isoteuflidin, or teuflidin
1	60.89	329/—	—/—	—	Teuflin

^aIdentified by comparison with a standard or reference compound.

n.d., Not determined or un-identified.

Table 3
Verbascoside and teucrioside content in *Teucrium*

Sample	Verbascoside (% dry weight)	Sample	Teucrioside (% dry weight)
TCA2312a	0.23	TCH2490	0.45
TCA2312	0.69	TCH2311	0.88
TCA420	5.5	TCH2722	0.94
TCA407	9.6	TCH138	1.47
		TCH137	1.56
		TCH2490	2.02
		TCH2708	2.30