Methods using liquid chromatography with UV detection (LC–UV), thin-layer chromatography (TLC), and digital photomicroscopy were developed to distinguish between the different species of Scutellaria lateriflora L. and its adulterants Teucrium canadense L. and T. chamaedrys L. Chemically, the 70% ethanol extract of S. lateriflora is characterized by the presence of flavonoids—predominantly baicalin, lateriflorin, dihydrobaicalin, and baicalein. The major compounds of the 70% ethanol extract of T. canadense are phenylpropanoids, with verbascoside as the most prominent, and a variable amount of teucrioside. Teucrioside is the major compound in T. chamaedrys, but it is not present in S. lateriflora. The presence of phenylpropane glycosides can therefore be used to distinguish between the S. lateriflora L. and the two Teucrium species by LC–UV and TLC. The abundant strap-shaped trichomes on the stem, as well as bristle-like trichomes on the leaf, are typically seen microscopically for T. canadense, whereas the waxy cuticle with numerous glandular scales is found in T. chamaedrys. These cell structures were used to determine the adulteration of S. lateriflora crude herb with either of the two Teucrium species.

Mead-dog skullcap (Scutellaria lateriflora L., Lamiaceae) is a perennial herb indigenous to North America, growing in wet places from Canada to Florida and westward to British Columbia, Oregon, and New Mexico. It derives its common name from the helmet-shaped upper lid of the seed pods. The aqueous extract of the flowering parts has been traditionally used by Native Americans as a nerve tonic and for its sedative and diuretic properties (1, 2). Work on the chemistry of S. lateriflora has begun only recently. Mono- and diterpenes (3, 4) have been reported, as well as several flavonoids (5; Figure 1).

Concerns about the safety of skullcap have been raised because of reports of hepatotoxic reactions after skullcap-containing preparations were ingested (6, 7). However, because adulterations of skullcap with germander [Teucrium sp. (8) and T. canadense L. (9)] have been described, it is not certain whether the hepatotoxicity is actually due to the skullcap (8). This point is underscored by the fact that the hepatotoxicity of germander is due to its content of diterpenes containing an oxidized furan ring (10) and the diterpenes isolated from S. lateriflora do not contain this moiety. The aim of this study was to find suitable chemical and microscopic markers to distinguish between skullcap and its potential adulterants.

Experimental

**Apparatus and Reagents**

(a) **Liquid chromatography (LC) system.**—The LC system consisted of an Agilent quaternary pump, a UV–Vis detector (DAD), and an automatic sample injector (Agilent 1100 Series, Agilent Technologies, Burlington, MA). Column used: Zorbax XDB C-18 (5 μm, 150 × 4.6 mm id) with a Zorbax XDB C-18 guard column from Agilent Technologies.

(b) **Mass spectrometer.**—Agilent 1100 Series LC mass selective detector (MSD) trap with electrospray ionization (ESI) interface.

(c) **Centrifuge.**—ICC Model CL (International Equipment Co., Needham, MA).

(d) **Balance.**—Mettler-Toledo AT261 DeltaRange (Mettler-Toledo, Greifensee, Switzerland).

(e) **Thin-layer chromatography (TLC) system.**—TLC was performed with a Linomat IV and Twin Trough Chamber...
from CAMAG (Wilmington, NC); a Spectroline Model CC-80 chamber (Spectronics Corp., Westbury, NY) was used for UV–Vis observations. TLC plates: 10 \( \times \) 10 cm silica gel 60 F254 HPTLC plates purchased from VWR Scientific Products (South Plainfield, NJ). Photographs were taken with a Kodak DC290 Zoom Digital Camera (Rochester, NY).

(d) **Digital photomicroscopy equipment.**—The microscopic analysis was performed with an Optiphot trinocular microscope from Nikon (Tokyo, Japan) and a Spot JR digital camera from Digital Camera Diagnostic Instruments, Inc. (Sterling Heights, MI).

(e) **Reagents.**—LC grade acetonitrile, methanol, and ethyl acetate, as well as reagent grade glacial acetic acid, were from Fisher Chemical Co. (Fairlawn, NJ). Trifluoroacetic acid was purchased from Sigma Chemical Co. (St. Louis, MO). Diphenylboric acid aminoethylester was obtained from Aldrich Chemical Co. (Milwaukee, WI) and polyethylene glycol from Polarchem Corp. (Garden Grove, CA). Formic acid and chloral hydrate were purchased from Spectrum (Gardena, CA).

(f) **Preparation of Sample Extracts**

(a) **General extraction procedure.**—Dried *S. lateriflora* L. herb and *T. canadense* L. herb were obtained from Blessed Herbs (Oakham, MA). Voucher specimens (Nos. 02004 and 01002, respectively) were deposited at the herbarium of Tom’s of Maine. Dried *T. canadense* was also collected and identified by R. Gauthier (Québec, Canada). A voucher specimen (No. 2002-126) was deposited at the Herbier Louis-Marie (Université Laval, Québec, Canada). Dried *T. chamaedrys* L. herb was collected and identified by H. Guinaudeau (Angers, France). A voucher specimen (No. 01003) was deposited at the herbarium of Tom’s of Maine. The above-ground plant material was ground and extracted with 70% alcohol (ratio 1:10). For the isolation of skullcap flavones, fresh *S. lateriflora*, obtained from Tom’s of Maine, was ground and extracted with 70% alcohol (ratio 1:10). The solution was mixed in a 1000 mL Erlenmeyer flask.

(b) **MP for TLC.**—50.0 mL ethyl acetate, 5.5 mL formic acid, 5.5 mL acetic acid, and 12.5 mL water were mixed in a 100 mL Erlenmeyer flask. Volumes were measured with graduated pipets.

(c) **TLC reagent preparation.**—Natural products (NP) reagent: 0.5 g diphenylboric acid aminoethylester was dissolved in 50 mL methanol. Polyethylene glycol (PEG) reagent: 2.5 g PEG was dissolved in 50 mL ethanol.

(d) **Microscopy reagent preparation.**—Chloral hydrate crystals (45 g) were dissolved in a mixture of 25 mL hydrochloric acid–water (1 + 8) to which 10 mL glycerol was added.

Preparation of Solutions

(a) **Mobile phase (MP) for LC.**—Solvent containing 0.05% trifluoroacetic acid was prepared by adding 0.4 mL trifluoroacetic acid to 800 mL solvent in a graduated cylinder. The solution was mixed in a 1000 mL Erlenmeyer flask.

Figure 1. Chemical structures of main flavonoids occurring in *S. lateriflora*.

Figure 2. LC–UV/MS analysis of 70% alcohol extract of *S. lateriflora* above-ground parts.
The 70% alcohol extract of dried above-ground parts of *S. lateriflora* was used for screening by LC–UV/MS. Comparison of the UV and MS spectra with reference standards allowed identification of baicalein (1), baicalin (2), scutellarin (3), wogonin (4), and lateriflorein (5) in the extract. Because some of the major peaks in the LC–UV trace of the fresh skullcap could not be identified with on-line methods, a 70% ethanol extract was fractionated and 3 flavone–glucuronides, ikonnikoside I (6), lateriflorin (7), oroxylin A-7-O-glucuronide (8), and dihydrobaicalin (9) were isolated as described earlier (5).

The LC–UV/MS analysis of a 70% ethanol extract is shown in Figure 2. The main compounds in the total ion current (TIC) trace and the UV chromatogram (λ = 280 nm) are the flavone–glucuronides 2 and 7 and dihydrobaicalin 9. The MS conditions, which were optimized by using a reference solution (100 µg/mL baicalin), gave the [M+H]+ ion as the base peak for flavone–glucuronides, and the MS/MS analysis showed the loss of a glucuronic acid moiety by a characteristic
Figure 4. TLC analysis using natural products reagent and polyethylene glycol reagent; UV at 365 nm. Reference standards have the following Rf values: lane 1, baicalein, 0.93; lane 3, baicalin, 0.41; lane 4, ikonnikoside I, 0.39; lane 6, verbascoside, 0.57; lane 8, teucrioside, 0.32. Lane 5 is extract of *T. canadense* with 2 bright green/white bands at Rf = 0.32 and 0.57 corresponding to reference standards in lane 6, Rf = 0.57 for verbascoside, and lane 8, Rf = 0.32 for teucrioside. Lane 7 is extract of *T. chamaedrys* with one bright green/white band at Rf = 0.32 corresponding to reference standard for teucroseide. Lane 2 is extract of *S. lateriflora* with a dark red band at Rf = 0.40 corresponding to baicalin and ikonnikoside I of lanes 3 and 4.

Figure 5. TLC analysis using UV detection at 254 nm. Reference standards have the following Rf values: lane 1, baicalein, 0.93; lane 3, baicalin, 0.41; lane 4, ikonnikoside I, 0.39; lane 6, verbascoside, 0.57; lane 8, teucrioside, 0.32. The *S. lateriflora* extract in lane 2 demonstrates presence of baicalin and ikonnikoside I. The presence of verbascoside and teucroseide is demonstrated in *T. canadense*, lane 5. Teucroseide is present in *T. chamaedrys*, lane 7.
Figure 6. (a) *S. lateriflora*, cross section of stem showing (A) thin-walled parenchyma of pith; (B) cortex; and (C) epidermis with flattened cells; 100× magnification. (b) *S. lateriflora*, cross section of leaf showing finger-like trichome with warty exine; 400× magnification.
Figure 7. (a) *T. canadense*, cross section of stem showing epidermal trichomes, epidermis, cortex, and parenchyma of hollow pith; 200× magnification. (b) *T. canadense*, surface of stem showing strap-shaped multicellular trichomes; 200× magnification.
Figure 8. (a) *T. chamaedrys*, cross section of stem showing (A) epidermis; (B) cortex; (C) pith comprised of thin-walled parenchyma; 100× magnification. (b) *T. chamaedrys*, surface of leaf showing waxy cuticle with numerous stomata and glandular scales; 400× magnification.
compounds in weak band was observed at Rf = 0.57 in the skullcap extract studied contained verbascoside and/or teucrioside. A very TLC chromatograms (Figures 4 and 5); both glycosides are suitable markers to detect any adulteration of confirmed by the LC–UV/MS analysis (Figure 3). Neither Teucrioside (11) occurred in only one of the lots analyzed. None of the flavones found in skullcap were detected. Teucrioside (11) has been reported to be one of the major compounds in T. chamaedrys as well (11), which was confirmed by the LC–UV/MS analysis (Figure 3). Neither 10 nor 11 occur in S. lateriflora; therefore, the phenylpropanoid glycosides are suitable markers to detect any adulteration of skullcap with germander. The differences in the chemistry of skullcap and its potential adulterants were also evident in the TLC chromatograms (Figures 4 and 5); both Teucrium species studied contained verbascoside and/or teucrioside. A very weak band was observed at Rf = 0.57 in the skullcap extract when the NP reagent was used (Figure 4), which might suggest the presence of verbascoside. The presence of verbascoside in skullcap, however, was definitely ruled out by UV detection at 254 nm (Figure 5). This method provides a simple and cost-effective alternative to LC–UV analysis to determine the presence of adulteration by germanders.

The microscopic analysis of S. lateriflora stems (Figure 6a) showed the following characteristic features: epidermis, cortex, and parenchyma of the pith. The epidermis is composed of a layer of compactly arranged cells, the cortex has thick-walled collenchymatous cells, and the pith has loosely arranged thin-walled parenchyma cells and a hollow center. Characteristics of the leaf are the lower epidermis, sinuous walls, and large glandular scales with 4 sections, finger-like covering trichomes, with a warted exine (Figure 6b) as well as anisocytic and diacytic stomata.

The microscopic analysis of T. canadense stems (Figure 7a) showed the following characteristic features: epidermis, cortex, and parenchyma of the leaf, and the waxy cuticle, and numerous glandular scales and stomata (Figure 8b).

The images obtained by digital photomicroscopy of the crude raw materials of the 3 different species of plants examined demonstrated that this method is a simple and elegant means of determining adulteration of skullcap with germanders. The mere presence of the abundant strap-shaped trichomes on the stem, the bristle-like trichomes on the T. canadense leaf, and the waxy cuticle with numerous glandular scales on the T. chamaedrys leaf, which are not present in S. lateriflora, distinguishes adulteration between germander and skullcap.

Acknowledgments

We thank O. Sticher (Swiss Federal Institute of Technology (ETH), Zurich, Switzerland) for providing an authentic sample of teucrioside.

References

(1) Burlage, H.M. (1968) Index of the Plants in Texas with Reputed Medicinal and Poisonous Properties, College of Pharmacy University of Texas, Austin, TX


