Fingerprint of Selected Salvia Species by HS–GC–MS Analysis of Their Volatile Fraction

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Abstract

Twenty species of *Salvia*, naturally grown or cultivated in Poland, are investigated by headspace gas chromatography–mass spectrometry analysis. The main components of the volatile fraction of *Salvia* species are identified as *a*-pinene, camphene, β-pinene, thujol, camphor, β-chamigrene, and cadina-3,9-diene. There are also the compounds that can be considered as chemotaxonomic markers, namely β-myrcene for *Salvia lavadulifolia*, β-phelandrene for *Salvia verticillata*, τ-terpinene for *Salvia stepposa*, and isocaryophyllene and caryophyllene for *Salvia officinalis*. Certain compounds (such as *o*-cymene present in *Salvia canariensis* and *Salvia stepposa*; β-trans-ocymene present in *Salvia lavadulifolia*, *Salvia sclarea,* and *Salvia amplexicaulis;* thujenone present in *Salvia staminea*, *Salvia atropatana, Salvia jurisicii,* and *Salvia officinalis;* and thujone present in *Salvia azurea, Salvia lavandulifolia, Salvia hians,* and *Salvia triloba*) can constitute chemotaxonomic advice for the aforementioned species. Also, the lack of certain compounds otherwise common in the individual sage species can be considered as chemotaxonomic advice (e.g., *Salvia sclarea* has no α-pinene and β-pinene; *Salvia lavadulifolia* lacks camphene; *Salvia triloba* lacks β-pinene and camphene; and *Salvia officinalis* lacks β-chamigrene, thujol, and cadina-3,9-diene).

Introduction

Genus *Salvia* L. (*Lamiaceae*), commonly known as sage, derives its name from the Latin verb "salvere," which means "to be in good health" or "to be well" (1). *Salvia* is one of the larger genera belonging to the subfamily *Nepetoideae* and contains ~ 900 species (2), which are widely distributed in the temperate, subtropical, and tropical regions of the globe but are rare in the Arctic or Alpine regions (3,4). A large number of the aromatic taxa make this family commercially important, owing to their odors, infusions, tinctures, and flavors that are used as components of herbal products (5). Genus *Salvia* makes nearly one quarter of the recognized genera of the *Lamiaceae*, and it is recognized around the world for having commercial, medicinal, and cultural importance, due to useful essential oils produced by *Salvia* foliage (6,7). A number of *Salvia* species have been cultivated as ornamental plants for their aromatic and aromatherapeutic properties or for the confectionery as culinary herbs (3). Approximately 30 *Salvia* species naturally grow or are cultivated in Poland (8). Medicinal applications of *Salvia* herbs are diverse due to a different pharmacological activity of certain species. Some of them are applied as alimentary tract stimulators and digestion regulators with additional antiseptic properties. Other *Salvia* species are known for their antipyretic, analgesic, and expectorant properties, and they are readily applied in therapy of influenza and the cold. Still some other species are used in therapy of psychoses, depressions, and neuroses due to sedative properties of the respective decoctions. Application of sage against menstruation disorders was also reported (9).

Essential oils are always a mixture of up to several hundred constituents, most of them being hydrocarbons and oxygenated compounds (10). They represent a small fraction of a plant's composition, yet they possess an interesting pharmacological activity. Terpenes are responsible for antiphlogistic, antihistaminic, and antiallergic properties of *Salvia* (11). For antibacterial and antifungal activity of essential oils, such compounds are responsible (e.g., α-pinene, β-pinene, borneol, and bornyl acetate) (12,13). Certain components of essential oils (α-pinene, β-pinene, and camphene) additionally show spasmolytic activity (14,15). Composition of essential oils from certain *Salvia* species is described in literature (16–23).

Chromatographic fingerprinting is one of the most popular methods in herbal medicine studies, and it has been widely introduced and accepted by the World Health Organization, Food and Drug Administration, European Medicines Agency, German Commission E, British Herbal Medicine Association, and Indian Drug Manufacturers' Association (24). According to its definition, a chromatographic fingerprint is a chromatogram that represents chemical characteristics of the herb (25). According to the majority of published methods, chromatographic fingerprints of herbs have been constructed based on a single chromatogram (26–29). These methods are usually focused on qualitative and quantitative determination of individual known compounds or of a small set of several compounds.

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Table I. Volatile Compounds in Salvia Species, Their Respective Retention Times, and Semi-Quantitative Evaluation of their Relative Contributions (%) to the Overall Volatile Fraction*.

Volatile Compound	α-Pinene	Camphene	β-Pinene	β-Myrcene	o-Cymene	Limonene	β-Phelandrene	β-trans-Ocymene	Eucalyptol	Cis-Ocymene	τ-Terpinene	*p*-Mentha-1,4-diene	Thujol	Thujenone	Thujone	Camphor	Borneol	Bornyl acetate	β-Chamigrene	Cadina-3,9-diene	Murolene	Isocaryophyllene	Caryophyllen
No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Retention time (min)	10.97	11.75	12.20	12.25	13.15	13.26	13.32	13.36	13.50	13.91	13.95	14.59	15.12	15.35	15.47	16.11	16.65	18.84	21.60	22.43	23.31	21.70	22.53
	Peak Height (%)																						
S. azurea	0.48	1.99	2.69			13.92			3.24	2.27	+		1.79		0.94				5.46	2.76	4.55		
S. lavandulifolia	7.22		16.15	2.82				++	21.33	1.74	+	2.47			13.08	19.03	3.77	1.63	0.15	1.02			
S. verticillata	10.72	2.39	2.49			5.85	+		0.80	1.54	+	2.06	2.18			5.23	0.53	0.60	2.17	2.33	1.60		
S. pratensis	1.32	17.42	2.47			2.35			3.39			+	9.11			3.06	+		1.87	2.46	1.29		
S. staminea	10.09	4.86	5.23			15.68			3.58	2.30	+		3.33	0.69					2.98	1.41			
S. deserta	35.35	4.01	13.02			7.45						3.59	2.79						0.44				
S. cadmica	1.72	14.91								2.42	+		8.98			2.45			25.26				
S. forskaohlei	6.94	5.97	55.79			9.07					1.01	3.09	3.21			0.88			1.80	0.80			
S. sclarea		23.36						1.63				+	12.31			2.74			3.63	+			
S. canariensis	16.42	0.02	4.84		0.01			18.97			+							22.49	14.78		0.03		
S. hians	1.26	2.67	3.13			9.95			1.71		1.25		0.98		0.50			6.00	22.05	2.61	2.57		
S. triloba	11.59								23.40						17.81	17.75							
S. glutinosa	+	15.95	11.97			10.50					+		7.02			1.65		2.10	5.58	3.47	+		
S. nemorosa	8.98	13.74	2.20							7.44	+	2.70	5.52			1.23			6.88	1.17	+		
S. tesquicola	9.54	16.91	3.15								8.05	3.02	6.15			+			3.42	+			
S. amplexicaulis	2.03	24.00	+					+				+	7.63			+			8.50				
S. atropatana	+	5.74	2.45			4.12			3.26				1.85	+					0.90				
S. stepposa	2.88	17.26	2.77		1.88						6.77		8.42			2.66			3.39	+			
S. jurisicii	+	12.53	+						1.46				14.75	6.20		6.04			+	+			
S. officinalis	0.02	+	0.40						23.72					17.52		18.22	2.42	0.22				7.19	4.91

* = estimated from the respective peak heights
\+ = data between limit of detection (LOD) and limit of quantification (LOQ)
++ = lack of adequate (i.e., enabling quantification) peak separation

The aim of this paper is to make a comparison of the volatile fractions originating from the selected *Salvia* species that grow and are cultivated in Poland in order to find similarities and differences in chemical composition and biological activity among particular species and to check chemotaxonomic markers for the individual species.

Materials and Methods

Plant material

Samples of *Salvia* species investigated in this study were collected in the Pharmacognosy Garden of Medical University (Lublin, Poland) in August 2007. These *Salvia* species are typical of the temperate climatic zone and are used for diverse purposes. Certain species (e.g., *S. officinalis, S. lavandulifolia, S pratensis, S. deserta, S. sclarea, S. canariensis, S. amplexicaulis,* and *S. atropatana*) reveal the well-pronounced pharmacological activity and hence, they are used as medicinal herbs. Some other species (*S. azurea, S. lavoandulifolia, S. cadmica, S. triloba,* and *S. glutinosa*) are used as fragrance spices or perfume components. Finally, *S. verticillata, S. nemorosa*, and *S. tesquicola* are the appreciated honey-yielding plants. Numerous *Salvia* species (e.g., *S. azurea, S. pratensis, S. hians,* and *S. jurisicii*) are grown as ornamental plants and decorate flower beds in many gardens worldwide. The botany specialists identified each investigated species, and voucher specimens are deposited in the herbarium of the Department of Pharmacognosy, Medical University (Lublin, Poland). The investigated species are listed in Table I.

Plant material was dried for 40 h in an oven with a forced air flow at 35ºC to 40ºC. Then the obtained dry material was stored in a refrigerator until the commencement of the analysis. Finally, 1 g of each plant species was weighed, powdered in a porcelain mortar, and placed in 10-mL glass vials stoppered with a silicon-teflon septum. Three replicates of each sample were processed in an identical way.

Headspace gas chromatography–mass spectrometry

The headspace gas chromatography–mass spectrometry (HS–GC–MS) analyses were carried out with use of a TRACE 2000 model GC with an MS TRACE model mass detector (ThermoQuest, Waltham, MA) equipped with a CTC Analytics model autosampler (Combi PAL, Basel, Switzerland) used in the headspace mode. Temperature and time of the headspace desorption were, respectively, 70ºC and 15 min. 0.5 mL of the headspace phase was introduced on to the DB-5 capillary column (30 m × 0.25 mm i.d., 0.25-µm film thickness; Agilent Technologies, Palo Alto, CA). Helium (p = 100 kPa) was used as carrier gas. Gradient analysis was run using the following temperature program: 40ºC (3 min); 40–150ºC (8ºC/min); and 150ºC (15 min). The temperature of the injector was kept constant at 150ºC. Mass spectrometer was fitted with an EI source operated at 70 eV. Identification of individual compounds was based on a comparison of the obtained mass spectra of the individual chromatographic peaks with those valid for the standards and available from the National Institute of Standards and Technology (Gaithersburg, MD) software library. A comparison was also carried out of the retention times valid for individual peaks from the *Salvia* samples with those of the known ether oils components. To this effect, we used pine oil, peppermint oil, eucalyptus oil, and juniper oil as the sets of the volatile standards (Apotheca Pacis, Rybnik, Poland).

The identified compounds are listed in Table I. Relative contributions of individual volatile compounds contained in each individual *Salvia* species to the overall volatile fraction were evaluated from the respective peak heights. As is the case with most chromatograms recorded by the tandem GC–MS system, the presented evaluation is of semi-quantitative importance only; this is because it does not take into the account individual ionization yields of individual volatile compounds.

Results and Discussion

Gas chromatograms of the investigated *Salvia* species have perceptibly differentiated profiles (Figure 1). Retention times of a volatile fraction are contained within a relatively wide interval ranging from 8–27 min. Most of the investigated species (e.g., *S. azurea, S. verticillata, S. pratensis, S. staminea, S. cadmica, S. sclarea, S. canariensis, S. glutinosa, S. nemorosa,* and *S. tesquicola*) have their volatile compounds distributed within a wide range of the retention times. Some other species (e.g., *S. lavandulifolia, S. deserta, S. forskaohlei, S. triloba, S. atropatana, S. stepposa,* and *S. jurisicii*) are rich in compounds characterized by low retention times, whilst *S. hians* is rich in compounds of higher retention times.

The identified volatile compounds are listed in Table I. Seven compounds appear in most of the investigated species, and these are α-pinene (present in 19 species), camphene (present in 18 species), β-pinene (present in 17 species), thujol (present in 16 species), camphor (present in 14 species), β-chamigrene (present in 18 species), and cadina-3,9-diene (present in 13 species). In Table II, we show the chemical structures of the aforementioned seven volatile compounds most frequently appearing in the examined *Salvia* species and their respective mass spectra.

There are some other compounds that are relatively rare with the investigated sage species. For the sake of an example, β-myrcene was found in *S. lavandulifolia*; β-phelandrene was found in *S. verticillata*; τ-terpinene was found in *S. stepposa,* and isocaryophyllene and caryophyllene were found in *S. officinalis*. These compounds can be regarded as chemotaxonomic markers for the aforementioned species. Such compounds as *o*-cymene (present in *S. canarien*sis and *S. stepposa*), β-trans-ocymene (present in *S. lavandulifolia, S. sclarea* and *S. amplexicaulis)*, thujenone (present in *S. staminea, S. atropatana, S. jurisicii* and *S. officinalis*), as well as thujone (present in *S. azurea, S. lavandulifolia, S. hians* and *S. triloba*) can be regarded as chemotaxonomic advice for the respective sage species.

Also the lack of certain (otherwise frequently occurring) compounds in some *Salvia* species provides chemotaxonomic advice for these particular species. In *S. sclarea*, no α-pinene and β-pinene can be found; *S. lavandulifolia* has no camphene; *S. triloba* contains no β-pinene and camphene, and *S. officinalis*

is deprived of β-chamigrene, thujol, and cadina-3,9-diene.

There are still other volatile components, which remain unidentified in our experiment, yet they frequently appear in many investigated sage species. For the sake of an example, component with the retention time equal to 26.01 min is present in fourteen *Salvia* species, and its relatively highest levels are observed with *S. azurea, S. pratensis, S. staminea, S. cadmica,* and *S. sclarea*.

Conclusions

In our HS–GC–MS study, we obtained fingerprint gas chromatograms of the volatile fraction from 20 *Salvia* species most frequently grown in Poland. Seven volatile compounds were identified as characteristic of the prevailing majority of the investigated species. With four species, unique volatile compounds

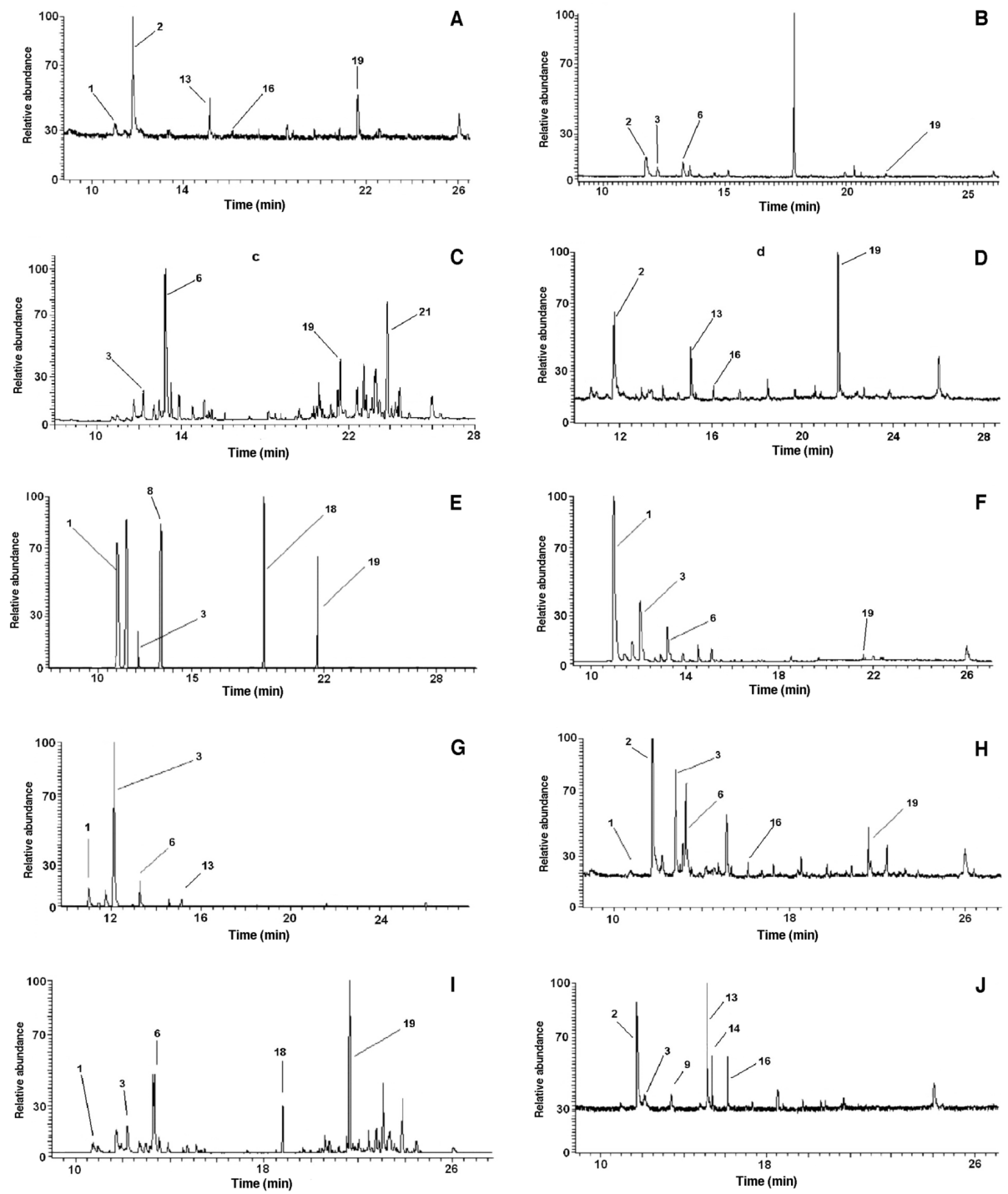

Figure 1. Fingerprint gas chromatograms of the volatile fraction determined by means of HS–GC–MS for the following Salvia species: *S. amplexicaulius*, A; *S. atropatana*, B; *S. azurea*, C; *S. cadmica*, D; *S. canariensis*, E; *S. deserta*, F; *S. farskaohlei*, G; *S. glutinosa*, H; *S. hians*, I; *S. jurisicii*, J; *S. lavandulifolia*, K; *S. nemorosa*, L; *S. officinalis*, M; *S. pratensis*, N; *S. sclarea*, O; *S. staminea*, P; *S. stepposa*, Q; *S. tesquicola*, R; *S. triloba*, S; and *S. verticillata*, T. Selected (i.e., most abundant and/or best separated) peaks are labeled according to the numbering attributed to them in Table I.

were found, enabling them to act as their respective chemotaxonomic markers. With 13 species, certain volatile compounds were identified, which (although not unique for these species) can be considered as their respective chemotaxonomic advice. With five species, an absence of the otherwise frequently occurring volatile compounds was observed, which can be considered as a respective chemotaxonomic advice, as well.

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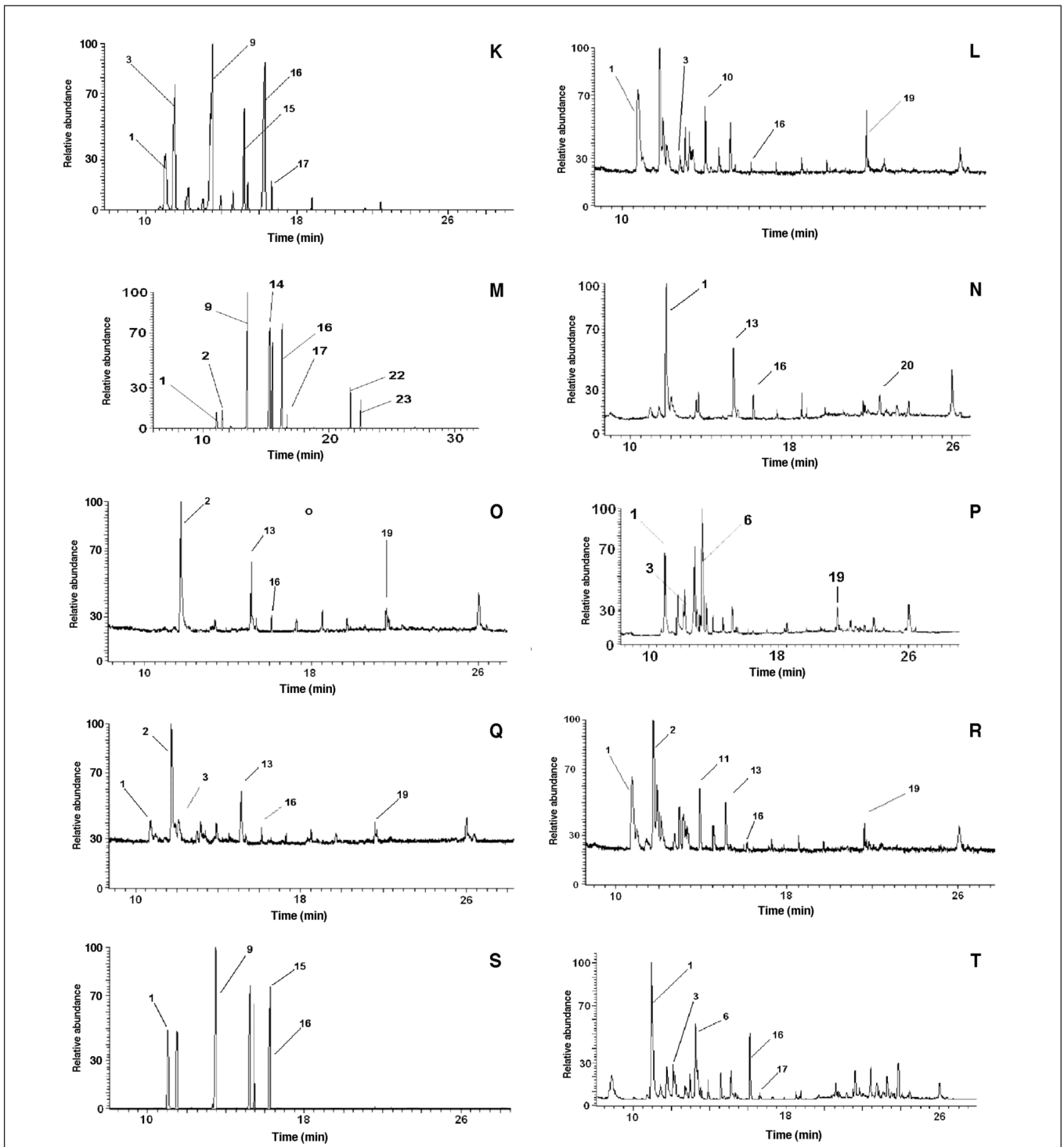
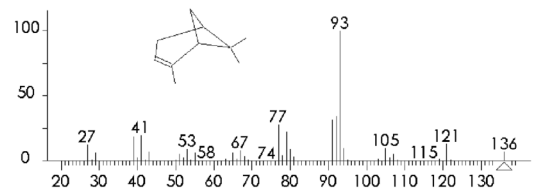


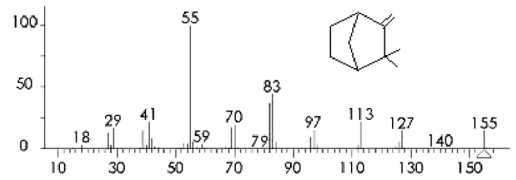
Figure 1. (Continued) Fingerprint gas chromatograms of the volatile fraction determined by means of HS–GC–MS for the following Salvia species: *S. amplexicaulius*, A; *S. atropatana*, B; *S. azurea*, C; *S. cadmica*, D; *S. canariensis*, E; *S. deserta*, F; *S. farskaohlei*, G; *S. glutinosa*, H; *S. hians*, I; *S. jurisicii*, J; *S. lavandulifolia*, K; *S. nemorosa*, L; *S. officinalis*, M; *S. pratensis*, N; *S. sclarea*, O; *S. staminea*, P; *S. stepposa*, Q; *S. tesquicola*, R; *S. triloba*, S; and *S. verticillata*, T. Selected (i.e., most abundant and/or best separated) peaks are labeled according to the numbering attributed to them in Table I.

Table II. Seven Volatile Compounds Most Frequently Appearing in the Salvia Species With Their Respective Structural Formulas and Mass Spectra

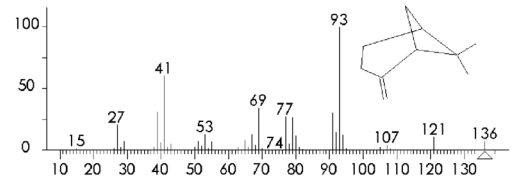
α-Pinene



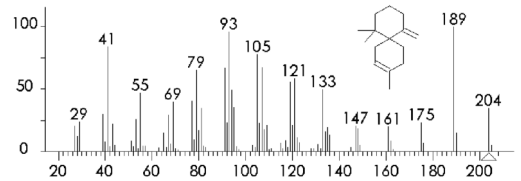
Camphene



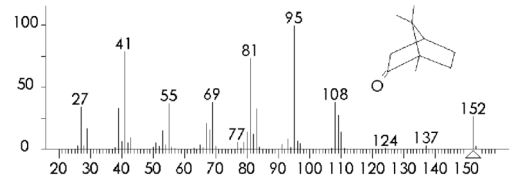
β-Pinene



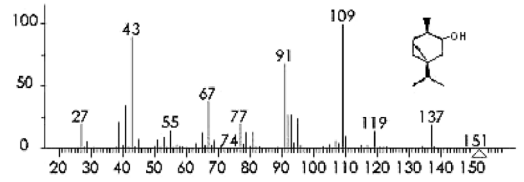
β-Chamigrene



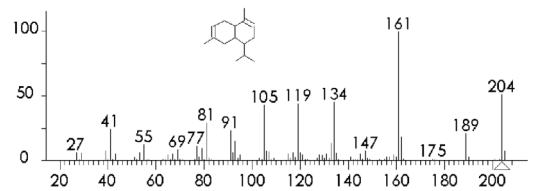
Camphor



Thujol



Cadina-3,9-diene



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