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Composition of the Essential Oils of Commercial Samples of *Salvia officinalis* L. and *S. fruticosa* Miller: A Comparison of Oils Obtained by Extraction and Steam Distillation†

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The essential oils of commercially available samples of leaves of *Salvia officinalis* L. and *S. fruticosa* MILLER obtained by steam-distillation and dichloromethane extraction were analysed by gas liquid chromatography. Although standardized conditions of sample preparation were employed, differences in the composition of the oils were found: steam distillation yielded a reduced amount of the less volatile compounds, and the accuracy of determination was significantly lower than in the case of extraction. The commercial samples, which differed considerably in the composition of their essential oils, were quite inhomogeneous in respect to their oil content partly due to the different ages of the leaves. Extraction of individual leaves of *S. officinalis* showed a decrease in the α-thujone content, with a corresponding increase in the relative amount of camphor, related to leaf age. Owing to the observed variability of the essential oil composition of *S. officinalis*, the relative contents of α-thujone, β-thujone and camphor have to be totalled in order to form a significant parameter for the characterization of *Salvia* species. This parameter varies between 45 and 68% in *S. officinalis* and between 4.8 and 15.9% in *S. fruticosa* with a small standard deviation. Consideration of this parameter together with the amount of 1,8-cineole (*S officinalis*—2.8 to 23%; *S. fruticosa*—55 to 75%) permits the differentiation between these species and respective mixtures.

Keywords: Salvia officinalis; Salvia fruticosa; essential oil; gas liquid chromatography

INTRODUCTION

Salvia officinalis L. and *S. fruticosa* MILLER (synonym—*S. triloba* L. fil) are frequently used as medicinal plants and spices. Numerous articles concerning the compositions of the essential oils of these species have been published (for example, *S. officinalis*—Ivanic *et al.*, 1978; Kedzia *et al.*, 1990; Koedam, 1982; Kustrak *et al.*, 1984; Piccaglia and Marotti, 1990, 1993; Pitarevic *et al.*, 1984, Putievsky *et al.*, 1986a, 1990: and *S. fruticosa*—Bayrak and Akgül, 1987; Putievsky *et al.*, 1986b; Catsiotis and Iconomou, 1984; Kustrak, 1987). Variations in the compositions of the essential oils were found to be considerable (Table 1) and this may be due to the quality of the plant material (influence of harvest time, different chemical types, employment of fertilizers etc.) as well as to the methods used for their analysis. The majority of the data was obtained for essential oils produced by steam distillation. Whilst the pH-value of the water used and the duration of the steam distillation process are of some importance, the boiling temperature (corresponding to the ion content of the water) and the degree of grinding have a significant effect on the results (Iconomou *et al.*, 1982). Owing to the possible formation of artefacts during steam distillation (Stahl, 1984) the isolation of essential oils by a simple extraction process must be considered to be more gentle. Further, extraction of only 50–200 mg plant material (depending on the content of essential oil) is sufficient for gas liquid chromatography (GC) permitting an examination of individual plants (Kastner *et al.*, 1992; Länger *et al.*, 1992, 1993; Mechtler *et al.*, 1994a,b). In contrast to the published method of Schmidt *et al.* (1990) which involves digesting the drug with 94% ethanol for 4 h, the application of dichloromethane enables rapid sample preparation (Kastner *et al.*, 1992; Mechtler *et al.*, 1994b). Based on this method, the chemical variability within individuals of *Salvia* species has been investigated, as well as the homogeneity of commercially available samples. The

Table 1. Compositions of the essential oils of *Salvia officinalis* and *S. fruticosa*[a]

Component	*Salvia officinalis*[b]		*Salvia fruticosa*[b]	
	Minimum[a] (%)	Maximum[a] (%)	Minimum[a] (%)	Maximum[a] (%)
α-Pinene	1.23	7.12	3.57	21.78
Camphene	0.91	9.45	0.39	8.06
β-Pinene	0	4.73	1.2	11.60
1,8-Cineole	5.62	25.5	41.96	74.39
α-Thujone	7.65	67.90	0	12.01
β-Thujone	4.27	46.92	0	6.34
Camphor	0.48	25.73	0.9	25.76
Borneol	0	16.53	0	5.25
Bornyl acetate	0.48	6.77	0	3.16
β-Caryophyllene	0	8.97	1.30	13.23

[a]Data obtained from the literature cited in the Introduction section.
[b]In order to compare data, the sum of the percentages of the components shown in the table has been normalized to 100 for each literature report considered.

† Dedicated to Professer Dr. M. Wichtl with best wishes on his 70th birthday.

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accuracy of the results obtained after extraction and steam distillation are discussed.

EXPERIMENTAL

Plant material. For intra-individual studies, leaves from plants cultivated in the botanical garden of the Institute of Pharmacognosy, Vienna, were collected. Twenty five commercially available samples declared as Fol. Salviae officinalis (one was adulterated with Fol. Salviae trilobae) and 11 samples declared as Fol. Salviae trilobae from several trading companies in Germany, Turkey and Austria were taken for comparison of the composition of the essential oil.

Distillation. Leaf samples (10 g) were distilled in an apparatus according to the Austrian Pharmacopoeia (ÖAB, 1990) with 400 mL saturated NaCl solution for 2 h. NaCl-saturated water increases the oil yield, reduces the duration of distillation and leads to better standardized results (Wichtl, 1971). The influence of the NaCl concentration of the ratio of the main compounds of the essential oil is of minor relevance (Iconomou *et al.*, 1982).

Extraction. Leaf samples (200 mg) were extracted with 1 mL dichloromethane for 10 min in an ultrasonic water bath.

Conditions of GC analysis. A Perkin Elmer Sigma 300 model chromatograph (Perkin Elmer Corp., Norwalk, CT, USA) was used fitted with a fused silica capillary column (SE 54 CB; 50 m × 0.25 mm i.d., Macherey-Nagel, Düren, Germany) and a filled liner injector (modified after Brandauer and Ziegler, 1982), the filled part (5 mm length) containing 3% OV101 on Chromosorb W AW DMCS (100/120 mesh). The carrier gas was nitrogen (flow-rate 4.8 mL/min) and the temperature was programmed from 60 to 270°C at a rate of 2°C/min. Samples (either 1 μL of the dichloromethane extract or 1 μL of a 1:1000 dilution of the steam distilled oil) were injected directly onto the liner using a 10 μL Hamilton syringe with split injection (1:5). Integration was carried out using Perkin Elmer Omega software. A typical chromatogram is shown in Fig. 1.

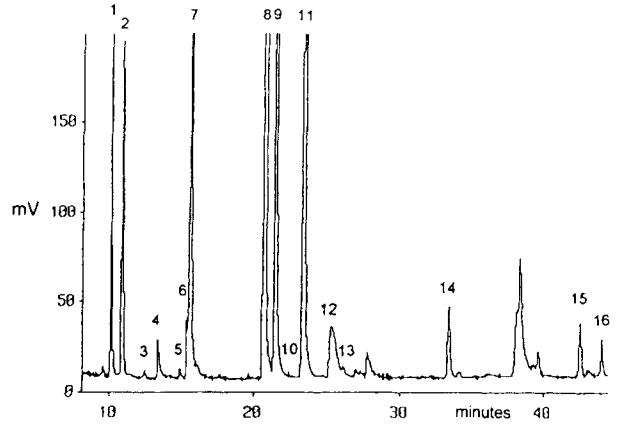

Figure 1. Typical GC chromatogram of the essential oil extracted from leaves of *Salvia officinalis*. Key to peak identity: **1**, α-pinene; **2**, camphene; **3**, β-pinene; **4**, myrcene; **5**, 3-carene; **6**, limonene; **7**, 1,8-cineole; **8**, α-thujone; **9**, β-thujone; **10**, thujol; **11**, camphor; **12**, borneol; **13**, terpinen-4-ol; **14**, bornyl acetate; **15**, β-caryophyllene; **16**, humulene. (For chromatographic conditions see Experimental section.)

Table 2. Coefficient of variability v of the amount of each component of the essential oil of *Salvia officinalis* determined after steam distillation ($n = 10$) or extraction ($n = 12$)

Component	v Extraction	v Steam distillation
α-Pinene	3.98	10.07
Camphene	5.1	13.46
β-Pinene	17.08	19.79
1,8-Cineole	2.05	12.55
α-Thujone	7.1	13.6
β-Thujone	6.80	15.16
Camphor	5.15	13.44
Borneol	11.52	17.93
Bornyl acetate	6.32	17.56
β-Caryophyllene	7.54	13.43

Calculation of data. In order to compare results in published papers, as well as our results, for a number of compounds, values have been standardized, ie the sum of the frequently cited compounds α-pinene, camphene, β-pinene, 1,8-cineole, α-thujone, β-thujone, camphor, borneol, bornyl acetate and β-caryophyllene has been normalised to 100. The relative estimated value of the standard deviation (S), the Pearson coefficient of variability v (Weber, 1980) was calculated from

$$v = \frac{S \cdot 100}{\bar{x}}$$

where $\bar{x}$ is the population mean.

RESULTS AND DISCUSSION

Comparison of oils obtained by steam distillation and by extraction

Ten random samples taken from an homogenous commercial sample were steam distilled. From the same material, three typical leaves were taken and each divided into four parts each of which were analysed by the extraction method. The reproducibility of the extraction method (calculated separately for each leaf) was considerably higher than that found for steam distillation even under strictly identical conditions (Table 2). Although thermal stressing during steam distillation did not form any artefacts, it resulted in a loss of less volatile compounds (Tables 3–5).

Homogeneity of commercial samples—intra-individual differences. The analysis of 50 randomly chosen leaves of a commercial sample of Fol. Salviae officinalis showed an enormous inhomogeneity, which was considerably higher than the inaccuracy of the method (Table 3), some leaves even showing more 1,8-cineole than thujone and camphor. However, microscopic examination (Länger *et al.*, 1991) of the non-glandular hairs of such leaves verified their identity. Apart from the known genetic variability of *S. officinalis* (Ch. Franz, personal communication), these inhomogeneities can be explained by intra-individual differences in the production of essential oil. From the top to the base of an individual plant, the relative contents of α-thujone and β-thujone decrease, while the amounts of camphor, α-pinene, camphene and borneol increase (Figs. 2 and 3). This gradient is detectable in commercial samples as well: camphor dominates in the essential oil of glabrescent leaves (from the base of an individual), while grey tomentose

Table 3. Inhomogeneity within a single commercial sample of *Salvia officinalis*

Component	Extraction: relative amount per leaf[a] (%)			Steam distillation:
	Minimum	Mean (n=50)	Maximum	Relative amount[b] (%)
α-Pinene	1.9	8.7	19.2	5.0
Camphene	0.7	4.1	10.0	1.7
β-Pinene	0.1	0.3	1.8	0.4
1,8-Cineole	10.9	24.1	43.1	18.7
α-Thujone	15.7	35.9	59.3	46.3
β-Thujone	4.9	11.7	25.8	18.6
Camphor	1.4	10.2	30.4	7.3
Borneol	0.4	2.7	10.8	1.5
Bornyl acetate	0.1	1.3	8.0	0.3
β-Caryophyllene	0.1	1.0	4.7	0.2

[a]Composition determined, using the extraction method, for 50 individual leaves taken from the sample (for protocol see Experimental section).
[b]Composition determined, using the steam distillation method, for a 10 g leaf sample (for protocol see Experimental section).

leaves (from the top) contain α-thujone as the main component (Table 6). However, the sum of the contents of α-thujone, β-thujone and camphor remains nearly constant.

Essential oil of commercial samples

S. officinalis. The relative amounts of the individual components of the essential oils from different samples of *S. officinalis* varied even under strictly standardized analytical parameters (Table 4). The dominating compounds were camphor (14 samples) and α-thujone (10 samples). The high relative standard deviations for α-thujone (31.5%) and camphor (36.3%) may cause problems in quality control analyses. However, the summation of the contents of α-thujone, β-thujone and camphor (45 to 68%) results in a more stable parameter (relative standard deviation—10.5%) and seems to be significant for the essential oil of Fol. Salviae officinalis.

Table 4. Relative percentage composition (range and mean values) of the essential oils of *Salvia officinalis* in 25 commercial samples determined by the extraction or the steam distillation method

Component	Extraction			Steam distillation		
	Minimum	Mean	Maximum	Minimum	Mean	Maximum
α-Pinene	0.7	6.0	13.6	0	3.5	8.0
Camphene	1.6	8.1	12.3	0	4.8	8.6
β-Pinene	0	0.4	1.8	0	0.3	1.2
1,8-Cineole	2.8	15.1	22.8	1.8	14.2	21.7
α-Thujone	7.5	23.0	41.9	13.1	28.0	48.5
β-Thujone	2.5	7.6	18.0	3.9	9.7	19.1
Camphor	14.4	29.7	47.4	7.3	31.7	50.2
Borneol	2.1	4.8	22.7	1.5	4.9	23.9
Bornyl acetate	0.4	2.3	9.8	0.3	1.6	5.7
β-Caryophyllene	0.6	3.0	15.9	0.2	1.3	9.7
Σ (Thujones+camphor)	45.3	60.3	68.3	56.2	69.4	81.4

Table 5. Relative percentage composition (range and mean values) of the essential oils of *Salvia fruticosa* in 11 commercial samples determined by the extraction or the steam distillation method

Component	Extraction			Steam distillation		
	Minimum	Mean	Maximum	Minimum	Mean	Maximum
α-Pinene	6.5	9.0	13.7	4.3	6.3	8.0
Camphene	0.7	3.6	6.9	1.0	2.6	4.2
β-Pinene	3.2	4.5	6.9	2.5	3.6	5.2
1,8-Cineole	55.8	65.3	74.4	63.0	69.9	80.8
α-Thujone	0.8	1.9	4.0	0.7	2.2	4.0
β-Thujone	0.9	1.5	3.0	1.2	2.0	2.9
Camphor	2.3	7.0	11.6	4.4	8.7	14.0
Borneol	0.8	2.0	3.4	0.2	1.4	3.1
Bornyl acetate	0	0.5	1.8	0.1	0.6	1.9
β-Caryophyllene	3.1	4.7	6.4	1.3	2.5	4.0
Σ (Thujones+camphor)	4.8	10.4	15.9	6.3	12.9	19.4

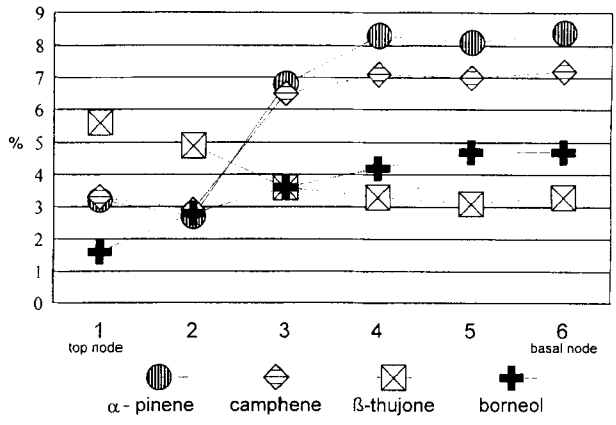

Figure 2. Relative amounts (%) of α-pinene, camphene, β-thujone and borneol in the leaves of an individual plant of *Salvia officinalis* (from top to the base).

S. fruticosa. The differences in composition of the essential oils from different samples of *S. fruticosa* were smaller than in those of *S. officinalis*: the relative standard deviation for 1,8-cineole, which dominated the essential oil (55 to 75%; Table 5) was 8.62%. A high relative content of 1,8-cineole is characteristic of the essential oil of *S. fruticosa* because of the small total amount of α-thujone, β-thujone and camphor (only 4.8 to 16%).

Mixtures of *S. officinalis* and *S. fruticosa*. Microscopic investigation of one commercial sample declared to be Fol. Salviae officinalis showed that it was adulterated with leaves of *S. fruticosa*. The content of 1,8-cineole was high (48%) but lower than in pure samples of *S. fruticosa*. The sum of the thujones and camphor (37%) was not in the range normally associated with *S. officinalis*. Beside the anatomical details, the amount of α-thujone (19%) and the ratio of 1,8-cineole, α-thujone and camphor excluded the

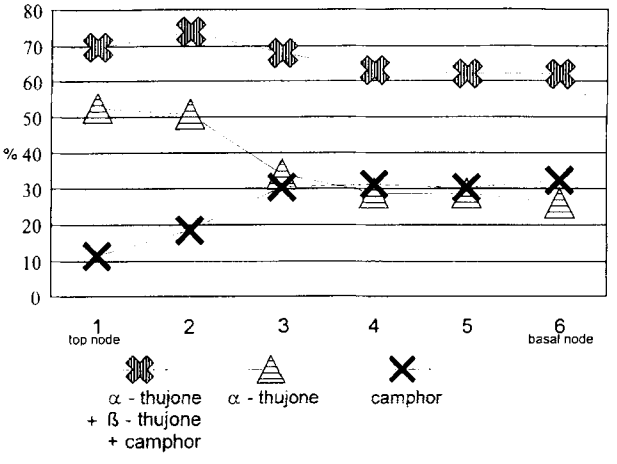

Figure 3. Relative amounts (%) of α-thujone, camphor and the sum of α-thujone, β-thujone and camphor in the leaves of an individual plant of *Salvia officinalis* (from top to the base).

Table 6. Relative amounts of α-thujone and camphor in individual leaves of different ages in commercial samples of *Salvia officinalis*

Leaf Indumentum	Relative amount (%)								
	α-Thujone			Camphor			Σ-(Thujones+camphor)		
	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum
A. Grey tomentose	11.3	27.6	53.2	2.2	26.1	43.2	46.3	62.7	80.7
B. Intermediate between A and C	8.8	25.2	35.7	14.2	28.2	44.6	52.8	59.2	68.5
C. Yellowish-green glabrescent	9.2	18.6	40.8	17.7	38.9	54.2	53.2	61.5	73.0

presence of *S. lavandulifolia* (characterized by traces of thujones with main compounds being 1,8-cineole and camphor; Lawrence *et al.*, 1970).

According to our experience, both extraction and steam distillation have their own specific advantages. Extraction should be preferred in the case of analyses of individual plants or single plant organs with the benefit of rapid sample preparation and small sample size. For quality control of commercial samples according to pharmacopoeias, steam distillation is recommended requiring about 10 g crude drug plus 2 h for isolation. Problems of the crystallization of camphor, 1,8-cineole and borneol in the condenser (Koedam, 1982) were not observed under the conditions used and therefore can be neglected.

Regarding the variation of the essential oil in commercial samples, the question arises as to which ratio between the compounds in the oil might be regarded as optimal. The demand for an α-thujone-rich essential oil in the case of *S. officinalis* probably has historical reasons only: the low thujone content of *S. lavandulifolia* (formerly treated as a subspecies of *S. officinalis*) allowed differentiation from the typical dalmatian sage. Although the toxic effects of thujone are evident (Teuscher and Lindequist, 1988) the concentrations found in Fol. Salviae are toxicologically irrelevant. Recent investigations (Harrer, 1993) showed that only 17% of the genuine thujone could be extracted with hot water, corresponding to 2.5 mg thujone per cup of tea (the most common preparation of Fol. Salviae). Owing to the scantly solubility of the compounds of the essential oil, other constituents (e.g., carnosol and rosmarinic acid) should also be considered for quality control.

In conclusion, GC analysis remains important for the differentiation of essential oils of different *Salvia* species. Whereas the oil of *S. fruticosa* is dominated by 1,8-cineole (60–80% of the steam distilled oil), *S. officinalis* is characterized by the summation of α-thujone, β-thujone and camphor as a constant parameter (56–81% of the steam distilled oil). This parameter, and the content of 1,8-cineole, also permits the detection of adulteration in *Salvia* samples.

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