Study of Clones of *Salvia officinalis* L. Yields and Chemical Composition of Essential Oil

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ABSTRACT: The essential oil of *Salvia officinalis* L. in five selected clones of different origins (France, Hungary, Portugal, Romania, Czech Republic) was studied. Yields of oils from dried leaves were excellent (2-3%), and higher than those previously reported. The α : β -thujone ratio varied according to origin. Overall, some of the oils were of high quality in terms of their α - and β -thujone and camphor contents. \bigcirc 1998 John Wiley & Sons, Ltd.

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KEY WORDS: Salvia officinalis L.; essential oil yield; chemical composition; α - and β -thujones

Introduction

The genus *Salvia* (sage) of the family Lamiaceae comprises nearly 900 species spread widely throughout the world, which correspondingly display marked morphological and genetic variations according to their geographical origin. Sage is an aromatic plant used as a flavour and food condiment, and in cosmetics, perfumes¹ and medicine.

The work described here concerns medicinal sage (*Salvia officinalis* L.). Numerous studies have been carried out on this species, including the recent work of Grella and Picci² and Belkamel³ on the effects of harvest date on chemical composition, of Putievsky⁴ and Perry⁵ on oils from cultivated clones from Dalmatia and Albania, of Tsankova⁶ on oils from Bulgaria, and of Pace⁷ on oils of Italian wild sage.

This work was carried out in cooperation with the French National Conservatory of Perfumery, Medicinal, Aromatic and Industrial Plants at Milly-la-Forêt, on cultivated plants. The aim was to select, out of some 30 different populations, those giving the best yields of essential oils to determine their chemical composition, and interpret the observed variations.

Experimental

Plant Material

The different populations studied were cloned by cuttings at the National Plant Conservatory at Milly-la-Forêt, France. The leaves were dried in shade for a day before extraction.

Extraction and Analysis

The essential oil was extracted by steam distillation for 4 h in a Clevenger-type apparatus as prescribed by the French Pharmacopeia. Analysis of the essential oils was performed by gas chromatography using a DELSI 121C apparatus fitted with a flame ionization detector and a CP WAX51 fused silica column (25 m \times 0.3 mm i.d., 0.10 µm film thickness, Chrompack, The Netherlands). Temperature programming was 5 min at 50° C, then 50° to 210°C at 2°C per min. The composition of the oils was expressed as percentage area by peak normalization, assuming all response factors are equal to 1. Identification of compounds was by GC-MS and, where possible, comparison of their linear retention indices with those of authentic compounds. For GC-MC a CP WAX 51 fused silica WCOT column (50 m \times 0.3 mm i.d.) was used with helium as carrier gas and coupled to a WG 70 mass spectrometer: ionization energy 70 eV. Temperature programming

Table 1. Yields of essential oil of Salvia officinalis L.from five clones to different origin

Origin	Ref.	Number of samples	Yield (%)	Average yield (%)		
France Hungary	B3S4 B3S7	9 9	1.3-2.5 2.2-3.1	2.05 2.50		
Portugal	B3S2	8	2.4-3.2	2.90		
Romania	B2S4	10	2.0 - 2.5	2.30		
Czech Republic	B1S5	9	1.8 - 2.7	2.20		

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Table 2.	Essential	oils of	Salvia	officinalis	L.
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Origin and reference Ide	Identification ^a	Original clones				Propagated plants					
		Portugal B3S2	Hungary B3S7	Romania B2S4	Czech Republic B1S5	France B3S4	Portugal B3S2	Hungary B3S7	Romania B2S4	Czech Republic B1S5	France B3S4
No. of samples							8	9	10	9	9
1 Tricyclene	MS,RI	0.15	0.04	0.06	0.06	0.08	0.1 - 0.2	0.1 - 0.4	tr	0.1	0.1 - 0.2
2 α-Pinene	MS,RI	4.83	4.91	3.36	0.75	1.41	5.4-6.6	1.9 - 2.7	5.7-9.0	1.0-1.3	0.8 - 2.9
3 α -Thujene	MS,RI	-	_	_	-	tr	-	tr	_	tr	tr-0.1
4 Camphene	MS,RI	4.43	2.60	2.61	2.64	3.07	4.2-5.3	3.9-5.9	2.2 - 2.9	2.1-3.8	2.0 - 3.2
5 β -Pinene	MS,RI	3.08	1.23	1.49	0.98	1.61	2.6-3.3	1.1 - 1.6	2.3 - 4.4	1.1-1.6	1.0 - 2.8
6 Sabinene	MS,RI	0.20	0.10	0.09	0.09	0.16	0.1 - 0.2	0.1 - 0.2	0.1 - 0.2	0.1	0.1 - 0.2
7 α -Phellandrene	MS,RI	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
8 Myrcene	MS,RI	0.67	0.38	0.04	0.24	0.81	0.8 - 1.0	0.1 - 1.0	0.4 - 0.8	0.3-0.8	0.4 - 1.0
9 α -Terpinene	MS,RI	0.05	tr	_	-	0.08	0.1	0.1 - 1.0	tr-0.2	tr-0.1	0.1
10 Limonene	MS,RI	1.08	0.55	0.36	0.41	1.26	1.3 - 1.7	0.6 - 1.5	0.8 - 1.4	1.0 - 1.4	0.5 - 1.5
11 1,8-Cineole	MS,RI	17.04	11.93	4.61	11.69	13.43	13.4-16.8	7.1-9.7	3.6-6.0	9.5-13.3	8.6-11.6
12 (<i>E</i>)- β -Ocimene	MS,RI	0.13	tr	_	tr	0.25	0.1 - 0.2	0.1 - 0.2	0.1 - 1.3	tr-0.1	0.1
13 y-Terpinene	MS,RI	0.13	0.09	_	0.04	0.25	0.2 - 0.3	0.2 - 0.4	0.1 - 1.0	0.1-0.3	0.1 - 0.5
14 (Z)- β -Ocimene	MS,RI	tr	tr	_	tr	tr	tr	tr	0.1-0.3	tr	tr-0.1
15 <i>p</i> -Cymene	MS,RI	0.25	0.16	0.35	0.15	0.13	0.1 - 0.2	0.1 - 0.2	0.3 - 0.4	0.1-0.2	0.1 - 0.2
16 Terpinolene	MS,RI	0.25	0.11	_	0.07	0.35	0.3 - 0.4	0.2 - 0.3	0.1 - 0.3	0.1-0.3	0.1 - 0.7
17 α -Thujone	MS,RI	1.94	25.10	22.80	0.66	20.90	1.1-1.5	19.6-24.3	18.9-26.6	0.5-0.9	22.2-31.9
18 <i>B</i>-Thujone	MS,RI	17.60	1.45	9.24	20.57	2.50	15.0-17.7	1.7-2.9	5.0-8.3	24.4-25.9	2.7-8.9
19 Camphor	MS,RI	30.79	24.17	32.90	31.79	22.00	27.0-32.2	23.8-27.9	18.2-27.3	20.8-27.1	15.8-24.0
20 Linalol	MS,RI	0.42	0.79	0.79	1.15	0.77	0.3-0.4	0.5 - 0.7	0.3-1.4	0.8 - 1.0	0.2-0.7
21 Bornvl acetate	MS,RI	1.90	1.37	1.21	0.62	1.55	0.8 - 1.9	2.8 - 3.9	0.6 - 1.4	0.6 - 0.9	0.4 - 2.8
22 <i>endo</i> -Bornyl acetate	MS,RI	_	_	_	_	_	tr-0.2		tr-0.4	tr	tr
23 β -Caryophyllene	MS,RI	2.40	6.01	0.73	4.90	4.59	3.5-4.3	1.7 - 2.4	1.0 - 1.6	4.8 - 8.0	1.0 - 4.4
24 Terpinen-4-ol	MS,RI	0.37	0.50	0.16	0.44	0.39	0.1-1.1	0.4-0.5	0.4–1.2	0.1-0.3	0.2-0.4
25 Aromadendrene	MS,RI	_	_	_	_	_	tr-0.4	tr	tr-0.4	0.2-0.3	tr-0.3
26 Umbellulol	MS,RI	1.19	_	0.39	3.20	7.26	tr-0.3	tr-0.2	0.1 - 0.2	0.1 - 0.2	0.1-0.2
27 α-Humulene	MS,RI	1.12	6.83	0.36	1.32	0.32	0.9-1.8	7.6-12.4	7.3-10.5		5.5-7.6
28 Thujyl alcohol	MS,RI	0.40	0.15	0.22	0.18	_	0.7-1.0	0.1-0.2	0.1-0.7	1.1-1.3	0.2-0.4
29 Umbellol isomer	MS	3.86	_	3.53	6.10	2.96	0.4-0.9	tr-0.1	0.1-0.4	0.1-1.0	tr-0.2
30 Borneol	MS,RI	0.46	1.57	0.65	0.22	0.56	1.7-3.7	2.9-4.3	1.8-3.3	2.8-3.7	1.9-4.5
31 iso-Borneol	MS,RI	0.20	0.47	0.18	0.11	tr	0.3-2.0	0.3-0.6	0.3-0.9	0.1-0.4	0.3-0.9
32 Myrtenol	MS,RI	0.08	0.08	0.11	0.11	0.08	0.3-0.6	tr	tr-0.2	0.1-0.3	0.1-0.6
33 <i>trans</i> -Carveol	MS,RI	0.10	0.08	0.22	0.09	tr	0.1-0.2	tr-0.1	tr-0.1	0.1	tr
34 <i>p</i> -Cymen-8-ol	MS,RI	-	0.10	0.05	0.04	tr	0.1-0.2	tr	0.1	0.1	tr
35 <i>trans</i> -Caryophyllene ox		0.17	0.47	0.35	0.45	0.33	0.2-0.5	0.1-0.2	0.1	0.3-0.6	0.1-0.3
36 Ledol	MS,RI	2.43	2.78	1.73	6.80	6.19	1.7-2.8	3.7-7.5	1.8–3.6	4.9-6.8	3.0-4.1
37 Caryophyllen-8-ol	MS,RI	0.52	1.40	-	1.46	2.47	0.1-0.9	1.1-2.6	1.3-4.3	0.9-1.6	1.9-4.1
α/β Thujone ratio		0.11	17.33	2.46	0.03	8.36					

 a^{a} MS = Mass spectral library. RI = linear retention index.

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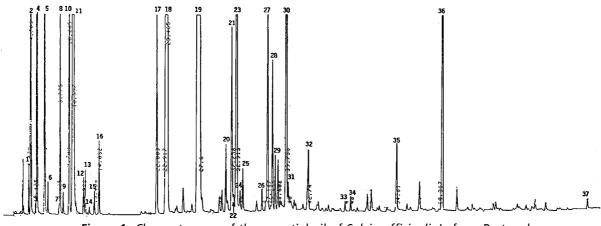


Figure 1. Chromatogram of the essential oil of Salvia officinalis L. from Portugal

was from 50 to 230° C at 3° C/min. In both cases, the samples were injected in split mode (0.05 µm, split 1/60), injector temperature 240°C.

Results and Discussion

Five populations from the National Plant Conservatory were selected, corresponding to five clones from France (Angers 85, ref. B3S4), Hungary (Budakalasz rose, ref. B3S7), Portugal (Coimbra rose, ref. B3S2), Romania (Iasi, ref. B2S4) and the Czech Republic (Brno 88, ssp. *minor*, var. alba, ref. B1S5) and propagated. Eight to ten samples of each population were analysed.

The yields were high (Table 1), 2-3% of essential oil (v/w of dry plant material), and higher than those reported in the literature, which are of the order of 1.5%.^{4,6}

Thirty-seven components were identified. The chemical compositions of the essential oils of these five populations of propagated plants and original clones, taken as reference, are given in Table 2. The chromatogram of one essential oil (from Portugal, ref. B3S2) is shown as an example (Figure 1).

Quality criteria defined on the basis of Yugoslav or Albanian oils require proportions of α - and β -thujones > 50% and of camphor < 20%.^{4,7,8}

The oils studied here presented high levels of *thujones*, although below 50%, and levels of *camphor* slightly above 20%.

Two essential oils, those from Portugal and the Czech Republic, are characterized by low levels of α -thujone and high levels of β -thujone. In this, and in their levels of camphor and 1,8-cineole, they resemble oils from Morocco.³

The three other populations, from Hungary, Romania and France, had more β -thujone than α thujone. The last two had chemical compositions close to the values reported by Lawrence⁹ for medicinal sage oil, and came nearest to meeting quality criteria.⁴

Monoterpene hydrocarbons represented only 10-12% of the oil from the French, Czech and Hungarian clones. These values are close to those for Albanian and Dalmation oils.¹⁰ The other two clones had higher levels (18–20%).

Lastly, ledol, a compound specific to medicinal sage oil,⁵ was found in all the clones (1.7-2.8%).

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