
The cis-thujone chemotype of *Salvia officinalis* L. essential oils

**Danutė Mockutė,
Ona Nivinskienė,
Genovaitė Bernotienė*,
Rita Butkienė**

*Institute of Chemistry,
Goštauto 9,
LT-2600 Vilnius, Lithuania
Fax: 370-5 261 018.
E-mail: albm@takas.lt*

The aerial parts of *Salvia officinalis* L. plants were collected in 5 gardens in Vilnius district. Essential oils were prepared by hydrodistillation of air-dried plants and analysed by GC and GC/MS methods. The main constituents of the oils were 1.8-cineole (6.8–8.2%), cis-thujone (14.8–19.0%), borneol (6.6–8.0%), α -humulene (7.6–8.7%), viridoflorol (7.2–8.2%) and manool (6.4–10.4%). The amounts of toxic cis- (the first major constituent) and trans-thujones and other limited compounds (α -pinene, camphene, limonene, 1.8-cineole, camphor, linalool and its derivatives, bornyl acetate and α -humulene) met the requirements of ISO 9909 (1999) standard. The largest part of essential oil comprised oxygenated terpenes (64.4–67.7%). The 89 identified compounds made up 97.5–98.2% of the oils.

Key words: Lamiaceae, *Salvia officinalis* L., chemical composition of essential oil, chemotype, 1.8-cineole, cis-thujone, borneol, α -humulene, viridoflorol, manool

INTRODUCTION

Essential oils of *Salvia officinalis* L. are used for healing different diseases [1, 2]. The healing power of α -thujone and 1.8-cineole chemotypes of the oils are presented in [1]. Both essential oils contain different amounts of α -thujone, 1.8-cineole, camphor, α -humulene, borneol, camphene, α - and β -pinenes alongside the compounds characteristic for each chemotype. Stimulant and antibacterial properties are exhibited by both oils. The oil of α -thujone chemotype has antiviral and antifungal properties beside the above [1]. The aerial parts of the sage in Lithuania are used in the mixtures of medicinal plants for healing digestion and circulation disturbances, bronchitis, angina, skin and other diseases [3]. Antioxidant activity of the essential oil (chemical composition was not given) produced by sage of the experimental garden of the Lithuanian Institute of Horticulture was the lowest in composition with other extracts (acetone, methanol, CO₂, water) [4, 5].

The chemical composition of the essential oil of the sage had been investigated in different countries [6, 7]. The data were reviewed in [7] and the sage commercial essential oils were divided in to 5 groups according to the amounts of the major constituents:

1. Camphor > α -thujone > 1.8-cineole > β -thujone;
2. Camphor > α -thujone > β -thujone > 1.8-cineole;

3. β -thujone > camphor > 1.8-cineole > α -thujone;
 4. 1.8-cineole > camphor > α -thujone > β -thujone;
 5. α -thujone > camphor > β -thujone > 1.8-cineole;
- The authors of [5] regarded the last group as the best choice with 5.3% of 1.8-cineole, 27.1% α -thujone, 6.2% β -thujone and 18.5% camphor.

The later investigations showed that some of the above main constituents were found in numerous essential oils of the sage [8–18]. The first major constituent of the volatile oil in Portugal was α -thujone [8, 9]. The amounts of 1.8-cineole, camphor, α - and β -thujones and other constituents depended on the collection time and the ratio of different plant parts in samples of sage used for essential oil preparation [9]. The composition of the flower, leaf and stem oils differed markedly in Portugal [9], Serbia and Montenegro [10]. The flower oils contained lower quantities of α -thujone, camphor and viridoflorol than the leaf oils. An opposite correlation was noted for borneol. Viridiflorol in the oils of the aerial parts [8] and of leaves [9, 10] was among the five major constituents. The leaves occupying different position in plants of *S. officinalis* \times *S. fruticosa* produced essential oils containing different amounts of the constituents [11]. Diterpene manool was the major constituent in some flower oils in Serbia and Montenegro [10]. α -Thujone was the first main constituent in Reunion Island [12], in some oils in Albania [13], Poland

[14] and New Zealand [15]. Some oils of the latter three countries contained camphor as the first main constituent. The amount of camphor exceeded that of α -thujone in Croatia [16] and Italy [17, 18]. The composition of the essential oil of *S. officinalis* L. ssp. *altissima* growing in Cuba differed from all the above mentioned oils [19]. Its main constituents were germacrene D (32.9%), β -caryophyllene (31.8%) and caryophyllene oxide (23.2%).

The standard ISO 9909 (1999) for the essential oils of sage *S. officinalis* L. [9] is (%):

α -Thujone	18.0–43.0	α -Pinene	1.0–6.5
β -Thujone	3.0–8.5	Camphene	1.5–7.0
Camphor	4.5–24.5	Limonene	0.5–3.0
1.8-Cineole	5.5–13.0	Linalool and its ester	< 1
α -Humulene	0–12.0	Bornyl acetate	< 2.5

The above standard covered nearly all essential oils investigated by authors of [7–18, 20]. The wide interval of the percentage of α -thujone (18.0–43.0%) and β -thujone (3.0–8.5%) given in the standard supposes the existence of the sage commercial oils with large amounts of toxic thujones [21]. Using drinks and meals with thujone for a long time caused seizures and dementia [21]. For example, the banned liqueur absinthe caused a lot of diseases. Preparations of *Salvia folium* with a reduced amount of thujone were prepared by authors of [22].

The content of thujones in the essential oils under study is at the lowest limit of the standard.

MATERIALS AND METHODS

The aerial parts of *Salvia officinalis* L., collected in 2002 in different localities of Vilnius district, were air-dried at room temperature (20–25 °C). Essential oils (0.7–1.4%) were prepared by hydro-distillation (2 h) of 10–25 g air-dried samples.

Analyses of the essential oils were carried out by GC and GC/MS. A HP 5890 II chromatograph equipped with FID and a HP-FFAP capillary column (30 m \times 0.25 mm) was used for quantitative analysis. The GC oven temperature was set at 70 °C for 10 min and then

programmed from 70 °C to 210 °C at a rate of 3 °C/min using He as a carrier gas (0.7 ml/min). The injector and detector temperatures were 200 °C and 250 °C, respectively. Analyses by GC/MS were carried out on an HP 5890 (Hewlett Packard) gas chromatograph equipped with a HP 5971 mass selective detector and a HP 7673 split/splitless injector. The separation was performed on a CP-Sil 8CB silica capillary column (50 m \times 0.32 mm; film thickness 0.25 μ m). The GC oven temperature was programmed as follows: from 60 °C (isothermal for 1 min) increased to 160 °C at a rate of 5 °C/min and to 250 °C at a rate of 10 °C/min, and the final temperature was kept for 5 min. The temperatures of the injector and detector were 250 °C and 280 °C, respectively. The flow rate of carrier gas (helium) was 1 ml/min. Mass spectra in electron mode were generated at 70 eV.

The percentage composition of the essential oils was computed from GC peak areas without correction factors. Qualitative analysis was based on the comparison of retention indexes on both columns and mass spectra with corresponding data in the literature [23, 24] and the computer mass spectra libraries (Wiley and NBS 54K).

RESULTS AND DISCUSSION

The amount of the first major constituent cis(α)-thujone varied from 14.8 to 19.0% and that of its trans-isomer from 3.0 to 3.4% in all essential oils of plant aerial parts collected in 5 gardens (Table). The above amounts of toxic ketones were at the lower limit of the standard ISO 9909 (1999) [7]. Both thujones did not exceed 22.4% of the oils. Near the same percentage of cis- and trans-thujones was found in the volatile oils of aerial parts of plants from Croatia [16], in the flower oils from Portugal [9] and in the leaf oils from Serbia and Montenegro [10].

Table. Chemical composition (%) of the essential oils of *Salvia officinalis* L. growing in Vilnius district*

No	Compounds	RI	A	B	C	D	E	Mean
1	2	3	4	5	6	7	8	9
1	Tricyclene	926	tr.	tr.		tr.		tr.
2	α -Thujene	931	tr.	tr.	tr.	tr.		tr.
3	α -Pinene	939	2.7	3.0	2.5	2.7	2.4	2.7
4	Camphene	953	2.3	2.8	1.7	2.1	1.4	2.1
5	β -Pinene	980	2.1	2.6	1.8	2.0	1.5	2.0
6	Myrcene	991	0.5	0.7	0.5	0.5	0.4	0.5
7	α -Phellandrene	1005	tr.	tr.	tr.	tr.	tr.	tr.
8	α -Terpinene	1018	0.1	0.1	0.1	0.1	0.1	0.1
9	p-Cymene	1026	0.3	0.1	0.2	0.1	0.3	0.2
10	Limonene	1029	0.6	0.5	0.5	0.7	0.6	0.6
11	1.8-Cineole	1033	6.6	8.2	7.9	8.2	7.6	7.7

Table (continued)								
1	2	3	4	5	6	7	8	9
12	γ -Terpinene	1062	0.2	0.3	0.2	0.2	0.1	0.2
13	Terpinolene	1088	0.1	0.1	0.1	0.1	0.1	0.1
14	cis-Thujone	1102	17.4	17.6	17.2	18.0	14.8	17.0
15	trans-Thujone	1114	3.0	3.3	3.4	3.4	3.3	3.3
16	Camphor	1143	4.0	4.0	3.8	4.1	3.4	3.9
17	trans-Pinocamphone	1160	0.2	0.2	0.2	0.2	0.2	0.2
18	Borneol	1165	7.0	8.0	7.0	7.9	6.6	7.3
19	cis-Pinocamphone	1173	0.1	0.1	0.1	0.1	0.1	0.1
20	Terpin-4-ol	1177	0.5	0.5	0.5	0.5	0.5	0.5
21	p-Cymen-8-ol	1183	0.1	0.1	tr.	0.1	0.1	0.1
22	α -Terpineol	1189	0.1	0.2	0.2	0.2	0.1	0.2
23	Myrtenol	1194	0.2	0.3	0.2	0.2	0.3	0.2
24	trans-Carveol	1217	0.1	0.1	0.1	0.1	0.1	0.1
25	cis-Carveol	1229	tr.	tr.	tr.	tr.	tr.	tr.
26	Dihydrolinalyl acetate	1275	tr.	0.1	0.1	tr.	tr.	tr.
27	Bornyl acetate	1285	0.3	0.3	0.1	0.2	0.1	0.2
28	p-Cymen-7-ol	1287	0.1	0.1	tr.	tr.	0.1	0.1
29	Thymol	1290	0.1	0.1	tr.	0.1	tr.	0.1
30	trans-Sabinylyl acetate	1291	0.0	0.1	tr.	0.1	tr.	tr.
31	Carvacrol	1298	0.1	0.1	0.2	tr.	0.1	0.1
32	Carvyl acetate	1337	tr.	tr.	tr.	tr.	0.1	tr.
33	α -Cubebene	1351	0.1	0.1	0.1	0.1	0.1	0.1
34	Eugenol	1356	tr.	tr.	tr.	tr.	0.1	tr.
35	α -Ylangene	1372	0.2	0.2	0.2	0.2	0.2	0.2
36	α -Copaene	1376	0.5	0.5	0.6	0.5	0.6	0.5
37	β -Bourbonene	1384	0.1	0.2	0.1	0.1	0.1	0.1
38	β -Cubebene	1390	tr.	tr.	tr.	tr.	tr.	tr.
39	γ -Caryophyllene	1404	0.1	0.1	0.1	0.1	0.1	0.1
40	α -Gurjunene	1409	0.1	tr.	tr.	0.1	tr.	tr.
41	β -Caryophyllene	1418	4.5	4.0	4.0	3.6	3.8	4.0
42	β -Gurjunene	1432	0.2	0.2	0.2	0.1	0.2	0.2
43	α -Guaiene ???	1438	0.1	0.1	0.1	0.1	tr.	0.1
44	Aromadendrene	1439	1.3	1.2	1.3	1.2	1.2	1.2
45	α -Humulene	1454	8.7	7.6	7.8	7.6	7.8	7.9
46	allo-Aromadendrene	1461	0.5	0.5	0.4	0.4	0.4	0.4
47	γ -Gurjunene	1473	0.1	0.1	0.1	tr.	tr.	0.1
48	γ -Murolene	1477	0.8	0.9	0.8	0.8	1.0	0.9
49	Germacrene D	1480	tr.	0.1	0.1	tr.	0.1	0.1
50	β -Selinene	1485	0.1	0.1	0.1	0.1	0.1	0.1
51	cis- β -Guaiene	1490	0.2	0.4	0.2	0.2	0.2	0.2
52	Viridiflorene	1493	1.1	1.1	1.3	1.1	1.2	1.2
53	α -Murolene	1499	0.2	0.2	0.2	0.2	0.3	0.2
54	trans- β -Guaiene	1500	tr.	0.1	tr.	0.1	tr.	tr.
55	γ -Cadinene	1513	0.4	0.4	0.4	0.3	0.4	0.4
56	cis-Calamenene	1521	0.1	0.1	tr.	tr.	0.1	0.1
57	δ -Cadinene	1524	1.1	1.1	1.3	1.1	1.5	1.2
58	Cadinadiene-1,4	1532	0.1	0.1	0.1	0.1	0.2	0.1
59	α -Cadinene	1538	0.1	0.1	0.1	0.1	0.1	0.1
60	α -Calacorene	1542	0.2	0.1	0.2	0.2	0.1	0.2
61	cis-Sesquisabinene hydrate	1545	0.1	0.1	0.1	tr.	0.1	0.1
62	β -Calacorene	1562	0.1	0.1	tr.	tr.	tr.	tr.
63	Germacrene D-4-ol	1568	0.1	0.1	tr.	tr.	tr.	tr.
64	Spathulenol	1576	0.2	0.3	0.5	0.4	0.6	0.4
65	Caryophyllene oxide	1581	0.9	0.9	0.9	0.5	0.9	0.8
66	Viridiflorol	1590	8.0	7.2	7.7	7.6	8.2	7.7
67	Humulene epoxide II	1606	2.0	2.1	2.4	2.1	2.6	2.2
68	β -Oplophenone	1607	0.1	0.2	0.1	tr.	0.1	0.1
69	1,10-di-epi-Cubenol ???	1614	0.3	0.3	0.3	0.3	0.3	0.3

The percentage of 5 compounds – 1.8-cineole (6.6–8.2%), borneol (6.6–8.0%), α -humulene (7.6–8.7%), viridiflorol (7.2–9.0%) and manool (6.4–11.4%) – was similar. The above compounds occupied the second to sixth places in the row of the main constituents. The seventh and eight main constituents were camphor (3.4–4.2%) and β -caryophyllene (3.6–4.5%).

The amounts of all limited monoterpenoids were close to the lowest limit of the standard ISO 9909. The percentage of limited monoterpene hydrocarbons α -pinene (2.4–3.0%), camphene (1.4–2.8%) and limonene (0.5–0.7%) met the requirements of the standard (Introduction). The quantities of oxygenated monoterpenes (beside cis- and trans-thujones) 1.8-cineole, camphor, dihydrolinalyl acetate (tr.–0.1%) and bornyl acetate (0.1–0.3%) also were close to the lower limit of the standard. Only one sesquiterpene hydrocarbon α -humulene was limited (Table) and the amount of it was close to the upper limit [9]. Nearly the same quantities of α -humulene had been found in leaf oils in Portugal [9], Serbia and Montenegro [10].

Borneol in the oils under study was among the main constituents (Table). The above compound in a lot of early investigated sage oils was found in the amounts < 6% [7–18]. The per-

Table (continued)								
1	2	3	4	5	6	7	8	9
70	Selina-3,11-dien-6- α -ol	1640	1.3	1.4	1.6	1.3	1.6	1.4
71	Caryophylla-4,8-dien-5-ol**	1641	0.3	0.3	0.3	0.2	0.3	0.3
72	epi- α -Muurolol	1642	0.1	0.1	0.1	0.1	0.2	0.1
73	α -Muurolol	1645	0.1	tr.	tr.	tr.	0.1	tr.
74	α -Cadinol	1653	0.2	0.1	0.2	0.2	0.3	0.2
75	14-Hydroxy-9-epi-caryophyllene	1664	0.5	0.5	0.5	0.4	0.5	0.5
76	β -Bisabolol	1671	0.4	0.3	0.4	0.4	0.5	0.4
77	Cadalene	1674	tr.	0.1	tr.	0.1	tr.	tr.
78	α -Bisabolol	1683	0.1	0.1	0.1	0.1	0.2	0.1
79	α -Trans-Bergamotol	1693	0.1	0.1	0.1	0.2	0.2	0.1
80	14-Hydroxy- α -humulene	1712	0.1	tr.	tr.	0.1	tr.	tr.
81	α -trans-Bergamotyl acetate	1798	0.3	0.2	0.2	0.3	0.3	0.3
82	epi-Laurene	1891	0.3	0.3	0.3	0.4	0.4	0.3
83	Rimuene	1894	0.5	0.4	0.6	0.5	0.8	0.6
84	m/z149 (Phthalate)	1900	3.0	2.4	2.6	3.2	3.9	3.0
85	Pimaradiene	1941	0.2	0.2	0.3	0.2	0.3	0.2
86	epi-13-Manool	1961	0.1	0.1	0.1	0.1	0.1	0.1
87	Manoyl oxide	1989	0.4	0.4	0.5	0.4	0.5	0.4
88	Manool	2056	8.0	6.4	8.8	8.4	10.4	8.4
89	Ferruginol	2362	0.2	0.2	0.4	0.4	0.5	0.3
Total			97.7	98.1	97.5	98.2	98.2	97.9
Monoterpene hydrocarbons			8.9	10.2	7.6	8.5	6.9	8.4
Oxygenated monoterpenes			39.9	43.4	41.0	43.4	37.6	41.1
Sesquiterpene hydrocarbons			21.0	19.7	19.8	18.4	19.8	19.7
Oxygenated sesquiterpenes			15.2	14.4	15.5	14.3	17.0	15.3
Diterpene hydrocarbons			1.0	0.9	1.2	1.1	1.5	1.1
Oxygenated diterpenes			8.7	7.1	9.8	9.3	11.5	9.3
* – Retention indexes on nonpolar column; tr. – < 0.1%; essential oils of plants collected in gardens: A – Čekoniškės, B – Balsiai, C – Kernavė, D – Sudervė, E – Salininkai.								
• ** – correct isomer not identified								

centage of borneol in some oils exceeded 6% [9, 10, 11, 14].

The chemical composition of the essential oils depended on the experimental conditions of steam or hydrodistillation and on the time of process. The higher percentage of oxygenated sesqui- and diterpenes was determined in the oils produced during a longer distillation time. Hydrodistillation of air-dried plants for 2 h produced oils containing oxygenated sesquiterpene viridoflorol and oxygenated diterpene manool in the same amounts as the other main constituents (1.8-cineole, borneol, α -humulene) (Table). Viridoflorol and manool had been found in the percentage < 6% in earlier investigated oils [7–18]. Some oils of sage growing in Portugal [8, 9] and in Serbia and Montenegro [10] contained marked amounts of viridiflorol (> 7%) and manool (> 6%). The chemical composition of the earlier studied sage essential oils to that of the oils under study (Table) was closest in the above countries [8–10].

The sage essential oils under study contained ~100 compounds beside the main constituents. Eighty-nine

compounds were identified. The largest part of the constituents comprised oxygenated terpenes (64.4–67.7%). The oxygenated monoterpenes made up 37.6–43.4%, oxygenated sesquiterpenes – 14.3–17.0%, and corresponding diterpenes – 7.1–11.5% (Table). The limits of variations of separate groups of terpenoids are markedly wider (differences between limits of corresponding groups: 6%, 2.7% and 4.4%) than for all oxygenated terpenes (3.3%). The amounts of hydrocarbons in the oil varied from 6.9 to 10.2% for monoterpenes, from 18.4 to 21.0% for sesquiterpenes and from 0.9 to 1.5% for diterpenes. The identified constituents made up 97.5–98.2% of the essential oils.

CONCLUSION

The five essential oils of cis-thujone chemotype produced by plants *Salvia officinalis* L. growing in Vilnius district met the requirements of the standard ISO 9909 (1999).

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D. Mockutė, O. Nivinskienė, G. Bernotienė, R. Butkienė

**SALVIA OFFICINALIS L. CIS-TUJONO CHEMOTIPO
ETERINIS ALIEJUS**

S a n t r a u k a

Vaistinės salvijos augalai buvo surinkti penkiuose soduose Vilniaus apylinkėse. Pagrindiniai eterinių aliejų komponentai buvo 1,8-cineolis (6,8–8,2%), cis-tujonas (14,8–19,0%), borneolis (6,6–8,0%) ir manolis (6,4–10,4%). Limituojamų junginių kiekiai eteriniuose aliejuose: cis- ir trans-tujonų, kamparo, 1,8-cineolio, α -humuleno, α -pineno, kamfeno, limoneno, linalolio bei jo darinių ir bornilacetato – visiškai atitinka standarto ISO 9909 (1999) reikalavimus. Aštuoniasdešimt devyni identifikuoti junginiai sudarė 97,5–98,2% tirtų eterinių aliejų. Dauguma (69,4–67,7%) lakiųjų junginių buvo oksiduoti terpenai.