

Chemical composition, antimicrobial activities and odor descriptions of various *Salvia* sp. and *Thuja* sp. essential oils

Chemische Zusammensetzung, antimikrobielle Aktivitäten und Geruchsbeschreibungen von verschiedenen *Salvia* sp. und *Thuja* sp. ätherischen Ölen

L. JIROVETZ, G. BUCHBAUER, Z. DENKOVA, A. SLAVCHEV, A. STOYANOVA, E. SCHMIDT

Summary

The chemical composition of various commercially available *Salvia* sp. (*S. officinalis* (two samples), *S. sclarea*, *S. lavandulifolia*) and *Thuja* sp. (*T. plicata*, *T. occidentalis*) essential oils were analyzed by GC and GC/MS. More than fifty volatiles were identified in all samples with the monoterpenes camphor, 1,8-cineole, fenchone, linalool, linalyl acetate, sabinene, sabinyl acetate, α -thujone and β -thujone as main compounds. These analytical results were correlated with olfactoric evaluations for reasons of quality control of these essential oils. Furthermore, all samples, some higher concentrated constituents, a phenolic reference compound and three classic antibiotics were tested against several microorganisms to get informations about the antimicrobial activity of each essential oil.

Keywords:

Essential oils, *Salvia* sp., *Thuja* sp., thujones, composition, antimicrobial activities, odor

Zusammenfassung

Die chemische Zusammensetzung von verschiedenen kommerziell erhältlichen *Salvia* sp. (*S. officinalis*, *S. sclarea*, *S. lavandulifolia*) und *Thuja* sp. (*T. plicata*, *T. occidentalis*) ätherischen Ölen wurden mit GC und GC/MS analysiert. Mehr als fünfzig flüchtige Inhaltsstoffe wurden in allen Proben identifiziert und die Monoterpene Kampfer, 1,8-Cineol, Fenchon, Linalool, Linalylacetat, Sabinen, Sabinylacetat, α -Thujon und β -Thujon als Hauptkomponenten identifiziert. Diese analytischen Resultate wurden mit olfaktorischen Bewertungen zur Qualitätskontrolle dieser ätherischen Öle korreliert. Weiters wurden alle Proben, einige höher konzentrierte Inhaltsstoffe, eine phenolische Vergleichskomponente und drei klassische Antibiotika gegen einige Mikroorganismen getestet, um Informationen über die antimikrobielle Aktivität jedes ätherischen Öles zu erhalten.

Kennwörter:

Ätherische Öle, *Salvia* sp., *Thuja* sp., Thujone, Zusammensetzung, antimikrobielle Aktivitäten, Geruch

1. Introduction

In continuation of an international project in the field of combined data interpretation of composition analysis, odor evaluation and antimicrobial activity testings [14, 23, 24, 43] of various aroma-samples, essential oils of sage (*Salvia officinalis* L., *Lamiaceae*) from Bosnia-Herzegovina and Egypt, Spanish sage (*Salvia lavandulifolia* Vahl, *Lamiaceae*), clary sage (*Salvia sclarea* L., *Lamiaceae*) from Russia, western red cedar wood-leaves (*Thuja plicata* Donn ex D. Don, *Cypresaceae*) from Canada and eastern white cedar wood-leaves (*Thuja occidentalis* L., *Cypresaceae*) from the Balkan-area were investigated.

Many papers about the composition of *Salvia* sp. and *Thuja* sp., their extracts and essential oils [4, 5, 8, 9, 11, 19-21, 27-30, 32, 35-40, 44, 47-50], the use of the essential sage and thuja oils for the flavoring of foods, e. g. alcoholic and non-alcoholic beverages, bitters,

ice-creams, candies and baked goods, chewing gums, condiments, meats and pickles [14, 18, 41, 44], the applications of these oils in perfumery and cosmetics [4, 6] as well as the biological, pharmacological and toxicological effects as fresh plant, herb, tea, extract and essential oil for the treatment of various diseases [3, 7, 12, 14, 15, 17, 20, 31, 34, 41, 42, 46, 50, 51] are available.

In addition, some data about biology, pharmacology, toxicology, odor and use in food-flavorings, perfumery and cosmetics as well as medical applications of main compounds (e. g. α - and β -thujone, camphor, 1,8-cineole, linalool and linalyl acetate) were published elsewhere [2, 4, 7, 3-16, 18, 23, 24, 31, 33, 41, 43, 47].

Although this immense number of data about sage- and thuja-samples exists, to the best of our knowledge, up to now no combined interpretation of data

from GC and GC/MS analysis, olfactive evaluation and antimicrobial testings (using agar diffusion and agar dilution method, both), including comparisons with antimicrobial data of some main compounds and commercial antibiotics, have been done on essential oils of *Salvia* sp. and *Thuja* sp. from various origin.

2. Materials and methods

2.1. Reference compounds

Camphor (W52,660-6), β -caryophyllene (W22,520-7), 1,8-cineole (W24,650-6), limonene (W26,330-3), linalool (W26,350-8), linalyl acetate (W26,350-5), α -terpineol (W30,452-2), α -thujone (89231), eugenol (W24,670-0) and tetracycline hydrochloride (achromycine hydrochloride – 25 g, T3383-25G) are products from Sigma-Aldrich Austria Co., Vienna; *Salvia officinalis*¹ (sage, *Lamiaceae*) was obtained from Aroma & Flavor Chemistry Department – National Research Center Giza (Egypt); terpinen-4-ol (800760), western red cedar wood leaf-oil (800177; *Thuja plicata* Donn ex D. Don, *Cypressaceae*, Canada; green leaves), eastern white cedar wood leaf-oil (800914; *Thuja occidentalis* L., *Cypressaceae*, Balkan-area, green leaves) *Salvia officinalis* L.² (sage, 801510; Bosnia-Herzegovina, leaves of flowering top of the plant), *Salvia lavandulifolia* Vahl (Spanish sage, *Lamiaceae*, 800283; leaves of the flowering top of the plant) and *Salvia sclarea* L. syn *Salvia sclarea* var. *turkestaniana* Mottet (clary sage, *Lamiaceae*, 800738; Russia, leaves of the flowering top of the plant) were purchased from Kurt Kitzing Co., Wallerstein; β -thujone (no product-number given) was delivered from Symrise Co. (former Dragoco Co.), Holzminden; Ciproxin^R 500 mg-tablettes (one tablette = 582 mg ciproflaxoxacine hydrochloride /water) bought from Bayer Austria Co., Vienna and Lidaprim^R-infusion-bottle (250 mg containing 0.8 g sulfametrol and 0.16 g trimethoprim) from Nycomed Austria Co., Vienna.

2.2. GC analysis

GC/FID analyses were carried out using a GC-14A with FID and C-R6A-Chromatopac integrator (Shimadzu, Japan), a GC-3700 with FID (Varian, Germany) and C-R1B-Chromatopac integrator (Shimadzu). The carrier gas was hydrogen; injector temperature 250 °C; detector temperature 320 °C. The temperature programme was: 40 °C/5 min to 280 °C/5 min, with a heating rate of 6 °C/min. The columns were 30 m x 0.32 mm bonded FSOT-RSL-200 fused silica, with a film thickness of 0.25 μ m (Biorad, Germany) and 30 m x 0.32 mm bonded Stabilwax, with a film thickness of 0.50 μ m (Restek, USA). Quantification was achieved using peak area calculations, and compound identification was carried out partly using correlations between retention times [1, 10, 22, 25, 26].

2.3. GC-MS analysis

For GC/MS measurements a GC-17A with QP5000 (Shimadzu) and Compaq-ProLinea data system (class5k-software), a GC-HP5890 with HP5970-MSD (Hewlett-Packard, USA) and ChemStation software on a Pentium PC (Böhm, Austria), a GCQ (Finnigan-Spectronex, Germany-USA) and Gateway-2000-PS75 data system (Siemens-Nixdorf, Germany, GCQ-software) were used. The carrier gas was helium; injector temperature at 250 °C; interface-heating at 300 °C, ion-source-heating at 200 °C, EI-mode was 70 eV, and the scan-range was 41-450 amu. For other parameters, see description of GC/FID, above. Mass spectra correlations were done using Wiley, NBS, NIST and our own library as well as published data [1, 22, 25].

2.4. Antimicrobial testings

The essential oil of sage, the key compounds and the reference compound were prepared as 20 % solutions of ethanol and dissolved in a 0.9 % NaCl solution (ratio of 1:10). As test microorganisms (colony-forming-units=cfu/cm³), Gram-(+)-bacteria *Staphylococcus aureus* ATCC 6538P (1x10¹³) and *Enterococcus faecalis* (clinical isolated, 1x10¹³); Gram-(-)-bacteria *E. coli* ATCC 8739 (2x10¹²), *Pseudomonas aeruginosa* G 28 (1.2x10⁹), *Klebsiella pneumoniae* (clinical isolated, 1x10¹³), *Proteus vulgaris* (clinical isolated, 3x10¹³) and *Salmonella* sp. (clinical isolated, 3x10¹²) as well as the yeast *Candida albicans* ATCC 10231 (1x10¹¹) – all products from the National Bank of Industrial Microorganisms and Cell Cultures, Sofia, Bulgaria – were used.

The antimicrobial activity was studied by two methods:

1. Agar diffusion disc method using 6 mm paper discs and quantities of 6 μ L of the sample. After cultivation of the bacteria and the yeast at 37 °C for 24 h the diameter of the inhibition zone (IZ) was measured.
2. Agar serial tube dilution method with results as minimum inhibitory concentration (MIC) as follows: The essential oil, pure and reference compounds were added to brine, containing 1.0 % (v/v) Tween 80 at the appropriate volumes to produce final concentrations of the samples in the range of 100-1.000 ppm; the Petri dishes were inoculated by pipetting 0.1 cm³ of the desired culture and 0.6 μ L of the samples as well as the reference compounds (the tablettes of Ciproxin^R were added as solution in saline at a quantity of 300 μ g) on paper discs (6 mm) and then incubated at 37 °C for 24 h.

2.5. Olfactive evaluations

All investigated samples were olfactically evaluated by a professional perfumer and two aroma-chemists; the aroma described in *Table 1* correlated with odor impressions published elsewhere [2, 4, 7, 16, 18, 33, 45].

Component	RI	So ¹	So ²	SI ³	Ss ⁴	Thp ⁵	Tho ⁶	Odor
ethyl isovalerate	840/1058	nd [§]	nd	nd	nd	0.03	0.01	intense fruity, apple-aroma on dilution
cis-salvene	856/1012	tr ^{&}	0.04	nd	nd	nd	nd	mild herbal
trans-salvene	867/1021	nd	0.01	nd	nd	nd	nd	herbal, green
tricyclene	927/1009	tr	0.64	0.39	nd	nd	0.12	piney, weak herbal
α-thujene	930/1025	nd	0.50	tr	nd	0.33	1.46	herbal, green, weak earthy
α-pinene	942/1036	0.20	6.48	4.91	0.04	1.41	3.33	pine-like, warm, herbal
β-fenchene	949/1057	tr	0.01	nd	nd	nd	tr	fresh, clean
camphene	952/1069	3.45	7.09	5.59	nd	2.48	2.55	camphoraceous, fresh
α-fenchene	957/1071	0.02	0.02	0.08	nd	0.37	2.04	fresh, minty
sabinene	972/1130	tr	0.01	1.27	nd	4.39	12.14	fresh, fruity, spicy
β-pinene	979/1122	1.55	3.74	4.59	0.05	0.11	1.14	resinous-piney, dry
myrcene	988/1158	1.13	1.97	2.48	1.21	1.42	4.05	weak citrus- and lime-like
ethyl 2-methylbutyrate	999/1302	nd	nd	nd	nd	0.09	0.05	powerful green-fruity
α-phellandrene	1002/1173	0.13	0.18	0.06	nd	0.12	0.12	citrus-like, weak peppery
δ-3-carene	1009/1147	0.09	tr	nd	nd	nd	0.99	sweet, lemon-note
p-cymene	1015/1273	tr	1.42	0.41	tr	0.63	2.37	citrus-like
α-terpinene	1017/1189	tr	0.21	0.08	nd	0.48	1.83	herbal, weak fruity, terpenic
limonene	1020/1206	1.91	1.87	5.02	1.32	1.14	2.36	lemon-like, fresh
β-phellandrene	1022/1214	nd	0.05	tr	nd	0.20	1.65	citrus-like, weak herbal-spicy
1,8-cineole	1025/1226	7.45	9.69	25.52	tr	0.05	0.08	eucalyptus-like, fresh
cis-β-ocimene	1027/1233	0.08	tr	0.09	0.51	tr	tr	warm-herbal, sweet
trans-β-ocimene	1042/1253	1.26	tr	0.07	0.93	tr	tr	warm-herbal, weak floral
γ-terpinene	1057/1250	0.13	1.66	0.31	nd	0.83	2.29	citrus-like, herbal
trans-sabinene hydrate	1060/1463	nd	tr	tr	nd	nd	1.09	spicy, weak fruity
terpinolene	1073/1282	0.34	0.87	0.25	tr	0.21	2.32	sweet-piney
fenchone	1081/1410	0.20	tr	tr	nd	15.12	12.87	camphoraceous, herbal
cis-sabinene hydrate	1090/1562	nd	0.09	0.13	nd	nd	0.12	spicy, weak fruity
linalool	1093/1515	0.51	0.31	2.39	20.98	tr	1.89	fresh-floral
α-thujone	1100/1440	22.19	21.48	0.06	nd	54.13	2.76	warm-herbal, minty
fenchol	1110/1576	0.17	tr	nd	nd	tr	0.22	camphoraceous, fresh
β-thujone	1119/1460	17.70	8.78	tr	nd	6.83	9.48	camphoraceous-herbal
camphor	1131/1518	25.11	18.08	29.17	nd	2.32	1.24	camphoraceous, fresh
trans-sabinol	1135/1666	nd	tr	0.83	nd	nd	0.82	spicy, fresh-fruity
β-terpineol	1138/1616	nd	nd	0.07	nd	nd	nd	floral, weak fruity
cis-sabinol	1147/1683	nd	nd	tr	nd	nd	0.32	spicy, weak fruity
camphene hydrate	1150/1603	nd	nd	nd	nd	0.15	0.17	fresh, clean, camphoraceous
borneol	1154/1684	1.60	2.88	2.19	nd	0.15	0.24	camphoraceous, earthy
p-cymene-8-ol	1163/1846	nd	nd	nd	nd	0.14	0.13	weak citrus-like
terpinen-4-ol	1168/1624	nd	0.04	0.54	tr	1.61	3.32	fruity, floral, terpenic
isoborneol	1156/1660	nd	nd	0.02	nd	nd	tr	camphoraceous, herbal
δ-terpineol	1159/1655	nd	nd	0.27	nd	nd	nd	floral, weak fruity
α-terpineol	1181/1717	nd	0.04	0.68	4.01	0.10	0.32	floral, liliac-like
estragole	1184/1652	nd	nd	nd	nd	0.40	nd	anise-like, sweet, phenolic-minty
nerol	1218/1777	nd	nd	nd	0.75	nd	nd	floral-fruity, sweet
fenchyl acetate	1221/1473	1.23	tr	nd	nd	1.21	0.27	camphoraceous, herbal
geraniol	1238/1802	nd	nd	0.35	3.22	nd	nd	sweet-floral, rose-like, fruity
linalyl acetate	1243/1542	0.08	nd	4.17	59.95	nd	1.24	floral, weak fruity
sabinyl acetate	1262/1657	nd	nd	3.41	nd	nd	16.55	fresh-spicy, weak fruity
bornyl acetate	1277/1608	0.41	0.74	1.38	nd	0.92	0.13	piney, balsamic, sweet
geranyl formate	1282/1692	nd	nd	nd	0.08	nd	nd	green-leafy, weak rose-like, sweet-fruity
thymol	1287/2098	nd	nd	nd	nd	0.26	nd	thyme-like, spicy
terpinyl acetate	1332/1697	nd	nd	0.99	tr	0.22	1.17	terpenic, weak floral-fruity
neryl acetate	1345/1707	nd	nd	nd	1.08	nd	nd	sweet-floral-fruity
α-copaene	1372/1511	nd	0.06	nd	0.29	nd	nd	weak spicy, herbal, woody
bourbonene	1388/1557	nd	nd	nd	0.13	nd	nd	green-woody, spicy
β-elemene	1401/1604	3.71	nd	nd	nd	nd	0.13	woody, weak sweet-floral
geranyl propionate	1424/1799	nd	nd	0.45	tr	nd	nd	sweet, rose-like, fruity
β-caryophyllene	1432/1617	2.70	6.18	1.16	1.31	1.61	1.23	dry, woody-spicy
α-humulene	1437/1681	4.21	2.53	0.36	tr	nd	0.19	woody, spicy
aromadendrene	1453/1653	nd	0.05	nd	nd	nd	nd	weak spicy-woody
germacrene D	1477/1716	nd	nd	nd	2.15	nd	nd	woody-spicy, weak herbal
trans, trans-α-farnesene	1502/1739	0.34	nd	nd	0.15	nd	nd	weak floral-fruity
δ-cadinene	1505/1769	tr	0.13	nd	nd	nd	1.29	weak fruity-woody, spicy
γ-cadinene	1508/1778	nd	nd	nd	nd	nd	0.22	spicy-woody, herbal
elemol	1541/2078	nd	nd	nd	nd	nd	0.75	spicy-woody
caryophyllene oxide	1576/1989	0.62	0.08	tr	0.14	0.23	0.13	warm-spicy, woody
viridiflorol	1588/2098	0.19	0.13	nd	nd	nd	nd	herbal, floral, woody
humulene oxide	1592/1972	nd	0.16	nd	nd	nd	tr	woody-spicy
sclareol	2223/2507	nd	nd	nd	0.48	nd	nd	- no data available

Tab. 1: Composition and olfactic evaluation[§] of the *Salvia sp.* and *Thuja sp.* essential oils including their retention-indices* and percentage[#]

[§] in accordance to published data elsewhere [2, 4, 7, 16, 18, 33, 45] and private olfactic evaluation data from pure aroma compounds (e. g. see [23, 24, 43])

* apolar/polar column

in relative %-peak area using GC with an apolar column (mean value of three analyses)

§ not detected

& trace compound (less than 0.01 %)

So¹ *Salvia officinalis* (Egypt) and So² (Bosnia-Herzegovina); SI³ *S. lavandulifolia*, Ss⁴ *S. sclarea*, Thp⁵ *Thuja plicata* and Tho⁶ (*Thuja occidentalis*)

3. Results and discussions

The essential oils were olfactorically evaluated as follows: *Salvia officinalis*¹ (Egypt): Characteristic sage odor with a fresh-camphoraceous and rosemary-pine-like top-note and herbal-minty thujone-base-notes; *S. officinalis*² (Bosnia-Herzegovina): Green-herbal, intense thujone-notes; *S. lavandulifolia*: Intense fresh-eucalyptus-top-note, camphoraceous (direction of spike-lavender), green-fresh-notes from sage; *S. sclarea*: Fresh-floral, intense lavender-note (linalool and linalyl acetate), herbal-spicy-woody in the background; *Thuja plicata*: Intense thujone-note with green-herbal side-notes; *T. occidentalis*: Fresh-spicy, green-herbal thujone-notes, woody-earthy in the background.

By means of gas chromatographic-spectroscopic analysis (GC/FID and GC/MS) of the essential oils about seventy constituents could be identified with the main compounds as follows (concentration higher than 3.0 %, calculated as relative %-peak-area using GC-FID with an apolar column) of the single samples (see Table 1): *S. officinalis*¹ (Egypt): Camphor (25.11 %), α -thujone (22.19 %), β -thujone (17.70 %), 1,8-cineole (7.45 %), α -humulene (4.51 %), β -elemene (3.71 %) and camphene (3.45 %); *S. officinalis*² (Bosnia-Herzegovina): α -Thujone (21.48 %), camphor (18.08 %), 1,8-cineole (9.69 %), β -thujone (8.78 %), camphene (7.09 %), α -pinene (6.48 %), β -caryophyllene (6.18 %) and β -pinene (3.74 %); *S. lavandulifolia*: Camphor (29.17 %), 1,8-cineole (25.52 %), camphene (5.59 %), limonene (5.02 %), α -pinene (4.91 %), β -pinene (4.59 %), linalyl acetate (4.17 %) and sabinyl acetate (3.41 %); *S. sclarea*: Linalyl acetate (59.95 %), linalool (20.98 %), α -terpineol (4.01 %) and geraniol (3.22 %); *T. plicata*: α -Thujone (54.13 %), fenchone (15.12 %), β -thujone (6.83 %) and sabinene (4.39 %); *T. occidentalis*: Sabinyl acetate (16.55 %), fenchone (12.87 %), sabinene (12.14 %), β -thujone (9.48 %), α -pinene (3.33 %) and terpinen-4-ol (3.32 %).

Using olfactoric data from elsewhere (see references cited in Table 1) the characteristic odor of the samples can be correlated to camphene, fenchol, camphor, borneol and fenchyl acetate (camphoraceous), limonene and 1,8-cineole (fresh), sabinene and sabinyl acetate (fresh-spicy), linalool and linalyl acetate (floral-fresh), α -pinene, β -pinene and terpinolene (pine-like) as well as α -thujone and β -thujone (thujone-like).

Results of antimicrobial testings (agar diffusion and agar dilution method in accordance to [13, 23, 24, 43]) of the different *Salvia* sp. and *Thuja* sp. essential oils against various microorganisms were as follows (see Table 2): Gram-(+)-bacterium *Staphylococcus aureus*:

High antimicrobial activity of both *S. officinalis* samples and *T. occidentalis*; Gram-(-)-bacterium *E. coli*: Very high activity of *S. officinalis* from Bosnia-Herzegovina and high activity of all other samples excluding *S. lavandulifolia*; Gram-(-)-bacterium *Proteus vulgaris*: Very high activity of *T. plicata* and high activity of both *S. officinalis* samples; Gram-(-)-bacterium *Pseudomonas aeruginosa*: Very high activity of *S. lavandulifolia* and *S. sclarea* as well as *T. plicata*; Gram-(-)-bacterium *Salmonella* sp.: Very high activity of *S. officinalis* from Egypt and *S. lavandulifolia*; Gram-(-)-bacterium *Klebsiella pneumoniae*: Very high activity of both *S. officinalis* samples and *S. sclarea*; Gram-(+)-bacterium *Enterococcus faecalis*: Very high activity of *S. lavandulifolia* and *T. occidentalis*; yeast *Candida albicans*: High activity only of both *S. officinalis* samples.

Correlations with the data of the high antimicrobial active, phenolic compounds eugenol and the synthetic antibiotics tetracycline hydrochloride and Ciproxin^R (these products and Lidaprim^R are known to have no effects against the yeast *C. albicans*) prove the effectiveness of the chosen testing methods, while Lidaprim^R (no effect against *P. aeruginosa* and *K. pneumoniae*) was found to have not the antimicrobial activity as expected.

Antimicrobial testings with the two main compounds of both sage samples, α -thujone and β -thujone showed high effects against the Gram-(-)-bacteria *P. aeruginosa* and *K. pneumoniae* and medium effects against *St. aureus*, *E. coli* and *C. albicans*.

Antimicrobial data of further constituents of the essential sage and thuja oils, published elsewhere [13, 23, 24, 43], such as camphor, β -caryophyllene, 1,8-cineole, limonene, linalool, linalyl acetate, terpinen-4-ol and α -terpineol were correlated to confirm our experience from previous studies [13, 23, 24, 43] in the field, that not only one or two compounds are responsible for antimicrobial activities of essential oils, but the whole composition with alternating synergistic and antagonistic effects of each volatile.

To compare the analytical and antimicrobial results of this investigation with reference-data of regulation, application and treatment of different samples (plants, plant parts, herbs, extracts and essential oils) of *Salvia* sp. and *Thuja* sp. as well as the target compounds α - and β -thujone, we can state that an use of all of these essential oils for flavoring of foods, as odor component in perfumery and cosmetic products and in medical preparations are possible without any danger, when not taken excessive and for a longer period (remember the word of Paracelsus: "the dosis renders the effect").

Compounds and Essential Oil	Inhibition Zones (IZ) in mm and Minimum Inhibitory Concentrations (MIC) in ppm of test-microorganisms									
	<i>Staphylococcus aureus</i> <i>Enterococcus faecalis</i>		<i>Escherichia coli</i> / <i>Proteus vulgaris</i>		<i>Pseudomonas aeruginosa</i> / <i>Salmonella sp.</i>		<i>Klebsiella pneumoniae</i>		<i>Candida albicans</i>	
	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC
Camphor ^{1,2,3}	13 / nt [#]	60 / nt	- / nt	- / nt	8 / nt	600 / nt	-	-	20	6
β -Caryophyllene ²	20 / nt	60 / nt	15 / nt	60 / nt	- / nt	- / nt	10	60	27	0.6
1,8-Cineole ^{1,2}	10 / nt	600 / nt	- / nt	- / nt	10 / nt	60 / nt	12	6	14	60
Limonene ³	8 / nt	60 / nt	6 / nt	0.6 / nt	- / nt	- / nt	6	0.6	14	6
Linalool ^{1,3}	19 / nt	60 / nt	9 / nt	6 / nt	8 / nt	600 / nt	9	60	16	60
Linalyl acetate ^{1,3}	11 / nt	60 / nt	- / nt	- / nt	8 / nt	600 / nt	-	-	20	6
Terpinen-4-ol ²	25 / nt	6 / nt	25 / nt	6 / nt	8 / nt	60 / nt	27	6	25	0.6
α -Terpineol ²	25 / nt	60 / nt	21 / nt	60 / nt	11 / nt	600 / nt	23	6	25	6
α -Thujone	20 / 18	60 / 60	10 / 8	60 / 60	8 / 8	600 / 60	12	600	14	60
β -Thujone	22 / 20	60 / 60	8 / 7	6 / 6	8 / 8	600 / 60	13	600	13	60
<i>Salvia officinalis</i> 1 ⁴	30 / 20	60 / 60	13 / 9	60 / 60	6 / 12	6 / 600	15	600	20	60
<i>Salvia officinalis</i> 2	13 / 10	60 / 60	10 / 9	600 / 60	- / 10	- / 60	15	600	13	60
<i>Salvia lavandulifolia</i>	25 / 11	0.6 / 600	10 / 7	0.6 / 8	9 / 8	600 / 600	10	60	20	0.6
<i>Salvia sclarea</i>	32 / 22	0.6 / 0.6	8 / 9	60 / 0.6	8 / 11	600 / 60	10	600	18	0.6
<i>Thuja plicata</i>	30 / 10	0.6 / 6	11 / 7	60 / 600	10 / 11	600 / 60	-	-	11	0.6
<i>Thuja occidentalis</i>	17 / 11	60 / 600	11 / -	60 / -	9 / 11	60 / 6	13	60	11	0.6
Eugenol	30 / 30	600 / 600	28 / 30	600 / 600	25 / 28	600 / 600	28	600	32	600
Ciproxin ^R	35 / 33	600 / 600	22 / 25	600 / 600	32 / 10	600 / 600	25	600	-	-
Lidaprim ^R	27 / 27	600 / 600	11 / 23	60 / 600	- / 8	- / 60	-	-	-	-
Tetracycline hydrochloride	15 / 22	600 / 600	11 / 13	600 / 600	15 / 10	600 / 600	20	600	-	-

Tab. 2: Antimicrobial activities of *Salvia sp.* and *Thuja sp.* essential oils and some main as well as reference compounds

[#]not tested against the microorganism until now, - no inhibition observed, ¹published elsewhere [23], ²published elsewhere [24], ³published elsewhere [43], ⁴partly published elsewhere [13]

Reference	Plant/plant part/compound	Data of regulation/application/treatment
[14, 41]	Thujone (α , β)	Foodstuffs / beverages: 0.5 mg/kg / 0.5 mg/L , alcoholic beverages with more than 25 % volume of alcohol: 5 mg/kg , alcoholic beverages with not more than 25 % volume of alcohol: 10 mg/kg , foodstuffs containing preparations on sage: 25 mg/kg , bitters: 35 mg/kg
[46]	Thujone (α , β) Cedar-leaf / sage oil	Flavoring of food with a maximum level of 0.5 mg/kg ; oral LD50: 0.25 g/kg oral-LD50: 0.83 g/kg / 2.6 g/kg
[18]	Sage / sage oil <i>Salvia lavandulifolia</i> oil <i>Thuja occidentalis</i> oil	Non-alcoholic beverages: 300 ppm ; baked goods: 170 ppm ; meats: 1.500 ppm / non-alcoholic beverages: 3.7 ppm ; ice-cream and ice: 16 ppm ; candy: 11 ppm ; chewing gum: 30 ppm ; condiments: 14 ppm ; meats: 110 ppm ; pickles: 2.4 ppm Non-alcoholic beverages: 2.0-11 ppm ; ice-cream and ice: 2.0-44 ppm ; candy: 20 ppm ; baked goods: 20 ppm ; condiments: 50 ppm ; meats: 40 ppm Non-alcoholic beverages: 0.01-0.50 ppm ; alcoholic beverages: 16 ppm ; ice cream and ice: 0.01-1.0 ppm ; candy: 12 ppm ; baked goods: 1.0-20 ppm ; meats: 15 ppm
[15]	<i>Salvia officinalis</i> drug	Whole leaf drug / cut leaf drug: 15 mL/kg / 10 mL/kg essential oil (water free drug)
[31]	<i>Salvia officinalis</i> tee drug	Gastro-intestinal application / inflammation of the mouth-area: max. dosage/day: adults 4-6 g Inflammation of the mouth-area: children 1-4: 1-3 g , 4-10: 3-4 g , 10-16: 4-6 g
[12]	<i>Salvia officinalis</i> drug	4-6 g/day herb or 0.1-0.3 g essential oil : infections (bacterium, fungus and virus), pharyngosis 2 tea-spoons (3 g) cut herb/cup water : cough, cramp, diarrhea, dysmenorrhoea, gastrostis 1-4 mL liquid herb extract : glossosis, inflammationsore throat, tonsilosis 1-4 g leaf, or in tea 3x/day or 1-4 mL liquid leaf extract (1:1 in 45 % ethanol) 3x/day : acne, infections (bacterium and yeast), cramp, dyspepsia, gingivosis, pharyngosis, stomatosis 2-3 tea-spoons (3.4-5.1 g) leaf in hot tea : angina, catarrh, colic, cough, odontosis, stomatosis boil 100 g leaf/liter wine two minutes: anorexia, asthma, infections (bacterium, fungus and virus), bronchitis, cramp, dermatosis, diabetes, diarrhea, enterosis, fever, gastrostis, gingivosis, halitosis, inflammation, laryngosis, mucosis, mycosis, rhinosis, stomatosis, wound-desinfection 2-4 table-spoons fresh leaf or 3-6 g dry leaf or 4.5 g dry leaf/2 mL alcohol/23 mL water : gas

Tab. 3: Some data of regulation/application/treatment for the use of thujones and thujone-rich drugs, extracts and essential oils

In conclusion, we can report that the essential oils of various *Salvia* and *Thuja* species were found to be especially rich in camphor, α -thujone, β -thujone, linalyl acetate, linalool, fenchone, sabinene, sabinyl acetate and 1,8-cineole as well as some further monoterpenic compounds, which are also responsible for the characteristic, especially fresh-camphoraceous, fresh-spicy, floral-fresh, pine- and thujone-like odor of the different samples. Also the antimicrobial activities of the essential sage and cedar wood oils against Gram-(+)-, Gram-(-)-bacteria and yeast are determined by many constituents with synergistic and antagonistic effects and cannot be correlated to a single compound. An application of all the samples in foods, medicinal, perfumery and cosmetic products is of very low risk, when taken in an appropriate dosage with regards to official regulations [14, 15, 41].

Acknowledgement

The authors acknowledge the help of Prof. Dr. *Ahmad Shalaby* and Dr. *Amr E. Edris*, Medicinal & Aromatic Plant Department, National Research Center Giza, for the identification and characterization of the plant material, the reference compounds from *Kurt Kitzing Co.* and *Symrise Co.*, the olfactive evaluation of the samples by *Wolfgang Höppner* (retired chief-perfumer of *Symrise* (former *Dragoco*) *Co.*, Hamburg) as well as the valuable discussion about the used antimicrobial testing methods with Prof. Dr. *Heinz Schilcher*, Emeritus of the "Freie Universität Berlin".

References

- [1] *Adams R.P.*: Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy. Allured, Illinois, 2001.
- [2] *Arctander St.*: Perfume and Flavor Chemicals (Aroma Chemicals). Arctander Publ., Montclair, 1969.
- [3] *Baricevic D., Bartol T.*: The biological/pharmacological activity of the *Salvia* genus. In: *SAGE–The Genus Salvia* (*Kintzios, S.E.*, ed.), Harwood Academic Publishers, Amsterdam, 2000, 143-184.
- [4] *Bauer K., Garbe D., Surburg H.*: Common Fragrance and Flavor Materials, 3rd Ed., VCH, Weinheim, 1997.
- [5] *BACIS* – Boelens Aroma Chemical Information Service, VCF Volatile Compounds in Food Database, TNO Nutrition and Food Research, Zeist, 2000.
- [6] *Boelens M.H.*: Chemical and sensory evaluation of three sage oils. *Perfum Flavor* 1997; 22: 19-40.
- [7] *Carta C., Moretti M., Peana A.*: Activity of the oil of *Salvia officinalis* L. against *Botrytis cinerea*. *J Essent Oil Res* 1996; 8: 399-404.
- [8] *Chalchat J., Michet A., Pasquier B.*: Study of the clones of *Salvia officinalis* L.: Yields and chemical composition of essential oils. *Flavour Fragr J* 1998; 13: 68-70.
- [9] *Couladis M., Tzakou O., Mimica-Dukic N., Jancic R., Stojanovic D.*: Essential oil of *Salvia officinalis* L. from Serbia and Montenegro. *Flavour Fragr J* 202; 17: 119-126.
- [10] *Davies N.W.*: Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases. *J Chromatogr* 1990; 503: 1-24.
- [11] *Dudai N. et al.*: Dynamics of yield components and essential oil production in a commercial hybrid sage (*officinalis* x *Salvia fruticosa* cv. *Newe Ya'ar* No. 4). *J Agric Food Chem* 1999; 47: 4341-4345.
- [12] *Duke J.A., Bogenschutz-Godwin M.J., duCellier J., Duke, P.-A.K.*: Handbook of Medicinal Herbs, 2nd Ed., 642-643, CRC Press, Boca Raton, 2002.
- [13] *Edris A.E., Jirovetz L., Buchbauer G., Denkova Z., Slavchev A., Stoyanova A.*: Chemical composition, antimicrobial activities and olfactive evaluation of *Salvia officinalis* L. (sage) essential oil from Egypt. *J Essent Oil Res* 2005; paper submitted.
- [14] *European Commission*: Council Directive 88/388/EEC, rev. 1, WGF/009/99, Flavourings for use foodstuffs and to source materials for their production, ANNEX II, Off J Eur Comm, Brussels, 1999.
- [15] *Europäisches Arzneibuch*: 4. Ausgabe, Grundwerk 2002, Band 2, Monographie – Salbeiblätter, *Salvia officinalis* folium, Verlag Österreich, 2002.
- [16] *Fazzalari F.A.*: Compilation of Odor and Taste Threshold Values Data, American Society for Testing and Materials, Philadelphia, 1978.
- [17] *Frohne D., Pfänder H.J.*: Giftpflanzen: ein Handbuch für Apotheker, Ärzte, Toxikologen und Biologen, Wissenschaftliche Verlags-GmbH Stuttgart, 1979.
- [18] *Furia T.E., Bellanca N.*: Fenaroli's Handbook of Flavor Ingredients, 2nd Ed., CRC Press, Cleveland, 1975.

- [19] *Gomes P., Ferreira M.*: Organ and season-dependent variation in the essential oil composition of *Salvia officinalis* L. cultivated at two different sites. *J Agric Food Chem* 2001; 49: 2908-2916.
- [20] *Hiller K., Melzig M.*: Lexikon der Arzneipflanzen und Drogen, Spektrum Akademischer Verlag GmbH, Heidelberg, 2000.
- [21] *Ivanic R., Savin K., Robinson F., Milchard M.*: Gas chromatographic examination of volatile oil from *Salvia officinalis* L. *Acta Pharm (Jugoslavia)* 1978; 28: 65-69.
- [22] *Jennings W., Shibamoto T.*: Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography. Academic Press, New York, 1980.
- [23] *Jirovetz L., Buchbauer G., Denkova Z., Stoyanova A., Murgov I.*: Antimicrobial Testings and Chiral Phase Gas Chromatographic Analysis of Lavandula Oils and Related Key Compounds. *EURO COSMET* 2004; 12(1): 30-33.
- [24] *Jirovetz L., et al.*: Antimicrobial testings and gas chromatographic analysis of pure oxygenated monoterpenes 1,8-cineole, α -terpineol, terpinen-4-ol and camphor as well as target compounds in essential oils of pine (*Pinus pinaster*), rosemary (*Rosmarinus officinalis*) and tea tree (*Melaleuca alternifolia*). *Sci Pharm* 2005; 73: 27-39.
- [25] *Joulain D., König W. A.*: The Atlas of Spectral Data of Sesquiterpene Hydrocarbons. E.B.-Verlag, Hamburg, 1998.
- [26] *Kondjoyan N., Berdaqué J.-L.*: A Compilation of Relative Retention Indices for the Analysis of Aromatic Compounds. Edition du Laboratoire Flaveur. Saint Genes Champanelle, 1996.
- [27] *Länger R., Mechtler Ch., Tanzler H., Jurenitsch J.*: Differences in the composition of the essential oil within an individual of *Salvia officinalis*. *Planta Med* 1993; 59: 635-636.
- [28] *Lawrence B.*: Progress in essential oil. *Perfum Flavor* 1994; 14(6): 87-98.
- [29] *Li Y., Craker L., Potter T.*: The effect of light level on essential oil production of sage (*Salvia officinalis*) and thyme (*Thymus vulgaris*). *Acta Hort* 1996; 426: 419-426.
- [30] *Mathe I., et al.*: Changes in the essential oil production of *Salvia officinalis* under climatic conditions of the temperature belt. *Planta Med* 1992; 58: A680.
- [31] *Meyer-Buchtela E.*: Tee-Rezepturen, 3. Ergänzungslieferung 2004, Salbeiblätter, Deutscher Apotheker Verlag, Stuttgart, 2004.
- [32] *Mockute D., Nivinskiene O., Bernotiene G., Butkiene R.*: The cis-thujone chemotype of *Salvia officinalis* L. essential oil. *Chimija* 2003; 14: 216-220.
- [33] *Mosciano G., Fasano M., Cassidy J., Connelly K., Mazeiko P., Montenegro A.*: Organoleptic characteristics of flavour materials. *Perfum Flavor* 1993; 18: 38-41.
- [34] *PDR[®] for Herbal Medicines* (LaGow, B., Chief-Editor), 3rd Ed., Thomson PDR, Montvale, 2004.
- [35] *Perry N., et al.*: Essential oils from Dalmatian sage (*Salvia officinalis* L.): Variations among individuals, plant parts, seasons, and sites. *J Agric Food Chem* 1999; 47: 2048-2054.
- [36] *Piccaglia R., Marotti M.*: Characterization of several aromatic plants grown in northern Italy. *Flavour Fragr J* 1993; 8: 115-122.
- [37] *Pino J.A.*: Essential oil of sage (*Salvia officinalis* L.) grown in Cuba. *J Essent Oil Res* 1997; 9: 221-222.
- [38] *Putievsky E., Ravid U., Dudai N.*: The influence of season and harvest frequency on essential oil and herbal yield from a pure clone of sage (*Salvia officinalis*) grown under cultivated conditions. *J Nat Prod* 1986; 49: 326-329.
- [39] *Putievsky E., Ravid U., Sanderovich D.*: Morphological observations and essential oils of sage (*Salvia officinalis* L.) under cultivation. *J Essent Oil Res* 1992; 4: 291-293.
- [40] *Radulescu V., Chiliment S., Oprea E.*: Capillary gas chromatography-mass spectrometry of volatile and semi-volatile compounds of *Salvia officinalis*. *J Chromatogr A* 2004; 1027: 121-126.
- [41] *Republik Österreich*: Verordnung über Aromen und deren Ausgangsstoffe, Bundesgesetzblatt II, Nr. 1998/42, Österreichische Staatsdruckerei, Wien, 1998.
- [42] *Roth L., Daunderer M., Kormann K.*: Giftpflanzen – Pflanzengifte, ecomed Verlagsgesellschaft AG & Co., KG, Landsberg, 1984.
- [43] *Schmidt E., et al.*: Antimicrobial Testings and Gas Chromatographic Analyses of Aroma Chemicals. *J Essent Oil-Bearing Plants* 2005; 8(1): 99-106.
- [44] *Seidemann J.*: World Spice Plants, Springer-Verlag, Heidelberg, 2005.

- [45] *Sigma-Aldrich*: Flavors & Fragrances, The Essence of Excellence. Sigma-Aldrich Co., Milwaukee, 2003.
- [46] *Tisserand R.*: The Essential Oil Safety Data Manual, Tisserand Aromatherapy Institute, Brighton, 1985.
- [47] *Tucker A., Maciarello M.*: Essential oils of cultivars of Dalmation sage (*Salvia officinales* L.). J Essent Oil Res 1990; 2: 139-144
- [48] *Vera R., Ming J.*: Chemical Composition of the essential oil of sage (*Salvia officinalis* L.) from Reunion Island. J Essent Oil Res 1999; 11: 399-402.
- [49] *Vernin G., Metzger J.*: Analysis of sage oils by GC-MS data bank: *Salvia officinalis* L. and *Salvia lavandulaefolia* vabl.. Perfum Flavor 1986; 11(5): 79-84.
- [50] *Velickovic D.T., Ristic M.S., Randjelovic N.V., Smelcerovic A.A.*: Chemical composition and antimicrobial characteristics of the essential oils obtained from flower, leaf and stem of *Salvia officinalis* L. originated from southern Serbia. J Essent Oil Res 2002; 14: 453-458.
- [51] *Wichtl M.* (ed.): Teedrogen. Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, 1989.

Adresse der Autoren:

*Oberrat Mag.pharm. Dr. Leopold Jirovetz
Univ.-Prof. Mag.pharm. Dr. Gerhard Buchbauer
Department of Clinical Pharmacy and Diagnostics
University of Vienna, Pharmacy-Center
Althanstrasse 14
1090 Vienna
Austria
e-mail: leopold.jirovetz@univie.ac.at
e-mail: gerhard.buchbauer@univie.ac.at*

*Univ.-Prof. Dr. Zapriana Denkova
Dr. Alexander Slavchev
Univ.-Prof. Dr. Albena Stoyanova
Department of Essential Oils
University of Food Technology
26 Maritza Boulevard
Plovdiv 4002
Bulgaria
e-mail: alstst@yahoo.com*

*Erich Schmidt
Kurt Kitzing Co.
Hintern Alten Schloss 21
86757 Wallerstein
Germany
e-mail: erich.schmidt@kurtkitzing.de*

Eingelangt am: 21.11.2005
Akzeptiert am: 26.01.2006

ernährung

ÖSTERREICHISCHE ZEITSCHRIFT FÜR WISSENSCHAFT, TECHNIK, RECHT UND WIRTSCHAFT

Offizielles Organ der Österreichischen Gesellschaft für Ernährung (ÖGE) und ihrer Sektionen und Zweigvereine, des Fachverbandes der Nahrungs- und Genussmittelindustrie Österreichs, des Schutzverbandes der österreichischen Lebensmittelindustrie.

Herausgeber:
Fachverband der Lebensmittelindustrie
A-1030 Wien, Zaunergasse 1-3

Baurat h. c. Ziv.-Ing. Dipl.-Ing. Otto Riedl
A-1190 Wien, Felix-Mottl-Straße 50

Wissenschaftlicher Beirat:

Generaldirektor Univ.-Prof. Dr. iur. et rer. pol. W. Barfuß
Univ.-Prof. Dipl.-Ing. Dr. nat. techn. E. Berghofer
Univ.-Prof. Dipl.-Ing. Dr. nat. techn. Dr. h. c. E. Brandl
Vizepräsident des OGH Hon.-Prof. Dr. K. Brustbauer
Univ.-Prof. Dr. med. P. H. Clodi
Univ.-Prof. Dr. med. W. Druml
Univ.-Prof. Dr. agr. I. Elmadfa
Univ.-Prof. Dr. med. J. M. Hackl
Univ.-Prof. Dr. med. K. Irsigler
OR Dr. L. Jirovetz
MR Dr. P. Kranner
Univ.-Prof. Dr. med. vet. J. Leibetseder
Ass. Prof. Dr. Peter Paulsen
Hon.-Prof. Dr. iur. K. Smolka
ao Univ.-Prof. Dr. I. Steiner
Univ.-Prof. Dr. med. R. Wenger

Chefredakteur: Dr. Michael Blass

Redaktion „Wissenschaft“:
Dipl.-Ing. Dr. Udo Pechanek
Mag. Marlies Gruber

nutrition

AUSTRIAN JOURNAL FOR SCIENCE, TECHNOLOGY, LAW AND ECONOMY

Österreichische Spirituosenzeitung

FÜR INDUSTRIE, GEWERBE UND HANDEL FACHBLATT FÜR DIE SPIRITUOSENERZEUGUNG, WEIN- UND OBSTBRENNEREIEN, FRUCHTSÄFTE UND SEKTERZEUGUNG SOWIE GÄRUNGSESSIGE

Offizielles Organ des Verbandes der Spirituosenindustrie und des Schutzverbandes Österr. Spirituosen-, Sekt- und Fruchtsafthersteller

Redaktion: Dr. Bruno Mayer

Verleger:
Fachzeitschriftenverlagsges. m. b. H.
A-1030 Wien, Schwarzenbergplatz 6
Telefon (01) 715 31 93 Telefax (01) 715 48 19
E-Mail: ernaehrung@dielebensmittel.at

Geschäftsführer: Dr. Bruno Mayer

Layout: Verena Meixner

Die „ernährung“ (nutrition) – ISSN 0250-1554 – erscheint elfmal jährlich. Nachdruck sämtlicher Artikel, auch auszugsweise, nur mit Quellenangabe, gegen Belegexemplar; Zitierung von wissenschaftlichen Beiträgen:

„ernährung“ (nutrition)

Jahresabonnement Inland € 72,-

Einzelpreis Inland € 10,-

einschließlich 10 Prozent MWSt.

Jahresabonnement Ausland € 90,-

Einzelpreis Ausland € 11,-

Anzeigen: Es gilt Tarifblatt 2006.

Anzeigenannahme:

Maria Ozelt

A-1030 Wien, Schwarzenbergplatz 6

Telefon (01) 715 31 93 Telefax (01) 715 48 19

E-Mail: ernaehrung@dielebensmittel.at

Hersteller: Ueberreuter Print und Digimedia, 2100 Korneuburg