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Capillary gas chromatography–mass spectrometry of volatile and semi-volatile compounds of *Salvia officinalis*

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Abstract

The essential oil and infusion of *Salvia officinalis* leaves have been widely applied in traditional medicine since ancient times and nowadays subjected to extensive research of their antibacterial, antiviral and cytotoxic properties. This paper shows chemical composition data of *S. officinalis* leaves essential oil isolated by steam distillation using a Clevenger-type apparatus. Also, the paper presents the chemical content of volatile and semi-volatile compounds of *S. officinalis* leaves infusion. The volatile and semi-volatile compounds of *S. officinalis* leaves infusion were isolated by solid-phase extraction (SPE) and liquid–liquid extraction with hexane and dichloromethane. SPE was carried out on 500 mg octadecylsilane (C_{18}) cartridges and elution with dichloromethane. Liquid–liquid extraction was performed with hexane and dichloromethane. The essential oil in dichloromethane and infusion extracts in hexane and dichloromethane were analyzed by gas chromatography coupled with mass spectrometry. The quantitative results obtained by solid-phase extraction and liquid–liquid extraction showed that SPE on C_{18} performed the highest recovery of the volatile compounds from infusion sample.

Keywords: *Salvia officinalis*; Essential oils; Semi-volatile organic compounds; Volatile organic compounds

1. Introduction

Numerous species of the genus *Salvia* (Labiatae) have been extensively used in popular folk medicine and many pharmacognostic researches intended to identify biologically active compounds responsible for their therapeutic effects [1,2].

Compounds from *Salvia* essential oil have been shown to exhibit high antibacterial activity against *Staphyloccocus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtils*, cytotoxic activity against Vero cells and virucidal activity against herpes simplex virus 1 and vesicular stomatitis virus [3,4]. According to these studies, the biological properties of *Salvia* essential oil were attributed mainly to camphor, 1,8-cineole and α-thujone and β-thujone.

The antioxidant activity of sage polyphenols, consisting of flavone glycosides and some rosmarinic acid derivatives were also evaluated [5,6].

Salvia officinalis essential oil is applied in the treatment of a large range of diseases such as, nervous system, heart and blood circulation, respiratory, digestive, metabolic and endocrine diseases, etc. Although the *S. officinalis* infusion is easily obtained and commonly used for the haemostatic, estrogenic, anti-perspiration, anti-neuralgic, antiseptic, hypoglycemic and many other therapeutic effects [7]. Its chemical composition was very little investigated and the scientific literature is poor in information concerning its chemical data [9,10].

The aim of our study was to achieve an efficient procedure for isolating the volatile compounds from plant leaves infusion. At the same time our paper tries to elucidate the chemical composition of *S. officinalis* leaves infusion.

2. Materials and methods

2.1. Reagents and chemicals

All solvents and reagents were purchased from Merck, Darmstadt, Germany: dichloromethane, *n*-hexane, methanol were SupraSolv for gas chromatography; cartridges for solid-phase extraction were LiChrolut RP-18 columns, 500 mg; anhydrous Na_2SO_4 granulated for organic trace analysis, was used; authentic standards were 1-octen-3-ol,

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cineole (eucalyptol), (1*S*)-(+)-borneol, linalyl acetate, bornyl acetate, (+)-camphor, all of them reag. Ph. Eur.; thujone was reag. DAB.

The *n*-alkanes C_6–C_{24} used for the determination of the retention Kovats retention index were from Fluka, Buchs, Switzerland.

2.2. Plant material

The plant material of *S. officinalis* was purchased from a local supplier. The leaves were separated from branches and manually grounded.

2.3. Essential oil extraction

Twenty grams of *S. officinalis* leaves were hydro-distilled in a Clevenger-type apparatus for 4 h. The essential oil was dried over anhydrous Na_2SO_4, stored in a dark glass bottle, and kept at 4 °C until analysis. The oil sample was diluted in dichloromethane (1/200) for GC analysis and 2 μl were injected.

2.4. Infusion sample preparation

Hundred millilitres of boiled water were poured over samples of 5 g of *S. officinalis* leaves. After approximately 30 min the cold extracts were filtered on glass fibre filter. The residue from the filter was rinsed three to four times with distilled water, and the final filtrated infusion was adjusted to 100 ml volume.

2.5. Solid-phase extraction of leaves infusion

A 500 mg C_{18} solid-phase extraction (SPE) cartridge was conditioned by eluting it with 2 × 4 ml dichloromethane, followed by 2 × 4 ml of methanol. The cartridge was allowed to dry after each flush. Then 2 × 4 ml of distilled water were passed through the cartridge (the cartridge should not be allowed to dry before sample application). By applying a slight vacuum, the obtained infusions were allowed to pass through the cartridge at a flow of 1–2 ml/min. The cartridge was then dried for 5–10 min with nitrogen. The compounds retained on C_{18} cartridge were eluted with

Table 1
Chemical composition of *Salvia officinalis* leaves essential oil

No.	Compound name	t_R (min)	Area (%)
1	*trans*-Salvene	4.398	0.06
2	α-*trans*-Ocimene	6.663	1.69
3	Camphene	7.161	1.66
4	β-Pinene	8.072	0.58
5	1-Octen-3-ol	8.657	8.50
6	ι-Phellandrene	9.079	0.22
8	γ-Terpinene	9.448	0.08
9	*p*-Cymene	9.762	0.07
10	1,8-Cineole	9.946	6.72
11	δ-Terpinene	10.846	0.14
12	*trans*-Sabinene hydrate	11.398	0.04
13	α-Terpinolene	11.723	0.08
14	α-Thujone	12.514	21.85
15	β-Thujone	12.839	5.51
16	Camphor	13.728	11.25
17	Isopinocamphone	14.140	0.10
18	1-Borneol	14.584	2.58
19	Terpinen-4-ol	14.866	0.32
20	Bornyl acetate	17.846	3.22
21	β-Caryophyllene	21.519	3.54
22	Aromandendrene	22.017	0.05
23	6-Oxobornyl acetate	22.115	0.10
24	α-Humulene	22.483	4.51
25	γ-1-Cadinene	23.025	0.06
26	Valencene	23.415	0.26
27	β-Cadinene	24.152	0.12
28	α-Farnesene	25.756	1.15
29	Veridiflorol	26.114	11.71
30	Citronellyl propionate	26.450	1.22
31	β-Elemene	26.981	0.09
32	Hydroxycaryophillene	27.121	0.27
33	Caryophillene oxide	27.208	0.33
34	α-Santalol	27.739	0.32
35	Manool	35.292	9.15

Identified 97.75% from total area.

2 × 1.5 ml of dichloromethane. For the quantitative analysis, the RP-18 column was eluted with 2 × 1.5 ml of solution of the internal standard (linalyl acetate), 200 ng/μl concentration.

2.6. Liquid–liquid extraction of leaves infusion

The 100 ml water extract (infusion) was re-extracted in a 250 ml separating funnel with 3 × 15 ml of dichloromethane. The combined extracts were dried over anhydrous sodium sulphate and then concentrated to 3 ml using a Rotavapor. Another 100 ml infusion were extracted in the same manner with hexane. For the quantitative analysis, the reunited liquid–liquid extractions were spiked with internal standard so that in the final solution, brought at 3 ml, the concentration of internal standard would be 200 ng/μl.

2.7. Gas chromatography–mass spectrometry

GC–MS analyses were carried out with a Fisons Instruments GC 8000 equipped with an electron impact quadrupole, MD 800 mass spectrometer detector. The electron ionization energy was 70 eV, ion-source temperature 200 °C and the interface temperature 280 °C. A split–splitless injection (split ratio, 1:30) at 280 °C injector temperature was employed.

A fused silica column 5% phenylpoly(dimethylsiloxane) (DB-5MS 30 m × 0.32 mm i.d. and 0.25 μm film thickness, J&W Scientific) was used. The oven temperature was programmed as follows: from 40 °C (3 min hold) raised at 5 °C/min to 160 °C (2 min hold), then at 10 °C/min to 280 °C and finally hold at 280 °C for 10 min. The carrier gas (helium) flow rate was 2 ml/min. Two microlitres of sample were injected. These conditions were applied both for essential oil and infusions samples. Data acquisition was performed with MassLab software for the mass range 30–600 u with a scan speed of 1 scan/s.

The identification of compounds was performed by comparing their mass spectra with the data from Adams [8], US National Institute of Standards and Technology (NIST, USA), WILEY 1996 Ed. mass spectral library and a personal library of 550 spectra.

Some of the compounds from essential oil or infusion extracts were confirmed by comparing their retention times with those of authentic standard substances. Linalyl acetate, a substance absent in the infusion extracts, was used as internal standard for efficiency evaluation of the proposed extraction methods.

2.8. Quantitative analysis

Standard solutions were prepared containing 100, 200, 300, 400 and 500 ng/μl of the following compounds: 1,8-cineole, α-thujone, β-thujone, camphor, 1-borneol, bornyl acetate, and the same quantity (200 ng/μl) of internal standard (linalyl acetate).

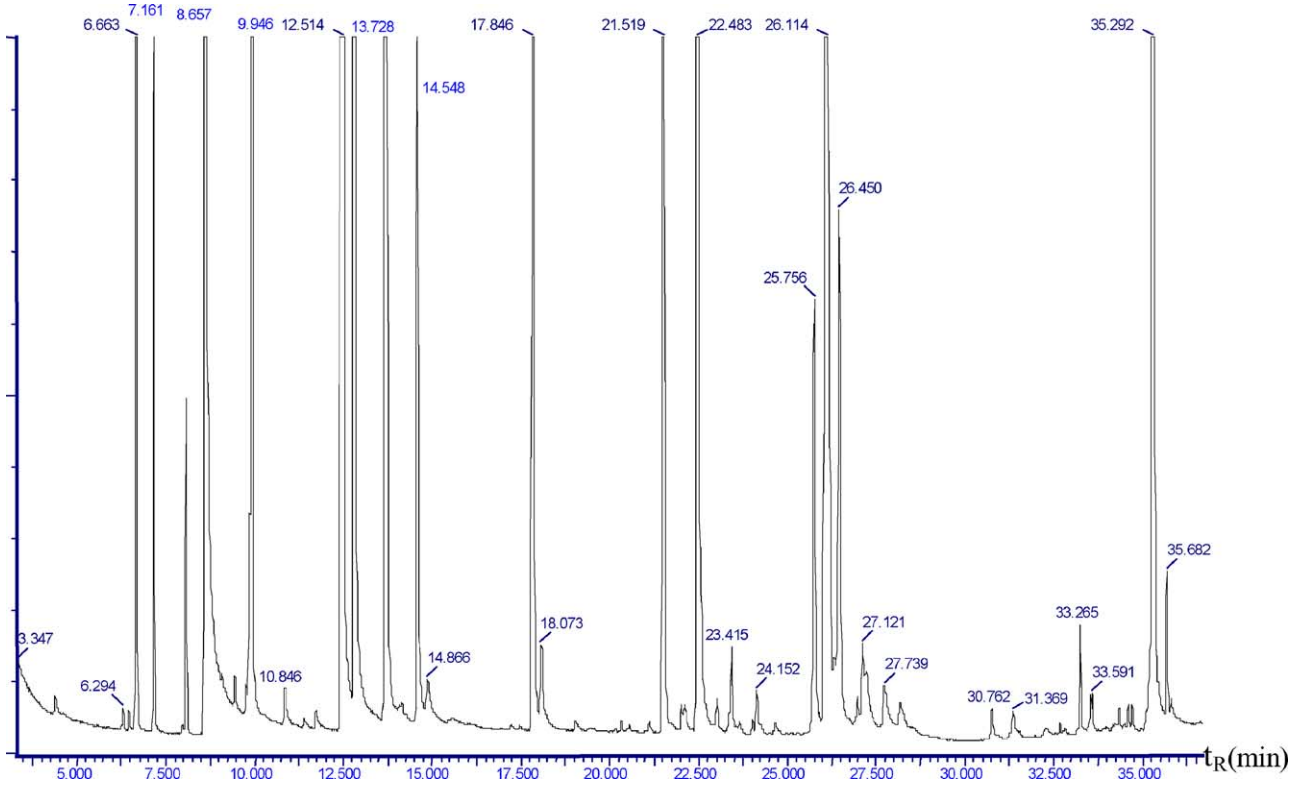

Fig. 1. Chromatogram of *Salvia officinalis* essential oil.

3. Results and discussion

In Fig. 1 is shown the essential oil chromatogram and in Table 1 are listed the identified compounds in the essential oil and their area relative to the total area. Thirty-five compounds were identified, having the total area of 97.75%.

The main components found in *S. officinalis* essential oil were: thujone (α and β, 27.36%), camphor (11.25%), 1-octen-3-ol (8.5%), 1,8-cineole (6.72%), compounds also reported in *Salvia fruticosa* essential oil [3].

The essential oil is rich in veridiflorol (11.71%), a sesquiterpenic alcohol and manool (9.15%), a diterpenic alcohol.

The following substances having quantities higher than 1% in essential oil were evidenced: monoterpenic hydrocarbons: ocimene (1.69%), camphene (1.66%); sesquiterpenic hydrocarbons: β-cariophyllene (3.54%), α-humulene (4.51%), α-farnesene (1.15%); and terpenic alcohols or their esters: 1-borneol (2.58%), bornyl acetate (3.22%).

In Fig. 2 is shown the infusion extract chromatogram obtained by SPE on C_{18} cartridge followed by elution with dichloromethane. In Table 2 are listed the identified compounds in infusion extracts resulted by separation on octadecylsilane and liquid–liquid extraction. It is obvious that through infusion the highly volatile compounds were lost. Therefore, the monoterpenic hydrocarbons (ocimene, camphene) occurring in essential oil were not found in infusion. Present in quantity of 8.50% in essential oil, 1-octen-3-ol is absent in infusions. On the other hand, the 1,8-cineole area is raised occupying the second place after monoterpenic cetones, such as, thujone and camphor.

The loss of highly volatile compounds, through infusion especially of the monoterpenic hydrocarbons, versus their presence in the volatile oil, can be easily explained by the difference between experimental conditions of the two isolation systems for the volatile compounds from the plant matrix. Therefore, in the Clevenger-type apparatus a co-distillation with water vapours of the volatile compounds is made, at a lower temperature than their boiling points. The infusion is made by extraction with water at its boiling temperature in an open extraction system, during which the most volatile compounds are lost due to direct volatilization and, simultaneously, to the co-vaporization with water vapours.

In compliance with researches of Sivropoulou et al. [3], thujone is the most cytotoxic compound, following by camphor and after that 1,8-cineole. In these circumstances it would be expected for the obtained infusion from sage to have significant cytostatic effects because the totalized ratio of these exceeded 64% of the volatile substances extracted from the infusion.

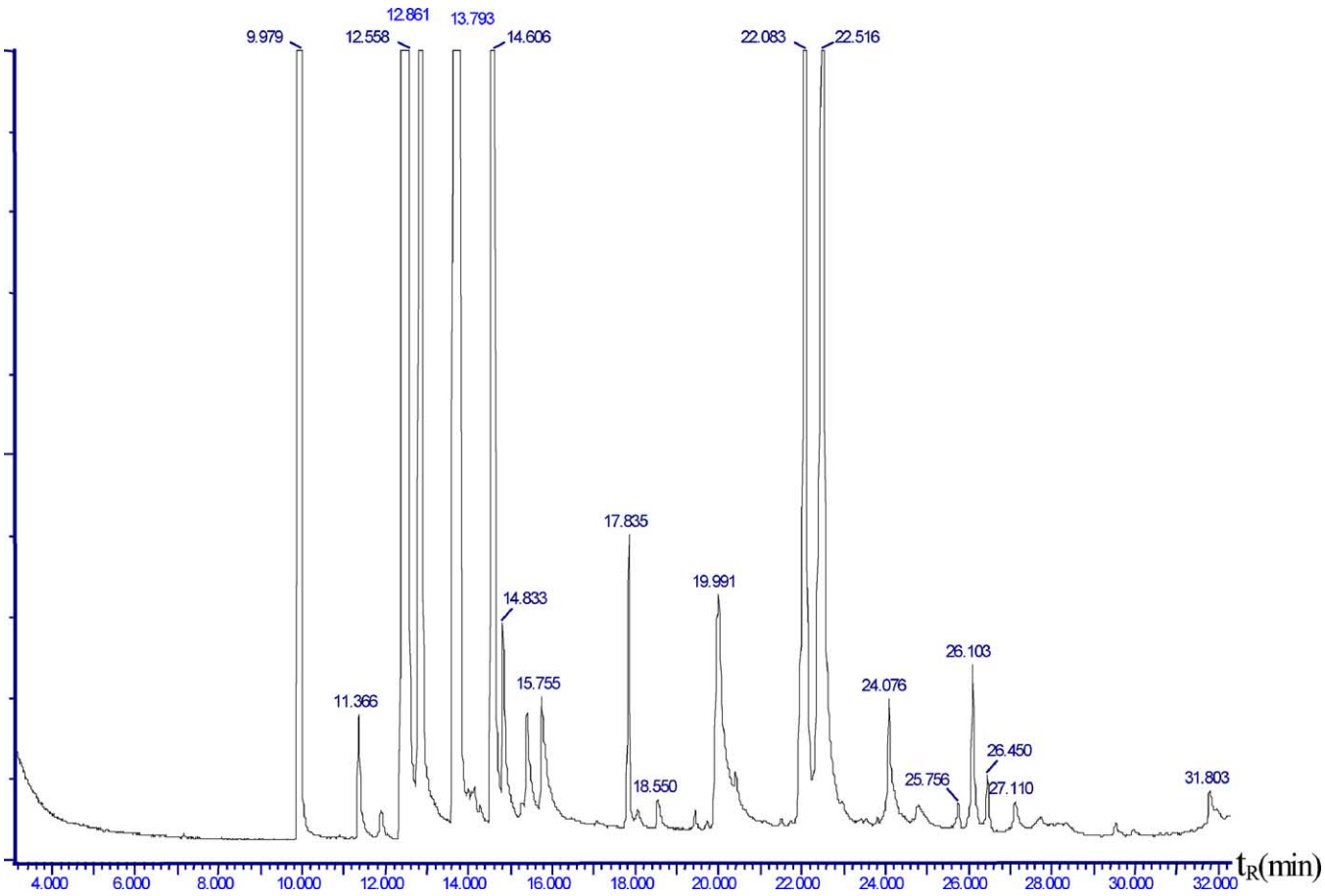

Fig. 2. Chromatogram of *Salvia officinalis* infusion extracted by SPE.

Table 2
Chemical composition of *Salvia officinalis* infusion extracts

No.	Compound name	t_R (min)	Kovats index (*I*)	Percentage from total area		
				SPE, C_{18}	Extraction with CH_2Cl_2	Extraction with hexane
1	1,8-Cineole	9.979	1037	16.16	10.66	11.37
2	α-Thujone	12.558	1117	25.78	22.61	23.95
3	β-Thujone	12.861	1127	7.07	4.58	5.13
4	Camphor	13.793	1153	24.94	25.88	24.93
5	Isopinocamphone	14.126	1162	–	0.19	–
6	1-Borneol	14.497	1173	5.38	5.66	5.62
7	Terpinen-4-ol	14.768	1181	0.43	0.47	0.30
8	*E*-1-Methoxy-7-methyl-1,6-octadiene	15.191	1194	–	0.42	–
9	α-Terpineol	15.397	1199	0.41	0.45	0.33
10	Dihydrocamphene carbinol	15.755	1210	0.53	1.74	0.81
11	Linalyl formate	16.044	1219	–	0.08	–
12	Bornyl acetate	17.835	1277	0.62	1.02	0.59
13	Naphthalene, decahydro-1,5-dimethyl	18.550	1299	0.10	0.97	0.48
14	*exo*-2-Hydroxycineole acetate	19.991	1344	1.73	–	2.74
15	6-Oxobornyl acetate	22.516	1423	7.99	9.18	10.59
16	12-Nor-caryophyll-5-en-2-one	24.076	1472	0.71	0.98	0.82
17	Dyhidroactinidiolide	24.792	1495	–	0.43	0.21
18	α-Farnesene	25.680	1524	–	–	0.13
19	Veridiflorol	26.103	1537	0.46	0.66	0.64
20	Citronellyl acetate	26.449	1547	–	0.21	0.20
21	Hydroxycaryophyllene	27.100	1571	0.17	0.30	0.13
22	Shyobunol	31.803	1721	0.20	3.29	0.73
Total				92.68	89.78	89.7

From the qualitative point of view, the three extraction procedures: SPE on C_{18}, liquid–liquid extraction with dichloromethane and hexane do not present significant differences, 6-oxobornyl acetate has a larger area in all three extracts than in the essential oil. Our experimental data could not allow us to explain this aspect.

For efficiency evaluation of the extraction methods of volatile compounds from infusion quantitative determinations were accomplished for the following compounds: 1,8-cineole, α-thujone, β-thujone, camphor, 1-borneol, bornyl acetate, using the method of internal standard. Linalyl acetate, a substance absent in the essential oil and infusion extracts of *S. officinalis* and whose chromatographic peak did not interfere with any other compounds of the sage extracts, was used as internal standard.

In order to do this, the standard solutions containing: 1,8-cineole, α-thujone, β-thujone, camphor, 1-borneol, bornyl acetate, according to Section 2.8, were used to obtained the calibration curves $A_{compound}/A_{IS}$ versus the ratio of the weight of the compound and the weight of the internal standard.

From the chromatograms of spiked infusion samples with internal standard the compound area and the internal standard area were determined and the quantity of every compound in mg/g of dry plant was calculated with the following formula:

$$\text{quantity (mg/g)} = f\frac{A_c V_f c_{IS}}{A_{IS} V_{inj} m}10^{-3}$$

where f is the relative response factor obtained from the slope of the calibration curve, A_c the area of the compound,

Table 3
Efficiency of liquid–liquid extraction relative to SPE[a]/C_{18}

Compound	Kovats index (*I*)	Recovery (%)		
		Plant material by SPE (mg/g) (100%)	Extraction with CH_2Cl_2	Extraction with hexane
1,8-Cineole	1037	0.660	55.34	46.35
α-Thujone	1117	1.020	73.54	58.71
β-Thujone	1127	0.244	68.37	61.86
Camphor	1153	0.964	73.06	53.34
1-Borneol	1173	0.216	71.30	58.26
Linalyl acetate	1251	–	–	–
Bornyl acetate	1277	0.024	75.10	60.50

[a] SPE recovery was considered 100%.

A_{IS} the area of the internal standard, c_{IS} the concentration of the internal standard (200 ng/μl), V_f the final volume of the sample (ml), V_{inj} the injection volume (μl), m the amount of the plant sample (g).

On a first estimation (measurement) by spiked samples with the same quantity of linalyl acetate, was emphasized the fact that the extraction on C_{18} resulted in the highest recovery of the *S. officinalis* infusion compounds. Consequently, an efficiency of 100% was assigned to C_{18} extraction and relative values of the main compounds recovery in extraction with hexane and dichloromethane were calculated in comparison to C_{18} extraction.

In Table 3 are given the obtained quantitative data, the values from the table representing the average of two determinations.

4. Conclusions

The experimental results concerning chemical composition of essential oil of *S. officinalis* are in agreement with the similar data from scientific literature. Though by infusion the highly volatile compounds were lost the main compounds in infusion extracts are the same as in volatile oil. The quantitative estimation shows that the highest efficiency was achieved by use of SPE on C_{18}. This procedure with its advantages: easy to use, quick and economic, may be a good method for isolating volatile compounds from plant infusions.

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