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Composition of Essential Oil and Its Content in Leaves and Gallenic Preparations Made of Sage (*Salvia officinalis* L.)

Zawartość i skład olejku eterycznego występującego w liściach i preparatach galenowych otrzymywanych z szałwi lekarskiej (*Salvia officinalis* L.).

Abstract. The aim of the investigations was to determine the contents and composition of OE found in leaves of the sage and in gallenic preparations: *Tinctura Salviae* and *Extractum Salviae 50*. The studies were conducted by GC/MS method. The findings showed that the main components of OE existing in *Oleum Salviae*, *Tinctura Salviae*, as well as in *Extractum Salviae 50* are: α- and β-thujone (36-41%), camphor (17-32%), 1.8-cinneole (10-14%), viridiflorol (5-8%) and borneol (3-7%). The content of these components in *Oleum Salviae* and *Tinctura Salviae* is essentially the same, but in *Extractum Salviae 50* is markedly different. This difference concerns above all a twice-larger content of camphor and borneol in the oil fraction which exists in *Extractum Salviae 50*. This content can result from sufficient solubility of monoterpenes in water-alcohol systems, which is connected with the presence of solubilizers which are triterpenes existing in sage leaves, as well as in investigated gallenic preparations.

INTRODUCTION

Essential oil obtained from sage (*Salvia officinalis* L.) has antiphlogistic, antibacterial and astringent properties; therefore, sage is one of the components of many herb mixtures. Medicines made of it may be applied both externally and internally (Newall et al., 1996; Wichtl, 1994). Besides pharmaceutical applications, sage is used as a spice (Klimek, 1957; Nowiński, 1983; Kostrzewa, 1983; Góra and Lis, 1998).

Leaves and leafed shoot tops with determined content of essential oil, which can range from 1 to 2.5% (FP IV demands not less than 1.0%) are the pharmacopoeal material. Twenty-four hours' fluctuations of its content are not big and the largest quantities accumulate in early morning hours. Both shortage and excess of moisture has unfavorable influence on the content of essential oil. Appropriate fertilization is also an important factor limiting the content of essential oil in the material (Szczyglewska, 1999). Essential oil from sage (*Oleum Salviae*) can consist of up to 50% of thujone, 15% of cinneole, and 8% of camphor and smaller amounts of borneol, pinene and other components (Kohlmünzer, 1998).

Essential oil produced from sage is applied in pharmaceutical, food and cosmetic industries (Klimek, 1957; Kostrzewa, 1983; Keville and Green, 1995; Newall et al., 1996). The chemical compounds contained in this essential oil show multidirectional pharmacological activity. Sage oil inhibits the growth of Gram-positive bacteria, e.g. oxygen rod-shaped bacteria from the genus *Bacillus* (Mazurella and Sicurella, 1960; Ross et al., 1980), micrococcus *Staphylococcus* and *Streptococcus* (Cade, 1957, Ross et al., 1980) and bacillus from the genus *Mycobacterium* (Mazurella and Sicurella, 1960). Sage oil also exerts an action on pathogenic yeastlike fungi from the genera *Cryptococcus* and *Candida* (Jelsenjak et al., 1987; Mazurella and Ligouri, 1958; Newall et al., 1996) as well as on mildew fungi from the genera *Aspergillus*, *Penicillum*, *Mucor*, *Rhizopus* (Mazurella and Ligouri, 1958; Ross et al., 1980) Poor or no activity of sage oil was observed towards Gram-negative rods from the genera *Escherichia*, *Enterobacter*, *Klebsiella*, *Salmonella* and *Pseudomonas* (Jelsenjak et al., 1987; Newall et al., 1996; Ross et al., 1980). Stronger activity of sage oil towards Gram-positive than towards Gram-negative bacteria was proved by microbiological investigations of Kędzia et al. (1990). Spray named Tymsal, which is the extract from sage and thyme, is nowadays available at pharmaceutical market. It can be applied in catarrh and inflammatory conditions of throat, larynx and oral cavity.

The aim of this work was to determine the content and composition of essential oil found in the leaves of sage (*Salviae folium*) and gallenic preparations: *Tinctura Salviae* and *Extractum Salviae* 50 produced from these leaves.

MATERIAL AND METHODS

1. Experimental material

The experimental material were sage leaves (*Salviae folium*) and gallenic preparations: *Tinctura Salviae* and *Extractum Salviae* 50 produced by "Herbapol-Klęka" Co. Moisture and the content of ash and essential oil were determined according to FP IV and FP V (1970, 1990).

2. Capillary Gas Chromatography — Mass Spectrometry

Quantitative and qualitative analysis of essential oil (*Oleum Salviae*) and essential oil components in gallenic preparations *Tinctura Salviae* and *Extractum Salviae 50* was performed by GC/MS method using an ITS-40 (Finnigan MAT, USA). The samples were injected (1µl) into DB-5 fused silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) by splitless injection. The injector temperature was 280°C. The column temperature was 35° for 2 min. and programmed to 280.

Identification of the compounds was performed on the basis of the MS Terpene Library of Finnigan MAT and confirmed by retention indices. The quantitative composition of essential oils was calculated assuming that those response factors were the same for each compound.

RESULTS

Moisture, ash and content of essential oil (OE) in the investigated material are shown in Tab. 1.

Tab. 1. Percentage content of moisture, ash and essential oil in sage leaves (*Salvia officinalis* L.)

Sample	Moisture	Ash	Essential oil
1	10.16	8.13	1.22
2	10.08	9.53	1.39
3	10.18	9.01	1.39
Mean percentage [%]	10.14	8.89	1.33

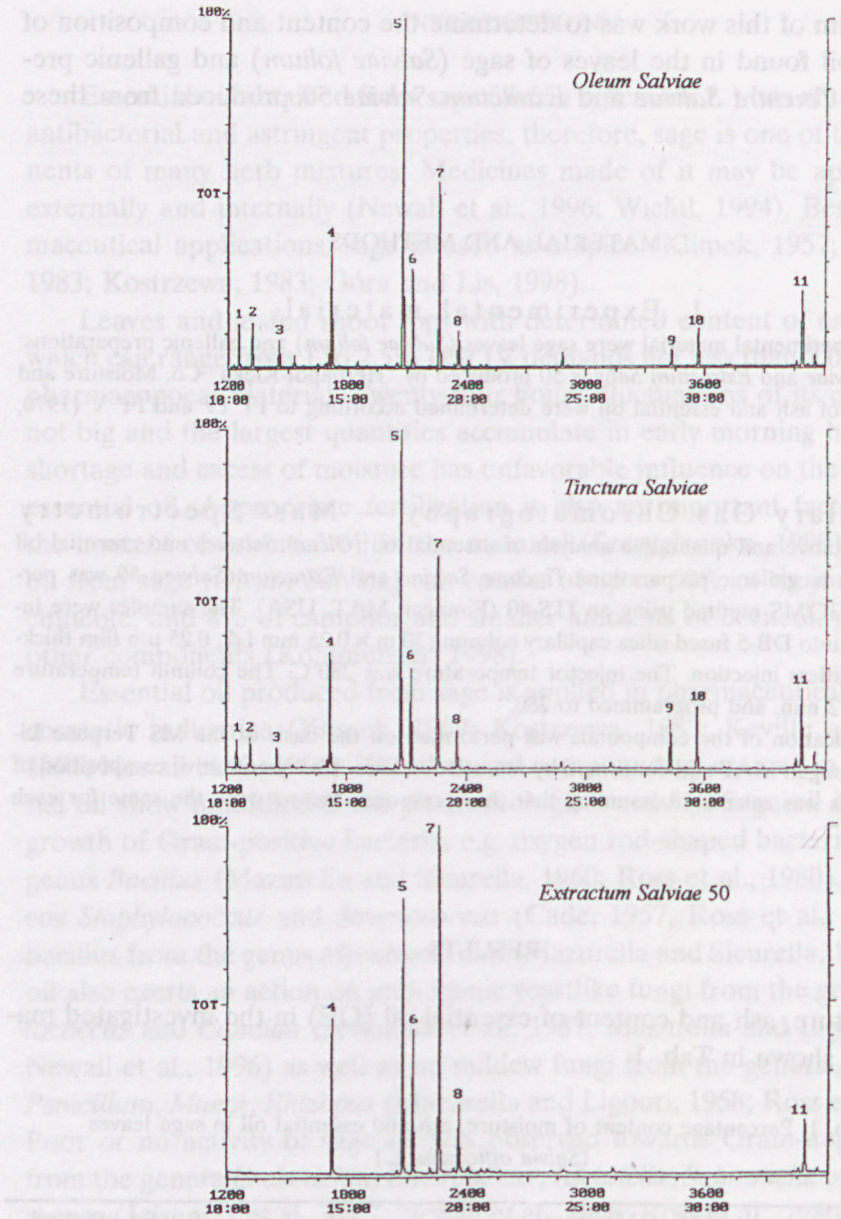

Fig. 1. Chromatograms GC/MS of essential oil components contained in *Oleum Salviae*, *Tinctura Salviae* and *Extractum Salviae 50* in the range of retention time 1200-4200 s. 1 — α-pinene; 2 — camphene; 3 — β-pinene; 4 — 1.8-cinneole; 5 — α-thujone; 6 — β-thujone; 7 — camphor; 8 — borneol; 9 — β-caryophyllene; 10 — α-humulene; 11 — viridiflorol

Tab. 2. A comparison of quantitative and qualitative composition of essential oil obtained from the sage and gallenic preparations: *Tinctura Salviae* and *Extractum Salviae 50* (GC/MS method in the range of retention time 1200-4200)

	Ol. Salviae		*Tinctura Salviae*		*Extractum Salviae 50*	
Compound	Retention time	Percentage	Retention time	Percentage	Retention time	Percentage
α-thujene	1282	0.165	-	-	-	-
α-pinene	1256	3.103	1253	2.004	-	-
Camphene	1323	3.174	1320	2.124	1320	0.114
β-pinene	1456	1.970	1453	1.413	-	-
Myrcene	1532	0.849	1531	0.235	-	-
p-cymene	1689	0.213	1686	0.285	-	-
Limonene	1706	1.099	1703	0.601	-	-
1.8-cinneole	1719	10.085	1717	9.719	1716	13.854
Linnalol + cis-sabinene hydrate	2050	0.426	2046	0.358	2046	0.348
α-thujone	2080	32.905	2074	30.11	2074	24.172
β-thujone	2128	8.075	2125	8.524	2124	12.747
Camphor	2260	16.639	2256	19.401	2256	32.210
Borneol	2356	3.481	2353	3.477	2354	6.976
Bornyl acetate	2884	1.235	2881	0.502	2879	0.218
β-caryophyllene	3433	1.535	3427	4.329	-	-
α-humulene	3567	3.603	3561	5.659	-	-
Viridiflorol	4091	7.422	4085	7.942	4088	5.441

As can be seen (Tab. 1), average humidity was about 10%, the content of mineral components was about 10%. The investigated material contained more than 1% of OE. It was compatible to the pharmacopoeia requirements.

Fig. 1 shows chromatograms of essential oils from the material and gallenic preparations. The composition, both quantitative and qualitative of these oils is shown in Tab. 2. By means of GC/MS 17, 16 and 9 compounds were determined, in the *Oleum Salviae*, *Tinctura Salviae* and the *Extractum Salviae 50*. In the *Oleum Salviae* 13 compounds with percentage content of more than 1% were found (α-thujone, camphor, 1.8-cinneole, β-thujone, viridiflorol, α-humulene, borneol, camphene, α-pinene, β-pinene, β-caryophyllene, bornyl acetate and limonene) in the *Tinctura Salviae* — 11 (α-thujone, camphor, 1.8-cinneole, β-thujone, viridiflorol, α-humulene, β-caryophyllene, borneol, camphene, α-pinene and β-pinene) and in the *Extractum Salviae 50* — only 6 (camphor, α-thujone, 1.8-cinneole, β-thujone, borneol and viridiflorol).

The main components in the essential oils from sage leaves and gallenic preparations are α- and β-thujone, and their content varies from 36% to 41%. The content of camphor is changing from 16% in the *Oleum Salviae*, to 19% in the *Tinctura Salviae* and 32% in the *Extractum Salviae 50*, respectively. In the case of *Extractum Salviae 50*, that means a double increase in comparison to the material. The same is with borneol, whose content in these oils is 3.5% and 7%, respectively. In the case of *Extractum Salviae 50*, there is a lack of β-caryophyllene and α-humulene and there is a different ratio of α- and β-thujone (2:1) in comparison to that in *Oleum Salviae* and *Tinctura Salviae* (4:1). The content of 1.8-cinneole, which is responsible for antiseptic properties of sage oil was within the range 10-14% in all oils. Viridiflorol was also found in essential oil and in gallenic preparations.

DISCUSSION

Many years' investigations on the composition of the essential oil found in leaves of different species of sage showed that the main and characteristic components are: α-thujone (2-46%), β-thujone (2,5-30%), camphor (2-34%) and 1.8-cinneole (2-30%) (Brieskorn and Dalferth, 1964; Ivanič and Savin, 1976; Kuštrak et al., 1984; Raic et al., 1985; Jelšenjak et al., 1987; Wagner and Bladt, 1996). Brieskorn and Dalferth (1964) found no thujone in *Salvia lavanduleafolia*. Other components of sage oil existing in the range from 2% to 8% are camphene, α-pinene, borneol, bornyl acetate, α-humulene and β-caryophyllene. Smaller amounts (in the range from 0.5 to 2%) of β-pinene, β-myrcene, linnalol and linnalyl acetate were also found (Brieskorn and Dalferth, 1964; Ivanič and Savin, 1976; Jelšenjak et al., 1987; Wagner and Bladt, 1996).

The present investigations proved these data. The main component (c.a. 40%) of *Oleum Salviae* is thujone and, as a matter of fact, its isomeric forms (α- and β-). Camphor (16.6%) and 1.8-cineole (10.1%) were also found in big amounts. α-humulene, borneol, camphen α-pinene, β-pinene, β-caryophyllene, bornyl acetate and limonene were found in the range from 1 to 4%. The essential oil also contains small amounts (less than 1%) of myrcene, p-cymene and α-thujene. It is worth to note the big amount of viridiflorol (7.4%) found in *Oleum Salviae*. This compound was first reported as a component of sage oil by Brieskorn and Dalferth. They investigated the essential oils obtained from three species of sage (*Salvia officinalis*, *Salvia lavan-*

dulaefolia and *Salvia triloba*) and viridiflorol was found in small amounts (0.35%) in *Salvia lavandulaefolia* only. Karl et al. (1982) found viridiflorol (from 3 to 9%) in *Salvia oficinalis* as well, which was also confirmed by the present results (from 5 to 8%).

In the gallenic preparations (*Tinctura Salviae* and *Extractum Salviae 50*) obtained from the sage leaves the components of sage oil were found. These compounds get into the preparations during the production process. The composition of essential oil derived from *Tinctura Salviae* is essentially the same as the composition of *Oleum Salviae*, but the composition of the oil obtained from *Extractum Salviae 50* is markedly different. The increase of the content of such monoterpenes as 1.8-cinneole, camphor and borneol in the essential oil fraction obtained from *Extractum Salviae 50* may be caused by their good solubility in water-alcohol systems, which can be connected with the properties of triterpene fraction. These compounds, and mainly ursulic acid, which is a natural solubilizer, enables solubility of monoterpenes mentioned above (Harborne, 1997). We found large amounts of ursulic acid both in the sage leaves and gallenic preparations (Ludwiczuk et al., 1999).

CONCLUSIONS

From the present investigations, it can be concluded that:

1. The qualitative composition of essential oil in *Oleum Salviae* and *Tinctura Salviae* is essentially the same. Only small differences were observed in quantitative composition and they concerned β-caryophyllene, whose amount was three times larger in *Tinctura Salviae,* and α-humlene, whose content was 1.5 times bigger.

2. A comparison of two different gallenic preparations showed that in *Tinctura Salviae*, which was obtained by extraction with 70° ethanol, 16 terpenes were found, and in *Extractum Salviae 50*, produced with 50° ethanol, 9 terpenes were detected.

3. The differences in solubility of monoterpenes, especially those which contain in their structure oxygen in keton, ether or alcohol forms (camphor, thujone, 1.8-cinneole and borneol), in water-alcohol systems, can be caused by a possibility of forming hydrogen bonds and the presence of solubilisers. In *Extractum Salviae 50*, there is twice as much camphor and borneol, an increased amount of 1.8-cinneole, and there is lack of β-caryophyllene and α-humulene.

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STRESZCZENIE

Celem prowadzonych badań było oznaczenie zawartości i składu OE występującego w liściach szałwi lekarskiej oraz w otrzymanych z nich preparatach galenowych: *Tinctura Salviae* i *Extractum Salviae 50*. Badania przeprowadzono metodą GC/MS. Wykazały one, że głównymi składnikami OE występującymi zarówno w *Oleum Salviae*, *Tinctura Salviae*, jak i w *Extractum Salviae 50* są: α- i β-tujon (36-41%), kamfora (17-32%), 1,8-cyneol (10-14%), wiridiflorol (5-8%) i borneol (3-7%). Zawartość tych składników w *Oleum Salviae* i *Tinctura Salviae* jest zbliżona, zaś w *Extractum Salviae 50* występują dość znaczne różnice w porównaniu do dwu poprzednich. Różnice te dotyczą przede wszystkim dwukrotnie większej zawartości kamfory i borneolu we frakcji olejkowej występującej w *Extractum Salviae 50*. Może to wynikać z dostatecznej rozpuszczalności monoterpenów w układach wodno-alkoholowych związanej z obecnością solubilizatorów, jakimi są triterpeny występujące zarówno w liściach szałwi lekarskiej, jak i w badanych preparatach.