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GC-MS ANALYSIS OF ESSENTIAL OILS FROM *SALVIA OFFICINALIS* L.: COMPARISON OF EXTRACTION METHODS OF THE VOLATILE COMPONENTS

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Abstract: In this paper, comparison of the volatile components composition in the samples obtained by hydrodistillation and solid-phase microextraction of *Salvia officinalis* was described. Different sample preparation techniques showed considerable differences in volatiles composition, especially with respect to sesqui- and diterpenoids. The comparison of the sage essential oil obtained by hydrodistillation in the Deryng and Clevenger type apparatus, according to the pharmacopoeial methods (FP VI and VII), showed the presence of the same terpenoids in both essential oils, however, the relative percentage composition of the components were different. These differences are caused by the different extraction times used in both methods. Since each essential oil to be admitted to medicinal use should meet requirements regarding the composition of major chemical components, the minimum time for the hydrodistillation of the essential oils from sage should be 1 h.

Keywords: *Salvia officinalis*, essential oil, hydrodistillation, Deryng apparatus, Clevengr-type apparatus, SPME

Common sage (*Salvia officinalis* L.) belongs to essential oil plants and is frequently used as medicinal plant and spice. Sage is reported to have wide range of biological activities, such as antibacterial, fungistatic, astringent, antiseptic, antifungal and digestive effects. Leaves are used in antiseptic and astringent herbal mixtures, Septosan and Dentosept, whereas essential oil distillated from sage can be helpful in aromatherapy (massage, bath, inhalation) and in bacterial infections, cough and bronchitis (1, 2). Some compounds present in the essential oil are microbiologically active (3). Daferera et al. (4) described the inhibitory activity of sage against *Botrytis cinerea*, *Fusarium* sp. and *Clavibacter michiganensis* microorganism. Essentials oils constitute multicomponent mixtures of mono-, sesqui- and diterpenoids or phenylpropane derivatives (phenols). Chemically, these can be hydrocarbons, alcohols, aldehydes, ketones, esters and ethers. Some essential oils contains sulfuric or nitric compounds, coumarins or other rare structures. The individual essential oil may be composed of several dozen of substances, however, usually a single compound responsible for a flavor and pharmacological activity dominates. The percentage of individual constituent in the essential oil is variable and depends on genetic (species, chemical plant variety) and environmental factors (climate, insolation, altitude) (5). Differences in the qualitative and quantitative essential oil composition can also be caused by the extraction procedure (6). The predominant method used for the essential oils extraction is hydrodistillation, whereas the most modern techniques are solid-phase microextraction (SPME) and microwave-assisted extraction (MAE) (7, 8). In Poland, the predominant apparatus used for the essential oils hydrodistillation is Deryng apparatus (9–11). The Deryng apparatus was recommended for essential oils distillation by Polish pharmacopoeias till volume VI (12). Polish Pharmacopoeia VII published in the 2006 (13) changed the hydrodistillation apparatus into Clevenger type. The Clevenger apparatus with its modifications is known and used all over the world for many years. Figure 1 shows the differences in construction of Deryng and Clevenger type apparatus.

The aim of this work is to compare the volatile components composition obtained from sage leaves

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by hydrodistillation using Deryng and Clevenger type apparatus and solid-phase microextraction (SPME) method.

EXPERIMENTAL

Plant material

The aerial parts of *Salvia officinalis* L. was collected in the Botanical Garden of Medical University in Lublin in June 2008. The voucher specimen has been deposited at the Herbarium of the Department of Pharmacognosy, Medical University in Lublin (TB062008S) and identified by Dr. Stanisław Kwiatkowski. Plants were air-dried, and for further analysis leaves were separated from the stem and powdered.

Hydrodistillation

Twenty grams of the powdered sage leaves were submitted to water-distillation in a Clevenger type apparatus with 400 mL water for 30 min. according to the Polish Pharmacopoeia VII (13). The hydrodistillation was also performed by use of Deryng apparatus with 400 mL after for 3 h according to the Polish Pharmacopoeia VI (12). The essential oil yields were measured. Subsequently, received essential oils were dried over anhydrous sodium sulfate and stored at 4°C until gas chromatographic determination of its composition.

Solid-phase microextraction (SPME)

The PDMS 30 µm fiber was used for the headspace extraction of 20.0 g powdered plant material placed in the tight tube. The extraction was carried out for 30 min. in the ambient temperature. Analytes were desorbed in the injector of gas chromatograph at 250°C.

GC-MS analysis

The analysis of the essential oils was performed using GCQ spectrometer (Thermo-Finnigan, USA) equipped with Restek RT-5 column (20 m × 0.18 mm and 0.2 µm stationary phase film thickness). Mass selective detector was operated in electron impact mode with ionization energy of 70 eV and mass range from 35 to 500 amu. Column temperature was initially kept at 50°C, then gradually increased to 280°C at 10°C/min rate. Injection temperature was 250°C. Carrier gas was helium at a flow rate of 1 mL/min. The components were identified based on the comparison of their retention indices relative to *n*-alkanes series and mass spectra with those of authentic samples, National Institute of Standards and Technology (NIST) and own libraries.

RESULTS AND DISCUSSION

The composition of the essential oils of *Salvia officinalis* leaves obtained by hydrodistillation using

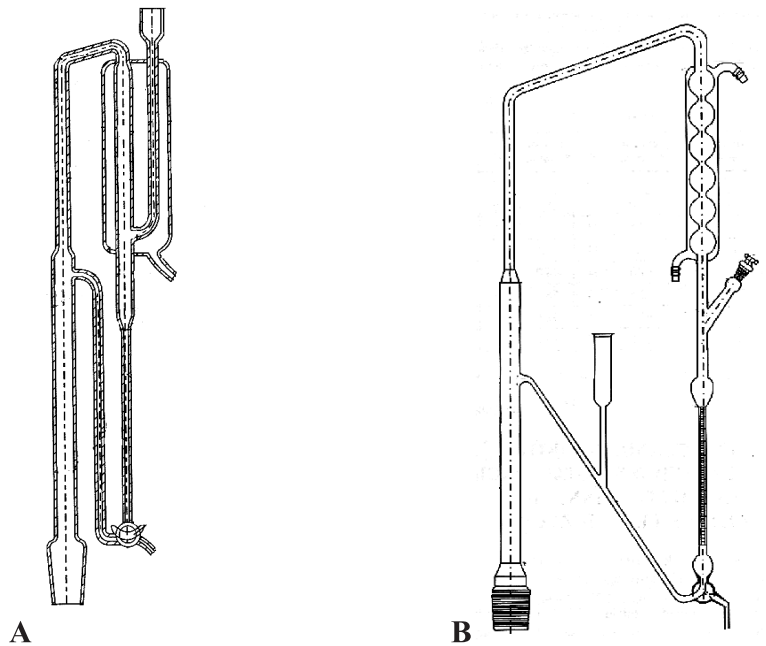

Figure 1. Pharmacopoeias apparatus for hydrodistillation of essential oils. **A** – Deryng apparatus, **B** – Clevenger type apparatus. (12, 13).

Table 1. Relative percentage composition of the essential oil from sage leaves received by different extraction methods.

No.	Compounds	RRI	Distillation apparatus		SPME
			Deryng	Clevenger type	
1.	(*Z*)-Salvene	849	0.4	0.3	0.2
2.	α-Pinene	933	2.6	2.5	0.7
3.	Camphene	947	3.9	4.1	1.3
4.	β-Pinene	977	0.3	0.3	0.1
5.	Myrcene	993	0.4	0.6	0.2
6.	*p*-Cymene	1026	1.0	1.3	0.5
7.	Limonene	1029	1.1	1.3	0.7
8.	1,8-Cineole	1031	7.3	9.4	10.4
9.	*trans*-Linalool oxide	1058	tr	tr	0.1
10.	*cis*-Linalool oxide	1072	tr	0.1	0.1
11.	Linalool	1086	0.2	0.4	0.4
12.	α-Thujone	1089	20.0	26.7	35.5
13.	β-Thujone	1103	7.8	10.0	12.4
14.	*trans*-Thujol	1120	0.1	0.2	0.1
15.	Camphor	1123	17.4	24.2	26.7
16.	Pinocamphone	1139	0.1	0.2	0.2
17.	Borneol	1150	1.6	2.4	1.4
18.	Terpinen-4-ol	1164	0.1	0.2	0.2
19.	Menthol	1172	0.1	0.1	1.1
20.	α-Terpineol	1176	0.1	0.2	0.2
21.	Myrtenol	1178	0.1	0.2	0.2
22.	Unknown	1209	0.1	0.2	1.3
23.	Bornyl acetale	1270	0.2	0.4	0.6
24.	Terpinen-4-ol acetate	1289	tr	0.1	0.4
25.	(*E*)-β-Caryophyllene	1423	0.5	0.6	0.6
26.	α-Humulene	1457	1.6	1.6	1.1
27.	*Allo*-Aromadendrene	1465	0.1	0.1	0.1
28.	Ledene	1499	0.1	0.1	0.1
29	Caryophyllene oxide	1584	0.1	0.1	0.1
30.	Viridiflorol	1592	15.4	6.0	-
31.	Humulene epoxide I	1599	0.8	0.5	0.1
32.	Humulene epoxide II	1605	1.1	0.7	0.3
33.	Humulene epoxide III	1616	0.2	0.2	0.1
34.	Unknown	1636	5.9	2.2	–
35.	Unknown	1640	1.2	0.4	–
36.	Sclarene	1943	0.1	tr	–
37.	Manool	2064	5.3	1.8	–
Total			**97.3**	**99.7**	**97.5**
Monoterpenoids			64.9	85.4	95.0
Sesquiterpenoids			27.0	12.5	2.5
Diterpenoids			5.4	1.8	–

tr – traces (< 0.1%); RRI – relative retention indices.

Clevenger type and Deryng apparatus were investigated by means of GC-MS analysis. Additionally, volatile components were extracted by use of SPME method and analyzed. Altogether, 37 compounds were detected, which are listed in Table 1 in order of their elution from the column.

Different sample preparation techniques, hydrodistillation and solid-phase microextraction, showed the differences in volatiles composition. SPME microextraction yielded 32 compounds, whereas hydrodistillation 37 compounds. Despite the fact, that the major components in all analyzed samples were the same (camphor and α-thujone) the relative amounts of the individual components varied. The major differences concern especially sesqui- and diterpenoids composition in the essential oils received after hydrodistillation and volatiles extracted by SPME. The content of these components in essential oils amounted from 14.3% for Clevenger type apparatus to 32.4% for Deryng apparatus. In volatiles extracted by SPME techniques, sesqui- and diterpenoids make up only 2.5%. The monoterpenoids content among all volatiles detected after SPME amounted to 95%, while in distilled essential oils 64.9% for Deryng and 85.4% for Clevenger type apparatus. This is because of the differences in the volatility of sesqui- and diterpenoids in comparison to monoterpenoids, and can be explained by the principles lying on the basis of both extraction methods. Solid-phase microextraction is solvent free sample preparation method, where a coated fiber is directly injected into the sample or into the headspace above the sample. Thereby, the volatile components are adsorbed to the fiber coating. During hydrodistillation, only steam-volatile compounds can be extracted. Highly volatile components can be lost during hydrodistillation and transformation of genuine compounds due to influence of heat, steam, and pH can occur as well (6, 14).

The essential oils yields measured after distillation according to Polish Pharmacopoeia VI (Deryng apparatus, 3 h) and VII (Clevenger type apparatus, 30 min.) was the same and amounted to 0.27% v/w. In both essential oils the same compounds have also been detected. The major volatile components were α-thujone, camphor, β-thujone, 1,8-cineole, viridiflorol and manool (Fig. 2). Despite these facts, the percentage composition of the essential oils components, calculated as the percent of peaks area, were different. The comparison of both hydrodistillation methods showed that the content of monoterpenes: 1,8-cineole, camphor and α- and β-thujone are clearly increased in the hydrodistillation by Clevenger type apparatus. On the other hand, the content of sesquiterpene alcohol, viridiflorol and diterpene alcohol, manool are decreased in comparison to Deryng apparatus. It is worth to mention that viridiflorol and manool have not been detected as the components of the volatiles received after SPME method, however, the contents of 1,8-cineole, camphor and α- and β-thujone were higher than received in distilled essential oils.

The differences in relative percentage composition of the essential oils obtained using Clevenger type and Deryng apparatus can be explained by the differences in construction of both distillation apparatus and different extraction time.

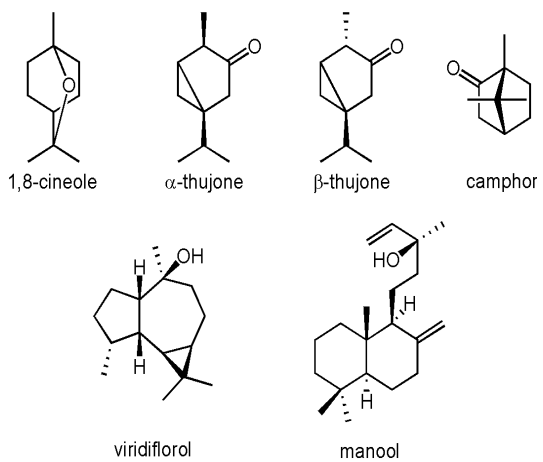

Figure 2. Structures of major compounds of sage essential oils

Table 2. Comparison of the major essential oils components of *Salvia officinalis* depending on distillation apparatus and extraction time

Compounds		Literature data				Own data	
		Clevenger 4 h (Tunisia) (15)	Clevenger 1 h (Brazil) (16)	Clevenger 2 h (Germany) (6)	Unger type, 3 h (Albania) (17)	Clevenger 0.5 h Poland	Deryng 3 h Poland
		I	**II**	**III**	**IV**	**V**	**VI**
1.	α-Pinene	2.89	3.07	1.5	4.35	2.5	2.6
2.	Camphene	4.07	4.40	2.2	**7.61**	4.1	3.9
3.	β-Pinene	4.41	**9.87**	1.7	0.94	0.3	0.3
4.	1.8-Cineole	**16.29**	**14.8**	5.6	**7.96**	**9.4**	**7.3**
5.	α-Thujone	17.76	**24.8**	**28.4**	**24.29**	**26.7**	**20.6**
6.	β-Thujone	**7.41**	3.97	5.9	4.03	**10.0**	**7.8**
7.	Camphor	**14.19**	10.9	**15.9**	**23.72**	**24.2**	**17.4**
8.	α-Humulene	4.37	**1.47**	**9.9**	2.83	1.6	1.6
9.	Viridiflorol	**4.63**	-	6.1	6.41	6.0	**15.4**
Sum of 4–7		**55.7**	**54.3**	**55.8**	**60.0**	**70.3**	**53.1**

– major components

– above ISO 9909 requirements

Table 2 showed comparison of the major essential oils components of *Salvia officinalis* depending on distillation apparatus and extraction time on the basis of our own and literature data. Since the quality of plant material (e.g., influence of harvest time, climatic conditions, different chemical types) as well as the method used for the analysis cause considerable variations in the composition of the essential oils, the comparison of the content of the individual compounds is very difficult. The summation of the contents of the major essential oil components: 1,8-cineole, α-thujone, β-thujone and camphor resulted in a more stable parameter, and seem to be significant for the essential oil from *S. officinalis*. Table 2 shows that among all cited literature data and our own studies, distillation by use of Clevenger type apparatus for 30 min. is characterized by the highest content of mentioned components (70.3%). Quite short distillation time makes the composition of the volatiles in this essential oil similar to those obtained after SPME (Table 1). The content of the same components in other samples was varied from 53.1 to 60.0% with quite low relative standard deviation (4.67%). Even through the extension of extraction time caused the lost of highly volatile components and the differences in the content of the individual components in essential oils from different countries, all these sage oils are comparable taking into account the sum of four major compounds. Taking these data into consideration, one can conclude that the type of distillation apparatus has insignificant effect on the quality of the essential oil from sage. However, too short distillation time cause considerable differences in the composition of essential oil from *Salvia officinalis*. The minimum time for the hydrodistillation of the essential oils from sage should be 1 h.

Each essential oil to be admitted to medicinal use should meet requirements regarding the composition of major chemical components. The composition of the essential oil of common sage, according to the ISO standard 9909:1997 should be as follows: α-thujone, 18.0–43.0%; β-thujone, 3.0–8.5%; camphor, 4.5–24.5%, 1,8-cineole, 5.5–13.0%; α-humulene, 0–12%; α-pinene, 1.0–6.5%; camphene, 1.5–7.0%; limonene, 0.5–3.0%, linalool and its esters, < 1% and bornyl acetate, < 2.5% (18). According to these requirements, the essential oils, V (Unger type apparatus, 3 h) and VI (Clevenger type apparatus 0.5 h) (Table 2) are characterized by to high amount of camphene and β-thujone, respectively. Other essential oils listed in Table 2 meet all these requirements.

The chemical composition of *Salvia officinalis* essential oils varies widely, and different chemotypes have been identified in consideration of their major constituents (19, 20):

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 > a-thujone > β-thujone
5. α-thujone > camphor > β-thujone > 1,8-cineole

Further authors (21) mentioned sesquiterpene alcohol, viridiflorol as the fifth very important constituent of sage essential oil. These authors describe new viridiflorol chemotype from Romanian *S. officinalis*, which in comparison to the other chemotypes is characterized by low amount of thujones, while viridiflorol content is ~20% (21). The essential oil received from the Polish sage belongs to the fifth chemotype.

CONCLUSIONS

In this paper the comparison of the sage essential oil obtained in the Deryng and Clevenger type apparatus, according to the pharmacopoeial methods (FP VI and VII), was done for the first time. In both essential oils the same compounds has also been detected, however, the relative percentage composition of the essential oils components were different. The extension of extraction time, according to the method described in Polish Pharmacopoeia VI (Deryng, 3 h), caused the lost of highly volatile components which are monoterpenoids, but on the other hand, more sesqui- and diterpenoids are present in the essential oil. Quite short distillation time in case of the method described in Polish Pharmacopoeia VII (Clevenger type, 0.5 h) makes the composition of the volatiles in sage essential oil similar to those obtained after SPME. Despite the fact that the concentration of the volatile components is higher in comparison to the Deryng apparatus, this essential oil do not meet ISO 9909 requirements regarding the composition of major chemical components. The minimum time for the hydrodistillation of the essential oils from sage should be 1 h.

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