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EXPERIMENTAL PAPER

Yield and chemical composition of essential oil from *Salvia officinalis* L. in third year of cultivation

GRAŻYNA ZAWIŚLAK

Department of Vegetables and Medicinal Plants
University of Life Sciences in Lublin
Leszczyńskiego 58
20-068 Lublin, Poland

e-mail: grazyna.zawislak@up.lublin.pl

S u m m a r y

The study upon sage (*Salvia officinalis* L.) has been carried out at the Experimental Section of Department of Vegetables and Medicinal Plants, University of Life Sciences in Lublin. The aim of present study was to evaluate the yield and chemical composition of essential oil extracted from sage leaves harvested from a three-year plantation. The sage herb harvest date in the third year of cultivation had a significant impact on the yield of plants. Higher yield of fresh and dry herb, dry leaves, and essential oil was achieved in August (the second harvest time) than in May (the first harvest time). Chromatographic examination indicated the presence of 50 chemical compounds in sage essential oil. It was a variable percentage of essential oil components depending on the raw material harvest time. The main components of the sage essential oil were: 1,8-cineole (16.08–18.04%), α-thujone (10.40–21.51%) and camphor (5.24–18.08%).

Key words: *sage, GC/MS, 1,8-cineole, α-thujone, camphor*

INTRODUCTION

Sage (*Salvia officinalis* L.) is an essential oil-producing plant. In its natural state, it occurs in the Mediterranean region [1-2]. In Poland, sage is grown in plantations [2-4]. In our climatic conditions, it grows quite well, although sometimes freezes during severe winters [5]. The sage leaf (*Salviae officinalis folium*) is a pharmacopoeial raw material [6].

According to Luwańska et al. [7], sage is used in the prophylaxis and in the treatment of rhinitis and inflammatory conditions of oral cavity as well as a component of formulations for oral hygiene. Sage leaf is one of the most important raw materials recommended for the prevention and treatment of dental disorders [8]. It can also be applied as an adjunct to the treatment of mild to moderate forms of Alzheimer's disease [9]. Sage also shows antiperspirant properties. Excessive sweating is a common symptom at patients with advanced cancer. Non-pharmacological method of the reduction of excessive sweating is drinking the sage infusion. So far, the mechanism of its action is not recognized [10].

Microbiological tests showed its high antimicrobial activity [11]. Antibacterial activity has been confirmed by many researchers [12-15]. Due to the microbiological activity of essential oil, it can be used as an ingredient in food products and pharmaceutical preparations [15]. Sage contains phenolic compounds that exhibit antioxidant activity [16-18]. Also sage essential oil exhibits antioxidant properties [19]. The oil components counted to oxygenated terpenes are distinguished by high activity [20].

The aim of present study was to evaluate the yield and chemical composition of essential oil extracted from sage leaves harvested from a three-year plantation.

MATERIALS AND METHODS

The study upon sage (*Salvia officinalis* L.) has been carried out at the Experimental Section of Department of Vegetable and Medicinal Plants, University of Life Sciences in Lublin.

PLANT MATERIAL

Experiments with sage were established from seedlings produced in a greenhouse. Seed material was obtained from PNOS Ożarów Mazowiecki (Poland). Seeds were sown on March 20 into the boxes filled with peat substrate. The first emergence appeared after two weeks. The seedlings were transferred into multipots and then planted in the field in mid-May at a spacing of 30 x 30 cm, in random blocks pattern, in 4 replications. The plot size was 2.16 m^2. Plants were grown on lessive soil developed from loess formations on Cretaceous marls. The cultivation stand was prepared in accordance with the recommendations of agricultural technology for sage. Treatments consisted of a manual weed control and soil loosening. No chemical plant protection treatments were applied, diseases and pests were not observed. In the first year of cultivation, sage was harvested once, while in the second – twice. The study was conducted in 2010–2011 on a three-year plantation. There were no plant fall-outs on the plantation as a result of their freezing during winter. The three-year-old plants were fertilized in early

April with full phosphorus and potassium rates, and half dose of nitrogen (P_2O_5 – 70 kg·ha^{-1} as granulated triple superphosphate, K_2O – 120 kg·ha^{-1} in a form of potassium sulfate, N 80 kg·ha^{-1} as ammonium nitrate in divided dose). The remainder of nitrogen fertilizer was introduced after the first harvest of herb – in mid-May. The harvest of the sage herb in the third year of cultivation was carried out in the first decade of May (first harvest) by cutting the plants at a height of 10 cm above the ground and at the end of August (second harvest) by cutting the plants at a height of 12 cm above the ground. Stems of sage in the third year of cultivation were heavily lignified at the bottom, therefore the cutting height was adjusted to the degree of their lignification.

After harvesting, the yield of fresh herb was determined. Sage herb was dried in a kiln at a temperature of 35°C for 5 days. After drying, the leaves were separated from stems to give a sage leaf, from which the essential oil was distilled.

The yield of essential oil obtained from the dried leaves was given in accordance with Farahani et al. [21].

Essential oil

Dry sage leaf was subject to essential oil content determination – distillation [22]; the distillation time was three hours, the size of the sample 20 g of dry leaves, and 400 ml of distilled water. After the distillation was complete, the oil was collected into the calibrated tube and volume of the oil was read after 30 minutes.

GC/MS analysis

Qualitative and quantitative composition of essential oil was determined by means of gas chromatography combined with mass spectrometry (GC/MS) using ITS-40 device (GC/ITMS system, Finnigan MAT, USA) equipped with DB-5 column (J&W, USA) of 30 m length, with diameter of 0.25 mm, and a stationary phase film thickness of 0.25 mm. The injector temperature was 280°C. A temperature gradient was applied: 35°C for 2 minutes followed by an increase of 4°C to 280°C. The qualitative analysis was based on MS spectra comparing them with the NIST/EPA/NIH spectra library [23]. The identity of ompounds was confirmed by retention indices found in literature data [24].

Statistical analysis

Results referring to the yields were statistically processed by means of variance analysis for the complete randomization pattern (single classification).

RESULTS AND DISCUSSION

The sage plantation is normally used in Poland for three years. In the first year, only one harvest is possible, while 2-3 harvests in the next 2–3 years as plants regrows [25]. The yield of fresh and dry herb as well as dried sage leaves in the third year of cultivation significantly depended on the time of harvest (tab. 1). Yield of fresh herb cut at the end of August (the second harvest date) was by about 27.35 kg greater than that harvested in the first decade of May (the first harvest date). Zawiślak [3] showed a significant effect of harvest time on the yield of fresh sage herb in the second year of the plantation performance. The author achieved higher yield of fresh sage herb in September (the second harvest date) than in May (the first harvest date). The yield of fresh herb from the second harvest in the third year of cultivation use was 228.17 kg·100m^{-2} (tab. 1), which was lower than that obtained from the two-year plantation, which was shown by Zawiślak [3].

Table 1.

Yield and content of essential oil in the leaves of sage (*Salvia officinalis* L.) in the third year of cultivation (2010–2011)

Harvest date	Yield of fresh herb (kg·100 m^{-2})	Yield of dry herb (kg·100 m^{-2})	Yield dry leaves (kg·100 m^{-2})	Share of leaves in dry herb (%)	Essential oil (%)	Yield of essential oil (g·100 m^{-2})
May	100.41 a	20.51 a	8.25 a	40.22	1.16 a	93.46 a
August	127.76 b	29.58 b	12.47 b	42.15	1.35 a	165.66 b
Σ	228.17	50.09	20.72	-	-	259.12

Dry herb yield obtained at the end of August was by about 30% higher as compared with that from the harvest carried out in the first decade of May (tab. 1). Similarly, the dry leaves yield was greater by 30% in August rather than in May.

Studies have shown that the yield of essential oil achieved from leaves harvested in May was significantly lower than that obtained in August and amounted to 93.46 g·100 m^{-2} and 165.66 g·100 m^{-2}, respectively. Research conducted by Kołodziej and Najda [26] showed that the yield of essential oil from sage growing in the first year, depending on the method of cultivation, was 3.58 g·m^{-2} (when sowing the seeds in the field) and 3.29·g 100 m^{-2} (cultivation from the seedlings). The yield of essential oil produced from the three-year plantation was slightly lower (tab. 1).

When analyzing data in table 1, it was found that the share of leaves in the dry herb was at a similar level both in the harvest in May and in August. Dry herb obtained in the third year of cultivation use contained about 60% of the stems, that are the waste material and are not used for pharmaceutical or cosmetic purposes. Study upon the three – year plants, as well as the analysis of the results by Zawiślak [3, 27] and Zawiślak and Dyduch [28] shown that obtained herb contained more stems that are not the herbal material, along with the sage aging.

Content of essential oil in dry sage leaf from the three-year plantation did not depend on the time of harvest of the raw material and ranged from 1.16 to 1.35% (tab. 1). Zawiślak [3] showed a significant correlation between the harvest time and content of essential oil in the second year of cultivation. According to the author, more oil was presented in leaves in September (second time harvest). Maric et al. [29] reported that the content of essential oil varied during plant development. Oil content in sage plants before flowering was 0.29–0.49%. In here presented experiment, the essential oil content before plant flowering (the first decade of May) was higher and amounted to 1.16% (tab. 1).

Chromatographic examination indicated the presence of 50 chemical compounds in sage essential oil, of which three were not identified at all (tab. 2). The main components of essential oil of sage grown on the three-year plantation were oxygenated monoterpenes that, independently on the time of harvest, accounted for over 50% of identified compounds. The low content of monoterpenes hydrocarbons in oil from leaves harvested in the first decade of May (9.07%) as compared to sage essential oil from the late summer (the end of August), was very interesting. It was also shown that the content of oxygenated sesquiterpenes in the essential oil from sage leaves in late August was twice as low (8.61%).

Table 2.

Percentage composition of the essential oil from the leaves of sage (*Salvia officinalis* L.) in the third year of cultivation (2010–2011)

Component	RI	Harvest date	
		May	August
Z-Salvene	860	tr.	tr.
E-Salvene	868	tr.	tr.
Tricyclene	931	tr.	tr.
α-Thujene	933	tr.	tr.
α-Pinene	940	4.41	5.65
Camphene	955	2.32	6.93
Sabinene	975	tr.	tr.
β-Pinene	980	2.34	2.13
Myrcene	988	tr.	tr.
α-Phellandrene	1006	tr.	tr.
α-Terpinene	1015	tr.	tr.
p-Cymene	1023	tr.	tr.
Limonene	1027	tr.	2.24
1,8-Cineole	1030	18.04	16.08
E-β-Ocimene	1042	tr.	tr.
γ-Terpinene	1052	tr.	tr.
cis-Sabinene hydrate	1063	tr.	tr.
Terpinolene	1075	tr.	tr.
Linalool	1088	tr.	tr.

trans-Sabinene hydrate	1090	tr.	tr.
α-Thujone	1095	21.51	10.40
β-Thujone	1104	6.49	2.69
trans-Pinocarveol	1123	tr.	tr.
Camphor	1131	5.24	18.08
trans-Pinocamphone	1142	tr.	tr.
Thujanol	1147	tr.	tr.
Borneol	1151	4.0	9.71
Terpinen-4-ol	1158	tr.	tr.
Myrtenol	1171	tr.	tr.
Bornyl acetate	1239	tr.	2.57
Myrtenyl acetate	1270	tr.	tr.
α-Cubebene	1287	tr.	tr.
α-Ylangene	1306	tr.	tr.
α-Copaene	1311	tr.	tr.
β-Burbonene	1317	tr.	tr.
Z-Caryophyllene	1333	tr.	tr.
E-Caryophyllene	1346	8.17	8.51
α-Humulene	1374	8.92	6.23
allo-Aromadendrene	1377	tr.	tr.
γ-Muurolene	1388	tr.	tr.
Viridiflorene	1400	tr.	tr.
α-Muurolene	1406	tr.	tr.
α-Cadinene	1417	tr.	tr.
α-Amorphene	1420	tr.	tr.
trans-Calamene	1424	tr.	tr.
α-Cadinene	1437	tr.	tr.
α-Calacorene	1440	tr.	tr.
Caryophyllene oxide	1472	tr.	tr.
Viridiflorol	1481	10.83	5.75
n.i.	1510	tr.	tr.
n.i.	1629	tr.	tr.
n.i.	1713	tr.	tr.
Manool	1810	6.62	2.86
Total		98.89	99.83
Groupe components:			
Monoterpene hydrocarbons		9.07	16.95
Oxygenated monoterpenes		55.28	59.53
Sesquiterpene hydrocarbons		17.09	14.74
Oxygenated sesquiterpenes		17.45	8.61

n.i. – not identified; tr. – trace (<0.05)

Tayoub et al. [30] found that the essential oil of *Salvia officinalis* L. contained monoterpenes in an amount of 57.3% and 41.7% of sesquiterpenes. The study showed higher content of monoterpenes in both the oil obtained from leaves during the May (64.35%) and August harvest (76.48%) (tab. 2). Most of the components identified in the sage essential oil belonged to oxygenated monoterpenes (1,8-cineole, α-thujone, camphor, β-thujone, borneol, bornyl acetals). A similar relationship was reported by Santos-Comes and Fernandes-Ferreina [31], as well as by Hayouni et al. [15].

On the basis of laboratory analyzes, it was revealed that 1,8-cineole was present in the highest quantity (tab. 2). The content of 1,8-cineole was maintained at a similar level both in the essential oil from leaves harvested in the first decade of May and at the end of August and it amounted to 18.04% and 16.08%, respectively. The compound was also present at a similar level at *Salvia officinalis* L. from Syria (18.3%) and it was also the dominant component [30]. The high content of 1,8-cineole in research by Hayouni et al. [15], which was 32.27%, is also interesting. In studies carried out by Bernotiene et al. [1], 1,8-cineole was present in essential oil from Lithuania at lower level (12.4–17.6%). According to Zawiślak [32], content of 1,8-cineole in the essential oil from the sage leaves in the first year of cultivation averaged to 9.03%. Zawiślak and Dyduch [33] demonstrated a variable content of 1,8-cineole in the essential oil from sage in the second year of cultivation, depending on the time of harvest. Essential oil from leaves harvested in May contained an average of 17.10% of 1,8-cineole, whereas in September – 9.43%. 1,8-cineole, as the main ingredient, was also present in the essential oil from *Salvia triloba* L. [14].

Also, α-thujone was the dominant component of the essential oil from the sage leaves harvested in the third year of cultivation. Oil from leaves harvested in May contained α-thujone at a level of 21.51% (tab. 2). A similar content of α-thujone was reported by Longaray Delamare et al. [14] - 24.8% and by Bozin et al. [13] - 19.9%.

According to Raal et al. [34], essential oil obtained from the leaves of sage grown in Estonia was characterized by a high content of toxic thujone. The study showed that the oil produced in our climatic conditions contained less thujone during late summer (late August) than in the first decade of May (tab. 2).

Content of camphor in the essential oil from sage leaf harvested in August was present at a level of 18.08% (tab. 2). Bozin et al. [13] reported that the oil originating from Serbia contained similar amounts of camphor (18.9%). Santos-Comes and Fernandes-Ferreina [31] determined camphor content in Portuguese oil from sage leaves at the level of 19.51%. Camphor was also a major component of essential oil derived from the sage leaves in the second year of cultivation in September, which averaged to 20.9% [33]. Ben Farhat et al. [17] demonstrated a highly diverse content of camphor (5.08–15.06%) in sage essential oil originating from Turkey, depending on the region of the raw material origin.

Studies have shown that sage essential oil from the first decade of May also contained twice as much viridiflorol (10.83%), β-thujone (6.49%), and manool

(6.62%). In late summer (at the end of August), camphor (18.08%), borneol (9.71%), and camphene (6.93%) were present in larger amounts than in May.

CONCLUSIONS

1. The sage herb harvest date in the third year of cultivation had a significant impact on the yield of plants. Higher yield of fresh and dry herb, dry leaves, and essential oil was achieved by harvesting sage at the end of August (the second harvest date), than in May (the first harvest date).
2. No significant effect of harvest time on essential oil content in the dry leaf of sage in the third year of cultivation, was observed.
3. Content of 1,8-cineole in sage essential oil remained at a similar level, regardless of the time of harvest of the raw material (18.04% - in May, 16.08% - in August).
4. Essential oil from the leaves harvested during the first decade of May contained more than twice as much α-thujone (21.51%) as those harvested in August (10.40%)
5. Essential oil from the leaves of *Salvia officinalis* L. in the third year of cultivation was characterized by a higher content of monoterpenes in May (64.35%) and in August (76.48%).

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PLONOWANIE I SKŁAD CHEMICZNY OLEJKU ETERYCZNEGO Z *SALVIA OFFICINALIS* L. W TRZECIM ROKU UPRAWY

GRAŻYNA ZAWIŚLAK

Katedra Warzywnictwa i Roślin Leczniczych
Uniwersytet Przyrodniczy w Lublinie
ul. Leszczyńskiego 58, 20-068 Lublin

e-mail: grazyna.zawislak@up.lublin.pl

S t r e s z c z e n i e

Badania nad szałwią lekarską (*Salvia officinalis* L.) przeprowadzono w Dziale Doświadczalnym Katedry Warzywnictwa i Roślin Leczniczych Uniwersytetu Przyrodniczego w Lublinie. Celem badań była ocena plonowania i składu chemicznego olejku z liści szałwii lekarskiej pozyskanego z plantacji trzyletniej. Termin zbioru ziela szałwii lekarskiej w trzecim roku uprawy istotnie wpłynął na plonowanie roślin. Większy plon świeżego i suchego ziela, suchych liści oraz olejku uzyskano w sierpniu (drugi termin zbioru) niż w maju (pierwszy termin zbioru). Badanie chromatograficzne wykazały obecność 50 związków chemicznych w olejku szałwiowym. Stwierdzono zmienny procentowy udział składników olejku eterycznego w zależności od terminu zbioru surowca. Głównymi składnikami olejku szałwiowego były: 1,8-cyneol (16,08-18,04%), α-tujon (10,40-21,51%) i kamfora (5,24-18,08%).

Słowa kluczowe: *szałwia lekarska, GC/MS, 1,8-cyneol, α-tujon, kamfora*