ESSENCIAL OIL CONTENT AND COMPOSITION DIFFERENT OF *Salvia officinalis* L. GENOTYPES CULTIVATED IN MOLDOVA

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Abstract. The quantitative and qualitative analysis of essential oil, extracted through hydrodistillation, from different genotypes of *Salvia officinalis* L. cultivated in Republic of Moldova was studied. The essential oil content analysed from 5 genotypes of *Salvia officinalis* L. varies according to the genotype and harvested phase. The highest content of essential oil was registered in the material harvested after flowers and seeds fall: 1.455-1.823% (s.u.) in shoots with leafs and 1.408-1.749% (dry matter) in leafs. GC-MS analysis of essential oil revealed that for different genotypes in the shoots with leaf there are registered 14-23 components, but in leafs - 17-25. The major components are represented by monoterpene ketones: α-thujone (21.2-38.8), β-thujone (5.877-16.201%), camphor (17.5-24.6%), followed by eucalyptol (6.47-11.2%).

Keywords: *Salvia officinalis*, essential oil, content, chemical components.

Rezumat. Genotipuri de *Salvia officinalis* L. cultivate în Moldova cu conţinut şi componenţă diferită a uleiului esenţial. S-a studiat analiza cantitativă şi calitativă a uleiului esenţial obţinut prin hidrodistilare din genotipuri de *Salvia officinalis* L., cultivate în Republica Moldova. Conţinutul de ulei esenţial evaluat la 5 genotipuri de *S. officinalis* evaluate variază în funcţie de genotip şi faza de recoltare atât în lăstarii cu frunze, cât şi în frunze şi inflorescenţe. Cel mai ridicat conţinut de ulei esenţial a fost atestat în materialul recoltat după scuturarea florilor şi seminţelor: 1,455-1,823% (s.u.) în lăstari cu frunze şi 1,408-1,749% (s.u.) în frunze. Analiza GC-MS a uleiului esenţial a demonstrat că la diferite genotipuri sunt identificaţi în lăstarii cu frunze de la 14 până la 23 componenţi, iar in cel din frunze de la 17 până la 25. Componenţii majori sunt reprezentaţi de monoterpene cetone: α-tujonă (21,2-38,8%), β-tujonă (5,877-16,201%), camforă (17,5-24,6%), urmată de eucaliptol (6,47-11,2%).

Cuvinte cheie: *Salvia officinalis*, ulei esenţial, conţinut, componenţi chimici.

INTRODUCTION

Salvia officinalis L. belongs to Lamiaceae family, a small evergreen bush used as medicinal, aromatic and spice plant from ancient times. Presently, there are used young shoots with leafs, flowers and essential oil. The pharmacological action of *Salvia officinalis* L. is attested as antiseptic and astringent, spasmolytic, anti-inflammatory haemostatic, expectorant, cicatrizing, heal wounds, antibiotic, bacteriostatic, antisudorific and tonic due to the essential oil of this plant (GONCEARIUC, 2008). The tea from leafs is used to treat different mouth diseases, as pharyngitis**,** atherosclerosis (HUBBERT *et al*., 2006) and has antioxidant activity (WALCH *et al*., 2011). It is also used in case of stress, irritation, skin cankers, abundant transpiration, rheumatism and for memory improvement (SCHOLEY *et al*., 2008). The *S. officinalis* leaf extracts are used in the treatment of Alzheimer disease in medium and moderate forms (AKHONDZADEH *et al*., 2003; DOS SANTOS-NETO *et al*., 2006) and for sure has an antihiperlipidemic effect (KIANBAKHT *et al*., 2011). It was noticed also an anticancer action (SIMIĆ *et al*., 2000; FORTES *et al*., 2003). Largely, there are used antiviral and antifungal characteristics of essential oil (ESCOP, 1997) in perfumery and aromatherapy (VOITKEVICI, 1999). In the Republic of Moldova some *S. officinalis* plantations are used for leaf production as a pharmaceutical product, others – to separate essential oil, with steam distillation. Our past investigations demonstrated that in *S. officinalis* leafs cultivated in Romania and Republic of Moldova, besides essential oil contains also flavones, triterpenice, phytosterols and polyholosides (DĂNILĂ *et al*., 2008).

This research represented the study of the essential oil content of different *Salvia officinalis* genotypes, at different harvesting phases, as well as oil components concentration at late harvesting phases, when the essential oil content is maximal. These results may indicate the most convenient phase for harvesting.

MATERIAL AND METHODS

There were investigated 5 genotypes of *S. officinalis* of different origin: the variety Miracol, created by us and approved, registered in the State Register of Moldova Republic; two genotypes largely cultivated in the South of the Republic of Moldova, district Cahul - Cahul-D (a variety created by the Botanical Garden Nikita, Crimea) and Cahul-M (originating from Russia); another two genotypes (G-1 and G-2) from the collection of Institute of Genetics and Plant Physiology. The samples were collected in the morning, corresponding to three developmental phases: before the formation of flower button (May 13 and 20, 2011), in the flowering time (June 2, 2011) and after flowers and seeds fall (July 29, 2011). The essential oil was separated from fresh collected material using hydrodistillation in Ginsberg apparatus and the oil composition was recalculate to dry mater. After distillation the essential oil was dried with Na_2SO_4 and was preserved in the freezer. Qualitative and quantitative composition of essential oil was determined by gas-chromatographic analysis in tandem with the mass spectrometry (GC-MS) for the separated samples of shoots with leaf, harvested only after the shaking of flowers and seeds. In these samples the content of essential oil was the highest. The

analysis equipment included: gas-chromatograph Technologies Agilent 7890 equipped with Selective Mass Detector with Quadrupol MSD Agilent Technologies 5975C, capillary column (30 м/0.25 мм/0.25 μм) with non-polar stationary phase HP-5 ms. The analysis was performed at a temperature of 250°C injector and detector - 280°C, using a temperature gradient from T1 = 70° (2 min), T2 = 200°C (5°C / min), T3 = 300°C (20°C / min, 5 min). Mobile phase: Helium 1ml/min, injected volume - 0.03 ml essential oil, split rate - 1:100. Identification of chromatographic peaks was performed using the software package AMDIS ™, coupled with the NIST database.

RESULTS AND DISCUTIONS

The evaluation of 5 genotypes of *Salvia officinalis* demonstrated the diversity of essential oil content at the different development phases. It was demonstrated that young shoots with leafs, for the majority of genotypes, accumulate a relatively low content of essential oil – 0.636 – 0.691% (dry matter), which depends of the shoot's growth phase (Table 1). The genotype Cahul-D (Fig. 1) contains only 0.511% (dry matter) of essential oil, but G-1 is the genotype with the highest content of essential oil – 0.905% (dry matter) before the floral buttons appears (May 20). At the third evaluation term, when the shoots have inflorescences, the essential oil content increased considerably for the variety Miracol (Fig. 3) – (0.981% (dry matter) and the genotype Cahul-M (Fig. 2) (0.818% (dry matter). In this phase it also increased the oil content for the genotype G-2 – 0.795 % (dry matter).

Table 1. Essential oil content in some genotypes of *Salvia officinalis*, 2011.
Tabel 1. Conţinutul în ulei esenţial la unele genotipuri de *S. officinalis*, 2011.

Variety, genotype	Essential oils content, % (dry matter)						
	May 13	May 20	June 2, 2011			July 29, 2011	
	shoots with leafs		shoots with leafs and inflorescences	shoots with leafs	inflorescences	shoots with leafs	leafs
variety Miracol	0.636	0.693	0.981	0.365	1.066	1.572	1.543
Cahul-D	0.691	0.511	0.682	0.646	0.644	1.619	1.536
Cahul-M	0.675	0.648	0.818	0.314	1.187	1.455	1.408
G-1	0.675	0.905	0.662	0.505	0.905	1.823	1.749
G-2	0.756	0.682	0.795	0.509	0.890	1.679	1.741

The highest content of essential oil was registered in inflorescences - 0.905-1.187% (dry matter), with exception of Cahul-D genotype, where the oil content does not differ significantly in the shoots with inflorescences and leafs, shoots and leafs, inflorescences. The amount of essential oil increased in all genotypes after fall flowers – May 29. The highest amount was registered for the genotypes G-1 and G-2, where the shots with leafs contain 1.823 and 1.679% (dry matter) respectively, but in leafs - 1.749 and 1.741% (dry matter). Similar content of essential oil – 1.6% (dry matter) was described (REABOTEAGOV *et al*., 2011) for the variety Predgornyi, created in the Botanical Garden Nikita, Crimea.

Figure 1. *S. officinalis* genotype Cahul-D. (original).

Figure 2. *S. officinalis* genotype Cahul-M. (original).

Figure 3. *S. officinalis* variety Miracol (original).

The GC-MS analysis of essential oil extracts from shoots with leafs, harvested at the end of July, revealed 14-23 components; in leaf samples – 17-25, which depend on genotypes (Table 2). The major compounds are represented by monoterpenes: α-thujone (21.2-38.8%), β-thujone (5.877-16.201), camphor (17.5-24.6%), followed by eucalyptol (6.47- 11.2%).

Thus, the variety Miracol and another four analysed genotypes have the same chemotype: **thujone/ camphor/eucalyptol** while, the concentration of major components, especial the minor compounds in extracted oil from leafs and shoots with leafs substantially differ for all genotypes. Other researchers (REABOTEAGOV *et al*., 2011) described varieties with two major components: β-thujone (39.5%), camphor (17.4%) and with significant concentration of monoterpenoxides – 1.8-cineole (10.5%) that was not detected in our studied genotypes. In the *S. officinalis* cultivated in Estonia and other European countries, 1.8-cineol is the major component of essential oil (RAAL *et al*., 2007). Romanian genotypes contain 12 components in the essential oil and the major one is α- thujone in the concentration of 31.23-52.86% (ONIGA *et al*., 2010).

In our genotypes the number of minor components of the essential oil from shoots with leafs, with the concentration bellow 1% vary from 1 (β-mircene, genotype G-2) to 11 (genotype Cahul-M), in the essential oil from leafs – from 5 (genotype G-2) to 12 (genotype Cahul-D) (Table 2). The number of minor components with the concentration 1% to 5% varies from 6 to 9 in the essential oil from shoots with leafs and leafs. These facts demonstrate the high variability of chemical components of the essential oil in the studied genotypes. Strong differences exist in the number and concentration of minor and major components.

Table 2. The biodiversity of essential oil components in the genotypes of *Salvia officinalis*, 2011.
Tabel 2. Biodiversitatea componenţei uleiului esenţial la genotipuri de *S. officinalis*, 2011.

Component	Variety Miracol		Cahul-D		Cahul-M		G-1		G-2	
	shoots/ leafs	leafs	shoots/ leafs	leafs	shoots/ leafs	leafs	shoots/ leafs	leafs	shoots/ leafs	leafs
α-Pinene	2.591	2.565	0.913	3.799	1.249	1.352	2.998	6.632	3.248	3.826
Camphene	2.418	2.613	1.327	2.57	1.781	1.582	2.445	3.076	2.252	1.704
Sabinene	0.253						0.326			
β-pinene	1.365	1.68	0.831	1.41	0.982	1.029	1.243	1.419	1.133	0.849
β-Mircene	0.778	0.669	0.513	0.719	0.695	0.662	0.949	1.162	0.885	0.86
o-Cymene								0.26		
Limonene	1.505	1.302	0.955	1.406	1.248	1.306	1.486	1.899	1.536	1.68
Eucalyptol	8.416	10.372	6.472	11.203	11.203	10.91	7.162	9.275	8.301	9.781
γ-terpinene	0.311			0.307	0.385		0.444	0.491		0.331
α-terpinene	0.489	0.317		0.369	0.384		0.411	0.484		0.527
Linalool	0.497	0.483		0.463	0.515	0.488	0.593	0.511		0.384
α-thujone	33.791	21.239	35.035	22.415	35.134	32.492	36.605	34.793	38.818	34.179
β-thujone	5.877	16.201	13.559	13.328	11.19	10.783	12.119	10.936	9.863	3.19
Isothujol				0.267						
cis-Sabinol	0.345			0.408	0.256					
Camphor	24.59	19.144	19.753	21.138	21.297	21.461	18.996	17.508	20.065	19.995
Borneol	3.302	3.472	3.685	4.541	2.573	2.935	1.56	1.365	1.654	2.125
4-Terpineol	0.538	0.554	0.817	0.694	0.685	0.689	0.593	0.507		0.542
α-Terpineol	0.285			0.314						0.24
Myrtenol				0.281						0.316
Bornylacetat	2.324	3.856	3.535	2.934	2.39	2.512	1.543	1.223	1.818	2.546
α-terpinylacetat		0.27		0.204	0.324					0.322
β-caryophilene	3.929	5.44	2.75	1.703	3.875	4.048	3.191	2.152	2.706	2.221
α-caryophilene	2.617	3.367	3.305	4.745	3.607	3.527	5.074	4.795	5.808	7.998
Caryophilene oxide		0.42			0.331					
Viridiflorol	3.063	3.653	5.607	3.924	4.17	3.791	1.476	1.511	1.913	3.638
Aroma dendrenoxid				0.379	0.336					0.576
Labdatriene	0.716	0.968	0.934	0.477	0.499	0.431	0.453			0.742
Components identifiend	22	20	16	25	23	17	20	19	14	23
Total %	**100.0**	**98.585**	**100.0**	**99.998**	**99.772**	**99.998**	**99.67**	**99.999**	**100.0**	**99.277**

The concentration of major components detected in the shoots with leafs – α-thujone varies from 33.791%, in the variety Miracol, up to 38.818%, for the genotype G-2. The same major component concentration in the essential oil is maximum in leafs and shoots with leafs for the genotype G-1(34.793%). For all analysed genotypes the concentration of α-thujone is higher in the shoots with leafs in comparison with leafs. Camphor concentrations vary from 18.996 % in the genotype G-1, up to 24.59% for the variety Miracol. For all genotypes, with exception of two genotypes from Cahul, the camphor concentration is higher in the essential oil extracted from shoots with leafs. The third major component – eucalyptol have the higher concentration (9.275-11.203) in the essential oil extracted from leafs. In the oil from shoots with leafs its concentration is 8.416 %, for variety Miracol and up to 11.203% for the genotype "Cahul-M".

The species *S. officinalis* is characterized through a high variability of chemical composition that depends on cultivation zone, variety, genotype, as well as on plant developmental stage. These conditions influence especially the essential oil and the number, concentration of its components. The oil amount is accumulated gradually, with plant

development, and increases the maximum in the phase of flowers and seeds fall. In our opinion this is the optimal harvesting phase.

CONCLUSIONS

1. Quantitative and qualitative analysis of essential oil extracted through hydrodistillation from *Salvia officinalis*, cultivated in Republic of Moldova was described in this work;

2. The essential oil content evaluated in 5 *S. officinalis* genotypes varies in function of genotype and harvesting phase in the shoots with leafs, leafs and inflorescences. The highest amount of essential oil was registered in the material harvested after flowers and seeds fall: 1.455-1.823% (dry matter) in the shoots with leafs and 1.408-1.749% (dry matter) in leafs;

3. GC-MS analysis of essential oil demonstrated that for different genotypes in the shoots with leaf are identified from 14 to 23 components, in leafs from 17 to 25. Major components are represented by monoterpenes ketones: α-thujone (21.2-38.8%), β-thujone (5.877-16.201%), camphor (17.5-24.6%), followed by eucalyptol (6.47- 11.2%).

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