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## ESSENTIAL OIL COMPOSITION OF GARDEN SAGE TEA FROM DIFFERENT ORIGIN

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Garden sage (*Salvia officinalis* L.) has been grown and utilized as medicinal plant since ancient times. Due to its soothing effect and reported antioxidant activity, garden sage tea is currently a favorite recreational drink of many people. However, garden sage tea with different origins may possess differential chemical profile of the essential oil and hence, may have dissimilar aroma, flavor, taste, and other qualities. The objective of this study was to evaluate oil content and composition of six garden sage teas with different origin (one from Greece, two from Turkey, two from Ukraine, and one from Czech Republic). The essential oil of these teas was extracted by hydro-distillation and analyzed by gas chromatography (GC). We found a wide variation in essential oil composition of the tested teas and especially of the percentage of major oil constituents of total oil content: 1,8 cineole (5-45 %),  $\alpha$ -thujone (2-35 %),  $\beta$ -thujone (1-13 %), camphor (16-42 %), borneol (2-12 %), terpineol (1-10 %), sabinylacetate (<1-14 %) and terpinylacetate (1-5 %). Such a wide variation in essential oil composition may be a result of the environmental conditions, or a result of including floral parts in teas, but may also suggest the presence of different chemotypes of garden sage. Consumers should be aware that different garden sage teas with different origin may have different composition and possibly different flavor, aroma, or antioxidant activity.

**Key words:** *Salvia officinalis* L., content and composition, medicinal herbs, tea quality, thujones, chemotypes, environmental conditions

**Introduction.** Garden sage (*Salvia officinalis* L.) is a perennial aromatic plant (subshrub), native to the Mediterranean region. *Salvia* spp. belonging to the *Lamiaceae* family, and includes about 900 species that grow under diverse environments in various regions all over the world (Hedge, 1984). Garden sage is cultivated in several European countries for production of essential oil or dry herbage, which is utilized in tea production (Blumenthal et al., 2000).

Garden sage was used in ancient Egyptian, Greek, and Roman medicines. Ancient Egyptians used it as a fertility drug. The Greeks used it to stop bleeding of wounds and to clean ulcers and sores, towards hoarseness and cough, enhancing memory functions, for gargles to treat sore throats and mouths (Zimmermann et al., 2011). Garden sage is still used in domestic medicine, typically as an herbal tea preparation – an infusion of dried sage leaves with boiling water (sage tea) (Dweck, 2000). Various literature sources noted that more than 60 different health conditions or diseases might be treated by garden sage or its derivatives. Garden sage has carminative, antispasmodic, antiseptic, astringent and antihidrotic properties. Pharmacognostical handbooks describe that traditionally sage has been used to treat flatulent dyspepsia, pharyngitis, uvulitis, stomatitis, gingivitis, glossitis (internally or as a gar-

gle/mouthwash), hyperhydrosis, and galactorrhoea (Veličković et al., 2003; Then et al., 2004; Barnes et al., 2007).

It is difficult to obtain correct and updated information of the origin of garden sage in tea production occurring in various countries. Some of the material is collected in the wild, whereas some comes from cultivated fields. This diverse selection presents challenges in ensuring consistency in supply and quality of garden sage tea. There is no information on how garden sage teas with different origin compare with respect to chemical composition. Therefore, the objective of this study was to evaluate essential oil content and composition of six garden sage teas with different origin (one from Greece, two from Turkey, two from Ukraine, and one from Czech Republic) which were sold under different trade names but for the same purpose.

The European Pharma may vary depending on origin. Couladis et al. (2002) analyzed content and composition of 9 populations of garden sage growing wild in Montenegro and two native populations in Serbia. Average content of essential oil in leaves was 1.41 % from the Montenegro specimens and 1.66 % from the Serbian samples. Dob et al. (2007) reported 0.9 % essential oil in garden sage collected in Algeria.

Table 1.

*Sage teas used in the experiment*

product name	producer/country of origin	abbreviation
<i>Sage natura</i> - Original sage herbal tea	Greek product	A
<i>Saga herbal tea</i> - Sage portioned tea	DOGUS/Turkey	B
<i>Lipton sage Terk</i> - Sage portioned tea	ADACAYI/Turkey	C
<i>Šalvij listja</i> - Original sage herbal tea	Ukraine	D
<i>Folia Salviae</i> - Original sage herbal tea	BIOLA/Ukraine	E
<i>Sage herbal tea</i> - Original Sage tea	SERAFÍN SOKOLOV/Czech Republic	F

The yield of essential oil of 25 indigenous population of garden sage in Dalmatia ranged from 1.93 to 3.70 %, with an average of 2.83 % (Jug-Dujaković et al., 2012). The lowest content of essential oil was reported by Hamrouni Sellami et al. (2012) in infrared dried garden sage harvested in Tunisia (0.39 %). Ristić et al. (1999) analyzed the essential oil of eight populations of garden sage native to Montenegro and reported on the relations of oil composition and geographic origin. The impact on content and composition of essential oil is noticed by different factors as genetics or external environmental influence which may be the reason for different quality of herbal teas.

**Material and Methods.** Commercially available sage tea products consisting of sage herb were purchased in 2010 in Czech Republic, Ukraine, Turkey and Greece. For the purpose of this article we choosed the abbreviations A – F for the samples identification (Table 1).

Samples were chosen to be representative of available herbal teas in those regions, each in three samples (packs). All herbal teas samples, 2 g of each, were hydrodistilled for 2 h using a distillation apparatus of Coocking and Middleton (Humphrey, 1992). All hydrodistillations were carried out in triplicate. The oil was extracted with hexane and then dried by under vacuum in a vacuum rotary evaporator (Rotavapor, R-3 Büchi) at maximum 30°C. Essential oil samples were measured on analytical scale, and the essential oil content was calculated as percent (grams of oil per 100 g of dried garden sage tea). The essential oil samples were kept at 4°C in the dark, until the gas chromatography analysis.

The oils were analysed by gas chromatography (GC-FID) using a Hewlett-Packard 5890 Series II with FID, a split-split less system for injection, an HP-5 capillary column (50 m long x 0.20 mm i.d.) for constituent separation, and nitrogen as a carrier gas. The operating conditions were an injection temperature of 150°C, a detector temperature of 250°C and a temperature program beginning at 90 °C (0 min), 10 °C/min to 150 °C (5 min), 5 °C/min to 180 °C (3 min), then 7 °C/min to a final isothermal 280

°C for 25 min. The carrier gas flow velocity was 274 mm/s; auxiliary gases were nitrogen at 30 ml/min, hydrogen at 30 ml/min and air at 400 ml/min. Sample were 1.0 µl each and a manually injected. Peak areas and retention times were measured by electronic integration with a Hewlett Packard 3396 Series II integrator. These parameters are generally used to ensure the identified components.

The individual essential oil constituents were identified by respective GC retention time (RT), with either those reported in literature (Jennings and Shibamoto, 1980; Davies, 1990; Adams, 2001; De Martino et al., 2009), calculated Kovacs retention index (Ri) and compared with pure standards purchased from Sigma Aldrich, Bratislava, Slovakia. The Kovacs retention indexes (Ri) were determined in relation to a homologous series of n-alkanes (C8-C24) under the same operating conditions. Component relative concentrations were calculated based on GC peaks without using correction factors. The percentage of components of the oils was computed by the normalization method from the GC peak areas, calculated as mean values of three injections for each of oil samples, without using correction factors. Finally each compound was reported as a percentage of total oil. Statistical analysis was done by using multiple range test ANOVA on the significant level  $p < 0.01$  (Table 2).

**Results and Discussion.** Overall, the highest essential oil content was in the sample A and the lowest was in the sample B (Table 1). The essential oil content of the tested garden sage varied from 0.6 to 2.0 % of the total dry mass (Table 2). The amount of essential oil in garden sage of analyzed samples was within the range reported in the literature (Baser, 2000; Veličković et al., 2002; Veličković et al., 2003; Stojanov, 1972). In the essential oils from the garden sage teas in this study, we estimated 8 major oil constituents: 1,8 – cineol,  $\alpha$ -thujone,  $\beta$ -thujone, camphor, borneol, terpineol, sabinylacetate, terpinylacetate (Table 2). However, there was a wide variation in essential oil composition between oils from different teas origin.

Table 2.

## Content and composition of essential oils

Compounds name	RT	Ri <sup>a</sup>	Samples of sage teas [% of total essential oil]					
			A	B	C	D	E	F
1,8 - cineole	12.8	1034	43-45 <sup>a</sup>	20-22 <sup>b</sup>	5-6 <sup>c</sup>	11-12 <sup>d</sup>	5-6 <sup>c</sup>	15-16 <sup>e</sup>
$\alpha$ - thujone	15.4	1102	2-3 <sup>a</sup>	3-4 <sup>b</sup>	13-14 <sup>c</sup>	33-35 <sup>d</sup>	22-24 <sup>e</sup>	22-24 <sup>e</sup>
$\beta$ - thujone	15.7	1114	1-2 <sup>a</sup>	1-2 <sup>a</sup>	2-3 <sup>b</sup>	6-7 <sup>c</sup>	12-13 <sup>d</sup>	8-9 <sup>e</sup>
camphor	16.7	1145	18-21 <sup>a</sup>	16-17 <sup>a</sup>	40-42 <sup>b</sup>	18-19 <sup>c</sup>	20-22 <sup>d</sup>	28-30 <sup>e</sup>
borneol	17.5	1167	2-3 <sup>a</sup>	3-4 <sup>b</sup>	10-12 <sup>c</sup>	5-6 <sup>d</sup>	6-8 <sup>e</sup>	4-6 <sup>f</sup>
terpineol	20.6	1189	6-7 <sup>a</sup>	9-10 <sup>b</sup>	2-3 <sup>c</sup>	1-2 <sup>d</sup>	2-3 <sup>c</sup>	1-2 <sup>d</sup>
sabinylacetate	24.3	1290	$\leq 1^a$	12-14 <sup>b</sup>	2-3 <sup>c</sup>	2-3 <sup>c</sup>	4-5 <sup>d</sup>	$\leq 1^a$
terpinylacetate	25.2	1346	$\leq 1^a$	3-4 <sup>b</sup>	2-3 <sup>c</sup>	2-3 <sup>c</sup>	4-5 <sup>d</sup>	$\leq 1^a$
essential oil content [% of total dried herb]			2.0 $\pm$ 0.2 <sup>a</sup>	0.6 $\pm$ 0.1 <sup>b</sup>	0.8 $\pm$ 0.1 <sup>cd</sup>	0.8 $\pm$ 0.1 <sup>cd</sup>	0.7 $\pm$ 0.1 <sup>bc</sup>	0.9 $\pm$ 0.1 <sup>d</sup>

Note: RT – retention time; Ri<sup>a</sup> - Kovats retention index on HP-5MS column; ANOVA - significant level  $P < 0,01$ ; a,b,c,d,e,f heterogenous groups (the same letters in row means no significant differences).

1,8 cineol varied from 5 % (samples C and E) to 45 % of a total essential oil (sample A);  $\alpha$ -thujone from 2 % (sample A) to 35 % (sample D);  $\beta$ -thujone from 1 % (samples A and B) to 13 % (sample E); camphor from 18 % (samples A and D) to 42 % (sample C); borneol from 2 % (sample A) to 12 % (sample C); terpineol from 1 % (samples D and F) to 10 % (sample B); sabinylacetate from  $\leq 1\%$  (samples A and F) to 14 % (sample B) and terpinylacetate also from  $\leq 1\%$  (samples A and F) to maximum 5 % (sample E). Couladis et al. (2002) studied garden sage and revealed also that the oxygenated monoterpenes (1,8 cineol,  $\alpha$  - and  $\beta$  - thujone, camphor, borneol and bornyl acetate) were the most abundant, but their quantities varied over a wide range. Ristić et al. (1999) noticed the thujone content in the samples collected in the northern geographical localities significantly increased (34.85 %) with the comparing of the samples collected in southern localities (13.50 %), while the percentage of camphor, borneol and caryophyllene decreased. Because of the long distances of the origin of samples analyzed in this study, we are able to observe the similar findings. The highest content of thujone was observed in the samples D,E and F, which were obtained from Ukraine (37 – 40 %) and Czech Republic (35 %), countries localized northern, and the lowest amount was in samples A, B and C which were from Greece (3 %) and Turkey (4 - 17 %), countries localized southly.

Baser (2002) created classification of *Salvia* spp. according its chemical compositions. On the base of this classification it is possible that some of sage teas, which we investigated, did not contain garden sage but different species from genus *Salvia*. Samples A nad B could consist *S. fruticosa* (syn. *S. triloba*)

as a 1,8-cineol/camphor chemotypic group. Samples D, E, F by chemotypisation belonged to thujone group which is typical for *S. officinalis*. Another possible explanation of the differences between investigated sage teas could be different individual characteristics and agroecological conditions of the respective regions.

Investigation of aromatic and medicinal plants by chemical analysis showed that its curative power depends on the content and amount of components in their essential oils. The quantity and composition of garden sage essential oil is affected by genotype (Putievsky et al., 1992), but also by environmental factors (Perry et al., 1999). Determination of treatment quality parameters of tea helps not only to protect consumers, but also highlights the need to increase domestic production quality of this unique herb (Šalamon, 2000).

There is evidence that the analysis of the essential oil of garden sage herbal teas sold commercially in different countries had dissimilar oil content and composition, which points on different quality. It is important to carry out the quality monitoring of garden sage (and other herbs) drugs as a raw material used in the pharmaceutical, cosmetic and food industry.

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