Chemical Variation in Essential Oils of *Salvia Officinalis* L. Ecotypes Cultivated in Albania

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Abstract: *Salvia officinalis* L. was cultivated in different geographic locations of Albania. Sage plants originated from imported seeds and wild Albanian plants. Around 30 chemical compounds were identified in the essential oils of all cultivated sage plants; the latter were found to be very rich in *camphen*, *β-thujone*, *α-pinene*, *eucaliptol*, rich to moderate in *β-pinene* and *camphor*, and less rich in *α-thujone* and *limonene*. Sharp differences in chemical composition patterns and content of individual chemical compounds were noticed between and within cultivation sites. Cultivated sage was poorer in *α-thujone* versus wild plants. Cultivated sage, of Albanian wild plants origin, was the richest in *α-thujone* (18.45%) versus imported seeds sage. *β-thujone* over-dominated *α-thujone* in all cultivated sage plants. Volatile characters of cultivated sage were indicative of the species but not of the geographic origin of plant material. Variation in essential oils composition and chemical compounds' content (biosynthetic pathways) in cultivated sage is related more to the genetic background than the environmental factors. If cultivating sage in Albania, then wild local ecotypes would be best to use as *α-thujone* is maintained at satisfactory levels, local natural base is preserved, unnecessary hybridization with imported seeds sage is prevented, and are more resistant and cost effective.

Key words: Sage, Headspace-GC/MC, chemical composition, variation, cultivation.

1. Introduction

Albania, a lush Mediterranean country with cool, cloudy, wet winters and hot, clear, dry summers and terrain mostly mountainous and hilly with small plains along the Adriatic and Ionian coast, has been endowed by nature with a vast and unique diversity of MAPs (Medicinal and Aromatic Plants). The latter represent nearly 10% of Albania's flora [1]. Around 30% of all known European species of plants are found in Albania [2, 3]. Over 90% of MAPs are grown and collected from the wild. They are processed in the country and exported as dried, essential oils and fresh aromatic sachets [2, 3]. MAPs collection is an important source of income for thousands of rural families living in remote areas of the country [2, 3].

Albania's MAP exports, during 2013, reached 11,221ton experiencing an increase of 15% against

previous year [4].

Sage continues to be Albania's major MAPs export item [2-4] positioning Albania for years as leader in sage supply to the US market. Albanian sage accounted for 84% (Fig. 1) of total sage imports, in tones to the US market during 2014 [5].

There has been a significant increase in direct sage exports from Albania to the US market [5] over the years (Graph. 1). The increase is also attributed to the in country value adding of sage prior to export including organic certification, "ground" and "whole leaf" quality, up to-date processing equipment and facilities, etc. And that, in turn, has commended higher export prices providing more income to the Albanian supply value chain.

The growing market demand for Albanian wild sage (dried/essential oils) and inappropriate harvesting practices make imperative the identification and implementation of sustainable and commercially competitive supply options for instance "in situ"

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Fig. 1 Albania's market share in Sage imports to USA.

Graph 1 Trend of Albania's sage exports to USA.

cultivation if feasible economically, qualitatively and eco-friendly.

Cultivated sage plants were examined in the study in order to (1) identify the chemical composition profiles of each cultivated sage ecotype, (2) examine the chemical variation among sage ecotypes cultivated in different geographic regions of Albania, and (3) contribute to the research work on *Salvia officinalis* L. in Albania.

2. Materials and Methods

Salvia officinalis L. was cultivated in seven different geographic locations of Albania being North (Lezhë/LE; Koplik/KO), Northeast (Dibër/DI), Center (Lushnjë/LU), South (Libohovë/LB) and Southeast

(Skrapar-Strëneç/ST; Skrapar-Jaupas/JP). Sage plants were obtained from imported seeds, from Holland and Germany, except for one location (Dibër) where vegetative cuttings from wild local populations were used. Five samples per location were randomly picked prior to flowering stage (early June), and then fully dried at room temperature to avoid any potential contamination that would affect samples' quality.

Samples were analyzed at the LGC Health Sciences Fordham, Cambridgeshire/UK (www.lgcgroup.com) using Headspace-Gas Chromatography/Mass Spectrometry method. Analysis of the headspace fraction of the sage plant material was carried out with an Agilent GC 7890 (HP7890/MSD) with a mass spectrometer detector 5973 (Instrument reference/SS-GCMS-4) coupled to a Gerstel MPS Headspace Injection.

Approximately 140-150 mg of sample (dry sage leaves) was accurately weighed into a 10 mL headspace vial and heated for 10 min at 90 °C. Then 1000 µL of the headspace fraction was injected to the GC. The GC analysis was carried out with aRxi 624Sil MS column of 30 m long, 0.25 mm i.d. and 1.4 µm film thickness. The injector temperature was 200 °C and the oven temperature was programmed at an initial temperature of 40 °C for 10 min. The temperature was then increased at a rate of 12 °C/min up to 190 °C and retained at this temperature for 5 min. An additional programme was needed to elute the less volatile compounds at a rate of 6 °C/min up to 225 °C and retained at this temperature for 5 min. The carrier gas was helium with 1 mL/min flow rate, constant; the split ratio was 1:5. The make-up gas was nitrogen. The liner was a 4 mm split liner containing glass wool; spectrum purge was 3 mL per minute. Detector type was MSD, SCAN mode, 50-1000 m/z and the mass spectra were taken at 70 eV from 35 to 650 m/z. The source and quadrupole temperatures were 230 °C and 150 °C respectively. Components were identified comparing the retention times and spectral data from

the NIST standard reference database. Due to the quantity of peaks generated during the analysis, peaks with areas > 0.1% have been considered.

3. Results

Upon examination of thirty-five cultivated sage samples, around 30 compounds were identified including monoterpenes (comprising about 70%), sesquiterpenes, alcohols, acids, sulphides, etc. A snapshot of major chemical compounds identified in the sage plants according to cultivation site is presented below (Table 1):

Essential oils of cultivated sage in the present study showed to be highly rich in *camphene*, *α -pinene*, *eucalyptol*, *β -thujone*, rich to moderate in *β -pinene* and *camphor*, and less rich in *α -thujone* and *limonene*. Other compounds such as *β -caryophyllen*, *α -phellandrene*, *borneol*, *bornyl acetate*, *carene*, *cymene*, *β -ocymene*, *sterene* were found in small to trace amounts. Sporadically other compounds were identified such as *azulene*, *naphthalene*, *panasinsene*, *terpinolene*, *isoamilene*, *methylthymetan*, *trans- β -amylene*, *cyclene*, *1,5-hexadiene*, *dimethyl sulphide*, *1,5,5-trimethyl*, *ethanethiol*, etc.

Table 1 Qualitative and Quantitative variation of major chemical compounds amongst cultivated Sageplants.

Samples	α -thujone	β -thujone	Camphene	Camphor	Eucalyptol	α -pinene	β -pinene	Limonene	α -phellandrene	Cyclopropane, 1,1-dimethyl-2-(2-methyl-2-propenyl)-	Borneol	(+)-4-Carene	1,5,5-trimethyl-6-methylene-cyclohexene
DIBËR	1	13.8	17.4	17.9	8.8	8.5	10.9	8.9	2.3	2.1	2.5	0.7	-
	2	18.5	2.4	18	9	18.6	13.3	8.1	3.5	1.5	0.2	0.6	-
	3	5.1	15	14.5	5.4	19	15.2	9.3	3	1.4	3	0.5	-
	4	9	15.1	13.4	5.6	23	9.9	9.6	2.8	1.5	0.3	1.6	-
	5	4.6	18.4	14.6	4.2	8.7	26	6.1	3.3	1.4	2.5	0.5	-
LEZHË	1	6.2	24.2	14.9	11	4.6	6.9	6.7	4.3	1.5	10.6	0.2	0.4
	2	5.6	24.5	15.2	11	3.6	7	7.2	4.4	1	10.2	0.2	-
	3	3.1	11.5	22.2	-	4.9	20.8	9.9	5.7	0.3	1.6	0.2	-
	4	9.7	18	15.8	11.6	11.3	9.4	6.4	5	1.2	5	0.2	-
	5	2.9	17.7	20.5	14.9	7.6	10.7	6.1	6.1	-	5.8	0.5	-
KOPLIK	1	4.8	22.5	7.07	5.1	910	28.5	5.9	3.4	0.3	4.3	-	-
	2	4.6	20.8	7.5	5.2	9.6	29.4	5.9	3.5	0.8	4.2	-	-
	3	0.6	6.2	21.4	-	17.5	0	7.2	6.7	0.3	2.8	0.6	0.2
	4	5.5	18.8	14.7	9.3	16.3	11.2	6.9	4.4	1.4	4.2	0.2	0
	5	4.5	14.6	19.4	10	13.8	14	4.8	4.9	0.3	5.9	0.4	0
LUSHNJË	1	7	20.2	10.4	6.2	11.1	-	6.6	3.3	1.4	3.4	0.3	23.4
	2	12.4	13.7	16	8.4	14.7	11.6	10	2.6	2.3	2.8	0.2	0.2
	3	2	13.2	23.4	13.2	6.2	16.4	-	4.7	0.2	2.7	0.7	0.2
	4	3	18.2	17.2	9	17.3	10.6	9	5.7	1.7	2.6	0.2	0.1
	5	6.1	19.7	16.2	9.3	12.8	10.5	-	3.7	0.2	5	0.3	0
STRËNEC	1	3.3	12.6	17.8	-	14.7	13.6	9.5	3.6	0.2	4.3	0.6	0.3
	2	3.4	19.7	15.9	10.1	14.8	10.2	7.2	4	0.3	7.6	0.2	0.6
	3	2.2	11.7	21.2	11.8	13.2	15	9.9	4.7	0.2	2.1	0.8	0.2
	4	4	21.2	9.85	5.4	12.3	19.8	7.6	2.3	0.23	8.5	0.3	-
	5	2.8	15	18.7	10.8	17.3	13.2	7.4	5	0.3	3	0.2	-
JAUPAS	1	4.7	14.7	21.9	12.9	7.5	13.8	7.8	4.6	2.2	4.4	0.3	-
	2	2.5	7.7	22	10.9	7.9	20	5.3	5.8	0.4	1.8	0.9	-
	3	2.5	8.9	-	-	7.7	15.6	5.1	6.1	0.3	1.8	0.6	-
	4	9.7	20.2	13.9	6.5	7.3	11.3	4.5	10.9	1.7	1.7	0.5	0.3
	5	1.8	8.4	28	-	5	18	4.6	7	0.3	2.41	0.5	-
LIBOHOVË	1	6.3	17.9	10.86	5.3	15.4	19.7	-	3.4	1.5	4.3	0.2	-
	2	11.3	13.4	16.2	6.6	11.6	14.7	11.9	2.8	2	4	0.3	0.2
	3	4	10.3	15.66	9.3	19.3	13.9	-	4.7	1.5	3.8	0.5	-
	4	5.7	10.1	12.1	6.5	16.3	25	12.3	4	1.2	1.6	0.2	-
	5	12.3	23	13	5.5	11.5	15.5	9	1.9	1.8	1.5	0.2	-

4. Discussion

Bellomaria [6], following previous work made by Garcia et al. (1975) and Cabo et al. (1987) [cit.7,8] described four groups of *Salvia* species based on the main components of their essential oils; species rich in *incineole* and *thujone* (*S. officinalis* L.), species rich in *camphor* and *cineole* (*S. lavandula* Vahl.), species rich in *linalool* and *linalyl acetate* (*S. sclarea* L.) and species containing *terpenoids* of different types. Results of the present study indicate that sage plants, even though cultivated, were quite rich in *cineole* and *thujone*; this is related to the species type, which in this case is *S. officinalis* L. Therefore, essential oils are important informative characters of a given species. The “cultivated” status may explain the high contents of particularly *camphene*, α - and β -*pinene*, which in wild sage populations are at much lower levels [9-11].

Franz [12] indicates that variation in the composition of essential oils within a given species appears to be a rule rather than exception; and is influenced by three major factors: (a) individual genetic variability, (b) plant parts and developmental stage, and (c) environment. All these factors influence plants' biosynthetic pathways, and subsequently the relative proportions of their compounds.

In the present study (Table 1), variation in the essential oils profiles of cultivated sage between locations was quite distinct, which one would think to be related to the environmental differences. However, significant differences were noticed even within the same cultivation site where content of a given chemical compound varied from zero to maximum levels; for instance α -*pinene*/Koplik (0-29.34%), *camphene*/Jaupas (0-28%), *camphor*/Lezhë (0-14.9%), β -*pinene*/Libohovë (0-12.22%). This variation may be explained with the origin of the plant material, which were imported seeds (breeding/selection). But, most importantly chemical differences were noticed even amongst sage plants, originating from and cultivated within the

same eco-zone of the wild ecotypes (Dibër case). These differences may be attributed to the age and developmental stage of the mother plants (ontogenesis), time of obtaining the cuttings, variability within the same plant population due to potential hybridization over the years (genetic background), etc.

It is worth mentioning extreme occurrence of a given compound, despite the geographic location of the cultivation site or origin of the mother plant. For example, *1,5,5-Trimethyl-6-methylene-cyclohexene* was absent in all sage plants under investigation except for Jaupas/JP2 where it reached a maximum of 26.62%, or (+)-*4-carene*, which was almost lacking in all cultivated sage plants but one, Lushnjë/LU1, where it reached a maximum level of 23.42% (Table 1). This is related to the genetic make up of each individual sage plant.

Sage plants cultivated in Dibër, originating from wild Albanian ecotypes, were the richest in *thujone* (total of α - and β -) of all locations reaching 20-31.08%. Whereas, Koplik and Lezhë in particular, which were the closest to Dibër geographically but imported seeds were used, generated much lower levels of it varying 6.8-27.27% in Koplik and 14.61-30.34% in Lezhë. Furthermore, sage plants of the Dibër location (local ecotypes used for cultivation) were the richest in α -*thujone* (single compound) of all sage plants of all sites under study (Table 1). There were differences in α -*thujone* content even between sage plants of this location, varying from 4.6-18.45% (up to 4 times), yet the Albanian ecotypes, even though brought to cultivation in lower altitudes and with less sun exposure, still generated much higher α -*thujone* amounts of all cultivated sage plants of all locations. This may be explained with the origin of the mother plants (*Albanian ecotypes* versus *imported seeds*) rather than the environmental factors.

Wild sage of Northern Albania (Lezhë, Koplik, Shkodër) is characterized by high amounts of α -*thujone* [9-11]. In the present study, cultivated sage

of imported seeds, generated much lower contents of α -thujone reaching 0.607-5.5% (Koplik) and 2.86-9.7% (Lezhë). Thus, the same environmental factors (same soil texture/structure and climate) generated essential oils profiles quite different from those of the wild populations. This once again emphasizes that genetic background dominates significantly the environmental factors.

Variation in α -thujone content between *cultivated sage* (present study) and *wild sage* [9, 10], for the same geographical locations, is presented graphically (Graph 2). It is quite noticeable the sharp difference in α -thujone content; this may be explained with the “status” of the sage plants (*spontaneous* or *cultivated*), which leads to plants genetic background of the plant material.

There has been long debate over the years on the side effects of thujone. Scientific Committee on Food of the European Council [13] indicates that α -thujone acts as a GABA blocker and is 3 times more potent than β -thujone, and that thujone causes epileptiform convulsions and seizures in humans. However, it is agreed that the database regarding thujone exposure via medicines should be expanded; yet current limits (up to nearly 7mg/day) are sufficiently protective for consumers [13-17]. Consumers and businesses’ education is vital; on the other hand meeting markets’ demand is important, too. Thus, if α -thujone content will be the commercial indicator of the sage batches for export from Albania, then wild sage, especially Dalmatian ecotypes of the north of Albania that are proved [9-11] to be rich in α -thujone, would be best to be used sustainably as source of supply (carry on the consolidated tradition of Albania in wild sage exporting). If cultivating sage, then northern α -thujone rich local ecotypes (versus imported seeds) would be an attractive/efficient option as α -thujone content is maintained at satisfactory levels, the wild local recourse base is preserved, unnecessary hybridization of Albanian sage population with imported seeds produced sage is avoided, and most importantly are

cost effective.

β -thujone over-dominated α -thujone in all cultivated sage plants under study, despite the geographic location and origin of plant material. Averaging the content of α -thujone of sage samples per each location, separately from β -thujone, the variation in α - and β -thujone content (in %) between locations is quite sharp (Graph 3). Therefore, if thujone low ecotypes (rich in β -thujone) are preferred, then cultivated sage can be considered as an option for supplying “thujone safe” sage products. However, one must make sure that there is a sound demand for Albanian cultivated sage products in order to best orient investments in time and capital.

Asllani [9, 10] and Schmiderer [11] provide sound evidence on the geographic origin of the Albanian sage based on the essential oils profiles. Wild Albanian

Graph 2 α-thujone content, wild vs cultivated sage.

Graph 3 Differences in α- and β-thujone in cultivated sage.

sage, North chemotypes, are quite rich in thujone (α -thujone over dominating β -thujone), poor in *camphor*, poor in *1,8 cineole*, and very poor in α - and β -pinene and *camphene*. South chemotypes are rich in *camphor*, rich in *1,8 cineole*, poor in *thujone* (in both α - and β -thujone), and very poor in α - and β -pinene and *camphene*.

The present study reveals that cultivated sage plants, despite the geographic location of the cultivation site (North, Northeast, Centre, South, Southeast) all followed the same pattern—considerably poor in α -thujone, very rich in β -thujone, relatively poor in *camphor*, rich in *1,8 cineole*, and very rich in α - and β -pinene and *camphene* (Table 1).

This pattern is quite different from what has been previously reported [9-11] on wild Albanian sage. Therefore, unlike wild sage, essential oils of cultivated sage do not allow for accurate characterization of plants' geographic origin. However, essential oils profiles may be informative characters in terms of the origin of the plant material (*wild* versus *cultivated*).

It was quite obvious that each sage plant had a unique chemical composition pattern of essential oils; thus each cultivated sage plant represented an individual sage chemo-type. This significant variation might be influenced somehow by various factors such as ontogenesis (in agreement with [18]), cultivation schemes such as harvesting time, sun exposure, altitude, irrigation, drainage, use or fertilizers, etc. (in agreement with [19]), and environmental factors (in agreement with [20]). But above all, it is related mainly to the quality of the plant material (genetic make-up). This finding is in agreement with [7, 21-24] which concluded that variation in the volatile oils pattern is more related to the genetic background of each individual (intrinsic factors) rather than to environment (extrinsic factors).

There is a need in Albania for a closer collaboration between the herb and spice industry, academic and research institutions and relevant stakeholders to fully explore and inform on the current state of the herb and

spice ecotypes and their respective profiles. Furthermore, training and education on the preservation of Albanian natural resources as well as the international markets' demand, requirements and trends is crucial for sustainable and competitive development of the Albanian herb and spice industry in the long run—"sustainable business is good business". There is an immediate need for the identification and monitoring the "commercially competitive initiatives" i.e. cultivation. Recent chaotic developments in the country have led to sage cultivation with no prior accurate technology and market knowledge. Even though sage cultivation might be an attractive option yet Albanian farmers/collectors and entrepreneurs would be best to consider the economics and ecological aspects of it.

5. Conclusions

Headpace GC/MS was very efficient in the chemical profiles' characterization of cultivated sage plants.

Cultivated sage plants were found very rich in *camphen*, β -thujone, α -pinene, *eucaliptol*, rich to moderate in β -pinene and *camphor*, and less rich in α -thujone and *limonene*.

Significant chemical variation existed *between* and *within* cultivation sites; the basis for this variation is related to the genetic background rather than environmental factors.

Chemical differences even amongst cultivated plants, originating from the wild Albanian ecotypes, are also explained with the genetic make-up of each individual plant rather than the environmental factors.

Hence, biosynthetic pathways (chemical composition profiles, relative proportions of each chemical compound) are mainly determined by the intrinsic factors (genetic background of each individual plant) rather than extrinsic factors.

Albanian sage ecotypes would be best to consider if/when cultivation activities undertaken due to similarity of their chemical composition profiles to the

wild sage and plants morphology, resistance to pest/diseases and harsh weather conditions.

Research work is needed to identify the current wild plant ecotypes/genotypes and their chemical profiling as well as appropriate genotypes, eco-zones and techniques for cultivation. This will greatly impact the preservation of Albanian authentic resource plants base and the competitiveness of the Albanian herb and spice export products.

It may be prudent that Albanian MAP exporters always declare status (“wild”; “cultivated”) of their sage export products especially as Albania wishes to join the European Union and that the international buyers have developed innovative traceability techniques (even at the DNA level).

Essential oils patterns of cultivated sage are not indicative of the geographic origin of the plant material; yet these secondary metabolites provide significant informative evidence to the academic and, in particular, the business community. Hence

- If *α-thujone rich sage* is preferred, then wild Albanian sage, especially from the North, would be best to (sustainably) collect/supply;

- If *α-thujone rich sage* is preferred, alternatively cultivated sage originating from local Albanian ecotypes would be best to work with;

- If *α-thujone low sage* is preferred, then cultivated sage can be an attractive option.

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