P 4. CHEMICAL CHARACTERISATION OF ESENTIAL OIL FOR NATURAL AND CULTIVATED SALVIA *OFFICINALIS FROM NORTH ALBANIA*

Aurel Nuro[1*], Bledar Murtaj[1], Aurora Nuro[2], Arjeta Murtaj[3], Aida Dervishi[4], Dhimitër Peçi[5]

[1] *University of Tirana, Faculty of Natural Sciences, Department of Chemistry*
[2] *"Faik Konica" non-Public High School, Tirana*
[3] *"Selman Daci", High School, Fushe-Kruje*
[4] *University of Tirana, Faculty of Natural Sciences, Department of Biotechnology*
[5] *University of Tirana, Faculty of Natural Sciences, National Center of Flora and Fauna*

E-mail: aurel.nuro@fshn.edu.al

ABSTRACT: This study present chemical data on essential oil samples of natural and cultivated *Salvia Officinalis* plant populations from North Albania. *Salvia Officinalis* is a widespread plant that is native in Mediterranean area and almost in all Albania territory. It is part of *Lamiaceae* family. Areal parts of *Salvia Officinalis* have been used since ancient times in culinary, cosmetics and traditional medicine. Sage has different properties (antiseptic, aromatic, carminative, estrogenic, stimulant, etc) and thought to have positive effects in human brain functions. *Salvia Officinalis* plants from Tropoja (five samples from natural habitat and four samples from cultivated areas) were selected in June 2019. The air-dried plant samples were cut in small pieces (1-2 cm). They were subjected of hydro-distillation for 4 hours using Clevenger type apparatus, recommended to European Pharmacopoeia, to obtain *Salvia Officinalis* essential oil. The chemical composition of the essential oils was obtained using GC/FID technique. VF-1ms capillary column (30 m x 0.33 mm x 0.25 um) were used for separation of its compounds. Main constituents (20 compounds) were found from 92.6% to 98.7% in all studied sage samples from Tropoja (North Albania) whether it was cultivated or natural plants. Their profile was the same between two population and similar to other studies from Mediterranean and Balkan area. It was as follow: alpha-Thujone > Camphor > Cineol > beta-Thujone > Camphene > Alfa-Humulene > beta-Caryophyllene > alpha-Pinene. Alpha-Thujone (the main constituent) was found in higher percentage in cultivated *Salvia Officinalis* plants because of agricultural areas used for their grow and farmer work. Plant harvesting time can influence in differences between constituents in sage samples.

Keywords*: Salvia Officinalis, Essential oil, alfa and beta Thujone, Camphor, Cineole, GC/FID.*

1. INTRODUCTION

In this study, natural and cultivated *Salvia Officinalis* plants from Tropoja, North Albania, were chemically characterized by using GC/FID technique. Albania is a reach country with medicinal plants due to appropriate Mediterranean climate. Around 3,200 various medicinal herbs, of which 350 species are exported ensuring that Albania develops its position as an important exporting country (Asllani, 2004). It's the largest exporter of *Salvia officinalis* and many other herbs mostly in Germany, USA, France, etc. The export of medicinal plants is important for Albanian economy. After 90' the medicinal herbs industry is decreased. Before 90' Albania earned about 3-4 times more than recent years. The green export success could help to improve the precarious economic situation, especially in rural areas. Local wild medicinal herbs have been seen as a means of overcoming a short-term emergency rather than as a stable economic sector. Wild plants collection and their farming is a considerable potential for developing the medicinal herbs industry. Collection areas and types of medicinal herbs according to their value should be clearly defined by the authorities and also keep statistics on volume, prices and trading companies (Kathe *et al*, 2003).

Salvia officinalis population is a member of Lamiacea family. It is native to the Mediterranean region and has naturalized in many places throughout the world. *Salvia officinalis* population is widespread in all Albanian territory. Albania is one of the main exporters of its (plant and essential oil) all over the world (Asllani, 2004; Kathe et al 2003). *S. officinalis* plants have been used since ancient times for snakebites, increasing women's fertility, and more. Also, it was called *Salvia salvatrix* (sage the savior). Sage is recommended as a diuretic, hemostatic, emmenagogue, and tonic. Areal parts of sage have many notable plant-derived chemical compounds, essential oils, minerals, vitamins that are known to have disease preventing, and health promoting properties. The prime biologically active component of common sage appears to be its essential oil. It contains mainly ketones; α-thujone, and β-

thujone. In addition, sage leaf contains numerous other compounds, including cineol, borneol, tannic acid; bitter substances like cornsole and cornsolic acid; fumaric, chlorogenic, caffeic and nicotinic acids; nicotinamide; flavones; flavone glycosides and estrogenic substances. Altogether, these compounds were known to have counter-irritant, anti-inflammatory, anti-allergic, anti-fungal and anti-septic properties (Kamatou et al 2008).

The main constituent, alfa-Thujone is GABA and Serotonin (5-HT3) receptor antagonist (Akhondzadeh et al 2003). It improves mental concentration, attention-span and quickens the senses; hence sage infusion has long been recognized as "thinker's tea." Fresh sage leaves are a good source of antioxidant vitamin such as vitamin-C. Vitamin C helps in the synthesis of structural proteins like collagen. Its adequate levels in the body help maintain integrity of blood vessels, skin, organs, and bones. It scavenges harmful, pro-inflammatory free radicals from the body.

2. MATERIAL AND METHODS

Sampling of *Salvia officinalis* from North Albania

Salvia Officinalis plants were selected in five natural stations and four cultivated areas of Tropoja region in altitude between 1200-1600 m above sea level. Sampling procedure was realized in June 2019. Areal parts of pplant samples were air dried in a dark room.

Isolation of *Salvia officinalis* essential oil

Dried plant material (50 g of *Salvia Officinalis*) was subjected to hydro-distillation for 6 hours, using a modified Clevenger-type apparatus to produce essential oil based on European Pharmacopoeia 3rd Edition. 2 ml Toluene was added to the balloon for isolation of *Salvia officinalis* essential oils. The oil was dried by anhydrous sodium-sulphate (Na_2SO_4) and kept sealed in dark glass vial at +4°C until use.

Apparatus and chromatography

Gas chromatographic analyses of *Salvia officinalis* essential oil were realized with a Varian 450 GC instrument equipped with a flame ionization detector and PTV injector. The temperature of PTV injector was 280°C. 1 ul of *Salvia officinalis* essential oil diluted in Toluene was injected in splitless mode. FID temperature was held at 280°C. Nitrogen was used as carrier (1 ml/min) and make-up gas (25 ml/min). Hydrogen and air were flame detector gases with 30 ml/min and 300 ml/min, respectively. VF-1ms capillary column (30 m x 0.33 mm x 0.25 µm) was used to separate compounds of *Salvia officinalis* essential oil. The oven temperature was programmed as follows: 40°C (held for 2 minutes) to 150°C (with 4°C/min), after that to 280 °C with 10°C/min and held for 2 minutes. The identification of the compounds was based on comparison of their Kovats indices (KI), their retention times (RT) and literature (Adams, 1995; David et al., 2010, Bozin et al., 2006). A mixture of n-alkanes from n-octane (C8) to eicosanes (C20) was used for calculation of Kovats indices (KI).

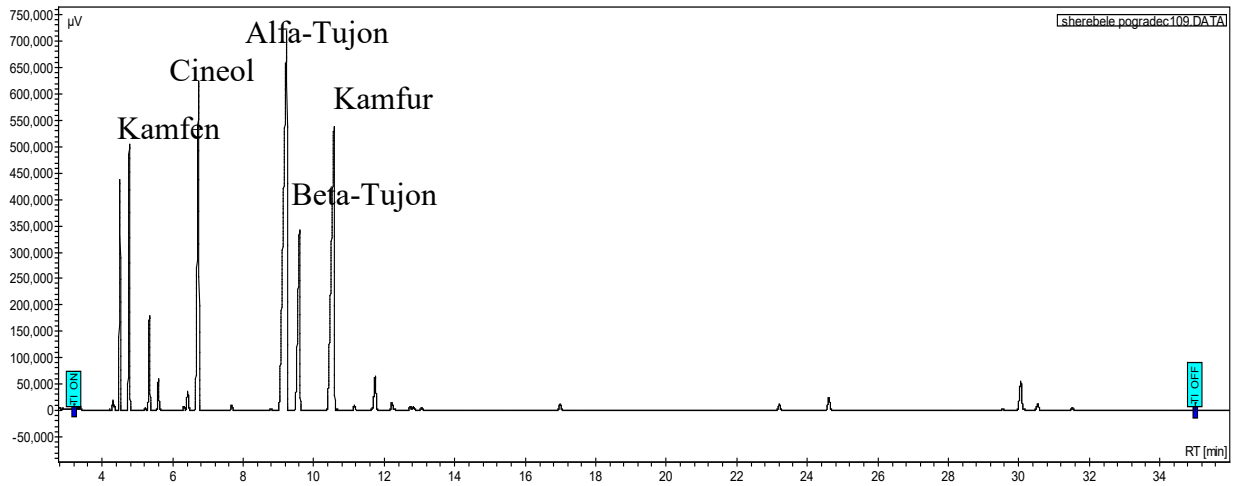

Figure 1. Main compounds of natural *Salvia Officinalis* essential oils from Tropoja

3. RESULTS AND DISCUSSION

Natural and cultivated *Salvia officinalis* samples from Noth Albania were analyzed, by using GC/FID technique. The data present average of 20 main compounds that were identified for all analyzed samples in both types. Note that, in chromatograms of *Salvia Officinalis* essential oil samples were detected more than 120 compounds. The peaks lower than 0.01% was not considered in this study. Percentage of 20 main compounds in all analyzed (natural and cultivated) *Salvia Officinalius* essential oil samples from North Albania was shown in Table 1. Their total in natural and cultivated samples was respectively 97.5% and 98.8%. It was observed the same profile for all analyzed samples (natural and cultivated). Profle of analyzed components for natural and cultivated *Salvia officinalis* samples was: alfa + beta-Thujone > Camphur > Cineole > Humulene > Camphene > beta-Caryophyllene (Figure 2). *Salvia Officinalis* essential oils were found to have higher concentrations for the total of alpha and beta-Thujone which are the prime biologically active component (Figure 3). The cultivated samples were shown the higher concentrations of these compounds (39.9%) because of these plants are grown in agricultural areas while natural samples in mountain areas (36.0%). Alfa-Thujone was the main component for both samples type (30.3% for natural and 35.6% for cultivated sage samples). This could be connected with the geological factors, the latitude, humidity, the harvesting time, etc. Camphor was the second compound with 19.1% for natural and 17.4% for cultivated samples. Cineole was found the third compound with 9.4% for natural and 9.7% and cultivated samples. The main group for both sample types were monoterpenes. Their total were 89.8% for natyral and 91.5% for cultivated samples (Figura 4). Seskuiterpenes (beta-Cariophyllene and alpha- Humulene) were found in high percentage in natural samples with 7.7%. Their content in cultivated samples was 7.3%. Figure 5 shown percentages of monoterpene groups in natural and cultivated *Salvia Officinalis* samples. The main group between monoterpenes was oxygenated (Cineole, Linalool, alpha-Thujone, beta-Thujone, Camphor, Borneol, Terpilen-4-ol, alfa-Terpineol, Bornil acetat) that were found from 68.2% (natural) to 71.8% (cultivated). Bicyclic monoterpenes (alpha-Pinene, Camphene and beta-Pinene) was the second group for natural and cultivated sage plants respectively with 12.0% and 12.3%. Monocyclic monoterpenes (alfa-Terpinene, Limonene, gama-Terpinene) were found higher in natyral samples (7.1%) than in cultivated one (5.7%). Myrcene (Aliphatic monoterpene) was around 1.3% and para-Cimene (aromatic monoterpene) was found with 0.3% for both sample types. Profile and leveles of main compounds found in natural and cultivated *Salvia Officinalis* samples from North Albania was the same with other reported studies from Mediterrean area (Daferera et al., 2000; Radulesku et al 2004; Kamatou et al, 2008).

Table 1. Percent of compounds dedected in analyzed *Salvia officinalis* essential oil samples from North Albania, June 2019

	Rt	Natyral	Cultivated
alfa-Pinene	4.32	4.19 ± 1.03	4.94 ± 1.27
Camphen	4.41	5.56 ± 0.94	5.11 ± 1.73
beta-Pinene	5.22	2.19 ± 0.73	2.33 ± 0.81
Myrcene	5.34	1.19 ± 0.42	1.17 ± 0.32
Limonene	6.41	0.27 ± 0.05	0.21 ± 0.04
alpha-Terpinene	6.47	6.32 ± 1.42	5.31 ± 1.63
Cineole	6.73	9.43 ± 3.21	9.65 ± 2.42
para-Cimene	7.33	0.32 ± 0.10	0.33 ± 0.07
gama-Terpinene	7.98	0.28 ± 0.07	0.15 ± 0.04
Cis-Sabinene hydrat	8.13	0.14 ± 0.04	0.23 ± 0.04
Linalool	8.44	0.17 ± 0.05	0.10 ± 0.03
alpha-Thujone	9.12	30.34 ± 3.25	35.62 ± 4.28
beta-Thujone	9.21	5.67 ± 1.27	4.29 ± 1.43
Camphor	10.52	19.12 ± 3.41	17.44 ± 3.62
Borneole	11.78	2.40 ± 0.93	2.43 ± 0.88
Terpinen-4-ol	12.21	0.47 ± 0.11	0.46 ± 0.06
alpha-Terpineole	14.43	0.25 ± 0.06	0.31 ± 0.05
Bornil acetat	16.95	1.47 ± 0.33	1.39 ± 0.35
beta-Cariophyllene	23.14	3.29 ± 0.64	3.40 ± 0.83

alpha-Humulene	24.65	4.42 ± 1.53	3.91 ± 0.94
Total		97.49	98.80
Total Monoterpene's		89.77	91.49
Monocyclic Monoterpene's		8.11	5.80
Bicyclic Monoterpene's		11.95	12.25
Aliphatic Monoterpene's		1.24	1.27
Oxygenated Monoterpene's		68.17	71.83
Aromatic Monoterpene's		0.30	0.34
Seskuiterpenes		7.72	7.31

Natural plants usually grow to higher altitudes where air temperatures are lower and soils are poorer. This may be the reason for the lower percentages of Thujones in them. Cultivated plants grow in fertile areas, under human care such as planting time, irrigation, ripening time, etc. This may be the reason for the higher percentages of Thujones in these plants. It should be noted that peoples prefer to use natural sage plants more often due to the better taste and effects they have. In many lands where sage is cultivated today, it has been used for crops, fruits and vegetables. So, cultivated plants may affected by previous use of pesticides, other chemicals and agricultural agromechanics.

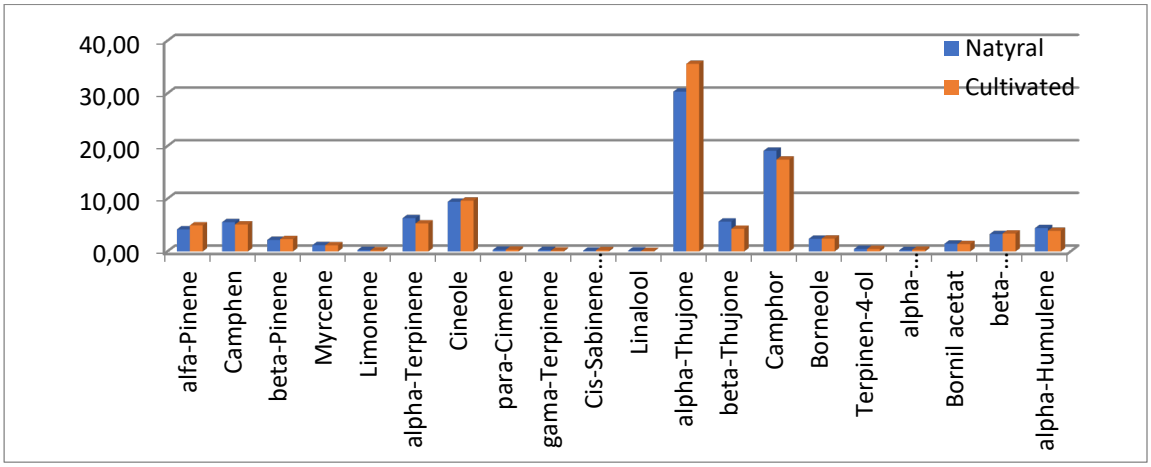

Figure 2. Profile of main constituents in natural and cultivated *Salvia officinalis* samples

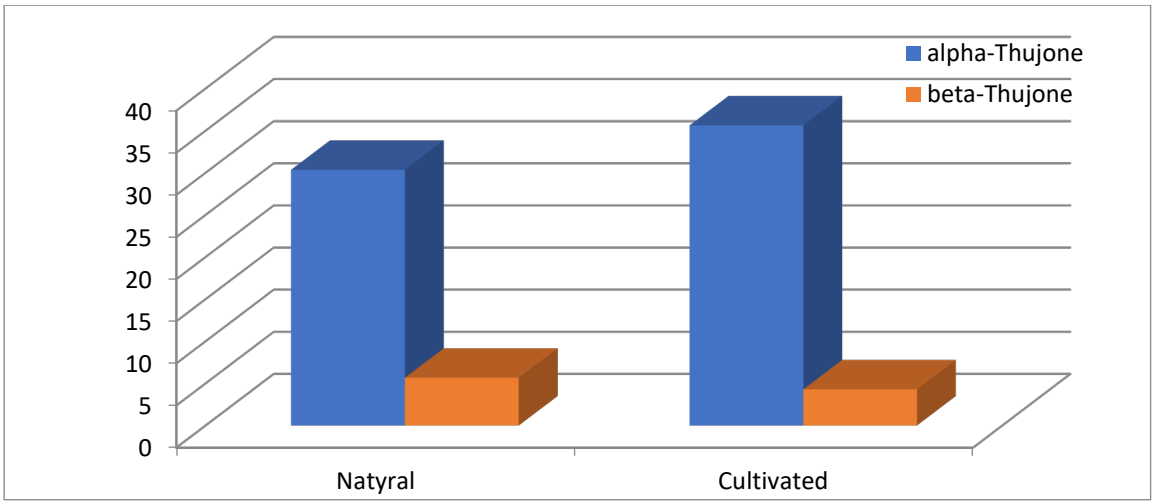

Figure 3. Percentage of Thujone's in natural and cultivated *Salvia officinalis* samples

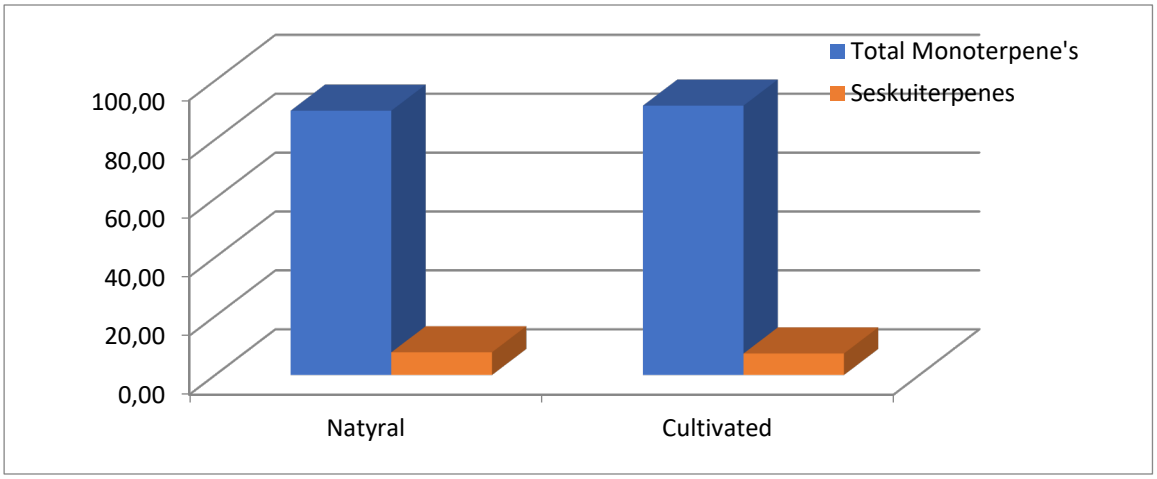

Figure 4. Monoterpenes and sesquiterpenes in natural and cultivated *Salvia officinalis* samples

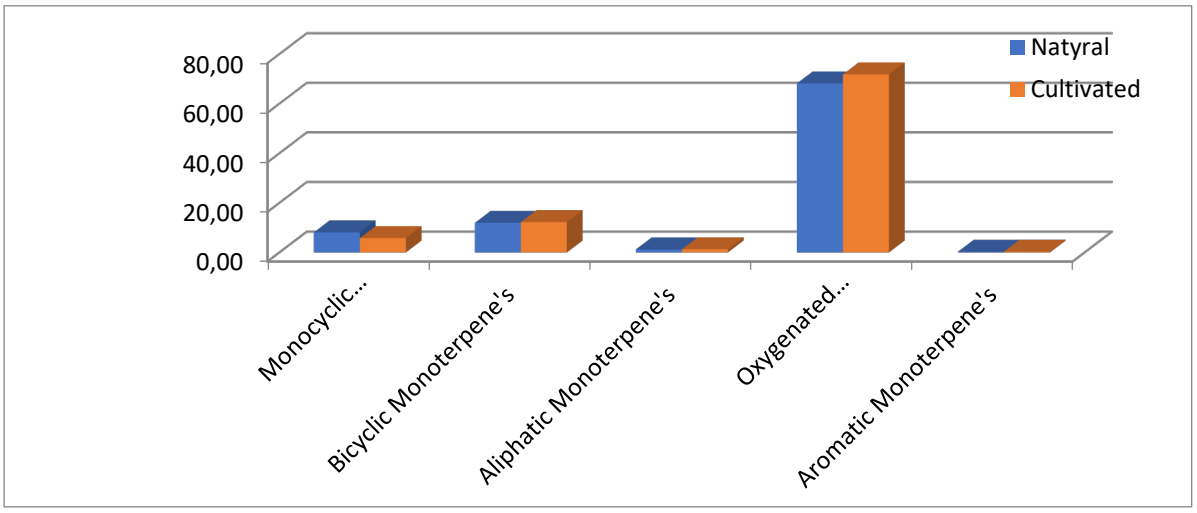

Figure 5. Distribution of monoterpenes in natural and cultivated *Salvia officinalis* samples

4. CONCLUSIONS

Natural and cultivated essential oil of *Salvia officinalis* samples from Tropoja (North Albania) were analyzed using GC/FID technique. Gas chromatography technique is recommended by the literature. Averages of results for both sample types present the total for 20 main compounds that were found for analyzed *Salvia Officinalis* essential oil. It was observed the same profile for all analyzed samples (natural and cultivated). α-Thujone, β-Thujone, Camphor, Cineol, Borneol, alfa-Terpinene, alfa-Pinene and beta-Pinene. *Salvia Officinalis* essential oils were found to have higher concentrations for the total of alpha and beta-Thujone that are the prime biologically active component. The cultivated samples were shown the higher concentrations of these compounds because of these plants are grown in agricultural areas that are more fertile and under the farmer care. Natural plants usually grow to higher altitudes where air temperatures are lower and soils are poorer. Differences could be connected with the geological factors, the latitude, humidity, the harvesting time, etc. The main group for both sample types were monoterpene's. The main groups between monoterpenes were oxygenated monoterpenes, bicyclic and monocyclic monoterpenes for both natural and cultivated sage plants. Aliphatic and aromatic monoterpenes were found in lower level. Profile and leveles of natural and cultivated *Salvia Officinalis* samples from North Albania was the same with other reported studies from Mediterrean area. The work would be complete if it continues for several years for shown better the differences between natural and cultivated *Salvia Officinalis* plants.

REFERENCES

Adams RP. (1995), Identification of essential oil components by gas chromatography/mass spectroscopy, Allured Publishing Cor-poration, Carol Stream: Illinois, USA.

Akhondzadeh S, Noroozian M, Mohammadi M, Ohadinia S, Jamshidi AH, Khani M. (2003). "Salvia officinalis extract in the treatment of patients ëith mild to moderate Alzheimer's disease: a double blind, randomized and placebo-controlled trial". *J Clin Pharm Ther* 28 (1): 53–9.

Asllani U., (2004) Esenca e bimëve aromatike e mjekësore të trevave Shqiptare

Bozin B., Mimica-Dukic N., Simin N., Anac-kov G. (2006), Characterization of the volatile composition of essential oils of some Lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils, J. Agric. Food Chem.,54, 1822-1828.

Council of Europe (1997). European Pharmacopoeia, 3rd edn. Strasbourg: Council of Europe.

Daferera D.J., Ziogas B.N., Polissiou M.G. (2000), GC–MS analysis of essential oils from some greek aromatic plants and their fungitoxicity on Penicillium digitatum, Jour-nal of Agricultural Food Chemistry, 48, 2576–2581.

David F., Scanlan F., Sandra P., Szelewski M. (2010), Analysis of essential oil compounds using retention time locked methods and retention time databases, Application, Agilent Technologies, 5988-6530EN.

Kamatou GPP, Makunga NP, Ramogola ËPN, Viljoen AM. South African Salvia species: A revieë of biological activities and phytochemistry. J Ethnopharmacol, 2008; 119: 664–672.

Kathe W., Honnef S. & Heym A., (2003) Medicinal and Aromatic Plants in Albania, Bosnia-Herzegovina, Bulgaria, Croatia and Romania. A study of the collection of and trade in medicinal and aromatic plants (MAPs), relevant legislation and the potential of MAP use for financing nature conservation and protected areas" WWF Deutschland / TRAFFIC Europe-Germany.

Radulescu V, Chiliment S, Oprea E. Capillary gas chromatography–mass spectrometry of volatile and semi-volatile compounds of Salvia officinalis. J. Chromatogr A, 2004; 1027: 121–126.