Proceeding Book of ISESER 2021

O 50. CHEMICAL CHARACTERISATION OF OREGANO VULGARE ESSENTIAL OIL FROM SOUTH-EAST ALBANIA

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ABSTRACT: In this paper were present chromatographic data of *Oregano vulgare* essential oil from Kolonja area located in South-East Albania. *Oregano vulgare* is a flowering plant of mint family (Lamiaceae). It is native to the Mediterranean region. *Oregano vulgare* can find almost in all Albania areas. Since ancient times it has been used in culinary and traditional medicine. Aeral parts of *Oregano vulgare* were sampled in the end of the June for a five years period (2015-2019) in Kolonja area. The air-dried leaves samples were subjected to European Pharmacopoeia apparatus (Clevenger type) for 4 hours to obtain *Oregano vulgare* essential oil. The leaves contain 1.43 - 1.74% essential oil. The chemical composition of the essential oils was analyzed using GC/FID technique. *Oregano vulgare* essential oil samples were injected in a Varian 450 GC. VF-1ms capillary column (30 m x 0.33 mm x 0.25 um) were used for separation of terpene compounds. Monoterpenes were in higher percentage in all studied *Oregano vulgare* essential oil samples between 87.5% to 89.4%. Aromatic monoterpenes (p-Cymene, Thymol and Carvacrol) were found as the main constituents (74.4 - 81.2%) because of fenolic type for this essential oil. Profile of *Oregano vulgare* was the same for all years despite the differences in atmospheric conditions and harvesting time. Profile and leveles of *Oregano vulgare* samples from Kolonja, South-East Albania was similar with other reported studies from Balkan and Mediterrean area. plants.

Keywords: *Oregano vulgare L., Essential oils, Terpene, Thymol, Carvacrol, Gas chromatography*

INTRODUCTION

Origanum vulgare is a flowering plant in the mint family (Lamiaceae). It was native in the Mediterranean region and almost in all Albanian teritory. It has purple flowers and spade-shaped, olive-green leaves. It is a perennial, although it is grown as an annual in colder climates. It does not survive the winter. Oregano is planted in early spring, the plants being spaced 30 cm apart in fairly dry soil. It will grow in a pH preferred range between 6.0 and 8.0. It prefers a hot, relatively dry climate, but does well in other environments.

Oregano is a culinary herb, used for the flavor of its leaves, which can be more flavorful when dried than fresh. It has an aromatic, warm, and slightly bitter taste, which can vary in intensity. Good-quality oregano may be strong enough almost to numb the tongue, but cultivars adapted to colder climates may have a lesser flavor. Factors such as climate, season, and soil composition may affect the aromatic oils present, and this effect may be greater than the differences between the various species of plants. Among the chemical compounds contributing to the flavour are carvacrol, thymol, limonene, pinene, ocimene, and caryophyllene.

Oregano contains polyphenols, including numerous flavones. The essential oil of oregano is composed primarily of monoterpenoids and monoterpenes, with the relative concentration of each compound varying widely across geographic origin and other factors. Over 60 different compounds have been identified, with the primary ones being carvacrol and thymol ranging to over 80%, while lesser abundant compounds include *p*-cymene, γ-terpinene, caryophyllene and δ-terpineol.

Organo vulgare is an herb with a wide range of pharmacological properties such as antimicrobial, gastrointestinal, and nervous system effects. Studies carried out on the chemical composition of the plant

have shown that the main chemical compounds present in *M. longifolia* essential oil are monoterpenes, particularly oxygenated ones. Essential oils derived from Organo have valuable pharmacological properties that have been investigated by many scientists around the world. Due to their antimicrobial, insecticidal, antifungal, and antibacterial activities, essential oils have been intensely screened and applied in the fields of pharmacology, medical and clinical micro-biology, phytopathology and food preservation (Daferera et al., 2000).

MATERIALS AND METHODS

Reactive and standards

Toluene of chromatographic grade and anhydrous Na2SO4 were purchased from Sigma Aldrich. A mixture of n-alkanes (Sigma Aldrich) from n-octane (C8) to eicosanes (C20) was used for calculation of Kovats indices (KI).

Sampling of *Oregano vulgare* L. from South-East Albania

Oregano vulgare samples were taken from population of Kolonja area, located in South-East Albania. Aeral parts (branches, leaves and flowers) of *Oregano vulgare* plants were sampled in the second week of the June for a five years period from 2015 to 2019 in four station of Kolonja area (Shtika, Starja, Borova, Barmashi and Leskoviku). Material plants were air dried in shadow for saving their morphological characteristics.

Isolation of *Oregano vulgare* esential oil

Dried plant material of *Oregano vulgare L.* were cut in small pieces (0.5 to 2 cm) before analyze. 50 g of plant material was subjected to hydrodistillation for 4 h, using a modified Clevenger-type apparatus to produce essential oil. 1 ml Toluene was added to the exctracting balloon for isolation of *Oregano* essential oils. The oil was dried by anhydrous sodium sulfate (Na_2SO_4) and kept sealed in dark glass vial at +4 ºC until use. Diluted essential oil in Toluene was used for GC/FID analyse.

Apparatus and chromatography

Gas chromatographic analyses of *Oregano vulgare e*ssential oil were realized with a Varian 450 GC instrument equipped with a flame ionization detector and PTV detector. The temperature of PTV injector was 260ºC. 1 ul of oregano essential oil diluted in Toluene was injected in splitless mode. A temperature for FID 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 isolate compounds of *Oregano vulgare L.* essential oil. The oven temperature was programmed as follows: 40°C (held for 2 minutes) to 150ºC (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, Konig et al., 1999; Bozin et al, 2006). Chromatogram of the *Oregano vulgare* essential oil for Leskoviku 2018 sample was shown in Figure 1.

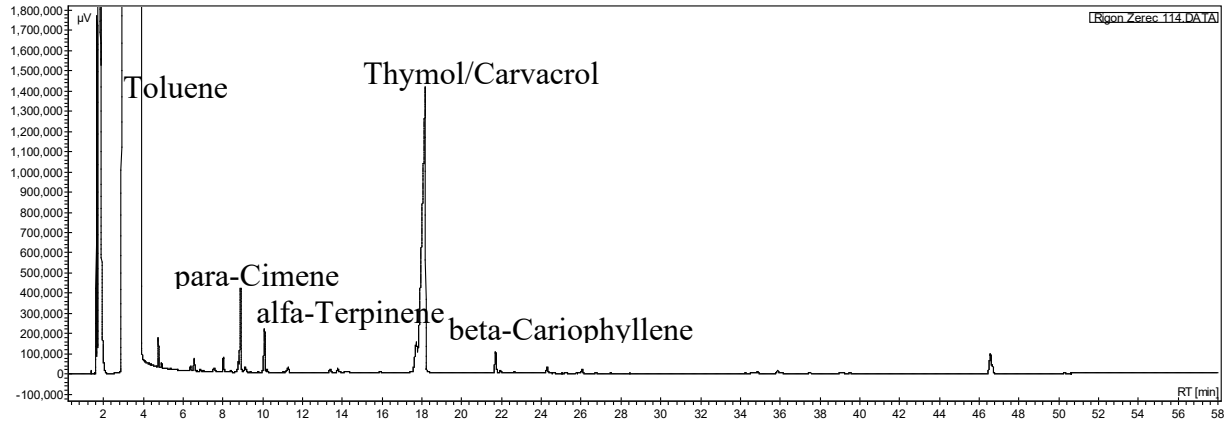

Figure 1. Chromatogram of *Oregano vulgare* essential oil from Kolonja, South-East Albania

RESULTS AND DISCUSSION

Essential oil samples of *Organo vulgare L.* from Kolonja area, located in South-East Albania were analyzed using GC/FID technique. All oregano samples were taken at Gramozi and Melesini mountains at an altitude of more than 1000 m above sea level. Average results of samples for one year were presented in this study. The data shown percentage for the total of peaks except for the peak of Toluene that was the solvent used for extraction. For individual *Organo vulgare* essential oil were found from 40 to 50 compounds. The peaks lower than 0.1% was not present in this study. The data present the total of 22 main compounds that were found for all analyzed organo essential oil. Their total (average value) were found from 92.6.0% (2015) to 97.4% (2018). Terpenes that were found in higher percentage were: Carvacrol, para-Cymene, gamma-Terpinene and beta-Caryophyllene.

Table 6 shows the average percentages of main components analyzed for Oregano vulgaris from the Kolonja area (2015 – 2019). Note that distribution of terpenes in *Oreganum vulgare* samples for a five years period it was almost the same for all analyzed samples. Samll differences were noted between the stations (analyzed samples in a year) and between data collected for one year. These differences could be because of geographic and geologic factors for studied areas. Latitude and the position of the plants in the places where they are growing could affect in their composition. Also, humidity, air temeperature, harvesting time and soil composition could affect in their composition as well as in their differences. Based in these data and chemical structure of terpenes were calculated the total of terpene groups. Figure 2 shows the distribution of the main compounds analyzed in oregano plants from South-East Albania. The main compounds found in *Oreganum vulgare* were: Carvacrol (57.3 - 76.2%) > Thymol (0.8 - 4.5%) > para-Cimene (5.2 - 14.2%) > gamma-Terpinene (2.4 - 3.9%) > beta-Caryophillene (2.4 - 4.9), etc. Other compounds are from 0.5 - 3%. This profile was similar with oregano essences from other areas of Albania. In essential oil of Kolonja area was noted a high percentage of Carvacrol and a very low percentage of Thymol.

Table 1. Averages of main compounds in analyzed Organo essential oil samples from South-East Albania, 2015 - 2019

	Rt	Year 2015	Year 2016	Year 2017	Year 2018	Year 2019
alfa-Pinene	4.32	0.09 ± 0.02	N.D.	0.81 ± 0.17	0.47 ± 0.19	0.53 ± 0.15
Camphene	4.41	0.78 ± 0.17	0.21 ± 0.05	0.30 ± 0.06	1.6 ± 0.52	0.67 ± 0.04
beta-Pinene	5.22	0.14 ± 0.04	N.D.	0.04 ± 0.01	0.25 ± 0.04	N.D.
Myrcene	5.34	0.42 ± 0.07	0.09 ± 0.02	0.07 ± 0.02	2.55 ± 0.52	0.52 ± 0.13
Limonene	6.41	0.78 ± 0.43	0.77 ± 0.17	2.56 ± 0.71	1.48 ± 0.35	1.55 ± 0.51
alfa-Terpinene	6.47	0.83 ± 0.11	0.38 ± 0.09	1.83 ± 0.48	0.99 ± 0.17	0.76 ± 0.15
para-Cymene	6.91	5.34 ± 2.01	5.15 ± 1.72	14.20 ± 4.82	6.13 ± 1.16	7.17 ± 2.16
gama-Terpinene	7.33	3.20 ± 1.38	2.37 ± 0.58	3.92 ± 1.16	2.18 ± 0.73	5.18 ± 1.18

Linalool	7.98	0.15 ± 0.02	0.57 ± 0.08	0.20 ± 0.05	0.62 ± 0.07	0.64 ± 0.14
Camphour	8.13	0.08 ± 0.01	0.50 ± 0.07	0.04 ± 0.01	N.D.	N.D.
Borneol	8.44	0.15 ± 0.03	N.D.	0.02 ± 0.01	0.68 ± 0.07	0.47 ± 0.09
Terpinen-4-ol	9.12	0.79 ± 0.21	N.D.	0.19 ± 0.04	0.69 ± 0.11	0.58 ± 0.09
alfa-Terpineol	9.21	N.D.	N.D.	0.23 ± 0.06	0.32 ± 0.10	0.61 ± 0.10
Bornyl acetate	10.52	0.45± 0.05	N.D.	0.34 ± 0.08	0.27 ± 0.07	N.D.
Carvone/Pulegone /Citroneol	11.82	0.42 ± 0.07	0.57 ± 0.08	2.77 ± 0.83	1.41 ± 0.53	1.47 ± 0.27
Thymol	12.01	0.74 ± 0.49	1.09 ± 0.27	2.87 ± 0.71	8.49 ± 1.83	6.49 ± 0.93
Carvacol	13.23	75.04 ± 7.25	76.23 ± 7.88	57.30 ± 11.42	65.9 ± 7.52	62.91 ± 8.63
beta-Cariophyllene	16.95	2.64 ± 0.82	2.42 ± 0.63	4.85 ± 1.13	2.91 ± 0.63	2.93 ± 0.53
alfa-Humulene	23.14	0.55 ± 0.08	5.73 ± 1.32	1.42 ± 0.43	0.44 ± 0.08	0.46 ± 0.07
Total		92.57	96.09	93.93	97.4	93.47
Total Monoterpene's		89.39	87.93	87.61	94.06	90.13
Monocyclic monoterpene's		4.81	3.53	8.31	4.64	7.64
Bicyclic monoterpene's		1.01	0.21	1.12	2.33	1.4
Aliphatic monoterpene's		0.42	0.09	0.07	2.55	0.55
Oxygenated monoterpene's		2.03	1.64	3.79	4.02	4.02
Aromatic monoterpene's		81.13	82.47	74.37	80.52	76.52
Sesquiterpene's		3.19	8.15	6.27	3.34	3.34

The terpenoic groups found in *Oreganum vulgare* samples were: aromatic monoterpenes, oxygenated monoterpenes, monocyclic monoterpenes, bicyclic monoterpenes, aliphatic monoterpenes and sesquiterpenes (Figure 3). Monoterpenes were found in higher level for all oreganum samples ranged from 87.6% to 89.4% The main monoterpene group found in all analyzed oregano samples was aromatic monoterpenes (Figure 4). Aromatic monoterpenes (para-Cimene, Thymol and Carvacrol) were found from 74.4 to 82.5%. These essential oils belong to phenolic types due to the high percentages of Carvacrol in them. Percentage of Carvacrol was found between 57.7% (2017) to 76.2% (2016). Its percentages are conected with humidity, air temperature, harvesting time in respective years. Oxygenated monoterpenes (Linalool, Kamfur, Borneol, Terpilen-4-ol, alfa-Terpineol, Pulegon, Carvon, Citroneol and Acetat bornili) were found in range from 1.6% to 4.1% (Figure 5). Even in lower percentage oxygenated monoterpenes are important for oregano taste and aroma. Note that Oreganum vulgare from Kolonja area is one of the most wanted in Albanian market. Monocyclic monoterpenes (alpha-Terpinene, Limonene and gamma-Terpinene) were the second group in oregano essential oil with 3.5 - 8.3% (Figure 6). Their profile for all samples was: gamma-Terpinene > alfa-Terpinene > Limonene. Figure 7 shows bicyclic monoterpenes in *Organo vulgare* essential oil samples of South-East Albania. Alfa-Pinene, Kamfene and beta-Pinene were found from 0.2 to 1.8%. Myrcene was representative of alicyclic monoterpenes (Figure 8). Its percentage was found from 0.1 - 0.5%. Figure 9 shows sesquiterpenes in analyzed Organo vulgare essential oil samples from Kolonja area (2015 – 2019). Beta-Cariophyllene and alfa-Humulene were found from 3.2% to 8.2%. Beta-Cariophyllene was found in higher percentage for all oregano samples except the sample of year 2016. The data obtained from analyzed samples of *Oreganum vulgare* of Kolonja area for a five years period showed that chemical composition of them was the same at the time because of their origin. Geographical position of oregano plants especially altitude, air temperatures, humidity and geological composition of the soil are important factors that affect directly in their chemical composition. These results were similar to other reported data of *Oreganum vulgare* essential oils from the Balkans and the Mediterranean area.

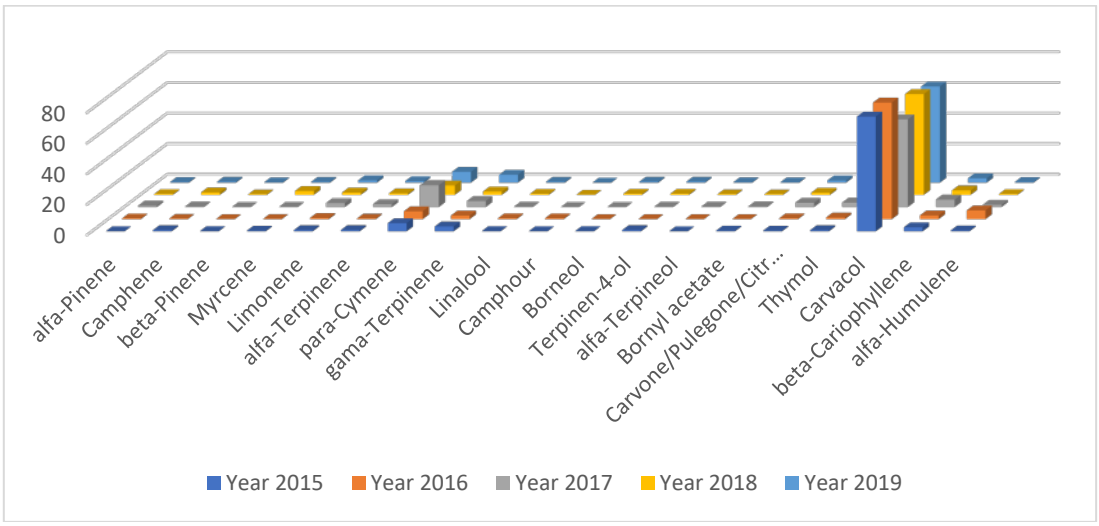

Figure 2. Distribution of terpenes in *Organo vulgare* essential oil samples from Kolonja area

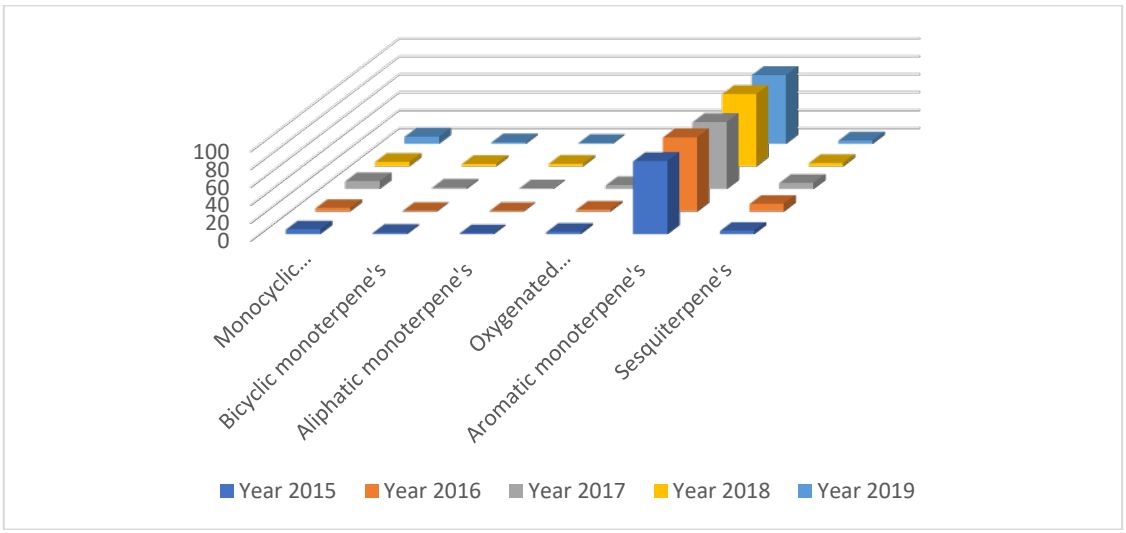

Figure 3. Main groups of terpenes in analyzed *Organo vulgare* essential oil samples

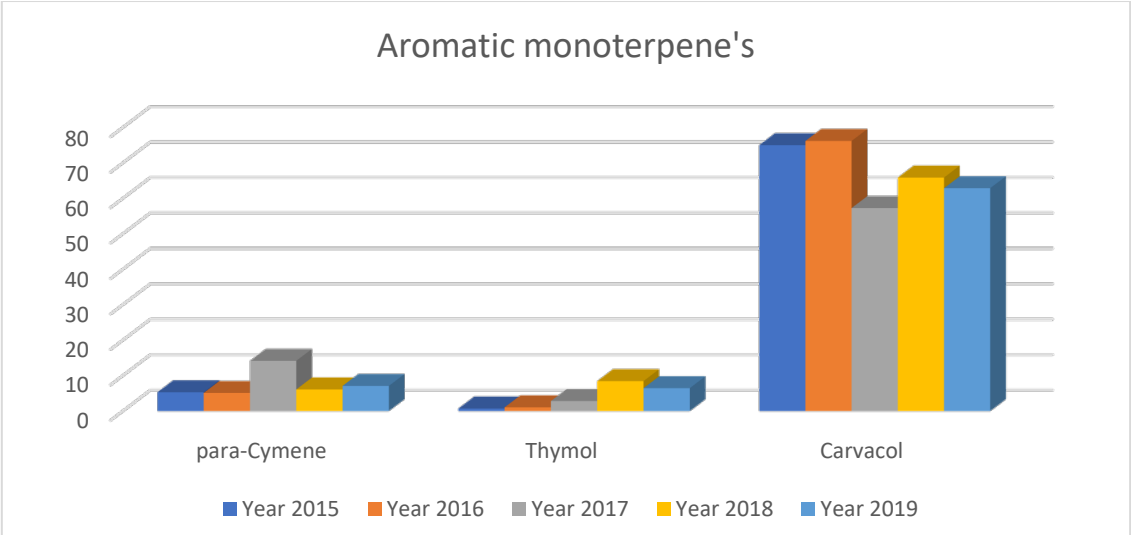

Figure 4. Aromatic monoterpenes in analyzed *Organo vulgare* essential oil samples

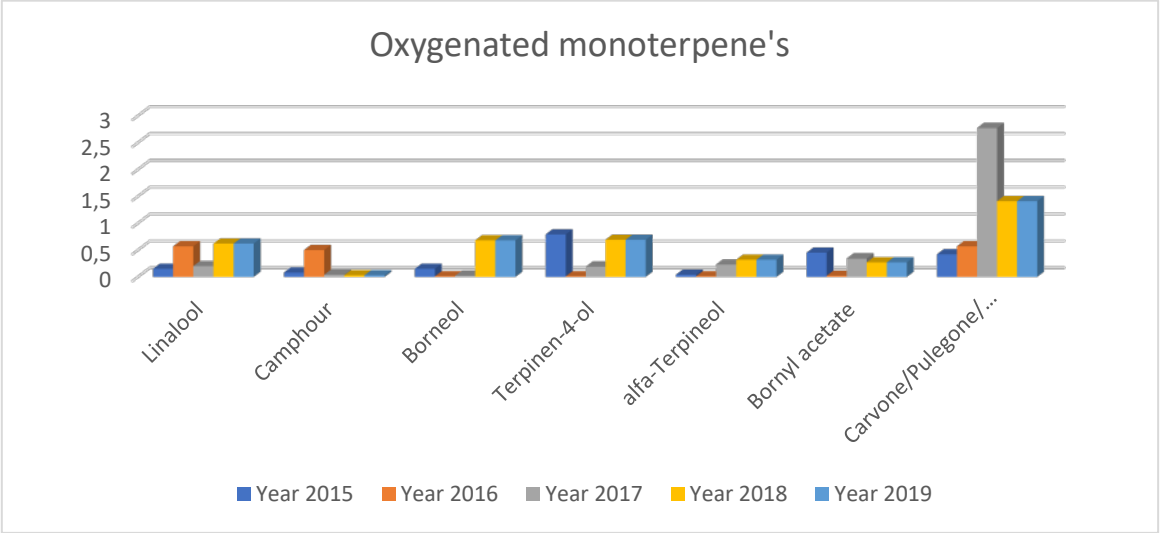

Figure 5. Oxygenated monoterpenes in *Organo vulgare* essential oil samples from Kolonja area (2015 – 2019)

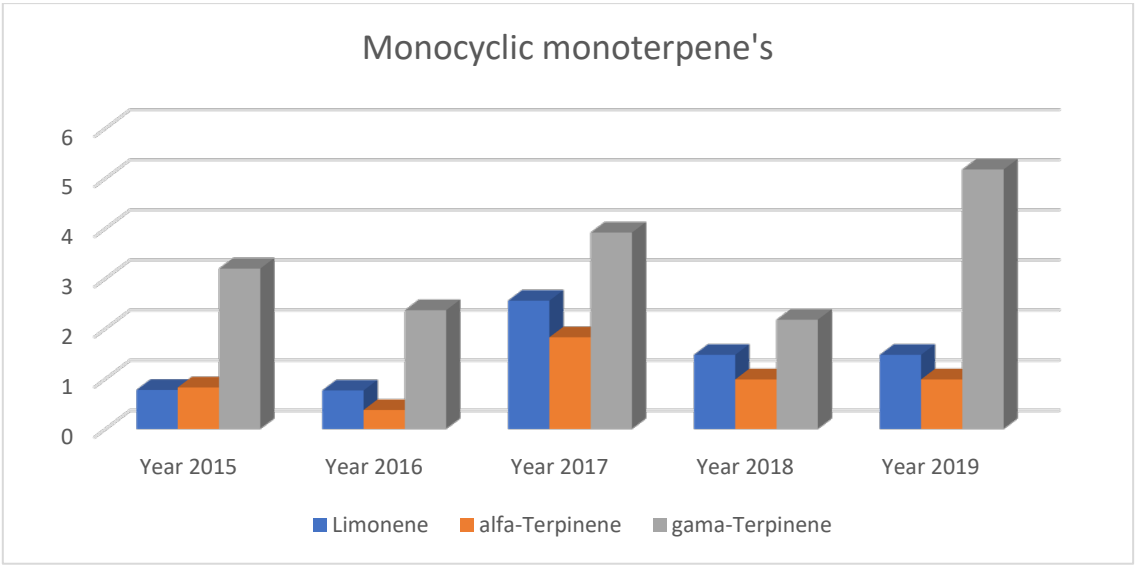

Figure 6. Monocyclic monoterpenes in analyzed *Organo vulgare* essential oil samples

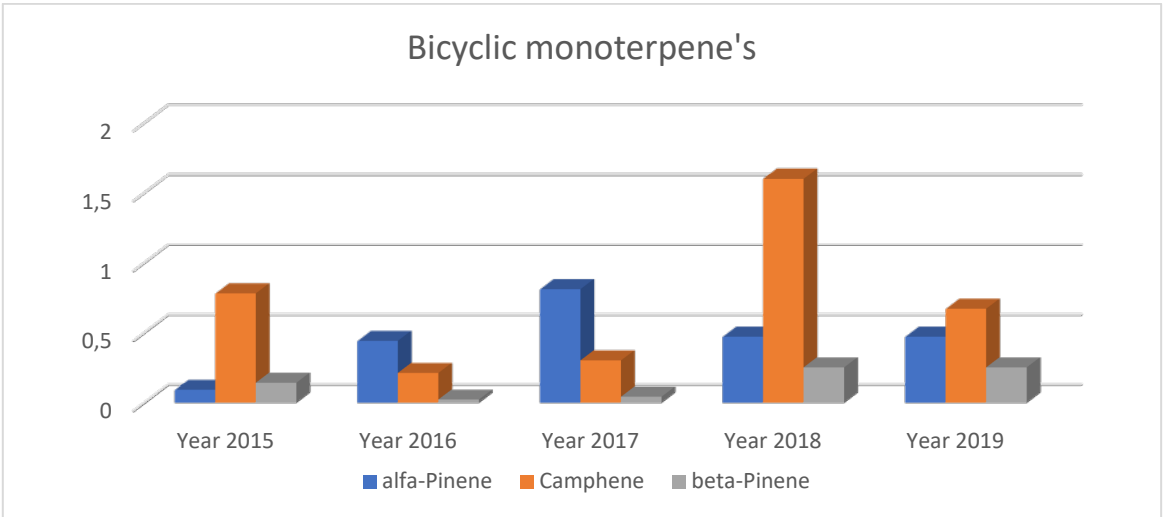

Figure 7. Bicyclic monoterpenes in *Organo vulgare* essential oil samples of South-East Albania

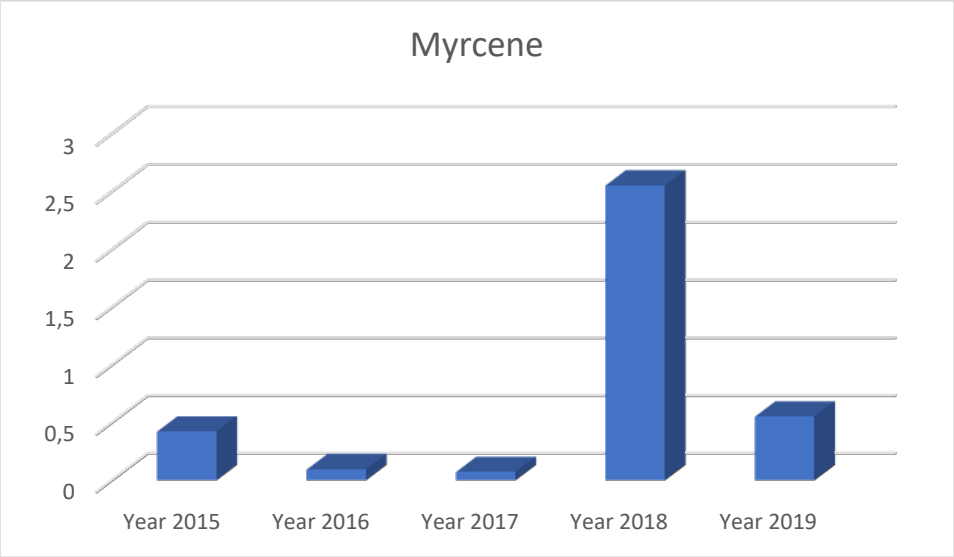

Figure 8. Aliphatic monoterpenes (Myrcene) in essential oil of organo samples

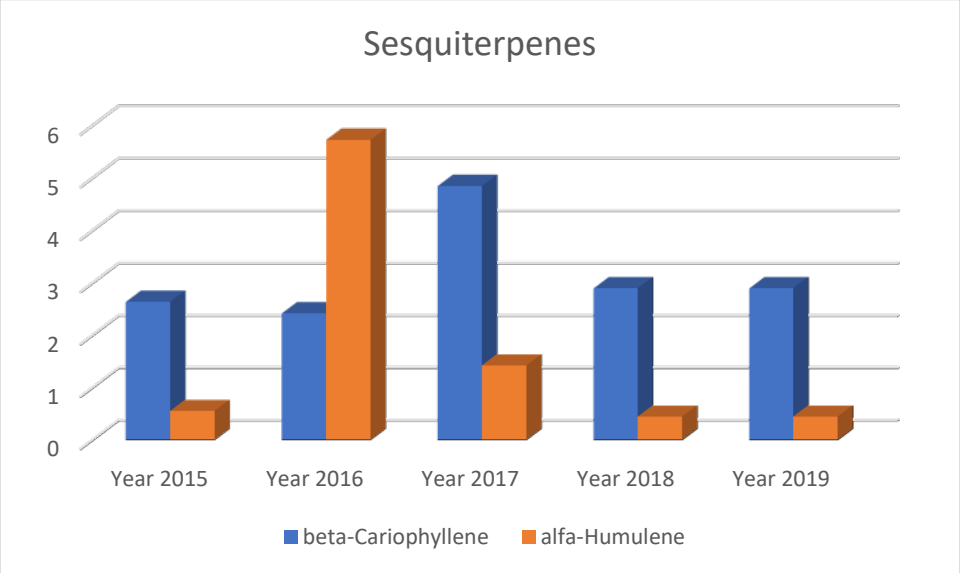

Figure 9. Sesquiterpenes in analyzed Organo vulgare essential oil samples from Kolonja area (2015 – 2019)

CONCLUSIONS

Essential oil of *Organo vulgare L.* samples from Kolonja area (South-East Albania) were analyzed using GC/FID technique. Oregano samples were taken at Gramozi and Melesini mountains at an altitude of more than 1000 m above sea level. Average results of samples (five stations) for one year were presented in this study. Chromatograms of *Organo vulgare* essential oil shows 40 to 50 compounds in total. The data present 22 main compounds found for all analyzed samples with a total of 92.6.0% to 97.4%. Carvacrol, para-Cymene, gamma-Terpinene and beta-Caryophyllene were identify in higher percentage for all analyzed samples. Monoterpenes were found in higher level for all oreganum samples. The main monoterpene group found in all analyzed oregano samples was aromatic monoterpenes. In essential oil of Kolonja area was noted a high percentage of Carvacrol and a very low percentage of Thymol. These essential oils belong to phenolic types due to the high percentages of Carvacrol in them. Distribution of terpenes in *Oreganum vulgare* samples for five years period it was almost the same for all analyzed samples. Samll differences were noted between the stations (between analyzed samples in a year) and between data collected for one year. These differences could be due to geographic position, geologic factors, humidity, air temeperature, and harvesting time. Profile and leveles of *Organo vulgare L.*

samples from South-East Albania was the comparable with other reported studies from Balkan and Mediterrean area.

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