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Genetic variation of oregano (*Origanum vulgare* L.) for etheric oil in Albania

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ABSTRACT

In this study are presented the results of studies for oregano in 16 locations (sites) of Albania for the content of the quantity of oils and their fractions. Albania is the one of the important diversity centers for Oregano because of their adaptation to diverse agro ecological conditions as a result of natural selection. The design of the experiment was based on split plot methods. From the total samples collected, were selected 16 mostly widespread samples of natural populations of origano (*Origanum vulgare* L. *sp. vulgare* and *sp. hirtum*). Essential oil isolation from oregano was performed by hydro distillation. Identification of the components was made by comparing mass spectra of components in essential oils with those from Nist, Wiley and Adams mass spectra libraries. The results show that the total oil content varies from 3.45% to 0.1%. The populations of Oregano in the part of southern Albania have a higher amount of etheric oil compared with those of the North part. The higher content of carvacrol in 55.63%, of the samples was determined in sites of the southern part of Albania, while in the other two investigated area central and north part it was 55.63% and 48.49% respectively. The same indicators were realised also for content of linalool and thymol, while North area has sites with high content of caryophyllen-oxide and *β*-pinen 3. But, in all sites, the oils of thymol and carvacrolit were dominant.

Keywords: Origanum; Essential Oil; Carvacrol; Thymol

1. INTRODUCTION

The genus *Origanum* is a member of the *Lamiaceae* family and has a complex taxonomy [1]. *O. vulgare* plays a primary role among culinary herbs in world trade [2]. It is distributed all over Europe, West and Central Asia up to Taiwan [3]. The use of *O. vulgare* as medicinal plant is believed to be due to biological properties of *p*-cymene and carvacrol. Bernáth [4] has noted that there are intraspecific taxa of oregano having no "oregano" character that is based on the presence of carvacrol. Oregano is the common name for a general aroma and flavour primarily derived from more than 60 plant species used all over the world as a spice [5]. Four main groups of plants commonly used for culinary purposes can be distinguished, *i.e.*, Greek oregano (*Origanum vulgare ssp. hirtum* (Link) Ietswaart); Spanish origano (*Coridohymus capitatus* (L.) [2]; Turkish oregano (*Origanum onites* L.); and Mexican oregano (*Lippia graveolens* HBK [6]. Origano is the commercial name of those *Origanum* species that are rich in the phenolic monoterpenoids, mainly carvacrol and occasionally thymol [7]. A number of chemically related compounds *i.e. p*-cymene; *γ*-terpinene, carvacrol methyl ethers, thymol methyl ethers, carvacrol acetates and thymol acetates; as well as *p*-cymenene, *p*-cymen-8-ol, *p*-cymen-7-ol, thymoquinone, and thymohydroquinone are present in the oil of *Origanum vulgare* which is extremely rich in essential oils (up to 7%) with carvacrol as a major constituent present in very high quantity (75% - 95%), followed by *p*-cymene (4% - 14%) and *γ*-terpinene (1% - 10%). It seems possible that the uses of the plant in traditional medicine can be attributed to the known biological properties of *p*-cymene and carvacrol [8]. Many of the studies confirmed the medicinal effects of oregano for human health. The *Origanum* species, which are rich in essential oils, have been used for thousands of years as spices and as local medicines in

traditional medicine [9]. About 20 European public institutions hold genetic resources of different species of oregano [10]. Marjoram (*Origanum vulgare* L.) is one of medicinal aromatic plants found wholesale almost in all areas of Albania, which is a perennial plant usually grows in dry area in smaller groups. Oregano plants are collected from natural habitats and used as raw materials in the pharmaceutical, cosmetic and food industry [11]. But many countries start to cultivate it in different areas. In botanical aspects, the oregano populations differ from one to another, that is they vary depending on the content and composition of essential oils [12]. From the quantity and quality of essential oils the values of this plant were determined [11,12]. The different results showed that the effects of oregano antioxidante are associated with high content of essential oils, thymol and carvacrols, and these are the main ingredients in oregano oil. The contents of Thymol and Carvacrol in oregano give it different properties [13]. According to the studies, carvacrol is a powerful bactericidal agent, and provides protection against mold and other common bacteria. The main objective in our study was to investigate the different regions and to find the variation for oil content in oregano plant populations.

2. MATERIAL AND METHODS

2.1. Collection Sites

The research expedition was organized in 2012 in the whole territory of Albania. During this expedition are identified and collected 62 accessions. At each location were taken of the 50 samples which derived a main representativ sample. From the total samples collected, were selected 16 samples mostly widespread of natural populations of origano (*Origanum vulgare* L. *sp*. *vulgare* and *sp. hirtum*). Those samples you perform analyzes for content of oils and their components. The confirmation of 53 essential oil was made to analyses: *β*-Pinene, *p*-Cymene, *γ*-Terpinene, Linalool, Terpinene-4-ol, Thymol, Carvacrol dhe Caryophyllene oxide. The overground parts of the flowering plants (20 - 25 cm from the top) were collected during the summer of 2012. The plant material was air dried, packed in paper bags and kept in a dark and cool place until analysis. Plant identity was verified and voucher specimens were deposited at the Institute of Pharmacognosy, Faculty of Pharmacy, Skopje.

2.2. Essential Oil Isolation

Essential oil isolation from oregano was performed by hydro distillation in all-glass Clevenger apparatus following this procedure: 20 g of the plant material was stored in 500 mL flask where 250 mL of water R was used as distillation liquid and 0.5 mL of xylene R was added in the graduate tube. The Distillation was performed for 2.5 h with a rate of 2 - 3 mL/min.

GC and GC-MS analyses: Agilent 7890A Gas Chromatography system equipped with flame ionization detector (FID) and Agilent 5975C Mass Quadrupole detector as well as capillary flow technology which enable simultaneous analysis of the sample on both detectors. HP-5 ms (30 m × 0.25 mm, film thickness 0.25 μm) capillary column was used. Operating conditions were as follows: *GC Method for essential oils*: oven temperature 60˚C (0 min), 3˚C/min to 240˚C (held for 1 min) and 10˚C/min to 280˚C (held for 1 min); helium as carrier gas at a flow rate of 1 mL/min; injector T = 220˚C and FID T = 270˚C. 1 μL of injection volume was injected at split ratio 1:1. The mass spectrometry conditions were: ionization voltage 70 eV, ion source temperature 230˚C, transfer line temperature 280˚C and mass range from 50 - 500 Da. The MS was operated in scan mode. *GC Method for Head Space*: oven temperature 60˚C, 20˚C/min to 280˚C; helium as carrier gas at a flow rate of 1 mL/min; injector T = 260˚C and FID T = 270˚C. 1000 μL of injection volume was injected at split ratio 1:1. The mass spectrometry conditions were: ionization voltage 70 eV, ion source temperature 230˚C, transfer line temperature 280˚C and mass range from 50 - 500 Da. The MS was operated in scan mode. *Head Space method*: Incubation Temperature 80˚C, Incubation Time 5.00 m:ss, Syringe Temperature 85˚C, Agitator Speed 500 rpm, Fill Speed 500 μl/s, Pullup Delay 500 ms, Inject to GC, Injection speed 500 μl/s, Pre Inject Delay 500 ms, Post Inject Delay 500 ms, Flush Time (m:ss) 0:10, GC Run time (m:ss) 10:00. *Identification of the components*: Identification of the components was made by comparing mass spectra of components in essential oils with those from Nist, Wiley and Adams mass spectra libraries, by AMDIS (Automated Mass Spectral Deconvolution and Identification System) and by comparing literature and estimated Kovat's (retention) indices that were determined using mixture of homologous series of normal alkanes from C_9 to C_{25} in hexane, under the same above mentioned conditions. The percentage ratio of the components was computed by the normalization method of the GC/FID peak areas and average values were taken into further consideration (n = 3).

2.3. Statistical Analyses

All statistical analyses were performed with the SPSS software (version 15.0, SPSS) [14]. Means values and variation coefficients were used in the statistical analyses. Effects of the studied traits were evaluated by ANOVA. In order to assess the differentiation of plants of oregano based on all variables that were measured, the Canonical Discriminate Analyses (CDA) was applied.

3. RESULTS

The our results for the oil content in the samples of 16 locality in Albania are presented in **Table 1**. The present collection of oregano showed appreciable genotypic variation in content of etheric oil compounds. In our results, the genetic variation varied from +167.53 to −93.42%.

The data which are showed in **Table 1** indicates that the total oil content varies from 3.45% (Vagalat-Delvin) to 0.1% (Baz-Burrel), which is one a wide variation between plants in different locality. **Figure 1** is presented the genetic variation compare with experimental average values μ at all locality collected in Albania. From the total locality (16), 50% or 8% locality showed postive values for oil content and 50% of locality are determined under experimental average value μ.

Table 2 showed the data of variation on the site of essential oils content in South part in Albania. Referring to the two main ingredients of etheric oil of oregano, thymol and carvacrol contents are higher in the South part (carvacrol = 68.4, thymol = 6.87) while in the central area the obtained results for content of carvacrol were 55.63% and thymol 5.30%. In the Northern part, the content with these two components were lower (carvacrol = 48.49%, and thymol = 4.19%) compare with south and central part of Albania. Referring to the average content of essential oil fractions, the sites of central area regions

Table 1. The oil content in different regions-locality in Albania.

Number	Regions-Locality	Content of etheric oil (EO) %	GV* %
1	Vagalat Delvinë	3.45	167.53
2	Mal i gjerë Girokastër	1.9	25
3	Llogara Vlorë	3.4	123.68
4	Qafë e Koshovicës Fier	2	31.57
5	Tozhar Berat	2.5	64.47
6	Cukalat Berat	3.2	110.52
7	Roshnik Beart	0.6	−60.62
8	Tregan Elbasan	0.6	−60.52
9	Kashar Tiranë	1.8	18.42
10	Shëngjergj Tiranë	0.4	−73.68
11	Shesh Tiranë	2.9	90.78
12	Baz Burrel	0.1	−93.42
13	Maqellarë Dibër	0.3	−80.26
14	Kala e Dodës Dibër	0.4	−73.68
15	Dragobi Tropojë	0.5	−67.1
16	Lepush Vermosh	0.4	−73.68
Average		1.53	

GV*: genetic variation.

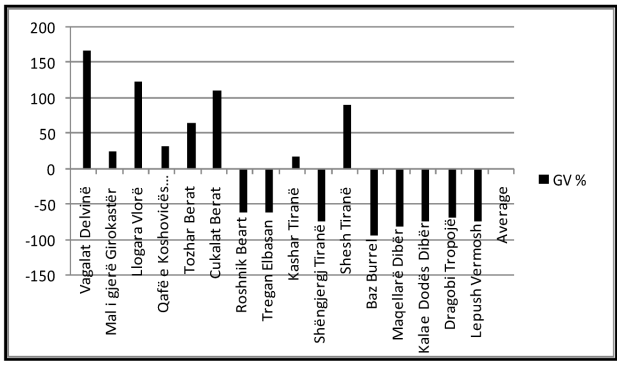

Figure 1. The genetic variation of etheric oil content.

compare to the south part distinct with higher of *p*-cymene (6.19%) or more +2.38% compare to the south part (3.81%), and +5.28% higher than north part regions (0.91%). Compared the average values for *p*-cymene 6.19% with experimental values μ (2.80%) the differences were +3.39% or expressed in relative values 121.07%, significantly higher. The differences between three regions for Caryophyllen oxide content are with higher significance. The total average values for this component are 2.11%, while the higher values are determined in north part 2.18%. Compare to average values the differences are +0.07% which are none significantly for level of probability. The central and south parts are characterized with lower quantity of Caryophyllen oxide content 2.18% and 1.96% respectively. The differences between these two regions are +0.22% or 10.42% higher for central part of Albania. With higher percentage of β-pinene is characterized the area in site of Northern Albania with average values of 0.34%. Also, the regions in the south par for these compounds are showed the lower content of β-pinene (0.15%). So, the differences among the two regions are +0.19% or expressed in relative values are 172.72%, significantly higher differences.

The content of γ-Terpinen in South part varies from 7.87% in the Qafa e Koshovices (Fier) to 0.19% in Berat Roshnik. In the north part, the content of γ-Terpinene ranges from 9.12% to 0.48%. The differences within locality inside one regions are +8.64%. The amount of the carvacrol in South part ranges from 82.76%. Amount of the carvacrol in south part was characterized with huge genetic variation which ranges from 82.76% to 0.66% (Tozhar-Berat). In Central part, the values also are with higher differences from 72.53 (Kashar-Tirane) to 33.875 (Shengjergj-Tirane). Results are presented in **Table 2**. **Table 3** describes the canonical discriminate functions, the eigenvalue, percentages of variation of each function and the cumulative variance of the three discriminate functions. This table also shows the stan- dardized elements of structure matrix. The three canoni- cal discriminate functions were significant ($p < 0.000$). It is important to point out the great relevance of the first

Table 2. Average values for essential oil content in Oregano at different part of Albania.

Content %	Regions of South part						Regions of Central Part					Regions of North part					
	Vagalat Delvinë	Mali gjere (Gjirokaster)	Llogara (Vlorë)	Qaf e Kosh. (Fier)	Tozhar (Berat)	Cukalat (Berat)	Roshnik (Berat)	Tregan (Elbsan)	Kashar (Tiranë)	Shengj (Tirane)	Shesh (Tiranë)	Baz (Burrel)	Maqellare (Diber)	Kala Dodes (Diber)	Dragobi (Tropojë)	Lepush (Vermosh)	x
β-Pinene	0	0	0	0.17	0.13	0.16	0	0	0.15	0.29	0.11	0.09	0.08	0.13	0.2	0.19	0.11
p-Cymene	2.94	2.15	3.21	7.87	7.34	3.7	0.19	0.28	9.12	0.18	5.91	0.24	0.3	0.31	0.4	0.58	2.80
γ-Terpinen	2.6	1.46	1.55	0.41	2.72	3.1	0.24	0	3.07	0.54	0.25	0.23	0.23	0.22	0.4	0.81	1.11
Linalool	0	0.19	0	0	1.69	0	1.21	0.54	0	0.92	0	7.56	2.25	0	1.5	2.91	1.17
Terpinen-4-ol	0.51	0.47	0.35	0.66	0.41	0.42	0.17	0.35	0.53	0.66	0.34	0.46	0.7	0.64	0.7	0.78	0.51
Thymol	35.5	5.67	1.27	1.08	0.66	1.25	2.77	14.7	0.9	4.37	1.25	5.87	2.96	4.12	4.9	3.11	5.65
Carvacrol	49	79.39	82.76	77.05	65.74	80.48	44.26	38.8	72.53	38.37	72.83	47.5	57.2	42.46	52	43.3	58.98
Caryophyllen oxide	0	1.12	1.34	2.14	0	0.2	3.05	0	1.33	3.94	2.01	2.91	4.89	5.51	3.2	2.19	2.11
Average																	9.06

Table 3. Summary statistics for canonical discriminant standardized functions.

Functions	1	2	3
Eigenvalue	17170.58	1130.05	11.21
Percentage of variation	99.9	0.1	0
Cumulative percentage	99.9	100	100
Canonical correlation	1	1	0.95
Functions at group centroides			
β-Pinene	−512.46	3.78	−1.397
p-Cymene	−228.43	−64.288	3.015
γ-Terpinene	−421.181	−6.484	−3.542
Linalool	−519.61	36.626	2.696
Terpinen-4-ol	−500.65	4.354	−0.932
Thymol	−179.23	−0.546	−3.709
Carvacrol	2812.387	5.395	0.152
Caryophyllen oxide	−450.798	21.162	3.716
df	7	7	7
Significance	<0.000	<0.000	<0.000
Elements of Structure Matrix			
Locality 1*	0.55*	0.83**	0.24
locality 2	0.078	−0.027	0.997**
Locality 3	0.52*	0.249	0.967**

*Largest absolute correlation between each variable and any discriminant function; *Locality 1 (South part of Albania); Locality 2 (Central part of Albania); Locality 3 (North part of Albania).

two discriminate functions justifying 99.9% and 0.1% of the variability. The first discriminate function showed a significant positive correlation with the locality 1 (0.55) and locality 3 (0.52) following by second and third discriminate function (0.83) and 0.99. But, the negative correlation (−0.027) was determined between second discriminate function and locality 2. Results are presented in **Table 3**.

4. DISCCUSION

The essential oil of oregano is composed of carvacrol and thymol as dominant components, followed by; terpinene, *p*-cymene, linalool, terpinen-4-ol and sabinene hydrate [5]. Results of various studies indicated that the antioxidant effects of oregano might be related to the dominant components, carvacrol and thymol, of the essential oil [15,16]. *Origanum vulgare* L. is the species with the highest variability in the genus *Origanum*. Nevertheless, diversity, genetic resources and potential for utilization of *O. vulgare* have not yet been fully explored so that extended research on oregano germplasm is necessary [17]. The oil content is an important indicator which associated with value of oregano. Also, Ayala [18] they reported different values in *Origanum spp* for oil content which varied to 8.8%. The samples from the southern part of Albania has a high amount of etheric oil compared with those of the North zone. The variation for the oil content was found also within an area. The different variation of essential oils in oregano were reported by numerous researches [10,19]. Obtained results by our study for analysis of the whole content of etheric oils showed that sites of southern part of Albania distinguished for higher content of *γ*-carvacrol on average values for with 68.4%, *γ*-Terpinene and 1.72%, Thymol and 6.87%. Gounaris *et al*. [20] in their research reported results for *γ*-Terpinene (10%), *p*-Cymene (5.98%), thymol (0.40%), Carvacrol (58.73%). A similar effect was also reported by Azizi [5] which showed results for *β*-pinene (0.1%), *p*-cymene (5.3%), *γ*-Terpinene (8.1%), thymol (0.3%), Carvacrol (77.4%). From these obtained data the content of the different fractions of essential oils is closely linked to the area where oregano is so widespread which have an ecological impact on their selection. According to Putievsky *et al.* [21] essential oil content of oregano was higher in full bloom stage than in the stage of start flowering. This hypothesis gives us some preliminary information that local populations that are selected and adapted to the agro ecological conditions for centuries are more tolerant to environmental stress [22]. An early study by Azizi *et al.* [23] for interactions factors showed that dry matter production and essential oil content of *Origanum vulgare* L. can be significantly affected by environmental and agronomical conditions including nitrogen fertilization and soil moisture regime, whereas percentage of main compounds of essential oil such as carvacrol, terpinene and *p*-cymene remained unaffected.

5. CONCLUSION

The content of the total amount of etheric oil is characterized with different variation in different regions. From our results, etheric oil with higher content was characterized in the south part in Albania. The quantity and content of oils fractions depend on the area where the samples were taken. So, carvacrol with higher content in 55.63% of the samples is coming from sites of Southern Albania, while in the other two areas, in Central and Northern area is 55.63% to 48.49% respectively. The same results were found for content of thymol, linalool's compounds, while North area had high content of caryophyllen-oxide and *β*-pinen. In all sites, the dominant oils were thymolit and carvacrol. In many cases, the essential components of oregano oil are determined by the genotype structure, but the environmental effects have a small effect. From the results of chromatographic analysis, it can be concluded that the populations have significantly changed in the composition of essential oils. The results of this study can be a good base for selection criteria to

establish the programs to improve the oregano plants.

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