Journal of Medicinal Plants Research Vol. 5(3), pp. 379-387, 4 February, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Chemical analysis of essential oil from Lebanese wild and cultivated *Origanum syriacum* L. (Lamiaceae) before and after flowering

Salam Zein[1], Sanaa Awada[1], Samar Rachidi[1], Amal Hajj[1], Elena Krivoruschko[2] and Hussein Kanaan[1]*

[1]Laboratory of Biotechnology of Natural Substances and Health products, Faculty of Pharmacy, Lebanese University, Beirut, Lebanon.
[2]Laboratory of Pharmacognosy. National University of Pharmacy, Kharkiv, Ukraine.

Accepted 29 November, 2010

Quantitative and qualitative analysis of components from wild and cultivated Lebanese *Origanum syriacum* before and after flowering were carried out. Fatty acids were extracted from leaves and identified by GC, minerals and trace elements were analyzed by atomic emission spectroscopy, and essential oils were extracted and identified by GC-MS. Sixteen fatty acids were obtained, whereas 2 essentials such as linoleic and alpha-linolenic, were dominating in leaves before flowering. 15 minerals were found at a larger amount in the cultivated population. Forty-one essential oils were identified, dominated by carvacrol in cultivated leaves and by thymol in wild leaves. This comparative study shows that *O. syriacum* was a source of nutrients and constituents for multiple pharmacological and agro-alimentary uses. Optimal conditions for obtaining these components were determined according to harvesting time, before or after flowering, in each wild and cultivated population.

Key words: *Origanum syriacum*, essential oils, fatty acids, trace elements, carvacrol, thymol.

INTRODUCTION

Lebanon is covered by a large number of plant species especially with medicinal properties. The floristic richness estimates 2600 plant species with a high percentage of endemic plant species (12%) in only 10452 Km^2 (Khouzami et al., 1996; El-Beyrouthy et al., 2008). One of the popular herbs used in folk medicine is the thyme "zaatar" beside its large culinary use as "mankouchi". Its taxonomic name is *Origanum syriacum* L. (Lamiaceae). *O. syriacum*, like many species of the genus *Origanum*, is found as a wild plant in the Mediterranean areas. It belongs to the Majorana section of Group B in Ietswaart classification (Skoula et al., 2002).

Dried *Origanum* species are used as stimulants, analgesics, antitussives, expectorants, sedatives, antiparasitics, antihelminthics, antirheumatics and gastrointestinal complaints in folk medicine (Baser, 2002;El-Beyrouthy et al., 2008; Loizzo et al., 2009). Recently, this plant has drawn more attention due to the antimicrobial, antifungal, insecticidal and antioxidative effects (Kusilic et al., 2004; Bakkali et al., 2008; Azizi et al., 2009). Also, the essential oil uses are more and more widespread as alternatives to synthetic chemical products to protect the ecological equilibrium. Many studies *in vitro* revealed new potential therapeutic effects of *Origanum*, in the treatment of Alzheimer's disease, as an anti-inflammatory agent in a cellular model of atherosclerosis, a suppressor of food bacterial growth and enterotoxin synthesis, and as a way of increasing the sensitivity of some antibiotics against resistant bacteria (Aslim et al., 2008; Ocana-Fuentus et al., 2010; De souza et al., 2010).

The composition of essential oil compounds of *O. syriacum* was the subject of several previous studies in

*Corresponding author. E-mail: hussein_kanaan@hotmail.com

Table 1. Geographical localization (Bazourieh-South Lebanon) of collected thyme determined by GPS receiver etrex vista® HCX Garmin. North to Road Map Pointer (91) Declutter 50.

Parameter	Wild *O. syriacum*	Cultivated *O. syriacum*
Elevation	150 m	150 m
GPS N	32° 45.015' 183°	33° 12.106' 216°
GPS E	035 15.472' 55.3	035 13.507' 70

Table 2. Soil analysis results (Soil laboratory- ministry of the agriculture).

Parameter	Wild *O. syriacum*	Cultivated *O. syriacum*
pH	7.8	8.08
Salinity Ms/cm	0.24	0.22
Organic matter %w.v	1.68	2.9
Phosphorus ppm	17	32
Nitrogen %	0.0112	0.0112
Total CaCO3 %	48	59.2
Sand %	70.6	79.6
Silt %	17	18
Clay %	20.4	12.4
Soil type	Sandy clay loam	Sandy clay loam

neighboring countries (Kamel et al., 2001; Abu Lafi et al., 2007, 2008; Lukas et al.,2009) and in Lebanon (Traboulsi et al., 2002; Hilan et al., 2006; Viuda-Martos et al., 2007). The green leaves of the *Origanum* herb are rich in essential oil which confers its characteristic and fragrance. The studies show that essential oil in *Origanum* is composed of carvacrol and/or thymol as dominant components. The extraction product can vary in quality, quantity and composition according to climate, soil composition, geographical location, seasonal variation, plant organ, age and vegetative cycle stage, harvesting time (Abu Lafi et al., 2007, 2008). No data on the fatty acids and trace elements composition of the aerial parts of *O.syriacum* in Lebanon has been reported.

The qualitative and quantitative analysis of *Origanum* composition diversifies the use of this natural product. Also, the variability of conditions of the analysis, allows being more specific about optimal quality and quantity of components. So, the aim of this study is to explore the constituents in both wild and cultivated *O. syriacum* collected from the south of Lebanon. We present a comparison analysis of fatty acids, minerals and essential oils compositions extracted from leaves in both populations before and after flowering and from blooming peaks.

MATERIALS AND METHODS

Plant material

Leaves samples of *O. syriacum* were collected in Bazourieh in the south of Lebanon, before flowering in April 2009, and in full bloom in June 2009. The identification of plant material was done according to the method of Ietswaart (1980). Voucher specimens of the populations were kept at Laboratory of Biotechnology of Natural Substances and Health products. All geographical conditions are presented in Table 1.Thyme leaves were air dried in the absence of light at room temperature for seven days for all the samples.

Soil analysis

The soil material was taken from the layer of 0 to 20 cm. It was analyzed by methods as indicated in the manual of Ryan et al. (1996), and adopted by the ministry of agriculture in Lebanon. The soil composition from wild and cultivated thyme is presented in Table 2.

Method for determining fatty acids composition

Extraction

1.3 g of dried leaves was placed into a hermetically sealed vessel, and 15 ml of a Folch solution was added. The mix was stirred regularly for 24 h, and then it was filtered through the paper filter into a 10 ml flask. 1 g of anhydrous sodium sulphate was added to the extract obtained, which was evaporated in the nitrogen stream at 25°C until dryness. 5 ml of methanol and 0.2 ml of chlorous acetyl were added to the residue and the flask was filled with nitrogen, and then it was boiled with the reflux condenser on the glycerin bath for 35 min at 65°C. The solution obtained was evaporated in the nitrogen stream to the final volume of approximately 0.5 ml. 1.0 ml of cyclohexane was added and the mixture was stirred for 10 min. After the complete stratification of the layers, the upper cyclohexane layer was used as a test sample.

GC analysis

Fatty acids were analyzed using GC-2014AF/SPL Shimadzu. The operating conditions were as follows: capillary column size was 25 m × 0.25 mm. Layer's density was 0.25 µm. the carrier gas flow rate was 1.0 ml He/min. Injector and detector temperatures were 240°C and 250°C, respectively. Split ratio was 1:50. The column temperature was held at 160°C for 5 min, and then raised to 225°C at 5°C/min for 10 min. The volume of sample injected was 1 µl. The content of each individual component was calculated by the internal regulation method.

Method for determining trace elements composition

Thyme dried leaves were analyzed by the atomic emission spectra based on the complete evaporation of a substance in the charge of the alternating current arc (the excitation source is IVS-28) and registration of emission by DFS-8 spectrograph. The method is based on the evaporation of the previously concentrated sample of the plant leaves from the graphite electrodes craters into the arc burning with the current force at 16 A, the tension at 220 V and the exposition for 60 s. the spectrum range is 250 to 350 nm. A set of graduated samples (artificial mixtures of the plants' composition) was used for transferring from the analytical signals values (darkening the lines of the elements determined) to the concentrations.

Method for determining essential oils composition

Extraction

One gram of the dry leaves was placed into a 22 ml Agilent vial (part number 5183-4536) with an open lid and a silicon seal. The hole was made in the seal where the air condenser, consisting of the common glass tube of 50 cm in length and 5 to 7 mm in diameter, was inserted. The water was added to cover half of the vial. The lid with the condenser was fixed and the vial was placed into the small sand bath with the fire-regulated heating. The heating degree was previously determined in order to avoid the boiling water from evaporating from the condenser, but rising not higher than 75% of its length. After boiling for an hour, the tube with the lid was removed. The condenser was washed twice by 1 to 2 ml of petroleum ether for collecting microquantities of essential oils adsorbed on its internal surface. The washing solution was collected into 12 ml Agilent vial (part number 5183-4536), where 10 to 15 mg of sodium sulphate was added to dry the essential oils and remove traces of moisture. The solution was evaporated by pure nitrogen to make the volume of 50 µl and then chromatographed. This method allows extracting the components of essential oils more completely for further qualitative analysis. It is of a particular importance in primary estimation of plant objects.

GC-MS analysis

The composition of the essential oils was performed on an Agilent Technology chromatographer 6890N coupled with mass-spectrometric detector 5973N.The operating conditions were as follows: the GC was equipped with a Quartz capillary column HP-5MS;30 m × 0.25 mm i.d.. The carrier gas flow rate was 1 ml He/min. Injection volume was 0.1 to 0.5 µl. split ratio was 1/50. The column temperature was held at 50°C, programming 4°C/min to 120°C, then 6°C/min to 250°C. Detector and evaporator temperature was 250°C.

The components of essential oils were identified by comparing the results of mass-spectra (m/Z) of the chemical substances included in the mixtures under research obtained in the process of chromatography and the data of mass-spectra library NIST02 (more than 174000 substances). The range of electronic scanning was 50 to 550 m/Z, and the electronic charge of mass-spectra was 70 eV. The retention time of the components was calculated by the control analysis of essential oils with addition of the normal alkanes mixture (C_{10}-C_{18}).

Calibration has been performed for approximate calculation of the content of the individual component in the sample. 0.5 mg of the substance has been proven to correspond to 25.10^8 area units. The sum of all peak areas of the components and the content of each substance (%) is given for each sample after the list of substances (under the name of "Sum of corrected areas"). Since the accurate weight of 1 g was taken for distillation, the content of volatile substances is 0.05 mg/1 g or 50 mg/kg. Then the content of each component accurately weighed is calculated according to the percent values given in the table.

RESULTS AND DISCUSSION

The focus of this study is to compare the composition of *O. syriacum* obtained from 2 populations, wild and cultivated, of the same region (Bazourieh) from the south of Lebanon. The results of quantification of fatty acid, minerals and essential oil components for each population were shown in Tables 3, 4 and 5, respectively. A chromatogram of carvacrol and thymol obtained after injection of essential oil from cultivated leaves before flowering is represented in Figure 1. The geographical localization was indicated by a GPS receiver and the same conditions of temperature and humidity were confirmed. Also similar soil type, a sandy clay loam, was identified through soil analysis.

Fatty acids

We studied fatty acids composition from leaves and bloom peaks from wild and cultivated *O. syriacum*. 16 fatty acids were identified (Cuvelier et al., 2004). Chromatography analysis of fatty acids methyl esters showed that myristic acid was the predominant component in both wild and cultivated leaves after flowering (61.50 and 79.07%, respectively) and in blooming peaks (84.15 and 76.33%, respectively). There was no trace of myristic acid before flowering. Myristic acid is particularly effective at causing the liver to synthesize cholesterol. It is mainly used for soap manufactures, flavorings, cosmetics, perfumes, also as raw materials in spices and in the pharmaceutical domain. Myristoleic acid was found at a lesser amount after flowering, it may have a cytotoxic effect against prostatic cancer (Iguchi et al., 2001).The predominant fatty acid in leaves before flowering was the α-linolenic acid (51.73% for wild and 49.34% for cultivated *Origanum*). Linoleic acid amount was less than 10%.

The human body can produce all but those 2 fatty acids out of the ones it needs. They must be supplied in diet. Hence, linoleic acid and α-linolenic acid are essential fatty

Table 3. Types of fatty acids contained in *O.syriacum* and their percentage concentration in leaves before and after flowering in wild (A) and cultivated (B) thyme and in blooming peaks.

No.	Compound	Common name	Systematic name	Percentage before flowering		Percentage after f lowering		Percentage blooming peaks	
				A	B	A	B	A	B
1	C14:0	Myristic	Tetradecanoic	-	-	61.504	79.070	84.156	76.334
2	C14:1	Myristoleic	cis-9-Tetradecenoic	0.487	-	25.306	9.529	6.800	11.884
3	C15:1		Pentadecenoic	-	-	-	0.714	0.706	-
4	C16:0	Palmitic	Hexadecanoic	14.420	15.122	5.18	3.312	2.608	3.485
5	C16:1n3	Palmitoleic, isomer		2.260	-	-	-	-	-
6	C16:1n6	Palmitoleic, isomer		1.208	1.195	-	-	-	-
7	C16:1n9	Palmitoleic, isomer	cis-9-Hexadecenoic	4.351	-	-	-	-	-
8	C17:1		Heptadecenoic	0.887	-	-	-	-	-
9	C18:0	Stearic	Octadecanoic	2.505	2.747	-	0.829	0.789	0.873
10	C18:1n9	Oleic	cis-9-Octadecaenoic	1.632	2.328	-	0.808	0.628	0.959
11	C18:2n9,12	Linoleic	cis,cis-9,12-Octadecadienoic	7.648	9.129	-	1.828	1.558	1.916
12	C18:3n9,12,15	α-Linolenic	cis, cis,cis-9,12,15-Octadecatrienoic	51.736	49.348	3.465	3.420	2.162	3.739
13	C20:0	Arachidic	eicosanoic	0.900	-	-	-	-	-
14	C20:1n13	Gadoleic		1.814	1.559	-	-	-	-
15	C24:0	Lignoceric	Tetracosanoic	8.219	6.523	-	-	-	-
16	C24:1	Selacholeic	cis-15-Tetracosenoic	1.573	2.280	-	-	-	-

acids for humans. In the body, essential fatty acids are primarily used to produce hormone-like substances that regulate a wide range of functions, including blood pressure, blood clotting, blood lipid levels, the immune response, and the inflammation response to injury infection. Palmitic acid had a higher amount before flowering in both wild and cultivated leaves (14.42 and 15.12%, respectively). Cardiovascular side effects were controversial.

These results allow the classification of the *O. syriacum* leaves before flowering for both 2 populations as source of essential fatty acids. It is not the same for leaves after flowering and blooming peaks. These results need to be confirmed by testing other populations of Lebanese *O. syriacum*.

Minerals

We studied minerals composition of wild and cultivated *Origanum*, and we identify 15 elements. It seems that the cultivated population, before and after flowering, has a higher amount of minerals than the wild population and the cultivated leaves before flowering have a higher amount of minerals than cultivated leaves after flowering. Blooming peaks are the poorest in minerals. Cultivated *Origanum* may be a dietary source of minerals and trace elements. For example, 1 g of cultivated leaves before flowering may contain 1.25 mg of Mn; 40 mg of Ca; 5 mg of Fe; 65 mg of K (recommended daily dose: 5 mg for Mn,1000-1500 mg for Ca; 10 to 18 mg for Fe; 2 to 4 g for K). Some others elements are detected as traces (Co<0.03; Cd<0.01; As<0.01: Hg<0.01).

Essential oils

It is reported that the great variability and diversity observed in the chemical composition of essential oil can be attributed to climatic (temperature, light, humidity), and soil variation, stage of vegetation, seasonal variation, harvesting time. Harvesting in May to June produces more essential oil than earlier in the year, namely January to March

Table 4. Trace elements compounds of wild and cultivated *O. syriacum* in leaves before and after flowering, and in blooming peaks (mg of trace element/100 g of plant).

No.	Element	Before flowering		After flowering		Blooming peaks	
		Wild	Cultivated	Wild	Cultivated	Wild	Cultivated
1	Fe	220	500	98	108	67	40
2	Si	450	1000	590	650	400	315
3	P	190	425	88	184	114	150
4	Al	110	250	107	119	67	70
5	Mn	55	125	19	20	17	16
6	Mg	335	750	285	325	200	225
7	Pb	<0.03	0.03	<0.03	<0.03	<0.03	<0.03
8	Ni	0.1	0.25	0.19	0.07	0.33	0.08
9	Mo	<0.03	<0.03	0.02	0.05	0.02	0.03
10	Ca	1790	4000	930	970	585	720
11	Cu	2.8	6.2	1.5	1.1	1.3	1.2
12	Zn	11	50	29	32	27	24
13	Na	224	300	390	215	270	160
14	K	3135	6500	2940	3240	2010	2530
15	Sr	0.56	1.25	2.9	3.2	2.0	2.2

(Abu Lafi et al., 2007, 2008). We noticed that *O. syriacum* essential oil was characterized by the presence of forty two components identified. The most abundant components were, as expected, thymol and carvacrol. The wild growing thyme was characterized by the dominant presence of thymol irrespective of the harvesting time, and the highest concentration (55.47%) was reached from leaves after flowering. Conversely, cultivated thyme revealed predominance of carvacrol from leaves by nearly amount of thymol and carvacrol (22.83 and 22.53%, respectively) from cultivated flowers. We suggested that this wild *Origanum* was of the thymol type. This result differs from some previous studies, where the carvacrol was the major component of the wild thyme, which has a distinct warm pungent taste, a distinctive property of carvacrol (Kusilic et al., 2004).

Other studies showed one of the 2 compounds to be either over dominating or to be both present in higher amounts. In this study the composition of essential oil is dominated by carvacrol and thymol followed by their precursors γ- terpinene and p-cymene, as well as α-terpinene, myrcene and caryophyllene. The percentage of γ-terpinene and p-cymene were increased during February and March while decreased during May. We remember that unstable genuine substances like E-sabinene hydrate and sabinene hydrate acetate were found in clearly smaller portions due to transformation to γ-terpinene under high temperature during distillation (Richter et al., 2007). A comparison of essential oils percentage is presented in Figure 2 for thymol and carvacrol and in Figure 3 for γ-terpinene, cymene, α -terpinene and myrcene. Octanol-3 essential oil range between 2.1 to 2.368% before flowering, and α – phellandrene (1.122 to 1.165%) was detected in flowers, and their amount is less than 1% for other samples. We suggested that leaves of *Origanum* after flowering are richer than others in both of thymol and carvacrol, while the blooming peaks are the poorest. Inversely , the sum of γ-terpinene, cymene are high in blooming peaks, leaves before flowering and full in leaves after flowering.

Because of the growing need for an extended usage of natural compounds like *Origanum* essential oils, the importance of obtaining these oils with standard properties are growing too. By this token, this study allows getting information about optimal harvesting times, parts of plants and essential oil composition. These results need to be tested in other Lebanese populations of *O. syriacum*, to confirm our results or to classify those populations according to their yielding essential oils, to allow future clinical or pharmacological uses.

Conclusion

The main constituents of *Origanum* essential oils were determined as thymol in wild plant and carvacrol in cultivated plant. Leaves after flowering contain the highest amount of thymol and carvacrol. Myristic acid was the dominant fatty acid after flowering, and α-linolenic acid was dominant before flowering in both wild and cultivated plants.

Fifteen minerals and trace elements were detected but in higher amount in the cultivated population. These analyses need to be extended to other Lebanese populations of *O. syriacum*, in order to obtain more

Table 5. Essential oil compounds of wild and cultivated *O. syriacum* in leaves before, after flowering and in blooming peaks.

No.	Compound	Before flowering				After flowering				Blooming peaks			
		Wild		Cultivated		Wild		Cultivated		Wild		Cultivated	
		RT*	%	RT*	%	RT*	%	RT*	%	RT*	%	RT*	%
1	Trans-2-hexenal	4.38	0.247	4.39	0.350	-	-	-	-	-	-	-	-
2	α-Tujene	6.10	1.638	6.08	0.607	5.61	0.038	5.63	0.988	5.63	1.684	5.64	1.783
3	α-Pinene	6.28	0.871	6.26	0.317	5.78	0.033	5.80	0.942	5.81	1.468	5.81	0.206
4	Camphene	6.67	0.148	6.66	0.057	-	-	6.17	0.137	6.18	0.200	6.18	0.206
5	Benzaldehyde	7.03	0.083	7.03	0.786	-	-	-	-	-	-	-	-
6	β-Pinene	7.46	0.112	7.45	0.087	-	-	6.94	0.238	6.94	0.247	6.94	0.300
7	1-Octen-3-ol	7.70	1.553	7.66	1.521	7.06	0.964	7.15	1.189	7.07	1.140	7.16	1.096
8	Myrcene	7.96	3.918	7.92	2.164	7.34	0.454	7.43	3.697	7.44	5.273	7.46	5.809
9	Octanol-3	8.21	2.139	8.16	2.368	7.51	0.480	7.61	0.921	7.54	0.119	7.62	0.888
10	α-Phellandrene	8.33	0.732	8.30	0.338	-	-	7.77	0.602	7.77	1.122	7.79	1.165
11	Δ^3-Carene	8.48	0.146	8.46	0.095	-	-	7.92	0.218	7.92	0.228	7.93	0.260
12	α-Terpinene	8.79	5.327	8.71	2.140	8.10	0.923	8.17	2.916	8.21	7.171	8.25	6.749
13	Cymene	9.14	10.171	9.07	5.530	8.42	4.615	8.54	6.033	8.55	9.337	8.59	9.126
14	Limonene	-	-	-	-	8.50	0.227	8.60	0.652	8.62	1.408	8.64	1.428
15	β-Phellandrene	9.24	1.019	9.13	0.360	-	-	-	-	-	-	-	-
16	Cis-Ocymen	9.40	0.373	-	-	-	-	9.13	0.150	-	-	-	-
17	Trans-Ocymen	-	-	9.69	0.112	-	-	-	-	-	-	-	-
18	γ-Terpinene	10.27	15.669	10.23	9.039	9.5	4.945	9.63	7.989	9.81	18.744	9.66	18.330
19	Trans-Sabinene-hydrate	10.54	1.361	10.47	2.453	9.73	0.871	9.88	3.497	9.90	1.062	9.94	1.321
20	Terpinolene	11.05	0.293	11.00	0.181	10.35	0.093	10.40	0.444	10.44	0.540	10.45	0.661
21	Cis-Sabinene-hydrate	-	-	-	-	-	-	10.74	0.175	-	-	-	-
22	Linalool	11.43	0.101	11.42	0.213	10.79	0.156	10.81	0.398	10.82	0.184	10.83	0.241
23	Borneol	13.63	0.085	13.63	0.206	-	-	12.99	0.191	-	-	12.99	0.188
24	Terpinene-4-ol	14.02	0.61	14.04	0.774	13.37	0.740	13.38	0.923	13.36	0.288	13.40	0.982
25	α-Terpineol	-	-	-	-	15.17	0.215	-	-	14.99	0.153	14.8	0.112
26	Methylcarvacrol	16.25	0.092	16.23	0.055	15.58	0.244	15.57	0.142	-	-	15.57	0.137
27	Cis-dihydro-carvon	-	-	-	-	-	-	16.9	0.164	-	-	-	-
28	Thymol	18.41	43.28	18.11	2.93	17.87	55.47	17.48	1.58	17.77	42.25	17.85	22.83
29	Carvacrol	19.34	4.26	18.74	58.88	18.42	26.03	18.21	61.76	18.41	4.62	18.22	22.53
30	Thymyl acetate	20.31	0.808	-	-	-	-	-	-	19.43	0.224	19.44	0.131
31	Carvacryl acetate	20.77	0.054	21.11	1.922	-	-	20.38	0.821	-	-	19.96	0.199
32	Caryophyllene	22.22	3.422	22.27	3.202	21.37	1.801	21.54	2.843	21.37	2.249	21.36	1.866
33	Aromadendrene	22.72	0.287	22.77	0.235	-	-	22.04	0.162	-	-	-	-
34	Humulene	23.17	0.516	23.28	0.433	22.38	0.331	-	-	22.36	0.258	22.35	0.243

Table 5. Continued.

35	Bicyclogermacrene	24.44	0.539	24.51	0.433	-	-	-	-	-	-	-	-
36	2-Methoxy-4-ethyl-6-methylphenol	26.14	0.068	26.20	0.317	-	-	-	-	-	-	-	-
37	Spatulenol	26.59	0.073	26.64	0.124	-	-	-	-	-	-	-	-
38	Ledene	-	-	-	-	-	-	23.71	0.112	-	-	-	-
39	Caryophyllene oxide	26.71	0.179	26.76	0.353	26.11	1.097	26.13	0.104	-	-	-	-
40	Humulen oxid	-	-	-	-	26.67	0.112	-	-	-	-	-	-
41	α-Muurool	-	-	-	-	27.32	0.158	-	-	-	-	-	-
42	α-Cadinol	-	-	27.88	0.171	-	-	-	-	-	-	-	-
Total non identified		0.065		0.33									

*: Retention time.

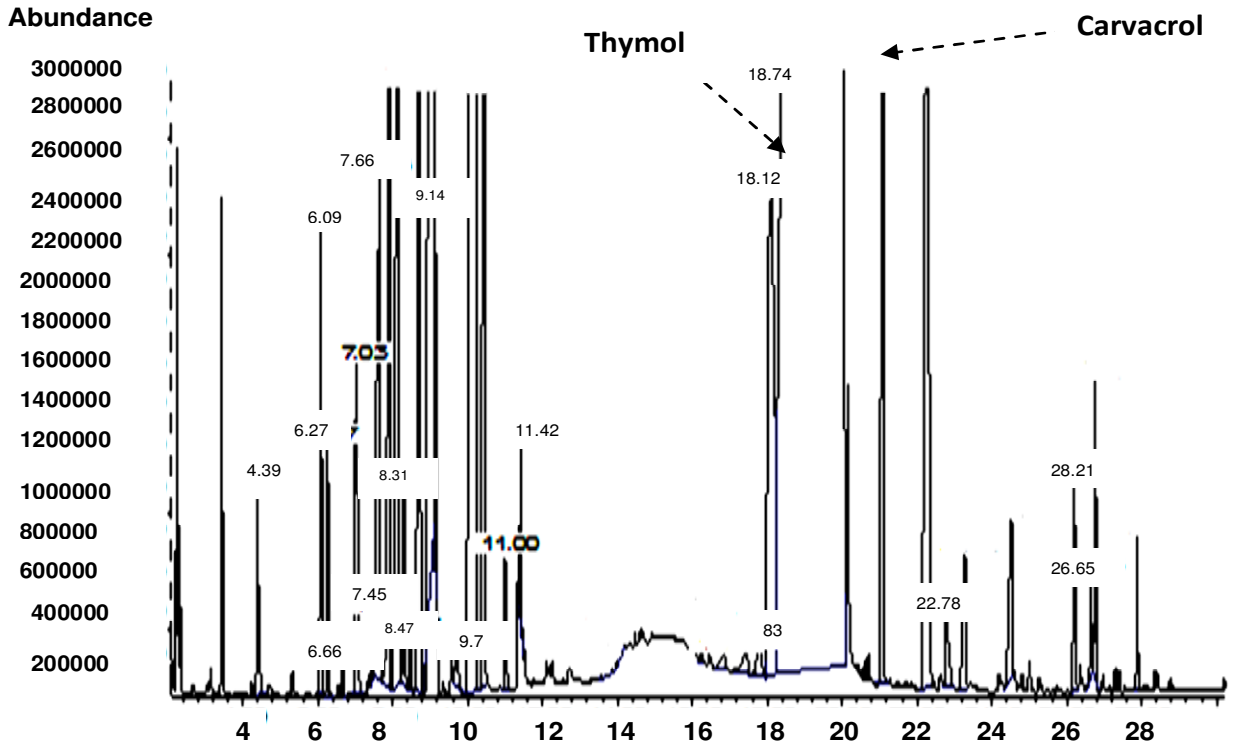

Figure 1. A chromatogramm of thymol and carvacrol obtained after injection of essential oil from cultivated *Origanum* before flowering.

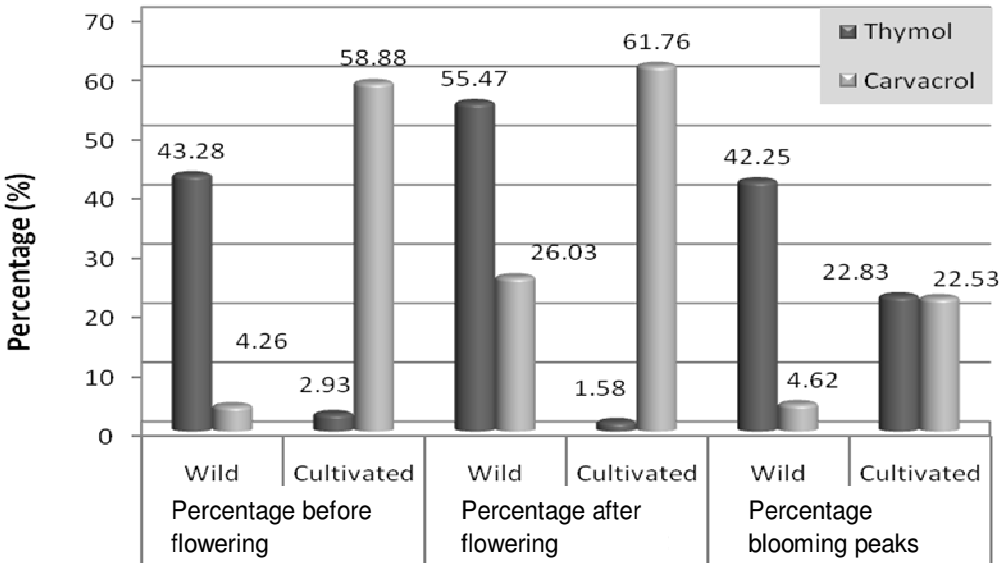

Figure 2. Comparison between thymol and carvacrol composition from leaves before and after flowering, and from blooming peaks in both wild and cultivated *Origanum*.

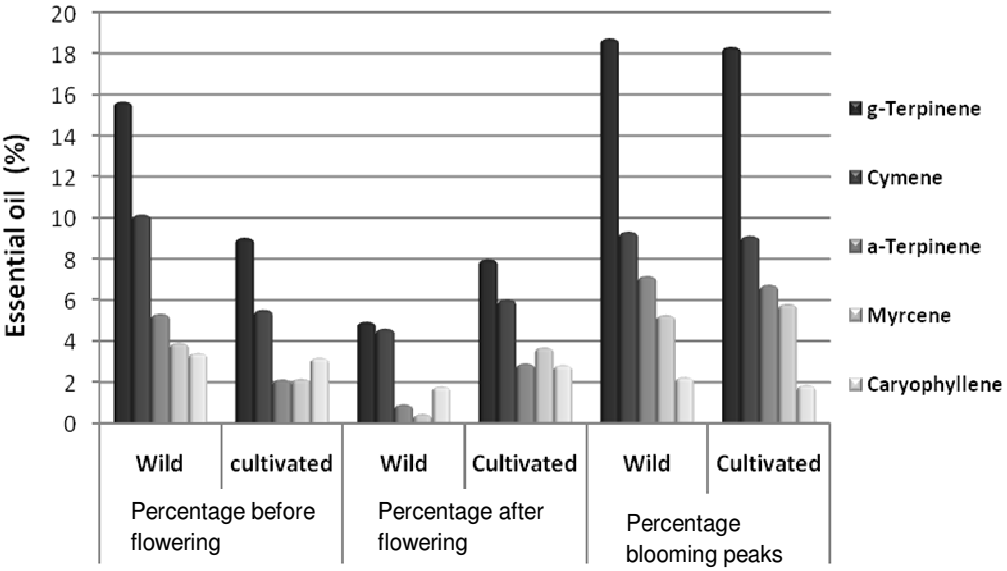

Figure 3. Representation of mainly essential oil components percentage in leaves before and after flowering and in blooming peaks.

possibility for clinical, nutritional or pharmacological applications.

REFERENCES

Abu Lafi S, Odeh I, Dewik H, Qabajah M, Imam A, Dembitsky VM, Hanus LO (2007). Natural compounds of Palestine flora. Comparison analysis by static headspace and steam distillation GC-MS of semivolatile secondary metabolites from leaves of cultivated Palestinian *Majorana syriaca*. Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Republic, 151: 21-29.

Abu Lafi S, Odeh I, Dewik H, Qabajah M, Hanus LO, Dembitsky VM (2008). Thymol and carvacrol production from leaves of wild Palestinian *Majorana syriaca*. Bioresour. Technol., 99: 3914-3918.

Aslim B, Yucel N (2008). *In vitro* antimicrobial activity of essential oil from endemic *Origanum minutiflorum* on ciprofloxacin-resistant Campylobacter spp. Food Chem., 107: 602-606.

Azizi A, Yan F, Honermeier B (2009). Herbage yield, essential oil content and composition of three oregano populations as affected by soil moisture regimes and nitrogen supply. J. Ind. Crop., 29: 554-561.

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008). Biological effects of essential oils – A review. Food Chem. Toxicol., 46: 446-475.

Baser KHC (2002). The Turkish Origanum species. In: Kintzios SE

(eds) The Genera Origanum and Lippia, Taylor and Francis, London and New York, pp. 109-126.
Cuvelier C, Cabaraux JF, Dufrasne I, Hornick JL, Istasse L (2004). Acides gras : nomenclature et sources alimentaires. Ann. Med. Vet., 148: 133-140.
De souza EL, De Barros JC, De Oliveira CEV, De Conceicao ML (2010). Influence of *Oreganum vulgarizes* L. Essential oil on enterotoxin production, membrane permeability and surface characterstics of Staphylocoque aureus. Int. J. Food Microbiol., 137: 308-311.
El-Beyrouthy M, Arnold N, Delelis-Dusollier A, DuPont F (2008). Plants used as remedies Antirheumatic and antineuralgic in the traditional medicine of Lebanon. J. Ethnopharmacol., 120: 315-334.
Hilan Ch, Sfeir R, Jawish D, Aitour S (2006). Huiles essentielles de certaines plantes medicinales Libanaises de la famille des Lamiaceae. Leb. Sci. J., 7: 13-22.
Iguchi K, Okumura N, Usui S, Sajiki H, Hirota K, Hirano K (2001). Myristoleic acid: a cytotoxic component in the extract from Serena repens, induces apoptosis and necrosis in human prostatic LNCaP cells. The prostate, 47: 59-65.
Ietswaart JHA (1980). Taxonomie Revision of the Genus Orignum (Labiatae). Leiden University Press: Leiden, Netherlands.
Kamel MS, Assaf MH, Hassanean HA, Ohtani K, Kassai R, Yamsaki K (2001). Monoterpene glucosides from *Origanum syriacum*. Phytochem., 58: 1149-1152.
Khouzami M, Bassil M, Fortunat L, Hayek A (1996). Etude de la diversité biologique du Liban. Minister of Agriculture, UNEP, Lebanon.
Kulisic T, Radoni A, Katalinic V, Milos M (2004). Use of different methods for testing antioxidative activity of oregano essential oil. Food Chem., 85: 663-640.
Loizzo MR, Menichini F, Conforti F, Tundis R, Bonesi M, Saab AM, Statti GA, Cindio B, Houghton, PJ, Menichini FR, Frega NG (2009). Chemical analysis, antioxidant, anti-inflammatory and anticholinesterase activities of *Origanum ehrenbergii* Boiss and *Origanum syriacum* L. essential oils. Food Chem., 117: 174-180.
Lukas B, Schmiderer C, Franz CH, Novak J (2009). Composition of essential oil compounds from different Syrian populations of *Origanum syriacum* L. (Lamiaceae). J. Agric. Food Chem., 57: 1362-1365.
Ocana-Fuentus A, Arranz-Gutierrez E, Senorans FJ, Reglero G (2010). Supercritical fluid extraction of oregano (O. vulgarie) essential oils: Anti-inflammatory properties based on cytokine response on macrophages-THP-1. Food Chem. Toxicol., 48: 1568-1575.
Ryan J, Gabaret S, Harmsen K, Rashid A (1996). A soil and plant analysis manual adapted for the west and north Africa region,.edn. ICARDA, Aleppo, Syria.
Richter J, Schellenberg I (2007). Comparison of different extraction methods for the determination of essential oils and related compounds from aromatic plants and optimization of solid-phase microextraction/gas chromatography. Anal. Bioanal. Chem., 387: 2207-2217.
Skoula M, Harborne JB (2002). The taxonomy and chemistry of Origanum. In: Kintzios (eds) The Genera Origanum and Lippia, Taylor and Francis, London and New York, pp. 67-108.
Traboulsi AF, Taoubi K, Hajj S, Bessiere JM, Rammal S (2002). Insecticidal properties of essential plant oils against the mosquito culex pipiens molestus. Pest Manage. Sci., 58: 491-495.
Viuda-Martos M, Ruiz-Navajas Y, Fernandez-Lopez J, Perez-Alvarez JA (2007). Chemical composition of the essential oils obtained from some spices widely used in Mediterranean region. Acta Chim. Slov., 54: 921-926.