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Investigation of *Origanum libanoticum* Boiss. essential oils chemical polymorphism by independent components analysis (ICA)

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The essential oils obtained from *Origanum libanoticum* Boiss., a plant endemic to Lebanon, were analyzed by GC/MS. Seventy compounds were identified, covering till 99.8% of the total oil composition. All samples were *p*-cymene and/or β -caryophyllene chemotype, with variable percentage of other compounds such as α -pinene, myrcene, α -phellandrene, limonene, etc. Compared to traditional drying method, lyophilized samples provided the highest essential oil (EO) yields and yields were higher at flowering stage (Chouwen: 0.33% in 2013 and 0.32% in 2014; Qartaba: 0.27% in 2013 and 0.37% in 2014). According to independent components analysis (ICA), date and site of harvest, altitude and drying technique had no effect on the variation of *O. libanoticum* EO chemical composition. An annual variation of EOs composition was observed since a particular variation in some major components concentration was revealed monthly and annually between 2013 and 2014.

Keywords: *Origanum libanoticum* Boiss., Essential oil, GC/MS, Independent components analysis, Harvesting date, Geographical location.

There are at least 250 000 species of vascular plants existing worldwide. Only a small number of species has been investigated both phytochemically and pharmacologically [1]. Lebanon occupies only 0.007 % of the world's land surface area and is a home to 2612 plant species compared to only 1600 species in Great-Britain which is 30 times more extended, and, 4200 species in mainland France, a country that is 55 times bigger than Lebanon [2-4]. The vegetation of Lebanon has the exceptionally high species-area ratio of 0.25 species per km², compared with a ratio of 0.0081 per km² for South Africa, 0.0044 for Brazil, 0.0021 for Egypt, 0.022 for Jordan, 0.015 for Spain, 0.017 for Syria and 0.011 for Turkey [5]. Relative to its size, Lebanon is recognized as a center of plant diversity and one of the Mediterranean region's mini-hotspots, sheltering high biodiversity richness including many rare and endemic plant species [6]. According to national floras, dating back to the early 1930s and 1960s, the country is shelter to almost 311 endemic species found in Lebanon, Syria and Palestine. A high percentage of endemic plant species (3.5%) is observed in Lebanon: among the 2612 wild species, 92 are endemics [7]. The genus *Origanum* (Labiatae) consists of 43 species belonging to 10 sections. It also has 18 natural hybrids [8]. *Origanum libanoticum* Boiss., known under the vernacular name "za'atar lebne", a wild cascading ornamental plant, endemic to Lebanon, is widespread throughout Lebanon, at various altitudes. It blooms from June till October on tall stems with cascading sprays of pink bracts and flowers.

Environmental factors were proven to affect the development of *Origanum* plant species. Many examples showed that the chemical composition of their essential oils (EOs) varies with geographical location, harvesting date, climate factors, soil composition, plant part, age, seasonality, vegetative stage, and so on [9-14]. The influence of those factors on the accumulation of distinct terpene metabolites in *Origanum* species defines its chemotypes.

We aimed, in this work, to investigate the yield and the chemical composition of the EO isolated from *O. libanoticum* and to seek for a possible chemical variability of its EO, for the first time, in relation to seasonal variations, drying method, geographical location and soil composition. We should point out that, no published work has presented a detailed characterization such as what is discussed in this paper. To fulfill this objective, sampling has covered two regions corresponding to two different altitudes: Qartaba (Jbeil District/Mount Lebanon Governorate) located at 1250 m and Chouwen (Keserwan District/Mount Lebanon Governorate) at 600 m.

The EO content increased from January to July (flowering stage) then decreased after flowering. The flowering stage was characterized by the highest EO yields. The highest yields were: 0.33% in 2013 (0.2 mL in 60.35 g of the dried herb) and 0.32% in 2014 (0.4 mL in 124.3g of the dried herb) for samples harvested from Chouwen and dried by lyophilization; 0.27% in 2013 (0.2 mL in 75.1 g of the dried herb) and 0.37% in 2014 (0.2 mL in 54.52 g of the dried herb) for samples harvested from Qartaba and dried by lyophilization. One-way ANOVA demonstrated the relation between EO yield and date of harvest ($p < 0.05$). Clearly the EO yield was influenced by vegetative stages and climate factors. Our results are in full agreement with most of previous works who reported that *Origanum* sp. EO content depends on the collection month and that the flowering stage increased the EO yield [15-16].

Student's *t*-test showed that the drying method of plant material influenced the EO yield ($p < 0.05$): lyophilized samples provided the highest yield values (Chouwen: 0.33% in 2013 and 0.32% in 2014; Qartaba: 0.27% in 2013 and 0.37% in 2014) (Figure 1).

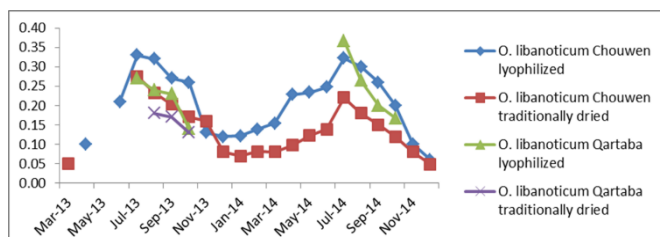


Figure 1: Monthly yields of essential oil (2013-2014) extracted from *O. libanoticum* harvested in Qartaba and Chouwen for lyophilized and traditionally dried plants.

Table 1 summarizes the altitudes of the two geographical locations and the main chemical parameters of the two soils. *O. libanoticum* harvested from Qartaba and Chouwen naturally grows in sandy soils. A statistically non-significant difference was noticed between altitude and oil content ($p > 0.05$). Region of harvest, elevation and temperature differences between the 2 sites probably did not influence the EO yield of the plants growing in the same edaphic conditions (similar soil characteristics).

Table 1: Altitude and soil parameters results at two Lebanese localities.

	Qartaba	Chouwen
Altitude (m)	1250	600
pH	7.94	7.98
Organic Matter (%)	2.83	1.93
P ₂ O ₅ (mg/kg)	12.73	11.1
K ₂ O (mg/kg)	142.06	88.59
N (%)	0.22	0.228

In total, 70 components were identified in the volatile oil of *O. libanoticum* plants collected from Qartaba and Chouwen, accounting for 99.8% of the total oil composition. Tables 2 and 3 gather the compositions of the EOs of the 53 samples of oregano collected during 2013 and 2014. The compounds were grouped into five classes: monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpenes hydrocarbons, oxygenated sesquiterpenes and others.

With reference to their EO composition, *Origanum* taxa may be characterized by the dominant occurrence of the following compounds:

- Monoterpene alcohol (linalool, α -terpineol, terpinen-4-ol) like the EO of *O. vulgare* subsp. *virens*, and sabinene hydrate, bicyclic monoterpenoid found in the EO of *O. majorana* (*Majorana hortensis* Moench.) [17];
- Phenolic monoterpenoids compounds, carvacrol and/or thymol, like the EOs of *O. syriacum*, *O. vulgare* subsp. *hirtum*, *O. vulgare* subsp. *viride*, *O. vulgare* subsp. *virens* and *O. onites* [16, 18-21];
- Sesquiterpenes (δ -elemene, germacrene D, β -bourbonene, β -caryophyllene) like the EO of *O. vulgare* subsp. *virens*, *O. vulgare* subsp. *gracile*, *O. vulgare* subsp. *viride* and *O. vulgare* subsp. *vulgare* [22].

In fact, carvacrol and sabinene hydrate are the two primary chemicals associated with culinary origanums. Carvacrol is responsible for the sharp, pungent flavor of the culinary oreganos [23-24]. Sabinene hydrate is linked to *O. majorana*'s sweet flavor and can occur heavily in its hybrids with *O. vulgare* subspecies (*O. xmajoricum*). *O. libanoticum*, unlike the other *Origanum* species, contained *p*-cymene and β -caryophyllene as major components followed by α -pinene, myrcene, α -phellandrene, limonene, 1,8-cineole, γ -terpinene, α -terpineol, thymol methyl ether, α -cubebene, α -copaene, geranyl acetate, β -bourbonene, germacrene D, bicyclogermacrene, β -sesquiphellandrene, δ -cadinene, spathulenol, caryophyllene oxide, octadecanol, docosane and tricosane and other minor components (camphene, α -terpinene, linalool, etc.). It should be pointed out that, unlike the only three previously described *O.*

libanoticum EO chemical composition, showing a predominance of β -caryophyllene (26.8%), caryophyllene oxide (22.6%) and germacrene-D (17.2%) [25], thymol methyl ether (32.8%), δ -cadinene (9.7%) and γ -terpinene (9.6%) [26], and, hexadecanoic acid (11.3%), thymol methyl ether (9.8%), (E)- β -caryophyllene (7.7%) and linalool (6.5%) [27], our results analyzing 53 samples, indicate that it is possible to attribute *O. libanoticum* growing wild in Qartaba and Chouwen to the *p*-cymene and/or β -caryophyllene chemotype. It was also noticed that the percentage of the dominance of *p*-cymene and β -caryophyllene varied from one month to another and between years.

Qualitative and quantitative analysis of the volatile oils extracted from 53 samples, harvested from the two Lebanese sites, throughout two years (2013 and 2014), was performed by GC/MS. The chemical constituents were submitted to multivariate analysis (ICA or independent components analysis), in order to study their intra-specific variation.

ICA-DA was applied on the original matrix **X** (peak areas) concatenated with the matrix **Y** (predefined groups) corresponding to year of harvest (two years vectors), extracted seven ICs. Thus, an ICA with seven ICs was applied to the matrix **X**. IC1 and IC6 were found to be informative since clear discriminations were observed. In IC1, samples harvested in 2013 (1) were characterized by the lowest proportions compared to the ones harvested in 2014 (2), which presented higher proportions (Figure 2). The source signal of IC1, in Figure 3, showed that β -caryophyllene is mainly at the origin of this discrimination. IC1 highlighted that samples harvested in 2014 contained more of β -caryophyllene, thymol methyl ether, germacrene D, bicyclogermacrene and δ -cadinene than the samples harvested in 2013.

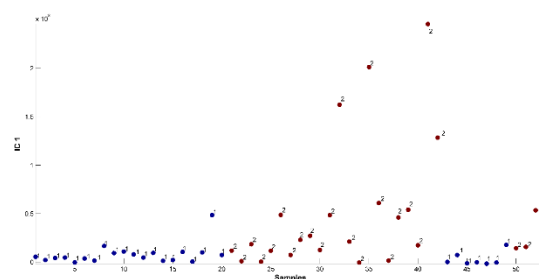


Figure 2: Proportions on IC1 for ICA-DA based on the year of harvest.

(1) Samples harvested in 2013

(2) Samples harvested in 2014

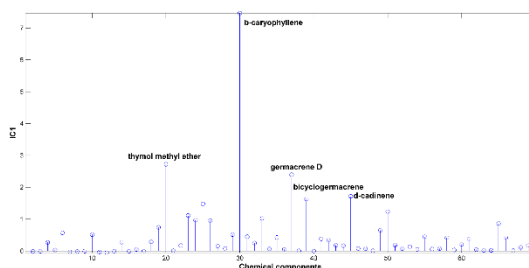


Figure 3: Source signal of IC1 for ICA-DA based on the year of harvest.

IC6 was also able to discriminate the samples (Figure 4). Looking at the extracted source signal in Figure 5, it can be seen that this discrimination is mainly due to β -sesquiphellandrene, thymol methyl ether, terpinen-4-ol, spathulenol, α -copaene and α -cubebene.

Samples harvested in 2013 (1) contained less of β -sesquiphellandrene, terpinen-4-ol, spathulenol, α -copaene and α -cubebene, and, more of thymol methyl ether than the samples harvested in 2014 (2).

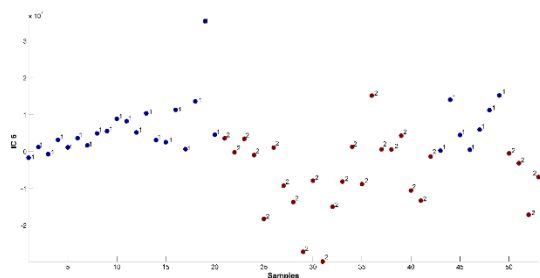


Figure 4: Representation of proportions on IC6 for ICA-DA based on the year of harvest.

- (1) Samples harvested in 2013
- (2) Samples harvested in 2014

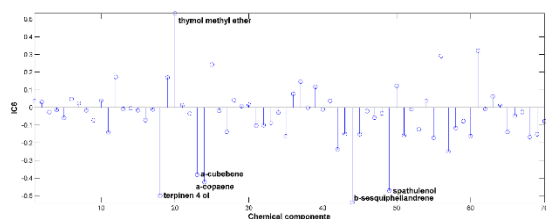


Figure 5: Source signal of IC6 for ICA-DA based on the year of harvest.

These discriminations highlighted the variation of EO chemical composition according to the year of harvest. The concentration of the main components (β -caryophyllene and *p*-cymene) defining *O. libanoticum* chemotype varied between 2013 and 2014. These variations were not observed in other *Origanum* species such as *O. syriacum* [16] and, were not only found among samples of different regions but also among samples of the same region with different months during 2 years reflecting the intraspecific variability.

Six ICs were extracted by ICA-DA applied on the matrix **X** of the peak areas and the matrix **Y** which presented by the two drying methods vectors. The six extracted independent components illustrated an overlap of the proportions, and showed that there is no discrimination between the samples traditionally dried and lyophilized. This result demonstrated that the two methods of harvest selected in this work, presented the same effect on the quantity and the quality of the *O. libanoticum* EO.

Discrimination was not detected between the different months of the year. An overlap of proportions is shown in the nine extracted independent components. So, date of harvest and vegetative stages had no effect on the variation of *O. libanoticum* EO chemical composition.

Two vectors (predefined groups) representing the two geographical locations were concatenated with the original matrix of peak areas. The application of ICA-DA allowed the determination of eight ICs as the optimal number. Samples presented superimposed proportions in the eight ICs which implied that the chemical composition of the EOs was not influenced by the site of harvest. *O. libanoticum* harvested from Qartaba and Chouwen naturally grows in sandy soils. Therefore, probably, neither the climatic factors associated to altitudes (such as temperature) nor the edaphic factors

which are similar in the 2 sites, had an influence on the EO chemical composition (Table 1).

In summary, wild populations of *O. libanoticum* collected throughout 2013 and 2014 from Qartaba and Chouwen produced EO characterized by the prevalence of *p*-cymene and β -caryophyllene. In addition, we demonstrated that the yield of EO varied according to the date of harvest and the drying method. Flowering phase was the most productive period. A comparison of the chemical composition of the EO of *O. libanoticum* at all stages of development throughout the 2 considered years revealed differences. However, ICA revealed that the chemical composition of the EOs was not influenced by the harvesting site, altitudes, drying techniques and date of harvest. It is worth noting that the combination of more than one factor (environmental conditions, temperature, light, humidity, ecological conditions, plant adaptation, etc.) could explain the variation observed in the EO main compounds concentration between the two years investigated. The investigations at chemotypes level and at the intraspecific chemical polymorphism are needed because biological activity of EOs depends on their chemical composition, the major compounds functional groups (alcohols, phenols, etc.) and the possible synergistic effects between the components. In this context, the value of an EO is its totum [28-29]. EO chemical composition varies in quality and quantity according to environmental and agronomic factors [30-31]. However, at variance with Al Hafi *et al.* (2016) [25], Arnold *et al.* (2000) [26] and Marrelli *et al.* (2016) [27] results, our samples were *p*-cymene and/or β -caryophyllene chemotype. Given the intraspecific polymorphism characterizing *O. libanoticum* EO and the variability affecting its chemical composition, which is observed from one month to another in our results, any determination of the EO biological activity must take into consideration, not only the chemical signature, but also its chemical variability. Therefore, larger scale and more long-term studies will allow room for validation and determination of the EO bioactivity. This will lead the research to an optimal harvest of the plant; a harvest adapted to the user's needs. Further studies conducted over several years on seasonality, genetics and growing conditions of wild and cultivated *O. libanoticum* should be performed in order to better understand the annual chemical variability observed. Furthermore, standardization of cultivation and propagation methods leading to efficient production and preservation of this plant is needed. Moreover, investigations of nutrient doses on quantitative and qualitative production of *O. libanoticum* EO compounds should be considered in future studies (each nutrient used in nutrient solution for the cultivation) in order to determine the optimal nutrient doses ensuring higher concentrations of the major EO compounds. Future investigations will focus the research on the biological activities of *O. libanoticum* EOs.

40	1504	1505	1758	1758	α -farnesene	1.4					1.3			1.9		
41	1507	1505	1741	1743	β -bisabolene		1.3	1.1	2.0	2.2	1.5		2.2	2.2		
42	1513	1513	1776	1776	γ -cadinene	0.4		0.4	0.4	0.4		1.1		0.7		
43	1524	1521	1780	1787	β -sesquiphellandrene			4.7	8.4							
44	1526	1522	1773	1773	δ -cadinene	2.6	4.3			5.6	4.7		4.7	5.0	4.7	
45	1539	1537	1745	1745	α -cadinene	0.2		0.1	0.2				0.6	0.3	0.4	
46	1541	1544	1918	1918	α -calacorene	0.1		0.2	0.3	0.3	0.7		0.3		0.3	
47	1544	1556	1854	1854	germacrene B	0.3		0.2	0.4	0.4	0.5				0.6	
48	1577	1577	2152	2150	spathulenol	1.7	3.5	3.3	5.7	10.4	11.6		1.1	3.0	13.4	
49	1581	1582	2008	2008	caryophyllene oxide	3.7	4.5	2.7	4.1	3.8			4.9	1.7	3.9	
50	1590	1592	2104	2104	viridiflorol			0.3	0.4	0.9	0.6		0.4			
51	1606	1599	2033	2025	<i>epi</i> -globulol			0.2	0.3	0.7			0.3			
52	1608	1608	2202	2071	humulene-1,2-epoxide	0.3		0.4	0.2	0.6	2.2		1.2	0.3	2.5	
53	1613	1618	2080	2083	1,10- <i>di-epi</i> -cubenol	0.6		0.3	0.5	0.4				0.9		
54	1640	1640	2188	2188	<i>T</i> -cadinol		1.1	1.5					2.3		4.7	
55	1641	1640	2316	2316	β -caryophylladienol	1.2		0.1	1.4	1.4				1.7		
56	1646	1640	2209	2209	<i>T</i> -muurolol	0.4			0.8	0.8	3.1			0.6		
57	1653	1653	2256	2255	α -cadinol	0.8	0.8	0.5	1.7	2.1	2.5		1.7	1.4	2.4	
58	1666	1665	2264	2264	intermedeol	0.4		0.3	0.4		1.4			0.5		
59	1675	1674	2170	2170	β -bisabolol	0.9	0.6	0.5	0.6	0.2	2.9		0.7	0.6	1.7	
60	1676	1675	2256	2256	cadalene	0.4	0.8	0.8		0.9			2.1	0.9	2.6	
61	1686	1685	2229	2229	α -bisabolol			0.3	1.2	1.9						
62	1697	1688	1953	1953	shyobunol	0.2			0.4	0.3						
63	1995	2000	2000	2000	eicosane				0.3				0.2			
64	2080	2077	2607	2607	octadecanol	0.4	0.7				6.1	14.3	5.2			
65	2088	2100	2100	2100	heneicosane	0.1	2.0	2.1	0.6							
66	2103	2111	2603	2622	phytol			1.5	0.3	0.2						
67	2187	2200	2200	2200	docosane	0.1	3.2	4.1	1.2						0.1	
68	2295	2300	2300	2300	tricosane	0.2	3.6	4.4	1.3	0.4	0.3	0.6	0.3		0.5	
69	2393	2400	2400	2400	tetracosane		2.1	1.9	0.6				0.1		0.3	
Monoterpene hydrocarbons						23.5	23.3	0.1	3.4	1.9	1.7	79.9	5.6	64.3	9.7	0.9
Oxygenated monoterpenes						11.5	8.4	4.2	8.8	10.3	7.5	0.0	10.5	18.3	11.7	8.5
Sesquiterpene hydrocarbons						48.5	36.7	33.4	59.2	53.0	45.1	2.4	54.3	12.5	55.6	51.4
Oxygenated sesquiterpenes						10.7	11.3	11.2	17.7	24.3	24.3	0.0	13.5	2.8	13.7	27.4
Others						4.9	17.0	17.8	7.8	4.6	16.5	14.9	13.0	2.0	7.1	7.3
Total identified						99.0	96.7	66.7	96.9	94.1	95.0	97.2	96.9	100.0	97.8	95.6

Harvesting date	Mar-13	Jul-13	Jul-13	Aug-13	Aug-13	Sep-13	Sep-13	Oct-13	Nov-13	Dec-13
Drying method	D1	D1	D2	D1	D2	D1	D2	D1	D1	D1

40	1504	1505	1758	1758	α -farnesene		0.6	1.4	1.0	0.9	0.5	0.3		1.1		
41	1507	1505	1741	1743	β -bisabolene	1.4							1.2			
42	1513	1513	1776	1776	γ -cadinene						0.3			0.3		
43	1524	1521	1780	1787	β -sesquiphellandrene	4.6										
44	1526	1522	1773	1773	δ -cadinene		1.7	3.8	2.5	2.1	1.5	0.9	2.5	2.3		
45	1539	1537	1745	1745	α -cadinene									0.1		
46	1541	1544	1918	1918	α -calacorene									0.1		
47	1544	1556	1854	1854	germacrene B									0.3		
48	1577	1577	2152	2150	spathulenol	7.7	0.5	1.0	1.5	1.6	1.9	0.3	1.5	2.0		
49	1581	1582	2008	2008	caryophyllene oxide	7.5	0.9	1.4	2.0	2.4	2.2	0.4	2.4	3.6		
50	1590	1592	2104	2104	viridiflorol									0.1		
51	1606	1599	2033	2025	<i>epi</i> -globulol									0.2		
52	1608	1608	2202	2071	humulene-1,2-epoxide	0.7					0.2			0.4		
53	1613	1618	2080	2083	1,10- <i>di-epi</i> -cubenol	0.7			0.4	0.4	0.4			0.4		
54	1640	1640	2188	2188	<i>T</i> -cadinol	2.1		0.3	0.5	0.6	0.7		0.6			
55	1641	1640	2316	2316	β -caryophylladienol	0.7								1.1		
56	1646	1640	2209	2209	<i>T</i> -muurolol							0.2	0.4	0.3		
57	1653	1653	2256	2255	α -cadinol	1.7		0.8	0.9	0.6	0.6	0.6		0.5		
58	1666	1665	2264	2264	intermedeol	0.6					0.2			0.3		
59	1675	1674	2170	2170	β -bisabolol	1.6			0.4	0.3	0.4		0.3	0.5		
60	1676	1675	2256	2256	cadalene	3.1		0.6	0.8	0.7	0.4	0.4	0.7	0.8		
61	1686	1685	2229	2229	α -bisabolol											
62	1697	1688	1953	1953	shyobunol	2.0			0.3	0.2		0.1		0.3		
63	1995	2000	2000	2000	eicosane	0.7						0.2				
64	2080	2077	2607	2607	octadecanol		2.1	6.5	4.9	9.0	3.1	2.5	1.6	0.9		
65	2088	2100	2100	2100	heneicosane							0.5		0.1		
66	2103	2111	2603	2622	phytol											
67	2187	2200	2200	2200	docosane							0.8		0.1		
68	2295	2300	2300	2300	tricosane	0.8						1.0		0.2		
69	2393	2400	2400	2400	tetracosane	1.0						0.5		0.1		
						Monoterpene hydrocarbons	15.5	87.2	55.0	26.8	37.9	27.5	50.7	39.5	34.0	26.7
						Oxygenated monoterpenes	6.2	10.8	11.5	10.4	10.6	13.1	13.5	3.6	15.4	14.1
						Sesquiterpene hydrocarbons	35.9	0.0	26.8	48.1	34.9	39.6	22.6	7.5	37.1	42.9
						Oxygenated sesquiterpenes	28.4	0.0	1.4	4.1	6.8	6.9	7.0	2.0	5.9	10.5
						Others	8.2	0.0	4.3	9.7	7.8	12.1	4.9	6.3	7.5	4.3
						Total identified	94.3	98.0	98.9	99.1	98.0	99.1	98.7	58.8	100.0	98.6

N°	Ri ^a	Ri ^b	Harvesting date													
			Jan-14	Feb-14	Mar-14	Apr-14	May-14	Jun-14	Jul-14	Aug-14	Oct-14	Nov-14	Dec-14			
			Drying method	D1	D1	D1	D1	D1	D1	D1	D1	D1	D1	D1	D1	
			Volatile compound													
1	924	924	1035	1035	α -thujene											

2	933	932	1076	1076	α -pinene	1.9			2.4	1.3	0.7						0.6
3	947	946	1076	1076	camphene												
4	973	969	1132	1132	sabinene				0.5	0.1	2.6	0.8					1.4
5	980	974	1118	1118	β -pinene								0.6				
6	991	988	1174	1174	myrcene				5.0	1.8	4.1	0.8	0.5	2.0			1.8
7	1003	1002	1188	1188	α -phellandrene			0.7									0.1
8	1009	1008	1157	1159	δ -3-carene												
9	1014	1014	1188	1189	α -terpinene				1.8	1.1	0.9	0.4					0.7
10	1024	1020	1280	1278	<i>p</i> -cymene	3.7	8.4	0.7	18.6	13.2	5.4	4.5	1.7	5.0			6.8
11	1025	1024	1203	1203	limonene				1.8		4.5	3.1	1.4	1.7		0.4	2.1
12	1027	1026	1213	1213	1,8-cineole	1.6	2.2		3.2	1.9	1.0						1.0
13	1043	1045	1269	1269	<i>Z</i> - β -ocimene												
14	1057	1054	1255	1256	γ -terpinene			1.3	6.0	3.9	3.0	1.7		1.8		2.4	
15	1086	1086	1265	1265	α -terpinolene												
16	1098	1095	1553	1553	linalool									0.4			
17	1174	1174	1611	1611	terpinen-4-ol				2.3	1.1	2.5		0.5	1.6	2.7	0.8	
18	1201	1186	1706	1706	α -terpineol			2.4		2.1	2.6	0.7	1.6	3.3	4.5	2.2	
19	1238	1232	1607	1609	thymol methyl ether			6.3	6.9	4.5	6.2	5.0	7.2	7.0	6.2	4.5	
20	1294	1289	2198	2198	thymol												
21	1300	1298	2239	2239	carvacrol								1.3	2.4			
22	1350	1345	1466	1466	α -cubebene			1.7	2.2	5.6	6.5	5.1	1.8	3.4	2.6	3.7	
23	1378	1374	1497	1497	α -copaene	1.0			0.9		2.8	4.5	7.7	1.5	5.8		
24	1379	1379	1765	1765	geranyl acetate	4.0	3.1	7.0	3.5	4.8	4.1	3.9		4.8		7.0	
25	1386	1387	1535	1535	β -bourbonene	2.2	2.4	3.7	2.2	0.7	1.1	1.7	1.9	4.0	2.6	4.2	
26	1389	1387	1547	1547	β -cubebene						0.2	0.3	1.3		0.3		
27	1391	1389	1600	1600	β -elemene					0.3		0.5				0.8	
28	1411	1410	1568	1568	α -cedrene	1.1		1.8	1.1	1.3	1.5	1.8	1.1	1.6	1.6	2.0	
29	1416	1417	1612	1612	β -caryophyllene	15.2	12.0	18.8	15.7	19.2	18.0	20.2	20.7	23.0	20.4	17.8	
30	1432	1431	1612	1612	β -gurjunene	0.7		1.2	0.9	0.9	0.9	1.3	1.3	1.1	1.3	1.2	
31	1438	1439	1628	1628	aromadendrene			0.5		0.7	0.3	0.9	0.5	0.5	0.8	0.7	
32	1454	1452	1668	1670	α -humulene	2.1	1.6	2.8	2.3	2.6	2.7	3.2	2.6	3.2	3.0	2.7	
33	1457	1454	1689	1689	<i>E</i> - β -farnesene								1.0				
34	1462	1469	1734	1734	<i>epi</i> -bicyclosquiphellandrene	0.9		1.3		1.8	1.2	1.6		0.8	1.6	1.9	
35	1478	1478	1704	1704	γ -muurolene					0.4	0.4	0.2			0.3		
36	1480	1484	1726	1726	germacrene D	4.5	3.2	3.9	3.3	4.3	3.5	7.1	6.7	4.3	3.8	4.7	
37	1483	1486	1784	1784	α -curcumene												
38	1497	1500	1756	1756	bicyclogermacrene	2.4	2.2	2.5	3.3	6.4	7.2	6.1	2.8	4.4	3.9	4.3	
39	1500	1500	1740	1740	α -muurolene												
40	1504	1505	1758	1758	α -farnesene	1.0		0.9		2.2	2.6	2.2					
41	1507	1505	1741	1743	β -bisabolene			1.3	2.0	0.9		0.7	1.6	1.6	1.7	2.3	
42	1513	1513	1776	1776	γ -cadinene			0.8	1.4	0.5	0.8	1.2			1.0		

43	1524	1521	1780	1787	β -sesquiphellandrene		3.5		4.7	5.7								
44	1526	1522	1773	1773	δ -cadinene	3.3		4.5			5.4	5.8	6.9	3.2	4.1	5.5		
45	1539	1537	1745	1745	α -cadinene					0.2		0.2			0.3	0.4		
46	1541	1544	1918	1918	α -calacorene						0.2	0.6				0.3		
47	1544	1556	1854	1854	germacrene B							0.3				0.5		
48	1577	1577	2152	2150	spathulenol	2.5	5.7	6.0	1.8	1.0	1.2	1.6	5.5	3.2	2.6	13.2		
49	1581	1582	2008	2008	caryophyllene oxide	3.7	5.7	6.5	3.0	1.3	1.7	1.8		4.8	3.9			
50	1590	1592	2104	2104	viridiflorol						0.6		0.5					
51	1606	1599	2033	2025	<i>epi</i> -globulol			0.6			0.3		0.2			0.6		
52	1608	1608	2202	2071	humulene-1,2-epoxide	1.0		1.0		0.7		0.2	0.8		0.8	1.9		
53	1613	1618	2080	2083	1,10- <i>di-epi</i> -cubenol							0.5						
54	1640	1640	2188	2188	<i>T</i> -cadinol	1.7	3.1		0.5	0.4	0.5		2.0					
55	1641	1640	2316	2316	β -caryophylladienol	1.1		2.9				0.7		1.4	0.2	4.8		
56	1646	1640	2209	2209	<i>T</i> -muurolol	3.2		0.7		0.4		0.6		0.6	1.2			
57	1653	1653	2256	2255	α -cadinol		2.0	1.6		1.3	1.1	1.2	1.1	0.8		3.0		
58	1666	1665	2264	2264	intermedeol			0.8		0.3								
59	1675	1674	2170	2170	β -bisabolol	1.5	1.3	1.2		0.8	0.5	0.7	0.8	0.6	0.3	1.3		
60	1676	1675	2256	2256	cadalene	3.1	1.8	1.2				1.3	1.7	0.5	0.5	2.9		
61	1686	1685	2229	2229	α -bisabolol			0.8				0.3			0.2			
62	1697	1688	1953	1953	shyobunol	2.1	1.0					0.4						
63	1995	2000	2000	2000	eicosane			2.3										
64	2080	2077	2607	2607	octadecanol	7.1	2.5					4.4	10.7	2.0	1.4			
65	2088	2100	2100	2100	heneicosane	1.3	3.9	1.5										
66	2103	2111	2603	2622	phytol	3.3	1.6											
67	2187	2200	2200	2200	docosane	1.2	6.9	2.4										
68	2295	2300	2300	2300	tricosane	2.5	6.8	2.5				0.4	0.5		0.4	0.6		
69	2393	2400	2400	2400	tetracosane	2.5	3.3	1.5								0.2		
Monoterpene hydrocarbons						3.7	13.4	1.4	38.9	24.4	18.2	10.0	2.8	9.2	15.9	0.0		
Oxygenated monoterpenes						1.6	2.2	8.7	12.4	9.7	12.3	5.7	11.1	14.3	14.5	7.5		
Sesquiterpene hydrocarbons						34.3	24.8	45.7	40.0	53.6	55.1	64.9	58.8	52.8	55.3	53.0		
Oxygenated sesquiterpenes						20.0	20.8	23.3	5.3	6.3	6.0	9.2	12.7	11.9	9.7	27.6		
Others						21.8	30.5	15.0	3.5	4.8	4.1	8.7	11.2	6.8	1.8	7.8		
Total identified						81.3	91.6	94.0	100.0	98.9	95.7	98.5	96.5	94.9	97.2	95.9		

Drying method (L: lyophilization; D: traditional drying). Harvest number (1: harvest at the beginning of the month; 2: harvest in the middle of the month). Retention index (Ri^a: Retention index calculated on a HP-5MS column; Ri^b: Retention index on a HP-5MS column from literature [16]; Ri^c: Retention index calculated on a HP Innowax column; Ri^d: Retention index on a HP Innowax column from literature [32-33]). t: trace (less than 0.05%). Blanks: not detected.

Table 3: Chemical composition of the essential oil of *O. libanoticum* harvested from Qartaba.

N°	Ri ^a	Ri ^b	Harvesting date	Drying method	Volatile compound	Jul-13		Aug-13		Sep-13		Oct-13		Jul-14		Aug-14		Sep-14		Oct-14	
						L1	D1	L1	D1	L1	D1	L1	D1	L1	D1	L1	D1	L1	D1	L1	D1
1	924	924	1035	1035	α -thujene			1.0	1.4			1.6	1.7	0.9							1.4
2	933	932	1076	1076	α -pinene	0.9		4.0	5.2	1.3	5.9	6.4	3.5	0.4							2.1
3	947	946	1076	1076	camphene			0.4				0.7									
4	973	969	1132	1132	sabinene	1.1		0.3	0.4			0.5	0.5	0.3	1.0						1.0
5	980	974	1118	1118	β -pinene	1.5		0.9	1.2			1.5	2.0	1.2		2.0		0.6			1.0
6	991	988	1174	1174	myrcene	1.3		2.2	3.4	t	3.8	3.7	2.2	0.8	1.2		0.4				2.1
7	1003	1002	1188	1188	α -phellandrene	8.2		0.8	2.1			2.1	1.4	0.7							1.5
8	1009	1008	1157	1159	δ -3-carene	0.5		0.4	1.1			0.8	0.6								
9	1014	1014	1188	1189	α -terpinene	2.5		2.1	3.2	2.4	3.6	3.3	1.9	0.5	0.4						2.6
10	1024	1020	1280	1278	<i>p</i> -cymene	24.8		25.2	40.9	41.5	43.1	40.9	23.5	4.1	2.3		2.9				28.6
11	1025	1024	1203	1203	limonene	6.2		6.9	11.8	6.5	12.5	11.3	6.7	1.0	1.0		0.3				6.7

12	1027	1026	1213	1213	1,8-cineole	4.8	5.2	7.1	3.0	8.2	6.8	4.3	0.7			5.6
13	1043	1045	1269	1269	Z- β -ocimene		0.2	0.3			0.3					
14	1057	1054	1255	1256	γ -terpinene	8.0	7.8	12.1	6.9	12.7	11.0	6.6	1.8	1.2	1.2	8.2
15	1086	1086	1265	1265	α -terpinolene		0.2	0.5			0.5					
16	1098	1095	1553	1553	linalool		0.2				0.4		1.0	0.4		1.5
17	1145	1141	1532	1532	camphor						0.2					
18	1174	1174	1611	1611	terpinen-4-ol		0.1	0.9			0.6		0.6	0.5		4.8
19	1201	1186	1706	1706	α -terpineol		0.9	0.6			1.3	1.8	1.8	1.2		
20	1238	1232	1607	1609	thymol methyl ether		3.4				t	1.8	5.3	3.1	4.3	
21	1300	1298	2239	2239	carvacrol		1.7	0.1			t		2.3			
22	1350	1345	1466	1466	α -cubebene		0.7	0.6	0.4		0.2	1.1	3.8	2.5	1.7	1.0
23	1378	1374	1497	1497	α -copaene		1.2				0.2	1.2	2.4	3.0	2.6	0.5
24	1379	1379	1765	1765	geranyl acetate		1.4				0.3	1.0	0.8	1.4	1.0	1.0
25	1386	1387	1535	1535	β -bourbonene		1.0				0.4	1.6	2.3	3.0	2.7	1.0
26	1389	1387	1547	1547	β -cubebene		0.1						0.4	0.5	0.5	
27	1391	1389	1600	1600	β -elemene		0.3						0.6	0.7	0.5	
28	1411	1410	1568	1568	α -cedrene		0.5					0.4	0.4	0.7	0.4	
29	1416	1417	1612	1612	β -caryophyllene	3.7	7.7	0.8		0.5	1.7	6.7	13.1	15.6	9.1	13.3
30	1432	1431	1612	1612	β -gurjunene		1.0				0.2	0.7	1.9	1.7	1.6	0.6
31	1438	1439	1628	1628	aromadendrene		0.8					0.3	1.1	0.9	1.0	
32	1454	1452	1668	1670	α -humulene		1.2				0.2	0.9	2.2	2.1	1.5	0.7
33	1457	1454	1689	1689	E- β -farnesene								0.2			
34	1462	1469	1734	1734	epi-bicyclosesquiphellandrene		1.0					0.5	1.9	2.0	2.0	0.8
35	1478	1478	1704	1704	γ -muurolene		0.2							0.2		
36	1480	1484	1726	1726	germacrene D	1.2	5.2	0.4			0.7	3.4	11.2	10.9	8.7	2.4
37	1483	1486	1784	1784	α -curcumene									0.1		
38	1497	1500	1756	1756	bicyclogermacrene	1.1	1.7	0.4			0.4	1.4	5.0	3.8	2.9	1.6
39	1500	1500	1740	1740	α -muurolene	1.0						0.7		0.2		
40	1504	1505	1758	1758	α -farnesene		0.7					0.3	1.4	1.4	1.0	0.6
41	1507	1505	1741	1743	β -bisabolene		1.1							0.7		
42	1513	1513	1776	1776	γ -cadinene		0.2						0.7	0.9	0.8	
43	1524	1521	1780	1787	β -sesquiphellandrene								0.6		0.4	
44	1526	1522	1773	1773	δ -cadinene	1.6	2.1				0.4	1.9	5.1	5.3	3.8	2.2
45	1539	1537	1745	1745	α -cadinene		0.2						0.4	0.3	0.4	
46	1541	1544	1918	1918	α -calacorene								0.2	0.2	0.3	
47	1577	1577	2152	2150	spathulenol		0.3				0.1	0.8	1.4	1.4	1.1	1.0
48	1581	1582	2008	2008	caryophyllene oxide		0.6				0.2	1.1	1.2	1.5	1.0	0.7
49	1590	1592	2104	2104	viridiflorol								0.4	1.1	1.5	
50	1606	1599	2033	2025	epi-globulol							0.4	1.0		0.3	

51	1613	1618	2080	2083	1,10- <i>di-epi</i> -cubenol	0.4						0.1				
52	1640	1640	2188	2188	<i>T</i> -cadinol	0.1				0.3	0.4		0.7			
53	1641	1640	2316	2316	β -caryophylladienol	0.2										
54	1646	1640	2209	2209	<i>T</i> -muurolol					0.3	0.8	0.9	0.6	1.0		
55	1653	1653	2256	2255	α -cadinol	0.5				0.8	1.8	1.8	1.7			
56	1666	1665	2264	2264	intermedeol		t									
57	1675	1674	2170	2170	β -bisabolol	0.1		0.1			0.3	0.7	0.3			
58	1676	1675	2256	2256	cadalene	4.5		t			0.5	0.5	0.3			
59	1686	1685	2229	2229	α -bisabolol						0.4					
60	1697	1688	1953	1953	shyobunol						0.2					
61	2080	2077	2607	2607	octadecanol	0.3		0.7		0.5	6.7	12.1	15.9	3.2		
62	2295	2300	2300	2300	tricosane	0.2					0.2	0.2	0.1			
63	2393	2400	2400	2400	tetracosane			0.3					0.3			
Monoterpene hydrocarbons						54.9	52.6	83.7	58.6	88.0	84.1	47.6	9.6	8.1	5.5	55.2
Oxygenated monoterpenes						4.8	11.5	8.7	3.0	8.2	9.3	7.9	11.6	5.1	4.3	11.8
Sesquiterpene hydrocarbons						8.5	26.8	2.2	0.4	0.5	4.4	21.1	55.0	56.8	41.9	24.9
Oxygenated sesquiterpenes						0.0	6.9	0.0	0.1	0.0	0.3	3.5	8.2	8.5	6.8	3.4
Others						0.5	1.4	0.7	0.3	0.0	0.8	7.9	13.1	17.4	1.3	4.1
Total identified						68.7	99.2	95.3	62.4	96.7	98.9	87.9	97.6	96.0	59.8	99.4

Drying method (L: lyophilization; D: traditional drying). Harvest number (1: harvest at the beginning of the month; 2: harvest in the middle of the month). Retention index (Ri^a: Retention index calculated on a HP-5MS column; Ri^b: Retention index on a HP-5MS column from literature [16]; Ri^c: Retention index calculated on a HP Innowax column; Ri^d: Retention index on a HP Innowax column from literature [32-33]). t: trace (less than 0.05.%). Blanks: not detected.

Experimental

Plant material and essential oil extraction: In order to cover all the vegetative stages for plants development, aerial parts of *O. libanoticum* were collected twice monthly throughout 2013 and 2014 from March 1st, 2013 and continued until December 1st, 2014 from Chouwen (34°4'56" N 35°45'43" E), at an altitude of 600 m and from Qartaba (34°05'58.70" N 35°48'46.39" E), at an altitude of 1250 m.

Botanical identification of the plant samples was carried out by Dr. Marc El Beyrouthy according to the New Flora of Lebanon and Syria as described by Mouterde (1983) [4]. A voucher specimen of each plant was deposited in the Herbarium of the Faculty of Agricultural and Food Sciences of USEK, Lebanon under the registry numbers MNIII185a for the Chouwen plant and MNIII185b for the Qartaba plant. In total, forty-two samples were harvested from Chouwen and eleven from Qartaba. Occasionally, *O. libanoticum* harvest could not take place, especially in Qartaba, due to climatological or seasonal obstacles. Fresh herb (150 g) was either air dried in the absence of light at 4°C for one month (traditional drying), or lyophilized using Virtis bench top "K" lyophilizer machine.

The EOs were isolated by hydrodistillation, performed for 3 hours using a Clevenger-type apparatus according to the method described in the European Pharmacopoeia (1997) [34]. EOs were collected, dried over anhydrous sodium sulphate and stored at 4°C in sealed brown vials.

Yield evaluation: The EO content was calculated as percentage of the dry mass. EO yield, expressed in mL/g, was calculated by measuring the volume of oil extracted per weight of dried/lyophilized plant material.

Soil analysis: Soil samples were collected from each site at a depth of 0-30 cm. Each sample was dried at room temperature, sieved at 2 mm and quartered before being analyzed at the Lebanese Agricultural Research Institute (LARI) Lebanon. Soil samples were taken during June 2014 at the time of flowering period of plants.

Essential oils analyses

GC analyses: The GC analysis was performed on a Thermo Electron Corporation apparatus fitted with a flame ionization detector (FID), a non-polar HP-5MS (5% Phenyl Methyl Siloxane) capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm) and a polar fused-silica HP Innowax capillary column (polyethylene glycol, 50 m × 0.20 mm i.d., film thickness 0.20 µm) in order to confirm the identification of the components. Helium was the carrier gas (0.8 mL/minute). The column temperature was initially set to 35°C before being gradually increased to 85°C at 5°C/minute, held for 20 minutes at 85°C, raised to 300°C at 10°C/minute and finally held for 5 minutes at 300°C. Diluted 1 µL samples (1/100, v/v in pentane) were manually injected at 250°C and in the splitless mode. Flame ionisation detection (FID) was performed at 310°C.

GC/MS analyses: The GC/MS analyses were performed using an Agilent gas chromatograph 6890 coupled with Mass Detector 5975. The 7683 B auto sampler injected 1 µL of each oil sample diluted in pentane (1/100 v/v). A fused-silica capillary column HP-5MS (30 m × 0.25 mm i.d., film thickness 0.25 µm) and a fused-silica HP Innowax polyethylene glycol capillary column (50 m × 0.20 mm i.d., film thickness 0.20 µm) were used in order to confirm the identification of the components. Helium was the carrier gas (0.8 mL/minute). The oven temperature program was identical to that described in 2.4.1. The mass spectra were recorded at 70 eV with an ion source temperature of 310°C and a transfer line heated to

320°C. The acquisition was recorded in full scan mode (50 - 400 amu).

Qualitative and quantitative analyses: Most constituents were identified by comparing mass spectra on both columns with those provided in the NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) and Wiley 275 libraries, our home-made library constructed with pure compounds and EOs of known composition (such as the EO of *Rosmarinus officinalis* L. from *Phytosun Aroms*, Plélo, France) and those from literature [35-36]. A further identification was achieved by comparing their retention indices (RI) on both polar and apolar columns with those from literature [36-37] or with those of standard compounds available in our laboratories, obtained from Sigma-Aldrich (Germany). Retention indices were determined in relation to a homologous series of *n*-alkanes (C₇ to C₂₅) analyzed under the same GC/MS operating conditions. Relative proportions of oil constituents were calculated from the GC peak areas corrected using the Probabilistic Quotient Normalization (PQN), using the median of all the chromatograms as reference signal for the correction [38].

Independent components analysis (ICA): theoretical background:

ICA is one of the most widely used blind source separation (BSS) techniques for revealing hidden factors that underlie sets of variables, measurements, or signals [39]. The goal of this statistical method is to extract the underlying source signals from a set of mixed signals, assuming that the original underlying source signals are mutually independently distributed. ICA looks for a linear transformation of non-Gaussian data that maximizes the statistical independence of the components and decomposes the measured overlapping signals of mixtures into a set of independent components (ICs) that are as independent as possible [40-41]. Maximizing independence means maximizing the non-Gaussianity of the signals.

The general model of ICA can be described as:

$$\mathbf{X} = \mathbf{A} \cdot \mathbf{S}$$

Where

\mathbf{X} ($n \times m$) is the matrix of observed signals that contains n measured signals of m variables each,

\mathbf{A} ($n \times d$) is a mixing matrix of unknown source signal proportions,

\mathbf{S} ($d \times m$) is the matrix containing the unknown source signals (the ICs).

ICA consists in estimating both matrices \mathbf{A} and \mathbf{S} knowing only \mathbf{X} . In this study the Joint Approximate Diagonalization of Eigenmatrices (JADE) algorithm was used in order to calculate a demixing matrix \mathbf{W} which approximates \mathbf{A}^{-1} . Among all the available algorithms, the JADE algorithm has proven to provide the most consistently reproducible and reasonable solutions [42].

The component signals (source signals: the rows of the matrix \mathbf{S}) are recovered from the measured mixed signals (peak areas of the chromatograms: the rows of the matrix \mathbf{X}) by:

$$\mathbf{S} = \mathbf{W} \cdot \mathbf{X} \text{ [43-44]}$$

The proportions, \mathbf{A} , can then be calculated by projecting \mathbf{X} onto \mathbf{S} :

$$\mathbf{A} = \mathbf{X} \cdot \mathbf{S}^T (\mathbf{S} \mathbf{S}^T)^{-1}$$

The ICA-DA procedure was used to determine the optimal numbers of independent components (ICs) to use in the ICA decomposition. In this procedure, a matrix \mathbf{Y} is created formed by vectors representing predefined groups corresponding to the different levels of the studied factors: geographical location of harvest (two sites implying two predefined groups), time of harvest (twelve months implying twelve predefined groups) and drying methods (two methods implying two predefined groups). The matrix \mathbf{Y} is concatenated with the original matrix \mathbf{X} of the peak areas in order to create a new matrix \mathbf{Z} on which ICA is applied with from 1 to n_{\max}

ICs. The number of ICs giving maximum discrimination of samples, based on the predefined groups, is then selected as the optimal number of ICs. The source signals extracted from the concatenated matrices contain contributions from both the MS variables and from the scaled groups matrix, so the corresponding proportions (scores) reflect not only the effect of the MS data, **X**, but also that of the predefined groups, **Y**. It is therefore necessary to recalculate the proportions using only the original matrix **X**, in

order to determine whether the MS variables selected by ICA-DA are able alone to discriminate the samples into the predefined groups.

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