Comparative analysis of the essential oil yield and chemical composition of cultivated *Origanum* spp. in Greece

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Abstract

Origanum vulgare spp. hirtum or commonly known as Greek oregano, is considered one of the best medicinal and aromatic plants worldwide. Its essential oil, mainly rich in carvacrol or thymol, makes the species of unique importance for culinary and pharmaceutical uses. The plant grows in the wild, maintaining its exceptional chemical characteristics, but it is also cultivated. Its cultivation is the solution to guarantee its supply and limit the overexploitation of wild populations. Nevertheless, apart from Origanum vulgare spp. hirtum, newly described varieties or other species merit further attention. Therefore, in this study, cultivated plants of O. vulgaris spp. hirtum, Origanum onites, and Origanum × intercedens were evaluated for their volatile profile using GC-MS analysis. Our results showed that all the Origanum species are rich in essential oil in which carvacrol is the most abundant constituent. In addition, new

data regarding *O. vulgare* spp. *hirtum* var. *Arethousa* and the hybrid *Origanum* × *intercedens*, add valuable information and prove that further exploitation of these two species is of great importance to the food, cosmetics and pharmaceutical industry.

Keywords: *Origanum* spp, *Origanum* vulgare spp hirtum var. Arethousa; *Origanum* × intercedens; cultivation; carvacrol

Introduction and background

The genus *Origanum* belongs to the Lamiaceae family. It is mainly distributed across the Mediterranean area [1], and it is considered one of the most important medicinal and aromatic plants of high commercial interest, due to its versatile uses that include preparations for medicinal, cosmetic, culinary, and food preservation purposes [2-4]. Plants of the genus have been classified according to the study of [1], which remains a hallmark regarding the *Origanum* genus classification. According to this study, the genus consists of 10 sections and 38 species, among which six subspecies that belong to *Origanum vulgare* spp and three varieties of *O. syriacum*. Plants of the genus are characterized by great morphological and chemical differences [1, 5], and several chemotypes have been described so far [5-7].

Origanum vulgare is the most popular among the species and belongs to sect. Origanum. As mentioned above, it consists of six subspecies separated based on morphological characters [1]. It is immensely cultivated in Europe and across the Mediterranean, and it is characterized by chemical polymorphism and a high yield of essential oil, which ranges from 0.8 to 44.1 mg/g of dry plant material [5]. The essential oil of the plant has been used since antiquity not only for culinary purposes but also due to its health promoting properties against common cold symptoms, skin and digestive disorders, as well as against inflammatory airway diseases [8]. Its medicinal properties are still recognized and are attributed to its high content of carvacrol and thymol, two phenolic compounds with remarkable presence in O. vulgare essential oil [7, 9], which also provide the plant's distinctive aroma [10]. The subspecies O. vulgare spp hirtum (Link) Ieswaart or commonly known as "Greek oregano", is native both to Greece and Turkey. Its characteristic intense flavor and aroma, make Greek oregano one of the most used spices for the preparation of Greek traditional dishes. The subspecies is characterized by the occurrence of two chemotypes, the carvacrol and thymol chemotypes [11], which are affected by the geography and climate of the collection area. Therefore, great differences have been recorded when examining species from different geographical areas [11, 12]. Harvesting of the plant takes place during summer (full bloom) and autumn [13, 14].

Nevertheless, the harvest period is important both for the essential oil yield and the concentration of the secondary metabolites presented. Kokkini et al., (1997) [14], observed that the essential oil yield is higher for plants collected at full bloom, with carvacrol being presented in great amounts. On the contrary during autumn harvesting, not only the production of essential oil is reduced, but also other compounds including p-cymene and γ -terpinene emerge as major constituents. Although the concentration of carvacrol and thymol may vary due to the influence of the collection site, for both harvesting periods these two isomeric phenols and their precursors, namely p-cymene and γ -terpinene, prevail [14]. The biosynthesis of thymol and carvacrol is affected by temperature, as lower ambient temperatures lead to increased production of thymol [15, 16]. Generally, Greek oregano is characterized by high carvacrol or thymol content (>65%) [17]. Higher amounts of carvacrol content have been reported for the O. vulgare spp hirtum var. Arethousa, a selection of the wild Origanum population found on the island of Ikaria. This variety possesses both a remarkable amount of essential oil (8% v/w) and an impressive amount of carvacrol which exceeds 90% [18].

Origanum onites is the second most commercial species of Origanum in Greece. It belongs to sect. Majorana and it is found in the Peloponnese, Aegean islands (Chios, Ikaria, and Crete) and Turkey [19]. Commercially it is sold under the name of Turkish oregano or Cretan oregano or Island oregano, which reflects its origin. Its essential oil is rich in carvacrol (69-93%) and reaches high essential oil yields (3-7 mL/100g) [20]. The high content in carvacrol, as well as the presence of borneol and the low abundance or absence of thymol, distinguish the species from that of O. vulgare spp hirtum (Link) Ieswaart [11, 20, 21]. In addition, its essential oil contains significant amounts of γ -terpinene, p-cymene and E-caryophyllene with concentrations ranging from 1-10% [9].

Origanum × intercedens is a hybrid of O. vulgare subsp. hirtum and O. onites (Ieswaart, 1980). It has restricted distribution and its essential oil yield accounts for 4.5-7%. Its carvacrol content is high (59-85%), thymol is found at low levels, while the presence of p-cymene and γ -terpinene is also important [22, 23]. Kokkini et al., (1993) [22], made an important observation by noticing the presence of methylthymol (2.8%) in the essential oil of this hybrid, a compound not found in O. onites but present in low amounts in some populations of O. vulgare spp. hirtum. However, the hybrid, despite its interesting chemical profile, has not yet been extensively studied or cultivated.

The aim of the present study is to chemically characterize samples of *Origanum* plants cultivated in different geographical regions of Greece. The volatile profile of six samples was elucidated by gas chromatography-mass spectrometry (GC-MS). Four samples belonged to the *O. vulgare* spp *hirtum*, one to the *Origanum onites* and one to the *Origanum x intercedens* hybrid. Two cultivars of the most commercial samples, namely *O. vulgare* spp. *hirtum* and *O. onites*, derive from organic crops. Chemical analysis of *Origanum vulgare* spp. *hirtum* var. *Arethousa* and *Origanum* × *intercedens*, contribute with additional information to the limited knowledge of the plants and are a motivation for their cultivation and further exploitation in the food and pharmaceutical industry.

Materials and methods

Plant material

To extract the essential oil, six samples of dried aerial parts from *Origanum* species were used. Each sample is referred hereinafter as OVH 1, OVH2, OVH3 and OVH4 for the species of *O. vulgare* spp. *hirtum* respectively, OO refers to *O. onites* and O×I to *O. × intercedens* (Table 1). Plant material was provided from suppliers in the local area except from samples OV3 and OxI, which were cultivated at the agricultural facilities of Agricultural University of Athens.

Table 1. Collection data of *Origanum* species.

Codification Plant part	Taxon	Collection site/period	Elevation (m)
OVH1 Leaves and flowers	Origanum vulgare spp. hirtum	Sykia, Imathia (Central Macedonia)/June 2024	335
OVH2* Leaves and flowers	Origanum vulgare spp. hirtum	Agyia, Chania (Crete)/July 2024	100
OVH3 Leaves and flowers	Origanum vulgare spp. hirtum var. Arethousa	Votanikos (Athens)/June 2024	70
OVH4 Leaves	Origanum vulgare spp. hirtum	Leonidio (Arkadia, Peloponnese)/June 2024	66
OO* Leaves and flowers	Origanum onites	Sitia, Lasithi (Crete)/May 2024	40
O×I Leaves and flowers	Origanum × intercedens	Votanikos (Athens)/June 2019	70

^{*}Organic cultivars

Essential oil collection

Volatile compounds were collected from flowers and leaves of the plant material using a Clevenger apparatus. Distillation took place for 3h. The collected essential oil was dried using anhydrous magnesium sulfate, filtered and stored at -20°C. Yield was calculated in mL/100 g of dry plant material.

GC-MS analysis

For the GC-MS analysis, a Bruker chromatograph 436-GC coupled to a mass spectrometry detector was used. Separation was performed on an OPTIMA 5MS capillary column (30 m length, 0.25 mm internal diameter, 0.25 μm film thickness). Samples (1 μL) were automatically injected using an integrated microsyringe. The injector and detector temperatures were set at 220 °C and 230 °C respectively, with an ionization energy of 70 eV. A temperature program was applied, starting at 60 °C and increasing to 250 °C with a rate of 3 °C/min, using He as the carrier gas at 1.0 mL/min in splitless mode. Mass spectra were recorded in the 45–400 m/z range. Data processing was carried out using Bruker MSWS software.

Compound identification was based on retention time and mass spectra comparison of the unknown compounds with Adams and NIST libraries. To further support our data, the Arithmetic Index (AI) was calculated using equation (1).

$$A.I.= 100n+100(tx-tn)/(tn+1-tn)$$
 (1)

Where t_n and t_{n+1} are the retention times of n-alkanes eluted before and after the studied compound, t_x is the retention time of the studied compound. The t_n , t_{n+1} are determined by analyzing a mixture of n-alkanes (C8-C24) under the same experimental conditions [24].

Results and discussion

The % yield (v/w) of the essential oil of the samples studied is presented in tables 2 and 3. Identified compounds are listed according to increasing A.I. Generally, plants of *O. vulgare* grown in Greece are characterized by numerous granular trichomes on their leaves and inflorescences [25], which might be one of the reasons for high essential oil yields.

All samples are of clear carvacrol chemotype. The carvacrol content ranged from 63.2 ± 0.6 to 89 ± 1 . with OVH1 sample having the highest % concentration followed by OVH3. Significant was also the presence of *p*-cymene and γ -terpinene, while regarding thymol, samples OVH2

and OVH4 presented a higher amount than OVH1 and OVH3. Sesquiterpene hydrocarbons were found with an abundance higher than 1% in quite all the samples, while *E*-caryophyllene concentration ranged from 0.23 ± 0.01 to 1.34 ± 0.01 . Plants of *O. vulgare* spp. *hirtum* collected from different geographic areas of Turkey [12] showed % yield between 1.3-6.5%, low % concentration of thymol on the contrary to carvacrol % concentration which reached 77.95%. *p*-cymene and γ -terpinene were also detected in considerable amounts, reaching 24.90% and 8.22%, respectively. The collection site played a vital role in the % yield and the final % concentration of the individual secondary metabolites detected in the study of [12]. On the other hand, the % yields of the samples studied herein were higher than those reported by [14] (% yields 1.0-1.6), confirming their statement that autumn cultivars result in lower yields of essential oil.

The sample from Leonidio region (Arkadia) showed the lowest yield. Generally, inflorescence is the plant part that accumulates the highest amount of essential oil; therefore, the low yield obtained from this sample is explained by the fact that the sample studied consisted only of leaves. Nevertheless, the % concentration of carvacrol remained high, indicating that leaves are a great source of carvacrol [26, 27]. In addition, it is important to state that genetic diversity and environmental varieties, including temperature and humidity, are factors that influence the qualitative and quantitative profile of a plant species [28, 29]. On the other hand, *O. vulgare* spp. *hirtum* cultivated in Ikaria Island showed a remarkable % yield, which reached 9%, while its carvacrol content was calculated to be over 93%. Thymol was not detected in this sample, and the % concentrations of *p*-cymene and γ-terpinene were relatively low [9] Arethousa is the first Greek oregano variety registered in the Greek National (and European) Catalogue of Cultivate Species. It is legally protected by the European Community Plant Variety Office (CPVO). Our results showed that cultivated plants showed a high % yield (8.9%), the highest among the samples *O. vulgare* spp. *hirtum* and comparable to that reported by [18].

Vokou et al., 1993 [19], examined plants from different regions of Greece and concluded that an inversely proportional relationship exists between % yield and altitude. In this report, the authors showed that as the altitude diminishes, the yield increases. This was also observed in our study, where OVH3 cultivated at low altitudes presented the highest % yield. Although OVH4 is found at quite an equal altitude, it seems that apart from the absence of flowers in the sample of our study, also the soil and climatic conditions of the geographical area influenced its % yield.

Table 2: Qualitative and quantitative analysis of the tentatively identified compounds from essential oils of O. vulgare spp. hirtum using GC-MS

Chemical compound	AI Literature	AI Experimental	% concentration	% concentration	% concentration	% concentration
-		-	OVH1±SD ¹	OVH2±SD	OVH3±SD	OVH4±SD
α -thujene ^{MH}	924	925	0.05 ± 0.07	0.67 ± 0.02	0.67 ± 0.02	0.62 ± 0.03
$lpha$ -pinene $^{ m MH}$	932	933	0.45 ± 0.06	0.36 ± 0.01	0.45 ± 0.02	0.42 ± 0.02
Camphene ^{MH}	946	947	tr^2	0.10 ± 0.00	tr	0.14 ± 0.01
1-octen-3-ol ^{AC}	974	972	tr	0.19 ± 0.01	0.12 ± 0.00	0.23 ± 0.00
eta -pinene $^{ m MH}$	974	976	tr	0.05 ± 0.04	0.09 ± 0.01	0.08 ± 0.01
3-octanone ^{AC}	979	979	_3	tr	tr	0.07 ± 0.00
β -myrcene ^{MH}	988	987	0.60 ± 0.08	0.71 ± 0.02	0.92 ± 0.02	0.95 ± 0.05
$lpha$ -phellandrene $^{ m MH}$	1002	1003	tr	0.11 ± 0.00	0.16 ± 0.001	0.15 ± 0.004
3-carene ^{MH}	1008	1010	tr	tr	tr	tr
$lpha$ -terpinene $^{ m MH}$	1014	1015	0.68 ± 0.07	0.88 ± 0.03	0.85 ± 0.02	1.24 ± 0.05
p-cymene ^{MH}	1020	1021	5.23 ± 0.51	4.75 ± 0.09	3.82 ± 0.11	7.91 ± 0.24
Limonene ^{MH}	1024	1027	0.24 ± 0.03	0.23 ± 0.01	0.27 ± 0.01	-
Sylvestrene ^{MH}	1025	1027	-	-	-	0.32 ± 0.02
cis - β -ocimene ^{MH}	1032	1033	tr	tr	-	0.10 ± 0.00
<i>trans</i> -β-ocimene ^{MH}	1044	1044	-	tr	tr	tr
γ-terpinene ^{MH}	1054	1055	2.46 ± 0.26	5.87 ± 0.10	3.94 ± 0.12	8.52 ± 0.27
cis-sabinene hydrate ^{OM}	1065	1063	tr	0.08 ± 0.00	0.16 ± 0.00	0.13 ± 0.02
Terpinolene ^{MH}	1086	1086	tr	tr	0.05 ± 0.04	0.09 ± 0.00
Linalool ^{OM}	1095	1095	tr	0.09 ± 0.00	tr	0.13 ± 0.00
Borneol ^{OM}	1165	1162	tr	0.24 ± 0.00	0.10 ± 0.04	0.39 ± 0.02
Terpinen-4-ol ^{OM}	1174	1174	0.37 ± 0.01	0.38 ± 0.02	0.45 ± 0.01	0.48 ± 0.01
<i>p</i> -cymen-8-ol ^{OM}	1176	1179	-	tr	-	tr
$lpha$ -terpineol $^{ m OM}$	1186	1186	tr	0.05 ± 0.04	0.10 ± 0.00	0.09 ± 0.00
<i>cis</i> -dihydrocarvone ^{OM}	1191	1191	tr	tr	0.08 ± 0.00	0.04 ± 0.03
trans-dihydrocarvone ^{OM}	1200	1198	tr	tr	tr	tr
cis carveol ^{OM}	1226	1217	-	tr	-	tr
Thymol methyl eher ^{OM}	1232	1230	-	0.22 ± 0.03	-	tr
Carvacrol methyl	1241	1239	-	-	-	0.54 ± 0.02

ether ^{OM}						
Thymol ^{OM}	1289	1286	0.09 ± 0.08	3.55 ± 0.04	0.10 ± 0.01	11.6 ± 0.2
<i>p</i> -cymen-7-ol ^{OM}	1289	1289	tr	-	-	-
Carvacrol ^{OM}	1298	1296	89.1 ± 1.3	79.8 ± 0.4	86.6 ± 0.4	63.2 ± 0.6
E -caryophllene $^{ m SEH}$	1417	1420	0.23 ± 0.01	1.02 ± 0.10	0.45 ± 0.01	1.34 ± 0.01
γ-elemene ^{SEH}	1434	1441	-	-	-	tr
$lpha$ -humulene $^{ m SEH}$	1452	1454	tr	0.11 ± 0.00	tr	0.16 ± 0.00
γ -muurolene $^{ m SEH}$	1478	1476	-	tr	-	-
Viridiflorene ^{SEH}	1496	1497	-	-	-	tr
eta -bisabolene $^{ ext{SEH}}$	1505	1507	0.41 ± 0.02	0.43 ± 0.01	0.55 ± 0.01	0.83 ± 0.02
δ -cadinene $^{ m SEH}$	1522	1523	-	tr	-	tr
Spathulenol ^{SEO}	1575	1576	-	tr	-	tr
Caryophyllene oxide ^{SEO}	1586	1582	tr	0.09 ± 0.00	tr	0.19 ± 0.01
%Yield (mL/100 g of dry plant material)			4.5	6.6	8.9	3.7
MH			9.71	13.73	11.22	20.54
OM			89.56	84.41	87.59	76.6
SEH			0.64	1.56	1.00	2.33
SEO			-	0.09	-	0.19
AC			<u>-</u>	0.19	0.12	0.30

¹Data are presented as mean \pm standard deviation (n=3)

²tr = traces < 0.05 %, AC: aliphatic compound, MH: monoterpene hydrocarbon, OM: oxygenated monoterpene, SEH: sesquiterpene hydrocarbon, SEO: oxygenated sesquiterpene

³Compound not found in the essential oil of the sample

Table 3: Qualitative and quantitative analysis of the tentatively identified compounds from essential oils of O. onites and O. \times intercendes using

2 GC-MS

Chemical compound	AI	AI	% concentration	% concentration
	Literature	experimental	OO±SD ¹	OxI±SD
α-thujene ^{MH}	924	925	0.50 ± 0.01	tr ²
α -pinene ^{MH}	932	933	0.67 ± 0.02	0.42 ± 0.03
Camphene ^{MH}	946	947	0.72 ± 0.02	tr
1-octen-3-ol ^{AC}	974	972	tr	tr
eta -pinene $^{ m MH}$	974	976	0.11 ± 0.01	0.18 ± 0.32
\dot{eta} -myrcene $^{ m MH}$	988	987	0.84 ± 0.05	0.42 ± 0.27
$lpha$ -phellandrene $^{ m MH}$	1002	1003	0.15 ± 0.01	0.12 ± 0.01
3-carene ^{MH}	1008	1010	tr	tr
$lpha$ -terpinene $^{ m MH}$	1014	1015	1.31 ± 0.05	0.89 ± 0.05
<i>p</i> -cymene ^{MH}	1020	1021	5.53 ± 0.18	8.33 ± 0.48
Limonene ^{MH}	1024	1027	0.37 ± 0.02	0.32 ± 0.01
cis - eta -ocimene $^{ m MH}$	1032	1033	0.47 ± 0.03	_3
$trans$ - eta -ocimene $^{ m MH}$	1044	1044	tr	-
γ-terpinene ^{MH}	1054	1055	7.46 ± 0.23	2.03 ± 0.11
<i>cis</i> -sabinene hydrate ^{OM}	1065	1063	tr	tr
Terpinolene ^{MH}	1086	1086	0.20 ± 0.01	0.13 ± 0.01
Linalool ^{OM}	1095	1095	0.20 ± 0.00	tr
cis-p-2-menthen-1-ol ^{OM}	1118	1118	tr	tr
$lpha$ -campholenal $^{ m OM}$	1122	1121	tr	-
<i>allo</i> -ocimene ^{MH}	1128	1126	tr	-
trans-p-2-menthen-1-ol ^{OM}	1136	1136	tr	tr
Borneol ^{OM}	1165	1162	3.53 ± 0.06	0.18 ± 0.01
Terpinen-4-ol ^{OM}	1174	1174	1.05 ± 0.03	0.50 ± 0.03
p-cymen-8-ol ^{OM}	1179	1179	tr	tr
α -terpineol ^{OM}	1186	1186	0.25 ± 0.01	0.06 ± 0.05
<i>cis-</i> dihydrocarvone ^{OM}	1191	1191	tr	0.06 ± 0.05
trans-dihydrocarvone ^{OM}	1200	1198	tr	-
cis-carvone ^{OM}	1239	1237	0.05 ± 0.04	tr
Carvacrol methyl ether ^{OM}	1241	1239	tr	-
Linalool acetate ^{OM}	1254	1251	0.09 ± 0.01	-
Bornyl acetate ^{OM}	1284	1282	tr	-

SEO			0.10	0.07
SEH			1.45	0.15
OM			79.69	86.93
MH			18.33	12.84
material)			3.1	4.0
%Yield (mL/100 g of dry plant			3.7	4.6
α -epi-cadinol ^{SEO}	1638	1639	0.15 ± 0.00	-
Caryophyllene oxide ^{SEO}	1582	1582	0.14 ± 0.01	0.07 ± 0.06
Spathulenol ^{SEO}	1577	1576	0.10 ± 0.00	tr
δ -cadinene $^{ m SEH}$	1522	1521	tr	-
γ-cadinene ^{SEH}	1513	1514	0.05 ± 0.04	-
eta -bisabolene $^{ ext{SEH}}$	1505	1507	0.74 ± 0.04	tr
Viridiflorene ^{SEH}	1496	1496	0.09 ± 0.00	tr
Germacrene D ^{SEH}	1484	1481	0.14 ± 0.00	-
$lpha$ -humulene $^{ m SEH}$	1452	1454	tr	-
Aromadendrene ^{SEH}	1439	1440	tr	tr
α -trans-bergamotene $^{ m SEH}$	1432	1436	tr	-
\acute{E} -caryophyllene $^{ m SEH}$	1417	1421	0.43 ± 0.02	0.15 ± 0.00
eta -bourbonene $^{ m SEH}$	1387	1386	tr	-
Geranyl acetate ^{OM}	1379	1377	tr	-
Carvacrol acetate ^{OM}	1370	1365	0.23 ± 0.01	tr
$lpha$ -cubebene $^{ m SEH}$	1348	1350	-	tr
Carvacrol ^{OM}	1298	1296	74.1 ± 1.0	86.0 ± 0.5
p-cymen-7-ol ^{OM}	1289	1289	tr	-
Thymol ^{OM}	1289	1286	0.19 ± 0.02	0.13 ± 0.01

⁴ Data are presented as mean \pm standard deviation (n=3)

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^{5 2}tr = traces < 0.05 %, AC: aliphatic compound, MH: monoterpene hydrocarbon, OM: oxygenated monoterpene, SEH: sesquiterpene

⁶ hydrocarbon, SEO: oxygenated sesquiterpene

^{7 &}lt;sup>3</sup>Compound not found in the essential oil of the sample

8 A similar profile was also shown in plants of O. onites and O. × intercendes. Oxygenated 36 monoterpenes are the most abundant fraction followed by monoterpene hydrocarbons. 9 Carvacrol content ranged from 74.0±1.0 to 86.0±0.5. Thymol was detected in low quantity; 10 p-cymene and γ -terpinene were found in considerable amount respect to other secondary 11 metabolites. Borneol was detected in both samples; however, its % concentration was higher 12 in the O.O essential oil. A comparison of the % sum concentration of the compounds families 13 for all the samples presented in this study is shown in Figure 1. A comparison of the amount 14 of the four characteristic constituents is presented in Figure 2A. Despite differences in their 15 16 % concentration (Tables 2 and 3, Figure 2B), their sum does not present significant deviations. Nevertheless, the contribution of p-cymene and γ -terpinene to the sum of the main 17 compounds is significant (Figure 2, diagram C). 18

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Samples collected from Greece and Turkey were analyzed by [11, 14], and their results showed that carvacrol prevails in both cases reaching 84.5% for the Greek and 71.2% for the Turkish sample. Furthermore, thymol was detected in low amounts, but borneol which % concentration was higher than that calculated in our study. Lower amounts of borneol ranging from 0.54 to 2.06%, were found in the study by [9]. The % yield and % concentration of carvacrol were within the range of our results; thymol was absent, while p-cymene and γ terpinene were detected in lower abundance. Origanum × intercedens is a natural hybrid found for the first time on the Aegean Island of Nisyros [22]. Although its chemical profile is close to OVH1 and OVH3 regarding the % sum of the characteristic compounds and the % sum in carvacrol and thymol, this species has not received much scientific interest yet; therefore, exploitation of the plant is very limited. Our results regarding the % yield of the essential oil (4.6 mL/100 g of dry plant material) and the % carvacrol concentration (86%), align with the studies of [22] and [23], where similar results were obtained. Differences were found regarding the % concentrations of p-cymene and γ -terpinene (higher concentration in our study) and the absence of thymyl acetate and methyl thymol, compounds identified in the sample of Nisyros. Concerning the organic cultivations (OVH2 and OO), thymol methyl ether was detected only in the essential oil of OVH2, while no other significant qualitative and quantitative differences were observed. On the other hand, O. onites, compared to all the samples studied, showed the lowest carvacrol and thymol content alongside the lowest sum of the characteristic compounds (Figure 2B-C). Its % yield was also relatively low. Nevertheless, our data provide an initial scenario regarding organic cultivation of Origanum spp since analysis of more samples from organic and conventional cultivations should be

performed to clarify whether organic cultivars have a positive or negative impact on the chemistry and yield of the essential oil of *Origanum* spp.

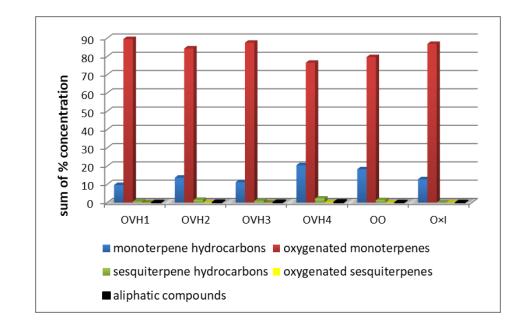


Figure 1: Concentration of the compounds' families expressed as a percentage of the total essential oil

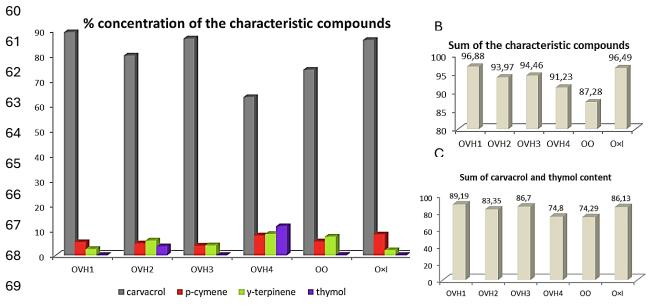


Figure 2: A: Concentration of the characteristic compounds in essential oils expressed as percentage of the total essential oil; B: Sum of the characteristic compounds expressed as percentage of the total essential oil; C: Sum of carvacrol and thymol expressed as percentage of the total essential oil

Conclusions

In conclusion, all the samples presented in this study belong to the carvacrol chemotype. The sample from the Imathia area (OVH1) and the variety Arethousa (OVH3) showed the highest carvacrol content, followed by the hybrid $Origanum \times intercedens$ a result that clearly shows the species potential for wider industrial uses. In all samples, oxygenated monoterpenes prevailed followed by monoterpene hydrocarbons. Regarding p-cymene, γ -terpinene and thymol, their % concentration was the highest among the compounds of the monoterpene hydrocarbons family, nevertheless the abundance differed significantly within the samples. Essential oil of OVH4 sample was obtained from the leaves of the plant. It had the greatest amount of thymol and the highest % concentration of p-cymene and γ -terpinene, while at the same time its % concentration of carvacrol was the lowest. Our results confirm previous studies and present new data regarding $Origanum \ vulgare \ spp. \ hirtum \ var. \ Arethousa$ and $Origanum \times intercedens$, therefore it supports the suitability of Greek cultivated species for essential oil extraction and other applications where high concentration of carvacrol is required.

References

- [1] J. H. Ietswaart, "A taxonomic revision of the genus Origanum (Labiatae)." Leiden Botanical Series 4.1 (1980): 1-153. https://doi.org/10.1007/978-94-009-9156-9
- [2] M. Sharifi-Rad, Y. Berkay Yılmaz, G. Antika, B. Salehi, T. B. Tumer, C. Kulandaisamy Venil, G. Das, J. K. Patra, N. Karazhan, M. Akram, Iqbal, et al., "Phytochemical constituents, biological activities, and health-promoting effects of the genus *Origanum*." *Phytother. Res*, 35(1), (2021): 95–121. https://doi.org/10.1002/ptr.6785
- [3] L. Bora, S. Avram, I. Z. Pavel, D. Muntean, S. Liga, V. Buda, D. Gurgus, & C. Danciu, "An Up-To-Date Review Regarding Cutaneous Benefits of *Origanum vulgare* L. Essential Oil." *Antibiotics*, 11(5), (2022): 549. https://doi.org/10.3390/antibiotics11050549
- [4] I. Rodriguez-Garcia, B. A. Silva-Espinoza, L. A. Ortega-Ramirez, J. M. Leyva, M. W. Siddiqui, M. R. Cruz-Valenzuela, G. A. Gonzalez-Aguilar, & J. F. Ayala-Zavala, "Oregano Essential Oil as an Antimicrobial and Antioxidant Additive in Food Products." *Crit Rev Food Sci Nutr.* 56(10), (2016): 1717–1727. https://doi.org/10.1080/10408398.2013.800832
- [5] B. Lukas, C. Schmiderer, & J. Novak, "Essential oil diversity of European Origanum vulgare L. (Lamiaceae)." *Phytochemistry*, 119, (2015): 32–40. https://doi.org/10.1016/j.phytochem.2015.09.008
- [6] H. Schulz, R. Quilitzsch, & H. Krüger, "Rapid evaluation and quantitative analysis of thyme, origano and chamomile essential oils by ATR-IR and NIR spectroscopy." *J. Mol. Struct.*, 661, (2003): 299-306. https://doi.org/10.1016/S0022-2860(03)00517-9
- [7] D. J. Daferera, B. N. Ziogas, & M. G. Polissiou, "GC-MS analysis of essential oils from some Greek aromatic plants and their fungitoxicity on *Penicillium digitatum*." *J. Agric. Food Chem*, 48(6), (2000). 2576–2581. https://doi.org/10.1021/jf990835x

- [8] A. Bouyahya, G. Zengin, O. Belmehdi, I. Bourais, I. Chamkhi, D. Taha, T. Benali, N. Dakka, & Y. Bakri, "*Origanum compactum* Benth., from traditional use to biotechnological applications." *J Food Biochem*, 44(8), (2020): e13251. https://doi.org/10.1111/jfbc.13251
- [9] G. Economou, G. Panagopoulos, P. Tarantilis, D. Kalivas, V. Kotoulas, I. S. Travlos, M. Polissiou, & A. Karamanos, "Variability in essential oil content and composition of *Origanum hirtum* L., *Origanum onites* L., *Coridothymus capitatus* (L.) and *Satureja thymbra* L. populations from the Greek island Ikaria." *Ind Crops Prod*, 33(1), (2011): 236-241.
- [10] M. Skoula, R. J. Grayer, G. C. Kite, & Veitch, N. C. "Exudate flavones and flavanones in *Origanum* species and their interspecific variation." *Biochem. Syst. Ecol*, 36(8), (2008): 646-654. https://doi.org/10.1016/j.bse.2008.05.003
- [11] S. Kokkini, R. Karousou, E. Hanlidou, & T. Lanaras," Essential oil composition of Greek (*Origanum vulgare* ssp. *hirtum*) and Turkish (*O. onites*) oregano: A tool for their distinction." *J. Essent. Oil Res*, 16(4), (2004): 334-338. https://doi.org/10.1080/10412905.2004.9698735
- [12] K. H. C. Baser, T. Özek, M. Kürkçüoglu, & G. Tümen, "The essential oil of *Origanum vulgare* subsp. *hirtum* of Turkish origin." *J. Essent. Oil Res*, 6(1), (1994): 31-36. https://doi.org/10.1080/10412905.1994.9698321
- [13] E. Skoufogianni, A. D. Solomou, & N. G. Danalatos, "Ecology, cultivation and utilization of the aromatic Greek oregano (Origanum vulgare L.): A review." *Not. bot. Horti Agrobot. Cluj-Napoca*, 47(3), (2019): 545-552. https://doi.org/10.15835/nbha47311296
- [14] S. Kokkini, R. Karousou, A. Dardioti, N. Krigas, & T. Lanaras, "Autumn essential oils of Greek oregano." *Phytochemistry*, 44(5), (1997): 883-886. https://doi.org/10.1016/S0031-9422(96)00576-6

- [15] A. J. Poulose, & R. Croteau, "Biosynthesis of aromatic monoterpenes: Conversion of γ-terpinene to p-cymene and thymol in *Thymus vulgaris* L." *Arch Biochem Biophys*, 187(2), (1978): 307-314. https://doi.org/10.1016/0003-9861(78)90039-5
- [16] M. K. Stefanakis, E. Touloupakis, E. Anastasopoulos, D. Ghanotakis, H. E. Katerinopoulos, & P. Makridis, "Antibacterial activity of essential oils from plants of the genus *Origanum*." *Food control*, 34(2), (2013): 539-546. https://doi.org/10.1016/j.foodcont.2013.05.024
- [17] A. Fleisher, N. Sneer, "Oregano spices and Origanum chemotypes." *J Sci Food Agric* 33, (1982): 441–446. https://doi.org/10.1002/jsfa.2740330508
- [18] G. Panagopoulos, "Chemotypic identification, spatial mapping, and evaluation of the production potential of aromatic and medicinal plants of the genera Origanum, Satureja, and Coridothymus on the island of Icaria." PhD diss., Agricultural University of Athens, 2012. https://doi.org/10.12681/eadd/28741
- [19] D. Vokou, S. Kokkini, & J. M. Bessiere, "*Origanum onites* (Lamiaceae) in Greece: Distribution, volatile oil yield, and composition." *Econ. Bot*, 42(3), (1988): 407-412. https://doi.org/10.1007/BF02860163
- [20] A. Stefanaki, C. M. Cook, T. Lanaras, & S. Kokkini, "The Oregano plants of Chios Island (Greece): Essential oils of *Origanum onites* L. growing wild in different habitats" *Ind Crops Prod* 82, (2016): 107-113. https://doi.org/10.1016/j.indcrop.2015.11.086
- [21] G. Economou, G. Panagopoulos, A. Karamanos, P. Tarantilis, D. Kalivas, & V. Kotoulas, "An assessment of the behavior of carvacrol–rich wild Lamiaceae species from the eastern Aegean under cultivation in two different environments." *Ind Crops Prod*, 54, (2014): 62-69.

- [22] S. Kokkini, & D. Vokou, "The hybrid Origanum× intercedens from the island of Nisyros (SE Greece) and its parental taxa; comparative study of essential oils and distribution." *Biochem. Syst. Ecol.* 21(3), (1993): 397-403. https://doi.org/10.1016/0305-1978(93)90031-L
- [23] Y. Gounaris, M. Skoula, C. Fournaraki, G. Drakakaki, & A. Makris, "Comparison of essential oils and genetic relationship of *Origanum*× *intercedens* to its parental taxa in the island of Crete. "*Biochem. Syst. Ecol.* 30(3), (2002): 249-258. https://doi.org/10.1016/S0305-1978(01)00079-5
- [24] R.P. Adams, "Identification of essential oil components by gas chromatography mass spectroscopy." Allured Publishing Corporation, Carol Stream, Ill (2007)
- [25] N. P. Gavalas, K. L. Kalburtji, S. Kokkini, A. P. Mamolos, & D. S. Veresoglou, "Ecotypic variation in plant characteristics for *Origanum vulgare* subsp. *hirtum* populations." *Biochem. Syst. Ecol.*, 39(4-6), (2011): 562-569. https://doi.org/10.1016/j.bse.2011.08.007
- [26] J. Radušienė, A. Judžintienė, D. Pečiulytė, & V. Janulis, "Chemical composition of essential oil and antimicrobial activity of *Origanum vulgare*." *Biologija*, 51(4) (2005).
- [27] Z. Özer, "Chemical Composition and Antioxidant Activities of Leaf and Flower Essential Oils of Origanum onites L. (Lamiaceae) Growing in Mount Ida-Turkey." *JOTCSA*. 7(3), (20200: 813-20. https://doi.org/10.18596/jotcsa.780334
- [28] R.S. Verma R. Padalia, A. Chauhan R.K. Verma A.K. Yadav H.P, Singh, "Chemical diversity in indian oregano (*Origanum vulgare* L.)." *Chem Bio* 7, (2010): 2054 2064.
- [29] E. Sarrou, E. Martinidou, L. Palmieri, I. Poulopoulou, F. Trikka, D. Masuero, M. Gauly, I. Ganopoulos, P. Chatzopoulou, & S. Martens, "High throughput pre-breeding evaluation of Greek oregano (*Origanum vulgare* L. subsp. *hirtum*) reveals multi-purpose genotypes for

different industrial uses." *J Appl Res Med Aromat Plants*, 37, (2023): 100516. https://doi.org/10.1016/j.jarmap.2023.10051