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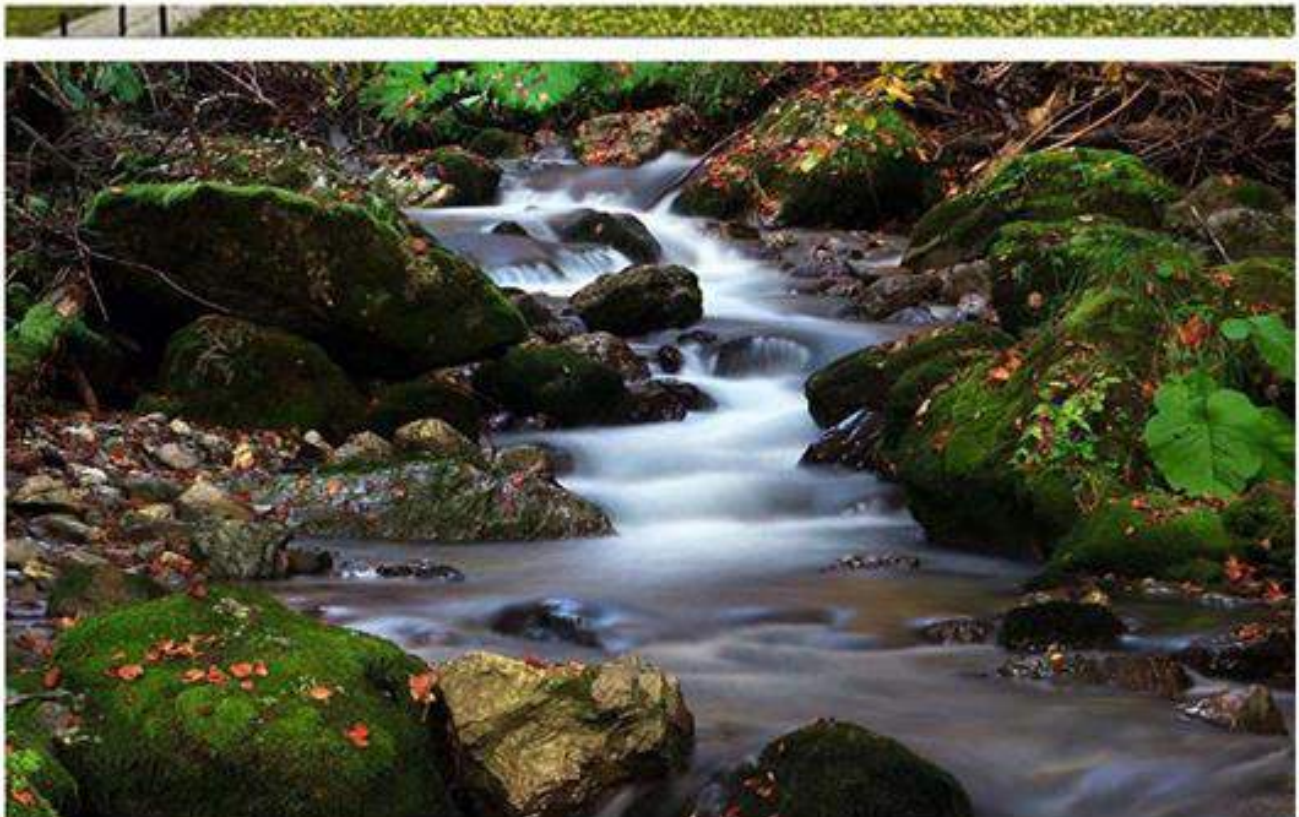


**GEA (Geo Eco-Eco Agro)**  
**University of Montenegro**  
28-31 May 2020, Podgorica, Montenegro



**GEA (Geo Eco-Eco Agro)**  
**International Conference**

# **Book of Proceedings I**



Podgorica, Montenegro, 2020

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GEA (Geo Eco-Eco Agro) International Conference - Book of Proceedings  
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# BOOK OF PROCEEDINGS I

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**Editor in Chief: Velibor Spalevic**

**Publisher: GEA (Geo Eko-Eko Agro), Faculty of Architecture – University, of Montenegro, Faculty of Philosophy - University of Montenegro,**

**Biotechnical faculty - University of Montenegro**

**Printing house: Artgrafika, Circulation: 250**

**Website: [www.gea.ucg.ac.me](http://www.gea.ucg.ac.me)**

**Photo front page: Zoran Ribo Raicevic**

ISBN 978-86-86625-29-8

ISBN 978-86-86625-28-1



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9 788686 625281 >

CIP - Каталогизација у публикацији  
Национална библиотека Црне Горе, Цетиње  
ISBN 978-86-86625-29-8 (Faculty of Architecture)  
COBISS.CG-ID 14162948 (print)

CIP - Каталогизација у публикацији  
Национална библиотека Црне Горе, Цетиње  
ISBN 978-86-86625-28-1 (Faculty of Architecture)  
COBISS.CG-ID 14113284 (electronic)

Article

# Chemical composition of *Origanum dictamnus* and *Origanum vulgare* ssp. *hirtum* from Greece

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**Abstract:** Most of the *Origanum* species are locally distributed within the Mediterranean region where they grow in the mountainous areas on the islands. Due to this, the rate of endemism is high, as in case of dittany of Crete (*O. dictamnus*). *O. vulgare* possesses the largest distribution area and can be found throughout the Mediterranean region, however, the yield and quality of the essential oil is controlled genetically and strongly affected by the environmental influences. *Origanum* essential oils predominantly containing carvacrol, are generally of superior quality and highly valuable raw material for food as well as in pharmaceutical industry. Essential oil of *O. dictamnus* contains 70.8% of carvacrol, while *O. vulgare* ssp. *hirtum* essential oil contains 78.5%. This study indicates the high quality of investigated *Origanum* species from Greece, and indicates it to be highly valuable raw material for food and in pharmaceutical industry.

**Keywords:** Greek oregano, dittany of Crete, essential oil, GC-MS, carvacrol

## 1. Introduction

Most of the *Origanum* species are locally distributed within the Mediterranean region where they grow in the mountainous areas on the islands. Due to this, the rate of endemism is high (about 70%). *O. vulgare* L. possesses the largest distribution area and can be found throughout the Mediterranean region, in most parts of the Euro-Siberian and the Irano-Turanian regions (Lukas, 2010). *O. vulgare* is an extremely variable species that includes six subspecies: ssp. *glandulosum*, ssp. *hirtum*, ssp. *gracile*, ssp. *virens*, ssp. *vulgare* and ssp. *viride*. It is known that subspecies differ significantly in morphological features as well as in content and essential oil composition (Mechergui *et al.* 2016; Kosakowska and Czupa 2018). The Greek oregano (*O. vulgare* L. ssp. *hirtum* (Link) Ietswaart, syn. *O. heracleoticum* L.) is the most commonly used commercial type of oregano. It grows wild throughout nearly across all of Greece (Goliaris *et al.* 2002). On the other hand, *O. dictamnus* L. also known as dittany of Crete, is native and endemic to the island of Crete, where it grows wild but is also cultivated (Varsani *et al.* 2017).

The yield and quality of the essential oil is controlled genetically and strongly affected by the environmental influences (Goliaris *et al.* 2002; Toncer *et al.* 2009). The main bioactive components of oregano are essential oil and phenolic components, generated from cymyl-pathway such as  $\gamma$ -terpinene, *p*-cymene, carvacrol and thymol (Lukas 2010; Stanojević *et al.* 2016). Their ratio represents the quality of the oil and indicates the aroma value. Oils containing predominantly carvacrol are generally of superior quality (Morsy 2017).

As a herbal tea, oregano is traditionally used for treating respiratory disorders, dyspepsia, painful menstruation, rheumatoid arthritis, scrofulosis and urinary tract disorders (Teixeira *et al.* 2013). It is also used as a culinary herb in gastronomy (Krigas *et al.* 2015). Oregano essential oil with high carvacrol content possesses good antimicrobial (Lesjak

*et al.* 2016; Leyva-López *et al.* 2017) and antioxidant properties (Karakaya *et al.* 2011; Stanojević *et al.* 2016). Because of this, oregano essential oil rich in carvacrol is a highly valuable raw material for food and in pharmaceutical industry (Ibišević *et al.* 2019).

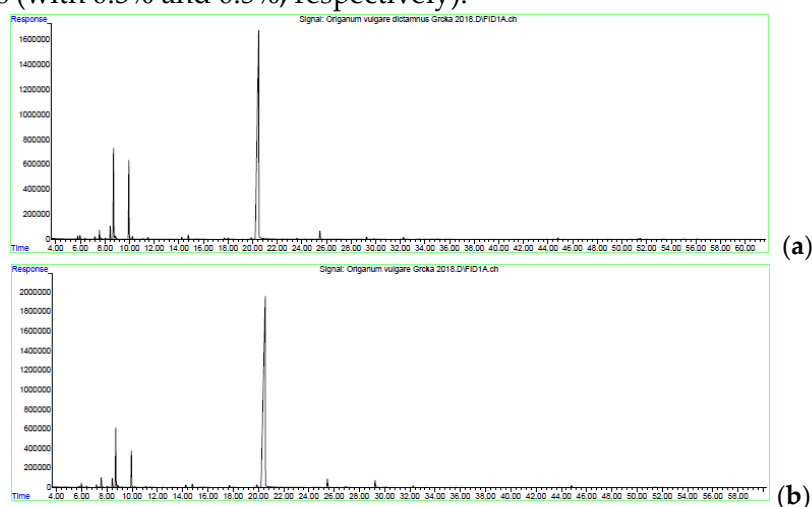
The aim of this study was to determine the chemical composition of essential oils obtained by hydrodistillation of *O. dictamnus* and *O. vulgare ssp. hirtum* commercial samples from Greece.

## 2. Materials and Methods

Dry commercial *O. dictamnus* and *O. vulgare ssp. hirtum* were purchased at a local market in Greece (producer Avramglou, Thessaloniki) in 2018. Air-dried aerial parts of *Origanum* were submitted to hydrodistillation (Clevenger apparatus, 3 h). Then, the essential oil was dried over anhydrous sodium sulfate and analyzed using an HP 5890 gas chromatograph coupled to an HP 5973 MSD and fitted with a capillary column HP-5 MS (30 m × 0.25 mm × 0.25 μm film thickness). Analytical conditions were as follows: helium was used as carrier gas; inlet pressure was 25 kPa; linear velocity: 1 ml/min at 210 °C; injector temperature: 250 °C; injection mode: splitless. MS scan conditions were: source temperature, 200 °C; interface temperature, 250 °C; electron energy, 70 eV; mass scanrange, 40–350 *amu*. Temperature program: 60 °C to 285 °C at a rate of 4.3 °C/min. The components were identified based on their linear retention index relative to C8-C32 *n*-alkanes, comparison with data reported in literature (Wiley and NIST databases). Percentage (relative) of the identified compounds was computed from GC peak area.

## 3. Results

A total of 35 compounds were detected in Greece *Origanum* essential oils (Table 1). In *O. dictamnus* 31 compounds comprised 99.1%, carvacrol being the dominant compound with 70.8%, followed by *p*-cymene (9.8%) and  $\gamma$ -terpinene (9.0%). A GC-MS chromatogram of *O. dictamnus* essential oil is shown in Figure 1a. The sum of the three above mentioned major essential oil constituents in *O. dictamnus* was 89.6%. In *O. vulgare ssp. hirtum* 28 compounds comprised 99.4%, the dominant compound was carvacrol with 78.5%, followed by *p*-cymene (6.8%) and  $\gamma$ -terpinene (4.4%). A GC-MS chromatogram of *O. vulgare ssp. hirtum* essential oil is shown in Figure 1b. The sum of these three major constituents was 89.7%. However, thymol as an isomer of carvacrol in both samples was present in low concentrations (with 0.3% and 0.5%, respectively).



**Figure 1.** A GC-MS chromatogram of *O. dictamnus* (a) and *O. vulgare ssp. hirtum* (b) essential oil

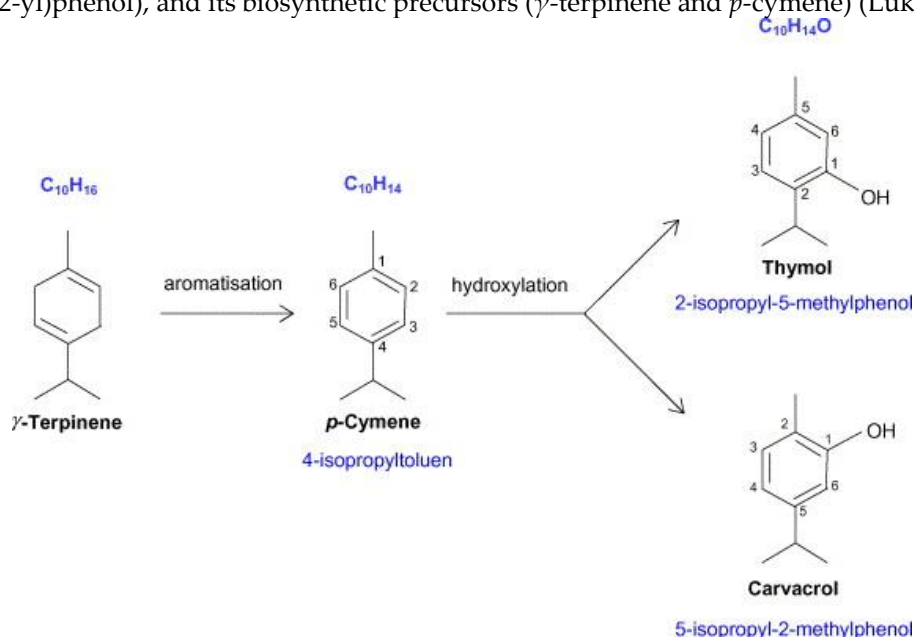
**Table 1.** Chemical composition of *O. dictamnus* and *O. vulgare ssp. hirtum* from Greece.

No	Compound	Rt	KI	<i>O. dictamnus</i>	<i>O. vulgare ssp. hirtum</i>
1	$\alpha$ -Thujene	5.786	918	0.3	0.1
2	$\alpha$ -Pinene	5.975	927	0.4	0.5
3	Camphene	6.378	935	0.1	0.1
4,5	$\beta$ -Pinene + 1-Octen-3-ol	7.174	971	0.4	0.4
6	3-Octanone	7.411	981	-	0.1
7	Myrcene	7.551	987	1.0	1.3
8	3-Octanol	7.672	990	0.1	-
9	$\alpha$ -Phellandrene	8.030	1005	0.1	0.1
10	$\delta$ -3-Carene	8.224	1010	0.1	0.1
11	$\alpha$ -Terpinene	8.425	1015	1.5	1.1
12	p-Cymene	8.697	1021	9.8	6.8
13	Limonene	8.854	1025	0.6	-
14	$\beta$ -Phellandrene	8.855	1025	-	0.5
15	cis- $\beta$ -Ocimene	9.160	1033	-	0.1
16	$\gamma$ -Terpinene	9.940	1052	9.0	4.4
17	cis-Sabinene hydrate	10.228	1059	0.4	0.1
18	Terpinolene	11.089	1081	0.1	0.2
19	trans-Sabinene hydrate	11.443	1090	0.1	0.1
20	Linalool	11.493	1091	0.3	-
21	Borneol	14.246	1159	0.3	0.4
22	Terpinen-4-ol	14.765	1172	0.7	0.6
23	$\alpha$ -Terpineol	15.371	1187	0.1	0.1
24	trans-Dihydro carvone	15.683	1196	-	0.1
25	Carvacrol, methyl ether	17.700	1241	0.2	0.3
26	Thymol	19.876	1290	0.3	0.5
27	Carvacrol	20.476	1302	70.8	78.5
28	$\alpha$ -Copaene	23.582	1374	0.1	-
29	trans-Caryophyllene	25.462	1418	1.2	1.3
30	$\alpha$ -Humulene	26.918	1452	0.1	0.2
31	$\beta$ -Bisabolene	29.250	1508	0.4	1.1
32	$\delta$ -Cadinene	29.854	1522	0.1	-
33	Caryophyllene oxide	32.251	1580	0.3	0.3
34	1,10-di-epi-Cubenol	33.520	1612	0.1	-
35	$\alpha$ -Cadinol	35.021	1654	0.1	-
<b>TOTAL</b>				<b>99.1</b>	<b>99.4</b>

Rt – Retention time; KI – Kovats Indices relative to *n*-alkanes on HP-5 MS

#### 4. Discussion

It is well known that essential oils of *Origanum* species is rich in cymyl-compounds (Figure 2), i.e. phenolic monoterpenoids, such as carvacrol (2-Methyl-5-(propan-2-yl)phenol) and thymol (5-Methyl-2-(propan-2-yl)phenol), and its biosynthetic precursors ( $\gamma$ -terpinene and *p*-cymene) (Lukas 2010).



**Figure 2.** Cymil biosynthetic pathway

Carvacrol was a predominant compound in all *O. dictamnus* samples (Economakis *et al.* 1999). Investigations with commercial samples of *O. dictamnus* from Crete showed that, apart from carvacrol (52.2%), they contained  $\gamma$ -terpinene (8.4%), *p*-cymene (6.1%), linalool (1.4%) and caryophyllene (1.3%), while the content of thymol was very low (0.5%) (Mitropoulou *et al.* 2015). Furthermore, wild population of *O. dictamnus* from Crete grown in Northern Greece contained carvacrol as the main compound in its essential oil (45.3-75.1%), followed by *p*-cymene (4.3-12.5%),  $\gamma$ -terpinene (0.4-9.5%) and linalool (0.8-13.4%) in percentage depending on distilled parts and year of cultivation (Argyropoulou *et al.* 2014).

The quantitative analysis of 23 essential oils obtained from *O. vulgare* ssp. *hirtum* all over Greece show that the major constituents are carvacrol (2.3-93.8%) and thymol (0.2-90.2%), accompanied by *p*-cymene (2.2-15.8%) and  $\gamma$ -terpinene (0-16.4%). Furthermore, carvacrol and thymol contents are highly negative correlated (Vokou *et al.* 1993). Therefore, *O. vulgare* ssp. *hirtum* is a chemically non-uniform species (Fleisher and Sneer 1982). There are three different chemotypes distinguished on the basis of the main compound in essential oil: thymol, carvacrol and intermediate one, contains both thymol and carvacrol (Stešević *et al.* 2018).

Carvacrol can be found in many aromatic plants including *O. dictamnus*, *O. vulgare* ssp. *hirtum*, as well as *O. majorana*, *Thymbra capitata*, *Satureja hortensis*, *S. montana*, *Thymus vulgaris*, *T. zygis* and *T. serpyllum* (Suntres *et al.* 2015). It is generally recognized as a safe food additive and used as a flavoring agent in baked foods, sweets, beverages and chewing gums (Mehdi *et al.* 2011). Furthermore, antimicrobial and antibiofilm activities of carvacrol against different bacteria and fungi responsible for human infectious diseases (Marchese *et al.* 2018), as well as anticancer properties (Mehdi *et al.* 2011; Ozkan and Erdogan 2011) characterize it as a natural remedy.

## 5. Conclusions

Because of the high content of carvacrol in the respective essential oils, *O. dictamnus* and *O. vulgare* ssp. *hirtum* from Greece have high quality and represent highly valuable raw material for food and in pharmaceutical industry.

**Acknowledgments:** The authors would like to express their gratitude to Herb Elixia Ltd., Belgrade, who was the initiator of this investigation.

**Conflicts of Interest:** The authors declare no conflict of interest.

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