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Evaluation of the Essential Oil Content of Cretan Dittany Cultivated in Northern Greece

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Abstract

Origanum dictamnus was cultivated at the Laboratory of Conservation and Evaluation of the Native and Floricultural Species (North Greece), far away from the island of Crete (South Greece) where it is growing wild. After one year of cultivation dittany barks/leaves and flowers were analyzed on the basis of their essential oils (yield and quality) for two subsequent years. The essential oils from different parts of the plants were obtained by hydrodistillation in a modified Clevenger-type apparatus, and their chemical analyses were performed by GC and GC-MS. The essential oil yield (1.05-3.14%) and content did not show a negative response to cultivation. Carvacrol was shown to be the main constituent in all samples (45.3-75.1%), with its percentages increasing at the second year of cultivation.

Keywords: *Origanum dictamnus*; Carvacrol; Essential oil; GC-MS; Cultivation

Introduction

Origanum dictamnus L. or 'Cretan dittany' is a white lanate subshrub, growing wild exclusively in the rocky slopes of mountainous Crete (Greece) [1,2]. It is well known since Aristotle's time for its curing abilities and it was widely used in Crete as a panacea for almost every illness [3-5]. It is characterized by the U.S.A. CFR (2009) as a safe spice for consumption and it is employed by the food industry as a natural additive, with flavouring, antioxidant or preservative role, thus holding great commercial value for the local Greek economy [6].

O. dictamnus is mainly commercialized for its essential oil. In most cases, carvacrol proved to be the major component, with *p*-cymene and *γ*-terpinene following in amounts [7-9]. Its content in carvacrol is considered very important, since this aromatic monoterpene holds a significant commercial interest. It is a known antimicrobial, antiseptic and antioxidant agent with low acute toxicity and weak genotoxic potential. It is approved by both the FDA and the European Commission as an additive [10]. Finding a stably and abundant rich source of carvacrol would be very important for the food industry. Carvacrol is a common component of the oils from oregano, thyme, marjoram and summer savory [3].

Nowadays the world demand for dittany's essential oil and especially its main compound, carvacrol is increasing. Impetuous collection of wild populations in the past, led to the rapid decrease and extinction of dittany from several areas of the island making its systematic cultivation crucial [3]. Furthermore, cultivation and production of dittany have nowadays ceased and scientific data concerning dittany's cultivation are sparse and based mostly on the farmers' experience. It must be emphasized that its cultivation is restricted to Crete, especially in Heraklion estate. In fact earlier authors reported that cultivation far away from Crete would be unsuccessful and it's not recommended [4,11].

The overall objective of the present research was to examine whether the Cretan dittany could be cultivated in a location other than Crete (South Greece), specifically in the Balkan Botanic Garden of Kroussia (North Greece) and to determine the effects of cultivation on essential oil yield and composition. The chemical composition of the essential oil of the cultivated plants was compared with the previously stated in the literature. Our focus was also centred on the carvacrol content of the plants since it has great industrial interest. The results could be very important because they could enforce the cultivation of this economically important plant in the continental Greece.

Materials and Methods

Plant material

Wild plants of *O. dictamnus* were collected at Emparos Mountain (alt. 430 m; island of Crete). The wild *O. dictamnus* was identified by Dr. Koureas D. (taxonomist) and a voucher specimen has been deposited in the Balkan Botanic Garden of Kroussia (Greece)-Laboratory of Conservation and Evaluation of the Native and Floricultural Species.

Propagation and conservation conditions: The plant has been asexually propagated by softwood tip cuttings of 3-5 cm, under mist. Cuttings were placed in propagation trays in a substrate of peat (Klasmann, KTS 1) and perlite (1:3 v/v) and maintained at bottom heat benches in a polycarbonate greenhouse. Soil temperature was kept at 18-21°C, while air temperature was 15-25°C, depending on weather conditions, and relative humidity 90% [12].

Young plants produced, were transplanted in 2 l pots in a mixture of peat (Klasmann, TS3) and perlite (2:1 v/v) and continue growing in the greenhouse. The temperature was different during the day and night and among the seasons (average temperatures day/night: winter 18/10°C, spring 25/10C, summer 32/20°C). The relevant humidity (RH) was kept at 55%. Drip irrigation system was applied for the plants and was dependant on the weather conditions (approximately 3 times/

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Cultivation time frame	Code of samples	Distilled parts	Date of collection	Yield (% v/w)
1st year	ord1	barks/leaves	9/9/2005	1.05%
1st year	ord2	flowers	9/9/2005	1.08%
2nd year	ord3	barks/leaves	11/7/2006	3.14%
2nd year	ord4	flowers	11/7/2006	2.69%

Table 1: List of cultivated *O. dictamnus* samples.

week, 400 ml/pot). Plants were fertilized with: a) 80% humic acid + 10% K_2O (1 g/l, 200 ml/pot), b) 20-20-20 N-P-K (3 g/l, 200 ml/pot).

The second year, plants were transferred at spring outside in a shaded (60%) nursery area. The pots were maintained on gravel for better drainage conditions. Irrigation was applied according to the weather conditions. Plant material for distillation was collected at two subsequent years (2005 and 2006) during the flowering stage.

Essential oil extraction

30 g of air-dried plant material of each collection were cut in small pieces, and the essential oils were obtained by hydrodistillation for 2 h in a modified Clevenger apparatus with a water-cooled oil receiver to reduce artifacts due to over-heating [13]. Distilled parts, their codes and dates of collection are presented in Table 1. The oils were taken in 2 ml of capillary GC grade *n*-pentane and dried over anhydrous sodium sulphate and subsequently stored at -20°C to minimize the loss of volatile compounds. Two oil samples for each collection were prepared and then analysed by GC and GC–MS analyses.

GC analysis

GC analyses were carried out on a Perkin-Elmer-8500 gas chromatograph with FID, fitted with a Supelcowax-10 fused silica capillary column (30 m × 0.32 mm (i.d.), film thickness: 0.25 μm). The column temperature was varied from 75°C to 200°C at a rate of 2.5°C/min. The injector and detector temperatures were programmed at 230°C and 300°C, respectively. The injection volume was 0.2 ml of the pure oil.

GC/MS analysis

GC-MS analyses were performed on a Hewlett-Packard 5973-6890 system operating in EI mode (70eV) and equipped with a split/splitless injector (220°C). A split ratio 1:10 and two different columns were used: a fused silica HP-5MS capillary column (30 m × 0.25 mm (i.d.), film thickness: 0.25 μm) and a HP-Innowax capillary column (30 m × 0.25 mm (i.d.), film thickness: 0.50 μm). The temperature program for the HP-5MS column was from 60°C (5 min) to 280°C at a rate of 4°C/min and for the HP-Innowax column from 60°C to 260°C at a rate of 3°C/min Helium was used as a carrier gas at a flow rate of 1.0 ml/min. The injection volume was 2 μl. The identification of the components was based on comparison of their mass spectra with those of the Wiley libraries [14] and with those described by the bibliography [15,16]. Retention indices (RI) for all compounds were determined according to Van den Dool and Kratz [17], using *n*-alkanes as standards. In many cases, the essential oils were subject to co-chromatography with authentic compounds (Fluka, Sigma).

Optical rotation

The $[\alpha]_D^{20}$ values were determined at 20°C at 589 nm in CH_2Cl_2 on Perkin-Elmer 341 Polarimeter.

Results and Discussion

O. dictamnus responded well to the conditions of cultivation. After producing young plants from asexual propagation they were transferred in the nursery. Their essential oils compositions were determined for two subsequent years, after one year of cultivation (1st year of cultivation) and after two years of cultivation (2nd year of cultivation).

Barks/leaves and flowers of *O. dictamnus* were collected during the flowering stage at two subsequent years of cultivation. The different plant parts were distilled independently and the essential oils were analyzed by GC and GC-MS. A list of the collected and distilled samples of *O. dictamnus* is provided in Table 1. The identified volatile components are listed in Table 2, together with their retention indices and percentages.

The four essential oils obtained were yellow in colour, with characteristic and picking odour. The yields (v/w) of all essential oils obtained were comparable to the ones described in the literature [18,19]. It is noteworthy that the yields of the essential oils increased impressively after two years of cultivation.

The four samples presented generally the same chemical profile. The essential oils consisted of a complex mixture of different substances, with oxygenated monoterpenes as the dominating

	Components[a]	RI[b]	RI[c]	ord1	ord2	ord3	ord4
1	α-thujene	921		0.3	-	-	-
2	α-terpinene	1012	1168	1.1	-	-	0.1
3	α-pinene	929		0.2	-	-	-
4	myrcene	982		0.3	0.2	-	-
5	δ-2-carene	1006	1142	-	0.5	-	-
6	*p*-cymene	1020	1255	**12.5**	**10.9**	**4.5**	**4.3**
7	γ-terpinene	1058	1234	9.5	4.1	0.4	1.6
8	*cis*-sabinene hydrate	1069	1445	1.6	1.7	1.5	1.8
9	terpinolene	1087		-	0.1	-	-
10	linalool	1097	1526	13.4	11.8	3.4	0.8
11	borneol	1166	1675	0.4	0.6	1.8	1.7
12	*cis*-dihydrocarvone	1187	1587	-	0.1	-	-
13	terpinen-4-ol	1177	1579	0.9	0.9	0.8	-
14	α-terpineol	1184	1677	0.2	-	-	-
15	carvacrol methyl ether	1242	1580	-	0.1	-	-
16	thymoquinone	1251		5.4	3.6	6.0	3.4
17	carvone	1253		-	0.1	-	-
18	thymol	1286	2162	0.8	0.5	-	0.6
19	carvacrol	1307	2193	**45.3**	**46.7**	**75.1**	**60.9**
20	carvacrol acetate	1371		0.2	0.1	-	0.3
21	α-copaene	1372	1472	-	-	0.2	-
22	*trans*-β-caryophyllene	1413	1572	4.1	4.0	1.0	2.9
23	α-humulene	1450	1648	0.2	0.3	-	0.2
24	β-bisabolene	1503	1700	-	0.4	0.2	0.2
25	caryophyllene oxide	1580	1971	0.9	2.2	0.5	3.8
	Total identified (%)			**97.3**	**89.2**	**95.4**	**99.8**
	$[\alpha]_D^{20}$			+1.25, (CH_2Cl_2, *c* 0.07)	+11.25, (CH_2Cl_2, *c* 0.20)	+0.35, (CH_2Cl_2, *c* 0.29)	+1.25, (CH_2Cl_2, *c* 0.25)

[a] Components listed in order of elution from a HP 5MS column.

Retention indices calculated against C_9-C_{24} *n*-alkanes on the HP 5MS column ([a]) and HP Innowax ([b]) capillary columns, respectively.

Table 2: Chemical and percentage composition of the essential oils of *O. dictamnus* cultivated in Northern Greece.

Grouped components	ord1	ord2	ord3	ord4
Monoterpene hydrocarbons	23.9	15.8	4.9	6.0
Oxygenated monoterpenes	**68.2**	**66.3**	**88.6**	**86.7**
Sesquiterpene hydrocarbons	4.3	4.7	1.4	3.3
Oxygenated sesquiterpenes	0.9	2.2	0.5	3.8

Table 3: Percentages of grouped components of the essential oils.

constituents (Table 3). However, the samples of the first year of cultivation were richer in substances. 18 substances were identified in sample ord1 and 20 in sample ord2. In contrast after the second year of cultivation 12 substances were identified in sample ord3 and 14 in sample ord4.

Among the identified compounds, carvacrol was the predominant compound in all samples (ord1: 45.3%; ord2: 46.7%; ord3: 75.1%; ord4: 60.9%). A considerable increase of its percentage composition is obvious at the second examination. We observe that between the first and second year of cultivation the percentage of carvacrol in barks/leaves increases by 65.8% and in flowers by 30.4%.

In addition, *p*-cymene is also present in considerable percentages (ord1: 12.5%; ord2: 10.9%; ord3: 4.5%; ord4: 4.3%). *γ*-Terpinene and linalool were also encountered in large percentages in the samples collected after the first year of cultivation.

It is clear from the results illustrated that after the first year of cultivation apart from carvacrol also its precursors, *p*-cymene and *γ*-terpinene, as well as linalool were found in appreciable percentages. On the contrary after the second year of cultivation the bulk of the essential oil consisted mostly of carvacrol.

The results were comparable with those previously published, for growing wild and cultivated in Crete plants. Carvacrol was also found to be the main compound in almost all previous investigations. However, so far, significant variations have been observed at its content. For wild populations the following data for carvacrol content have been reported: 82.3% and 66.6% [20]; 71.9% [8]; 67.8% from bracts and 46.3% from leaves [19]; 72.1% and 64.1% [21]; 64.1% [22] and 51.7% [6]. Other papers refer to cultivated populations on the island of Crete and the reported carvacrol percentages are 58.9% [20], 62.4% [23], 55.1% [7] and 42.9% [6]. Hydroponic cultivations in Crete under different conditions also revealed carvacrol as main compound ranging from 29.1% to 89.0% [19,24,25]. The only publication that refers to cultivation of dittany in a place other than Crete is from Figuérédo, Cabassu, Chalchat, and Pasquier [26]. The authors cultivated it in France and they determined that its carvacrol content was 70.0%. In contrast, in one case of cultivated *O. dictamnus*, thymol (78.0%) was the dominant constituent of its essential oil, while carvacrol was totally absent [22]. Also, the examination of other samples revealed high percentages of *p*-cymene (26.0-48.2%) and thymoquinone (13.0-22.9%), while carvacrol was present in significant smaller quantities (2.9-6.3%) [9].

Conclusion

The medicinal uses of dittany oil are well known. Especially its main compound, carvacrol has many industrial uses. Thus, its commercial value has given the impetus to its systematic cultivation. Until now the only records of dittany cultivation were from the island of Crete, where it is also growing wild. Previous authors mention that the chemical profile and consequently the properties of dittany essential oil are affected in cultivated populations growing out of the island of Crete and its cultivation was not recommended [4,11]. According to our results, the chemical content of *O. dictamnus* was not affected negatively by the cultivation. In contrast it resembled the ones mentioned in the literature. Carvacrol was in all samples the main compound. In fact after the second year of cultivation its percentage increased to 75.1% (bark/lvs) and 60.9% (fl), while in previous studies on cultivated dittany essential oil was found 42.9% [6], 62.44% [23], 64.1% [18] and 72.1% [21]. This feature was accompanied by the decrease of the percentages of its precursor molecules, *p*-cymene and *γ*-terpinene. Perhaps, the plants were well adapted to the new climate conditions.

To the best of our knowledge this is the first time that *O. dictamnus* is cultivated in Northern Greece. The present study reveals undoubtedly that under concrete conditions the cultivation of the plant is feasible without negative affect on its essential oil composition. On the contrary, carvacrol content increased the second year of cultivation. The here in results could create the background for cultivation of the plant in large scale and consequently the augmentation of its industrial use.

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