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Volatile Constituents of Bracts and Leaves of Wild and Cultivated *Origanum dictamnus*

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Abstract: **The chemical composition of the volatile constituents from bracts and leaves of wild and hydroponically cultivated *Origanum dictamnus* were analysed by GC and GC-MS. Three different levels of nitrogen (100, 150, 200 mg/l), were used in the nutrient solution for the cultivation, using the Nutrient Film Technique (N.F.T). Carvacrol was the predominant compound in all cases. The essential oils were investigated for their antimicrobial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Escherichia coli*, and *Pseudomonas aeruginosa*.**

Origanum dictamnus L. or "Cretan dittany" of the Lamiaceae family is a gray green wolly herb, up to 25 cm high, endemic on Crete (Greece), with ovate or round leaves, and large purple bracts. Wild plants are found on the rocky slopes of mountainous Crete. However, it is also cultivated because of its therapeutic properties (1, 2) and its essential oil which has been known since antiquity. It is known under the common names "dictamos" and "erontas". The essential oil from a mixture of leaves and bracts of the plant has been studied (3). Information concerning the oil compounds from leaves and from bracts, and the hydroponic cultivation have not been reported. However, Davtyan (4) reported the essential oil of the substrate cultivation of various medicinal and aromatic plants (5, 6). The Nutrient Film Technique (N.F.T.), is a widely used technique for commercial vegetable production (7, 8). Information concerning the potential of N.F.T. for the yield and the composition of the essential oil on greenhouse production of aromatic plants is limited. Wees and Stewart (9) reported that basil, oregano, parsley and thyme were grown successfully by N.F.T. The purpose of our investigation, was to make a qualitative and quantitative analysis of the essential oils of leaves and bracts of hydroponically cultivated and wild *O. dictamnus*. Three different concentrations of nitrogen (100, 150, 200 mg/l) were used in the nutrient solution in order to test whether nitrogen affects the growth, the yield and the chemical composition of the essential oils of the leaves and bracts of *O. dictamnus*. It has been established in earlier experiments, that dittany plants were grown successfully and with a remarkable yield (10, 11).

Materials and Methods

The leaves and bracts from the wild *O. dictamnus* were collected by Dr. C. Demetzos and C. Economakis in Chania (Malaxa, Crete, Greece), during the middle of July in 1997 and specimen was identified by Dr. D. Perdetzoglou (University of Athens). A voucher specimen (Perdetzoglou et Demetzos No 1643A, ATPH), has been deposited in the Laboratory of Pharmacognosy, University of Athens (Greece). The cultivation was carried out in an unheated greenhouse, using shoot cuttings which were obtained from a single dittany plant of the wild population used in the present study. The N.F.T. system originally designed for lettuce cultivation, is described in a previous paper (12). All solutions contained potassium 150 mg/l and phosphorus 25 mg/l, together with adequate levels of the other nutrients. The solutions were analysed weekly for phosphorus, nitrogen and potassium, with appropriate adjustments made when necessary. In order to maintain a good equilibrium of nutrients the solutions were renewed fortnightly. A pH of 6 was maintained by addition of nitric and phosphoric acid. Likewise the electrical conductivity was maintained between 1.2 and 1.5 mS/cm by a daily addition of a "complete stock" solution. Shoot cuttings, 7–10 cm long, were obtained from a single dittany plant of the

Table 1 Chemical and percentage composition of the essential oils of bracts and leaves of cultivated and of wild *O. dictamnus*.

	Compounds	RI	RI*	1	1*	2	2*	3	3*	4	4*
1	tricyclene	921	1010	0.23	0.04	0.24	0.72	0.27	0.15	1.80	0.15
2	α-pinene	926	1024	0.15	0.03	0.17	0.46	0.19	0.11	1.00	2.14
3	camphene	939	1061	0.04	0.01	0.04	0.03	0.03	0.03	0.37	1.42
4	sabinene	966	1130	0.05	0.04	0.07	0.11	0.07	0.04	–	0.34
5	β-pinene	976	1119	0.09	0.16	0.17	0.24	0.14	0.11	0.91	0.24
6	myrcene	988	1156	0.42	–	0.34	–	0.53	–	2.50	–
7	oct-1-en-3-ol	978	1440	0.18	1.95	0.08	0.56	0.11	0.22	–	1.28
8	3-octanol	990	1395	0.04	0.11	0.09	0.25	0.08	0.07	–	1.10
9	α-terpinene	1010	1187	0.37	0.10	0.31	0.30	0.46	0.07	1.88	0.99
10	*p*-cymene	1019	1267	1.22	3.81	2.02	6.63	2.00	5.64	3.11	4.17
11	limonene	1022	1206	0.11	0.09	0.15	0.34	0.17	0.08	0.57	0.23
12	γ-terpinene	1055	1250	1.73	1.22	1.32	1.36	1.85	0.72	11.75	6.77
13	sabinene hydrate (*trans*)	1059	1455	0.53	0.84	0.82	0.53	0.86	0.70	0.97	0.95
14	linalool	1098	1534	–	2.58	–	0.20	–	0.13	0.30	3.76
15	camphor	1143	1514	0.30	2.58	0.31	0.60	0.09	0.27	2.20	1.70
16	terpinene-4-ol	1166	1612	2.21	2.40	1.86	0.32	0.42	0.06	2.74	5.36
17	carvone	1234	1710	–	5.30	–	2.15	–	1.62	–	2.30
18	thymoquinone	1247	1756	–	6.37	–	7.68	–	6.98	–	5.67
19	thymol	1268	2174	9.13	2.46	0.10	1.43	0.16	1.88	0.10	6.55
20	carvacrol	1312	2208	72.70	60.94	87.10	53.60	89.0	45.60	67.87	46.30
21	NI	1325	2220	–	1.06	–	4.63	–	6.26	–	4.13
22	(*Z*)-caryophyllene	1407	1597	5.97	0.42	0.78	2.21	0.93	5.15	1.67	1.73
23	NI	1432	2250	–	0.04	–	2.10	–	8.34	–	0.08
24	β-bisabolene	1509	1734	0.55	0.14	0.15	1.70	0.14	1.90	–	0.37
25	δ-cadinene	1513	1754	–	0.15	–	0.90	–	1.50	–	0.09
Total identified (%):				96.02	91.74	96.12	82.32	97.50	73.03	99.74	93.61

RI and RI*: Retention indices on DB-5 and CP-WAX-52 CB columns were calculated according to Van den Dool and Kratz (15).
1, 2, 3 and 1*, 2*, 3*: Essential oils of bracts and leaves of cultivated *O. dictamnus*, using 100, 150, 200 mg NO_3-N/l respectively.
4 and 4*: Essential oil of bracts and leaves of wild *O. dictamnus*.
Compounds were listed according to their R_t to the DB-5 column.

Table 2 Antimicrobial activity of the essential oils of cultivated and of wild bracts and leaves of *O. dictamnus* L.

MICs (μg/ml) of the ess. oils and of standard antibiotic and of carvacrol										
Microorganism	1	1*	2	2*	3	3*	4	4*	S	Carvacrol
S. aureus	500	300	700	300	800	250	600	300	100	300
S. epidermidis	550	350	600	300	850	300	600	300	100	300
S. hominis	600	330	650	350	700	330	700	300	100	300
E. coli	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	200	300
Ps. aeruginosa	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.T	300

1, 2, 3 and 1*, 2*, 3*: Essential oils of bracts and leaves of cultivated *O. dictamnus*, using 100, 150, 200 mg NO_3-N/l respectively.
4 and 4*: Essential oils of bracts and leaves of wild *O. dictamnus*.
S: Streptomycin; N.T: not tested; N.A: Not Active (MIC values > 1000).

wild population used in the present study. After a month, thirty two rooted cuttings selected for their uniformity, were placed 25 cm apart in each of the N.F.T. channels. Two channels were used, for each of the three nitrogen levels examined (i.e., 100, 150, 200 mg/l). In July, when most of the plants were at the flowering stage, ten plants for each treatment were sampled. Their shoots were weighed and placed in a forced-air oven at 40 °C, until they had reached a constant weight. The dried shoots of the plants belonging to the same treatment were mixed. Bracts and leaves were separated and were subjected to hydrodistillation for 3 h, and the essential oils were obtained. Air-dried bracts (1.5 g) and leaves (4 g) from the wild plant were separated and were subjected to hydrodistillation for 3 h. All the oils obtained were dried over anhydrous Na_2SO_4 and stored under refrigeration. The essential oils were analysed using capillary GC and GC/MS system operating in the EI mode. The GC analysis was carried out using a Perkin-Elmer 8500 FID instrument, and a DB-5 fused silica column (30 m × 0.32 mm and 0.25 μm film thickness) as well as a CP-WAX-52 CB column (60 m × 0.32 mm and 0.25 μm film thickness). The columns were temperature programmed as follows: 50 °C for 5 min and 50–250 °C (3 °C/min) with helium as the carrier gas. GC/MS was carried out using Hewlett Packard (HP) 5973 mass selective detector, and a HP-5 MS fused silica capillary column of a 30 m × 0.25 mm (0.25 μm film thickness). The column was temperature programmed as follows: 50 °C for 5 min, then the temperature was increased to 280 °C, at a rate of 3 °C/min. Mass unit conditions: ion source 230 °C, ionization energy 70 eV, electron current 1435 μA. Identification of constituents was based on comparison of retention indices with those of authentic samples (13) and on the basis of their mass spectral fragmentation using the Wiley 138 I/NBS, GC-mass spectrometry library. The microbial strains were from the American Type Culture Collection. Standard antibiotic streptomycin was used in order to control the sensitivity of the test microorganisms. The MICs of the essential oils were determined by microdilution assay, as recommended by NCCLS (14). Minimum inhibitor concentrations (MICs) of streptomycin as well as of carvacrol were also determined in parallel experiments. MIC was defined as the lowest concentration that inhibited visible growth.

The yield of the essential oils of the leaves and bracts of wild *O. dictamnus* was 1.1 % and 0.8 % (v/w) and that of the hydroponically cultivated was 4 % and 3.5 % (v/w), respectively. The percentage of the essential oils of the leaves and bracts was not affected by the level of nitrogen in the nutrient solution. The qualitative and quantitative analysis of the constituents of the oils of the bracts showed qualitative differences, whereas in the case of the leaf oils only quantitative differences were observed (Table **1**). In the wild specimens, sabinene, oct-1-en-3-ol, 3-octanol, carvone, thymoquinone, β-bisabolene and δ-cadinene, were identified only in the essential oil of leaves, whereas in the essential oil of the bracts these compounds were totally absent. On the contrary, myrcene was only found in bracts but not in leaves of the wild specimen. Tricyclene, myrcene, α-terpinene, γ-terpinene and camphor were in higher percentages in the bracts of the wild plant, whereas α-pinene, camphene, 3-octanol, γ-terpinene, linalool, terpinene-4-ol and thymol, were also in higher percentages in the leaves of the wild plant, than in the three cultivated specimens in both cases. Carvacrol was the predominant compound in all cases, and the respective yields are shown in Table **1**. Quantitative differences among the three cultivated specimen in the case of the bracts were the amount of thymol and (*Z*)-caryophyllene, whereas in the case of the leaves were the amount of linalool, camphor, terpinene-4-ol and carvone (Table **1**).

Qualitative differences were also observed (Table **1**) among the cultivated specimens. Carvone, thymoquinone, and δ-cadinene were not identified in the essential oils of bracts, whereas in the essential oils of leaves these compounds were found in large amounts. The results of the antimicrobial activity are shown in the Table **2**. All the oils from the bracts of wild and cultivated *O. dictamnus* showed a weak antistaphylococcal activity. In contrast all the oils from the leaves of wild and cultivated specimen showed a marked antistaphylococcal activity. The oils are considered as non-active against Gram (−) bacteria (*E. coli* and *Ps. aeruginosa*) because the respective MIC values exceeded 1000 μg/ml.

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