

## SUPPLEMENTARY MATERIAL

### Composition, antibacterial, antioxidant and antiproliferative activities of essential oils from three *Origanum* species

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The essential oils from *Origanum dictamnus*, *O. libanoticum* and *O. microphyllum* were analyzed by GC-MS, finding carvacrol, p-cymene, linalool,  $\gamma$ -terpinene and terpinen-4-ol as major components. The antioxidant activity by DPPH and FRAP test and the antiproliferative activity against two human cancer cell lines, LoVo and HepG2, were investigated, showing that the essential oil of *O. dictamnus* was statistically the most inhibitory on both the cell lines while all the oils exerted a weak antioxidant activity. Furthermore, the samples were tested against ten Gram-negative and Gram-positive bacteria; all the oils were active on Gram-positive bacteria but *O. dictamnus* essential oil was the most effective (MIC = 25-50  $\mu$ g/ml) showing also a good activity against the Gram-negative *E. coli* (MIC = 50  $\mu$ g/ml). Data suggest that these essential oils and particularly *O. dictamnus* oil could be used as valuable new flavours with functional properties for food or nutraceutical products.

**Keywords:** *Origanum* species; essential oil; antibacterial; antioxidant; antiproliferative.

#### Experimental

##### *Plant material*

Aerial parts of *O. dictamnus* and *O. microphyllum* were both collected at the full flowering stage on rocky grounds on June 2005 in the Crete island, Greece, respectively on Mavri summit, Mt. Ida (1700 m a.s.l.) and on the Mt. Omalos (1150 m a.s.l.). Aerial parts of *O. libanoticum* were collected at the full flowering stage on June 2006 on a rocky ground near Laklouf (700 m a.s.l.), Lebanon. Voucher specimens (leg. & det. N. Arnold s. n., confirm. Th. Raus) were deposited in the Herbarium of the Botanischer Garten, Berlin Universität.

##### *Chemicals*

DPPH (2,2-diphenyl-1-picrylhydrazyl); 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ); acetic acid; sodium acetate; hydrochloric acid; ferric chloride; sodium carbonate; Folin & Ciocalteu's phenolic reagent; sodium nitrite; aluminium chloride; sodium hydroxide; 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX); gallic acid; quercetin; tert-butyl-4-hydroxy toluene (BHT) purchased

from Sigma Chemical Co. (St. Louis, MO) and methanol purchased from Carlo Erba (Milano, Italy). RPMI 1640 medium, Dulbecco's Modified Eagle's Medium (DMEM), foetal bovine serum, L-glutamine, penicillin/streptomycin, trypan blue, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich S.p.a. (Milano, Italy).

#### ***Essential oils isolation***

The air-dried samples were crushed, then hydrodistilled for 3 h as showed in Formisano et al (2008) using a Clevenger-type apparatus according to the method recommended in the current *European Pharmacopoeia* (6<sup>th</sup> edition, 2008) to obtain the following essential oils: a dark yellow oil for *O. dictamnus* (yield w/w 1.12%), a yellow oil for *O. libanoticum* (yield w/w 0.16%) and a reddish oil for *O. microphyllum* (yield w/w 0.27%). The oils were dried over anhydrous sodium sulphate and stored under N<sub>2</sub> at +4°C in the dark until tested and analysed.

#### ***GC and GC-MS analysis***

Analytical GC was carried out on a Perkin-Elmer Sigma 115 gas chromatograph fitted with a HP-5 MS capillary column (30m X 0.25 mm), 0.25 mm film thickness, as described previously (Formisano et al. 2008).

#### ***Identification of compounds***

Most constituents were identified by gas chromatography by comparison of their retention indices ( $K_i$ ) with those of the literature (Jennings & Shibamoto, 1980; Davies, 1990) or with those of authentic compounds available in our laboratories. The retention indices were determined in relation to a homologous series of *n*-alkanes (C<sub>8</sub>-C<sub>24</sub>) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 02, Wiley 275 libraries and our home-made library or with mass spectra from literature (Jennings & Shibamoto, 1980; Adams, 2007). Component relative percentages were calculated based on GC peak areas without using correction factors.

#### ***Antibacterial activity***

##### ***Bacterial strains***

Ten bacterial strains, selected as representative of the class of Gram+ and Gram-, were tested: *Bacillus cereus* (ATCC 11778), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Streptococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Proteus mirabilis* (ATCC 25933), *Proteus vulgaris* (ATCC 13315), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhi* Ty2 (ATCC 19430). The strains were maintained on Tryptone Soya agar (Oxoid, Milan, Italy); for the antimicrobial tests, Tryptone Soya broth (Oxoid, Milan, Italy) was used.

##### ***Antimicrobial activity assay***

The antibacterial activity was evaluated by determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) using the broth dilution method (Barry, 1976; Koneman, Allen, Janda, Schreckenber, & Winn, 1997). In order to facilitate the dispersion of the oil in the aqueous nutrient medium it was diluted with Tween 20 at a concentration of 10%. Each strain was tested with sample that was serially diluted in broth to obtain concentrations ranging from 100 µg/mL, to 0.8 µg/mL. The sample was previously sterilized with Millipore filter of 0.20 µm. The sample has been stirred, inoculated with 50µL of physiologic solution containing  $5 \times 10^6$  microbial cells, and incubated for 24 h at 37°C. The MIC value was determined as the lowest concentration of the sample that does not permit any visible growth of the tested microorganism after incubation. Control containing only Tween 20 instead of the essential oil was not toxic to the microorganisms. As positive control were used cultures containing only sterile physiologic solution Tris buffer. MBC was determined by subculture of the tubes with inhibition in 5 mL of sterile nutrient broth. After incubation at 37°C the tubes were observed. When the germs did not grow, the sample denoted a bactericidal action. Oil samples were tested in triplicate.

#### ***Antiproliferative effects against human tumor cell lines***

##### ***Cell culture***

Human colon carcinoma LoVo cells (ATCC CCL-229) were grown in RPMI-1640 medium supplemented with 1% antibiotic solution (penicillin/streptomycin), 1% L-glutamine and 10% fetal bovine serum (FBS), and incubated at 37°C under 5% CO<sub>2</sub>. Cell monolayers were subcultured onto 96 well culture plates ( $5 \times 10^4$  cells/well) used for experiments 24 h later. Cells counts and viability were performed using a standard trypan blue cell counting technique.

Hepatocarcinoma cell line HepG2 (ATCC HB-8065) was grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 1% antibiotic solution (penicillin/streptomycin), 1% L-glutamine and 10% fetal bovine serum (FBS). Cells were subcultured onto 96 well culture plates ( $3 \times 10^4$  cells/well).

##### ***Evaluation of antiproliferative activity***

Cells were treated with serial concentrations of samples. One hundred µL/well of each concentration were added to obtain final dilutions ranging from 2.5 to 100 µg/mL. Treatments never exceeded 0.5% of the solvent (DMSO), the percentage that was used to treat control wells.

After treatments, culture plates were kept at 37°C with 5%, v/v, CO<sub>2</sub> for 24 and 48 h. Cell viability was then assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Conforti et al., 2010). Briefly, after the period of incubation (24 and 48 h), medium was removed and 100 µL of 0.5% (w/v) MTT (Sigma), dissolved in phosphate-buffered saline (PBS), were added to each well and allowed to incubate for further 4 h. 100 µL of DMSO were then added

to each well to dissolve the formazan crystals. Absorbance values at 570 nm were measured using a microplate reader (GDV DV 990 B/V, Roma, Italy). Each experiment was carried out in quadruplicate.

#### ***Cell morphology microscopy***

Changes in cell morphology were visualized using an inverted microscope (AE20 Motic; Motic Instruments, Inc., VWR, Milan, Italy). Images were captured on a VWR digital camera (VisiCam 3.0 USB camera, Milan, Italy).

#### ***Antioxidant activity***

Essential oils were dissolved in methanol in order to obtain 1 mg/1 mL solutions, which was chosen as appropriate concentration for assessing antioxidant activity after preliminary studies of the different concentrations. For each antioxidant assay, a Trolox aliquot was used to develop a 0.5-10 mmol/l standard curve. All data were then expressed as Trolox Equivalents (mmol/L) and antioxidant activity referred to as Trolox Equivalents Antioxidant Capacity (TEAC).

#### ***Free Radical Scavenging Ability (DPPH method)***

The ability of essential oils and extracts to scavenge the DPPH radical was measured using the method of Brand-Williams (Brand-Williams, Cuvelier & Berset, 1995). Aliquots (40  $\mu$ L) of samples were added to 3 ml of DPPH solution ( $6 \times 10^{-5}$  mol/l) and the absorbance was determined at 515 nm after 60 min.

#### ***Ferric reducing/antioxidant power (FRAP method)***

The total antioxidant potential of extracts were determined by using the ferric reducing antioxidant power (FRAP) assay of Benzie & Strain (1996). A solution of 10 mmol/L TPTZ in 40 mmol/L HCl and 12 mmol/L ferric chloride was diluted in 300 mmol/L sodium acetate buffer (pH 3.6) at a ratio of 1:1:10. Aliquots (40  $\mu$ L) of sample solutions were added to 3 mL of the FRAP solution and allowed to react for 60 minutes before reading the absorbance at 593 nm.

#### ***Statistical analysis***

All experiments for the evaluation of antiproliferative activity were carried out in quadruplicate. Data were expressed as mean value  $\pm$  SEM. The concentration providing 50% inhibition compared with the control ( $IC_{50}$ ) was calculated by nonlinear regression using GraphPad Prism Software (San Diego, CA, USA). Statistical significance was assessed with a one-way analysis of variance (ANOVA) using SigmaStat Software (Jantel Scientific Software, San Rafael, CA). Significant differences among means were analyzed using Tukey's multiple comparisons test. Differences were considered significant at  $P < 0.05$ .

#### **References**

Adams RP. 2007. Identification of Essential Oil Components by Gas Chromatography/Mass

- Spectroscopy, fourth ed. Carol Stream, Illinois, USA: Allured Publishing Co.
- Barry A. 1976. The Antimicrobial Susceptibility Test: Principles and Practices. Philadelphia: Lea and Febiger.
- Benzie IF, Strain JJ. 1996. The ferric reducing ability of plasma (FRAP) as measurement of 'antioxidant power': the Frap assay. *Anal. Biochem.* 239:70–76.
- Brand-Williams W, Cuvelier ME, Berset C. 1995. Use of free radical method to evaluate antioxidant activity. *Lebensm. Wiss. Technol.* 28:25–30.
- Conforti F, Tundis R, Marrelli M, Menichini F, Statti GA, De Cindio B, Menichini F, Houghton PJ. 2010. Protective effect of *Pimpinella anisoides* ethanolic extract and its constituents on oxidative damage and its inhibition of nitric oxide in lipopolysaccharide-stimulated RAW 264.7 macrophages. *J. Med. Food.* 13:137-141.
- Davies, N.W. (1990). Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases. *J. Chromatogr.* 503:1-24.
- Formisano, C., Rigano, D., Senatore, F., Bruno, M., Rosselli, S., Raimondo, F. M. & Spadaro, V. (2008). Chemical composition of the essential oils of *Centaurea sicana* and *C. giardiniae* growing wild in Sicily. *Nat. Prod. Commun.* 3:919-922.
- European Pharmacopoeia 6.0, (2008). Determination of essential oils in herbal drugs, 2.8.12, pp. 251–252.
- Jennings W, Shibamoto T. 1980. Qualitative Analysis of Flavour and Fragrance Volatiles by Glass capillary Gas Chromatography. New York: Academic Press.
- Koneman EW, Allen SD, Janda WM, Schreckenber PC, Winn WC. 1997. Color Atlas and Textbook of Diagnostic Microbiology. Philadelphia, PA: Lippincott-Raven pub, pp. 784-785.

**Table S1** – Chemical composition of the essential oils from aerial parts of *Origanum dictamnus* (D), *O. libanoticum* (L), *O. microphyllum* (M).

LRI <sup>a</sup>	LRI <sup>b</sup>	Component	D	L	M	I <sup>c</sup>
800	800	Octane		0.2		1, 2, 3
854	1209	( <i>E</i> )-2-Hexenal		0.2		1, 2
925	1035	$\alpha$ -Thujene	0.4			1, 2
938	1032	$\alpha$ -Pinene	1.7		3.0	1, 2, 3
945	1056	Camphene			2.2	1, 2, 3
973	1132	Sabinene		1.9	<b>7.5</b>	1, 2
978	1118	$\beta$ -Pinene		0.5		1, 2, 3
990	1244	2-Pentylfuran		0.3		1, 2, 3
992	1195	2-Dehydro-1,8-cineole			0.2	1, 2
993	1173	Myrcene	1.3	1.9	1.4	1, 2, 3
1001	1146	$\delta^2$ -Carene			1.4	1, 2
1005	1150	$\alpha$ -Phellandrene		0.4	0.3	1, 2, 3
1013	1189	$\alpha$ -Terpinene			2.0	1, 2, 3
1020	1187	<i>o</i> -Cymene		1.1	0.6	1, 2
1025	1278	<i>p</i> -Cymene	<b>32.7</b>	0.5	1.0	1, 2
1029	1218	$\beta$ -Phellandrene	0.6	0.1	0.8	1, 2, 3
1030	1205	Limonene	0.6	0.3	0.8	1, 2, 3
1049	1262	( <i>E</i> )- $\beta$ -Ocimene		0.1		1, 2
1057	1256	$\gamma$ -Terpinene	<b>12.4</b>	1.1	<b>4.2</b>	1, 2, 3
1059	1557	<i>cis</i> -Sabinene hydrate		0.2	2.6	1, 2
1076	1477	<i>cis</i> -Linalool oxide (furanoid)		0.4		1, 2
1080	1282	<i>p</i> -Cymenene	0.5			1, 2
1086	1265	Terpinolene	0.4	0.2		1, 2, 3
1093	1474	<i>trans</i> -Sabinene hydrate	0.9		<b>7.1</b>	1, 2
1097	1553	Linalool	<b>7.8</b>	6.5		1, 2, 3
1145	1532	Camphor				1, 2, 3
1165	1502	<i>iso</i> -Menthone		1.1		1, 2
1167	1718	Borneol	0.4		1.6	1, 2, 3
1176	1611	Terpinen-4-ol	0.9	0.4	<b>16.2</b>	1, 2, 3
1179	1763	Napthalene		0.5	0.1	1, 2, 3
1183	1758	<i>cis</i> -Piperitol			0.5	1, 2
1186	2078	Octanoic acid			0.1	1, 2, 3
1189	1706	$\alpha$ -Terpineol		1.6	2.5	1, 2, 3
1207		<i>trans</i> -Piperitol			0.3	1, 2
1238	1662	Pulegone			0.2	1, 2
1239	1607	Thymol methyl ether		<b>9.8</b>		1, 2
1242		Carvone			0.1	1, 2
1245	1975	Carvacrol methyl ether	0.4		0.7	1, 2
1255	1857	Geraniol		0.5		1, 2, 3
1278	2190	Nonanoic acid			0.2	1, 2, 3

1284	1597	Bornyl acetate			0.9	1, 2
1287		Dihydroedulan II			1.1	1, 2, 3
1291	2471	Indole		2.3	0.3	1, 2
1293	1942	4-Hydroxy-2-methyl acetophenone	1.6	1.5		1, 2
1293	2198	Thymol	0.4		<b>4.3</b>	1, 2, 3
1299	2239	Carvacrol	<b>14.7</b>	1.1	<b>13.3</b>	1, 2, 3
1313	2073	<i>p</i> -Mentha-1,4-dien-7-ol			0.2	1, 2
1336	1495	Bicycloelemene		1.5	0.7	1, 2
1343		Piperitone		0.2		1, 2
1348	1466	$\alpha$ -Cubebene		0.2		1, 2
1353	2186	Eugenol		0.2	0.3	1, 2, 3
1356	1849	$\alpha$ -Longipinene		0.7		1, 2
1358	1787	( <i>E</i> )- $\beta$ -Damascenone			0.3	1, 2
1377	1497	$\alpha$ -Copaene	1.2	3.2		1, 2
1382	1400	$\alpha$ -Funebrene diepialfacedrene 469-61-4		0.5		1, 2
1385	1558	$\beta$ -Bourbonene		1.3		1, 2
1387	1600	$\beta$ -Elemene				1, 2
1398		$\alpha$ -Elemene			0.2	1, 2
1408		$\alpha$ -Gurjunene			0.8	1, 2
1415	1612	( <i>E</i> )- $\beta$ -Caryophyllene	<b>3.5</b>	<b>7.7</b>	<b>4.0</b>	1, 2, 3
1432	1612	$\beta$ -Gurjunene (Calarene)		0.4		1, 2
1432	1650	$\gamma$ -Elemene		2.3	1.8	1, 2
1437	1628	Aromadendrene		0.1	0.2	1, 2
1453	1867	( <i>E</i> )-Geranylacetone			0.2	1, 2
1454	1596	$\alpha$ -Guaiene			0.2	1, 2
1455	1689	$\alpha$ -Humulene		1.4	0.6	1, 2
1463	1662	<i>allo</i> -Aromadendrene		0.4	0.5	1, 2
1465	1782	Sesquiphellandrene		0.8		1, 2
1477	1726	Germacrene D		3.0		1, 2
1478	1704	$\gamma$ -Muurolene		0.6		1, 2
1487	1679	$\alpha$ -Amorphene			0.3	1, 2
1490		<i>cis</i> $\beta$ -Guajene				1, 2
1491		Bicyclosesquiphellandrene				1, 2
1492	1756	Bicyclogermacrene			1.0	1, 2
1503	1740	$\alpha$ -Muurolene			0.2	1, 2
1506	1758	( <i>E,E</i> )- $\alpha$ -Farnesene		0.2		1, 2
1508	1715	$\beta$ -Himachalene				1, 2
1510	1743	$\beta$ -Bisabolene	1.0	0.5		1, 2
1515	1776	$\gamma$ -Cadinene		2.1	0.8	1, 2
1519	1839	(1 <i>S</i> )- <i>cis</i> -Calamenene	0.4			1, 2
1526	1773	$\delta$ -Cadinene	0.3		0.7	1, 2
1532	1799	Cadina-1,4-diene		0.4		1, 2
1536		( <i>E</i> )- $\alpha$ -Bisabolene Z1740				1, 2
1541	1918	$\alpha$ -Calacorene		0.1		1, 2
1577	2150	Spathulenol		2.3		1, 2
1579	2008	Caryophyllene oxide	2.3	2.0		1, 2, 3

1587	2098	Globulol			1.1	1, 2
1591	2104	Viridiflorol			0.1	1, 2
1621	2324	Caryophylladienol II	0.7		0.2	1, 2
1636	2183	$\gamma$ -Eudesmol				1, 2
1640	2316	Caryophylladienol I			0.2	1, 2
1642	2209	T-Muurolol	0.6			1, 2
1650		<i>allo</i> -Aromadendrene oxide I		0.3	0.2	1, 2
1652	2235	$\alpha$ -Cadinol				1, 2
1689	2276	( <i>Z,E</i> )-Farnesol	0.2			1, 2
1692	2367	Vulgarol B	0.2	0.3		1, 2
1694		Pentadecan-2-one	0.3			1, 2
1698	1947	Shyobunol				1, 2
1769	2713	Tetradecanoic acid			0.2	1, 2, 3
1835	2131	Hexahydrofarnesyl acetone			0.3	1, 2
1923	2386	( <i>E,E</i> )-Farnesyl acetone		0.6		1, 2
1950	2622	( <i>Z</i> )-Phytol		0.4		1, 2
1957	2931	Hexadecanoic acid	0.7	<b>11.3</b>	1.0	1, 2
2023	2354	Octadecanal		0.3		1, 2, 3
2100	2100	Heneicosane				1, 2, 3
2122	3157	( <i>Z,Z</i> )-9,12-Octadecadienoic acid		2.7		1, 2, 3
2200	2200	Docosane				1, 2, 3
2400	2400	Tetracosane				1, 2, 3
2500	2500	Pentacosane	0.2	0.8	0.2	1, 2, 3
2600	2600	Hexacosane		0.2		1, 2, 3
2700	2700	Heptacosane		1.7	0.2	1, 2, 3
2800	2800	Octacosane		0.3		1, 2, 3
2900	2900	Nonacosane		0.5	0.3	1, 2
3000	3000	Triacotane		0.2		1, 2
3100	3100	Entriacontane		0.7		1, 2
3200	3200	Dotriacontane		0.2		1, 2
		<b>Total compounds</b>	<b>89.3</b>	<b>87.3</b>	<b>94.5</b>	
		<b>Monoterpene hydrocarbons</b>	<b>50.6</b>	<b>8.1</b>	<b>25.2</b>	
		<b>Oxygenated monoterpenes</b>	<b>10.0</b>	<b>10.9</b>	<b>32.4</b>	
		<b>Sesquiterpene hydrocarbons</b>	<b>6.4</b>	<b>27.4</b>	<b>12.0</b>	
		<b>Oxygenated Sesquiterpenes</b>	<b>3.8</b>	<b>4.6</b>	<b>1.8</b>	
		<b>Phenolic compounds</b>	<b>15.5</b>	<b>11.1</b>	<b>18.6</b>	

LRI<sup>a</sup> = Linear Retention Index on a HP5 MS column; LRI<sup>b</sup> = Linear Retention Index on a HP Innowax column

I = Identification; 1 = linear retention index; 2 = MS; 3 = Go-GC



**Table S2.** MIC and MBC values ( $\mu\text{g/ml}$ ) of essential oils from *Origanum dictamnus* (D), *O. libanoticum* (L) and *O. microphyllum* (M) and MIC of reference antibiotic.

<b>Bacterial strain</b>	<b>D</b>	<b>L</b>	<b>M</b>	<b>G*</b>
<i>Bacillus cereus</i> ATCC 11778	25 (50)	50 (100)	50	1,56
<i>Bacillus subtilis</i> ATCC 6633	25	50 (100)	50 (100)	1,56
<i>Staphylococcus aureus</i> ATCC 25923	50	100	50	3,12
<i>Staphylococcus epidermidis</i> ATCC 12228	25(50)	50 (100)	50 (100)	6,25
<i>Streptococcus faecalis</i> ATTC 29212	50 (100)	100	100	>100
<i>Escherichia coli</i> ATCC 25922	50(100)	100	50 (100)	3,12
<i>Proteus mirabilis</i> ATCC 25933	100	>100	100	100
<i>Proteus vulgaris</i> ATCC 13315	100 (>100)	>100	100	100
<i>Pseudomonas aeruginosa</i> ATCC 27853	>100	>100	>100	12,5
<i>Salmonella typhi</i> Ty2 ATCC 19430	100	>100	100	>100

\* Gentamicin

**Table S3.** Reducing capacity (FRAP test) and radical scavenging activity (DPPH test) of *O. microphyllum* (M), *O. dictamnus* (D) and *O. libanoticum* (L) ( $n = 5$ ;  $P < 0.001$ ).

	DPPH (mmol TE/L)	FRAP (mmol TE/L)
<i>M</i>	0.04± 0.05	0.50± 0.01
<i>D</i>	0.15± 0.04	0.52± 0.02
<i>L</i>	0.02± 0.05	0.57± 0.04
<b>BHT</b>	2.50± 0.01	3.90± 0.02
<b>Thymol</b>	0.67± 0.05	3.25± 0.04
<b>Carvacrol</b>	0.72± 0.03	3.01± 0.07

TE = mmol of Trolox Equivalents