Check for updates

NPC Natural Product Communications

2018
Vol. 13
No. 9
1183 - 1187

Potential Health Benefits of *Origanum heracleoticum* Essential Oil: Phytochemical and Biological Variability among Different Calabrian Populations

Mariangela Marrelli[a], Fabrizio Araniti[b], Maria Rosa Abenavoli[b], Giancarlo Statti[a] and Filomena Conforti[a,*]

[a]*Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, I-87036 Rende, (CS), Italy*
[b]*Department of Agraria, University "Mediterranea" of Reggio Calabria, I-89100 Reggio Calabria, Italy*

filomena.conforti@unical.it

Received: December 12th 2017; Accepted: July 25th, 2018

The potential health benefits of the essential oils of six different populations of *Origanum heracleoticum* L. from Calabria (Italy) were assessed, together with the evaluation of the influence of the site of collection on their metabolic profile and biological activity. Different terpenoids were identified, being thymol, carvacrol and *o*-cymene among the most abundant ones. Hierarchical cluster analysis allowed the identification of two main groups, which were discriminated by the altitude of collection. The antioxidant activity was tested by means of two *in vitro* assays: DPPH and β-carotene bleaching test. Samples EO-4 and EO-3 were the most active in protecting linoleic acid from peroxidation, with IC_{50} values of 4.00 and 4.68 µg/mL after 30 minutes of incubation. The anti-inflammatory potential was assessed through the evaluation of the capacity to inhibit NO production in lipopolysaccharide (LPS)-induced murine macrophage RAW 264.7 cell line. All essential oils induced a dose-depending inhibitory effect. An excellent activity was demonstrated for sample EO-4, followed by sample EO-3 (IC_{50} values equal to 32.77 and 49.48 µg/mL, respectively). Observed biological properties were correlated to the phytochemical content of analyzed samples.

Keywords: Oregano, Antioxidant activity, Anti-inflammatory activity, Nitric oxide.

Oregano is one of the most important spices in world trade. The name "oregano" refers to a number of species belonging to different families, whose essential oils (EOs) are characterized by a typical flavor originating from the high amounts of carvacrol [1]. *Origanum vulgare* L. is the species commonly known as oregano in most countries [2-4] and is present in the whole of Italy. *Origanum heracleoticum* L. (Lamiaceae) is instead typical of the South of the country [5].

Different studies dealt with the phytochemical variability of EOs from wild *Origanum heracleoticum* from different regions of Italy. A number of studies have shown that this species is a very variable taxon both in morphological and in chemical features, with an EO the main components of which are phenols, *p*-cymene, and γ-terpinene [6-8]. The antibacterial activity of the EO and the antioxidant potential of both extract and EO have also been explored [9-12]. We already tested the anti-inflammatory potential of the ethanolic extract of this plant through the evaluation of the inhibitory activity on nitric oxide (NO) production in LPS-stimulated RAW 264.7 macrophages [13].

The aim of this work was to evaluate the potential health benefits and applications of the EO of this species, particularly the antioxidant and anti-inflammatory potential. The influence of the site of collection on metabolic profile and biological activity of the EOs of six different populations of *O. heracleoticum* from Calabria (Italy) was also assessed.

The harvest times, the altitude and GPS coordinates of the location as well as the EO yield (percent of dry weight) of *O. heracleoticum* samples are reported in Table 1. The yield ranged from 3.47 to 5.43% and it was significantly higher in the samples collected at the highest altitudes (Table 1).

Table 1: Locality, altitude, collection date and EOs yield of the 6 wild populations of *O. heracleoticum* collected in Calabria (Southern Italy).

Sample	Locality	Altitude (m. a.s.l.)	GPS coordinates	Collection date	Yield (%)
EO-1	Sant'Eufemia d'Aspromonte	420	38°16'00.4"N - 15°51'07.8"E	July	3.65
EO-2	Podargoni	500	38°09'48.1"N - 15°46'57.7"E	July	3.47
EO-3	Bagaladi	460	38°01'21.4"N - 15°49'12.5"E	July	3.94
EO-4	Longobucco	784	39°27'05.4"N - 16°36'56.6"E	August	5.23
EO-5	Rogliano	660	39°10'14.0"N - 16°19'31.5"E	August	5.43
EO-6	Acri	720	39°29'24.0"N - 16°22'28.2"E	August	4.85

The relative abundances of the chemicals identified in the EOs of the 6 populations of *O. heracleoticum* collected at two different altitudes are reported in Table 2.

A wide array of terpenoids were identified. In particular, 29 terpenes were found, among which the two monoterpenic phenols thymol and carvacrol were among the most abundant ones together with *o*-cymene. Fifteen sesquiterpenoids were also found, among which caryophyllene, muurolene and cadinene were the most abundant ones.

To verify how location influenced the metabolic profile, the EOs isolated from 6 populations of *O. heracleoticum* were evaluated through GC-MS and then data were analyzed through unsupervised Principal Component Analysis (Figure 1).

The PCA score plot, which allowed the separation of the 6 populations basing on their metabolite profiles, is reported in Figure 1-A, whereas the PCA loading plot where the metabolites contributing in score-plot samples separation were highlighted is reported in Fig 1-B. The separation of the EOs belonging to different populations was achieved using the principal components PC1 vs PC2, which explained a total variance of 51.2%. In particular, PC1 explained the 31.5% of the variance while PC2 the 19.7%. Principal component analysis pointed out a clear separation

Table 2: Chemical composition of *O. heracleoticum* EOs.

Monoterpenes	RT	EO-1	EO-2	EO-3	EO-4	EO-5	EO-6
α-Thujene	09.90	14.46	-	-	2368.91	53.03	-
α-Pinene	10.07	375.79	524.34	106.45	3388.29	220.70	397.27
Camphene	10.50	47.54	50.06	13.68	404.69	-	40.33
β-Pinene	11.20	41.39	100.47	15.05	642.82	41.11	75.12
β-Myrcene	11.54	472.25	1063.98	118.08	6199.70	445.71	804.92
Sabinene	11.77	12.41	79.01	18.82	-	-	-
Δ-3-Carene	11.88	52.14	67.63	16.37	424.42	-	47.89
α-Terpinene	11.99	181.62	477.50	116.65	251.29	-	-
o-Cymene	12.14	8119.62	7450.03	3304.13	48175.94	6348.5	6888.6
Limonene	12.20	434.74	470.30	111.03	2515.31	230.34	302.94
Eucalyptol	12.24	112.05	74.04	22.75	809.58	60.35	57.47
β-Ocimene	12.34	6.54	177.80	16.25	783.18	152.88	164.14
γ-Terpinene	12.65	23.65	4570.53	750.77	19132.72	1170.81	2199.87
trans-4-Thujanol	12.78	43.73	196.66	91.31	1212.04	154.72	140.32
Linalool	13.18	1315.05	614.19	320.60	-	725.91	355.83
trans-p-2-Menthen-1-ol	13.46	14.26	35.12	21.27	-	-	-
cis-2-p-Menthen-1-ol	13.67	13.48	-	17.40	-	-	-
Myrtenol	13.73	19.87	43.51	16.13	406.19	39.22	74.68
Borneol	13.97	208.64	271.16	125.55	2435.39	297.50	551.85
4-Terpineol	14.07	1099.06	805.82	901.76	3384.45	653.01	432.95
p-Cymen-8-ol	14.14	95.69	-	39.92	236.68	36.50	34.55
α-Terpineol	14.20	413.19	212.38	334.61	1150.75	201.73	246.77
Carvone	14.25	37.32	-	86.09	463.43	-	-
3-methoxy-*p*-Cymene	14.56	737.8	671.48	69.38	2732.95	1519.11	400.93
Carvacrol-methyl-ether	14.64	3275.21	2657.99	2908.22	19843.72	2839.54	2900.29
Carvenone	14.87	38.55	-	-	507.28	57.34	155.03
3-*p*-Cymenol	14.99	413.08	265.27	113.94	1728.28	289.79	252.28
Thymol	15.06	10781.78	11145.27	6814.88	54459.34	13146.99	9104.01
Carvacrol	15.14	4568.63	3311.01	15117.63	68379.03	6374.46	12494.85
Sesquiterpenes							
Ylangene	15.72	59.31	53.96	51.76	443.07	57.21	34.41
α-Copaene	15.75	138.73	93.87	139.30	964.29	115.55	79.94
β-Caryophyllene	16.10	1192.22	1696.70	1182.44	16107.17	2500.99	1625.29
α-Bergamotene	16.13	43.92	82.763	40.06	633.74	63.99	75.34
Aromadendrene	16.23	45.52	52.10	45.20	362.70	96.99	57.49
α-Humulene	16.33	157.39	223.63	153.76	2518.07	442.70	268.93
γ-Muurolene	16.45	2028.02	2488.83	2716.3	10824.34	1893.07	1364.28
α-Muurolene	16.57	157.08	79.54	164.15	1079.27	164.43	80.91
β-Bisabolene	16.71	472.80	448.56	517.23	3853.02	525.29	395.92
Δ-Cadinene	16.74	1137.75	1108.69	1235.82	7096.58	1049.77	675.96
α-Calacorene	16.92	71.85	39.476	99.57	386.20	-	-
Spathulenol	17.20	49.87	40.90	703.89	5005.14	350.74	205.10
Cadinol	17.64	66.23	92.56	136.55	1075.13	168.15	111.18
α-Cadinol	17.76	33.68	117.97	83.74	568.03	90.86	95.71
Guaiazulene	17.94	98.72	-	-	-	-	-
Other compounds							
Methyl-2-methylbutanoate	04.43	54.29	562.53	-	-	-	218.57
Octan-3-one	11.45	31.71	-	5.15	-	-	-
3-methyl-6-hydroxybenzo-c- dihydrofuran	15.51	127.02	272.54	45.36	946.11	136.82	67.67

among EO-1 – EO-6. Although well separated, populations EO-1 – EO3, which were collected at the same altitude and in closer locations, tended to group together (Figure 1-A) and a similar trend was observed also on population EO-5 - EO-6. Only the population EO-4 was highly separated by the other populations.

On the other hand, using hierarchical cluster analysis, two main groups were observed (Figure 1-C), which can be classified as populations collected at an altitude of ~ 450 above s.l (EO-1 - EO-3) and populations collected at an altitude of ~ 720 above s.l (EO-4 - EO-6) (Figure 1-C).

The PCA loading plot reported in Figure 1-B allowed identifying the chemical compounds that contributed to samples separation. In particular, the PC1 was largely affected by γ-terpinene, ß-ocimene, α-terpinene and α-calacorene, whereas the PC2 was mainly affected by camphene, ß-pinene, linalool and spathulenol.

The radical scavenging activity of the different EOs was evaluated using DPPH test. Samples showed a modest radical scavenging activity, being EO-4 the most effective one, with an IC_{50} value of 320.9 µg/mL (Table 3). The antioxidant activity was also evaluated by means of a second assay, the β-carotene bleaching test. Sample EO-4, together with sample EO-3, was the most active one in protecting linoleic acid from peroxidation, with IC_{50} values of 4 and 4.68 µg/mL after 30 minutes of incubation (Figure 2) Also after 60 minutes of incubation the biological activity was significantly high (IC_{50} values equal to 6.91 µg/mL for sample EO-4 and 15.39 µg/mL for sample EO-3).

Essential oil EO-4 was the most interesting one. Its antioxidant activity could be related to the highest content of the two monoterpenoid phenols thymol and carvacrol [14,15].

The anti-inflammatory potential was tested through the evaluation of the capacity to inhibit NO production in lipopolysaccharide (LPS)-induced murine macrophage RAW 264.7 cell line. The presence of nitrite, a stable oxidized product of NO, was determined in cell culture media after 24 h by the Griess reagent. Dinitrogen trioxide (N_2O_3) generated from acidified nitrite (or from the autoxidation of NO) reacts with sulfanilamide to yield a diazonium derivative. This reactive intermediate interacts with N-1-naphthylethelene diamine to yield a colored diazo product that absorbs strongly at 540 nm [16]. The inhibitory activity observed at the concentration of 100 µg/mL is reported in Figure 3.

All samples induced a dose-depending inhibition of NO production. An excellent activity was observed for essential oil EO- 4, collected

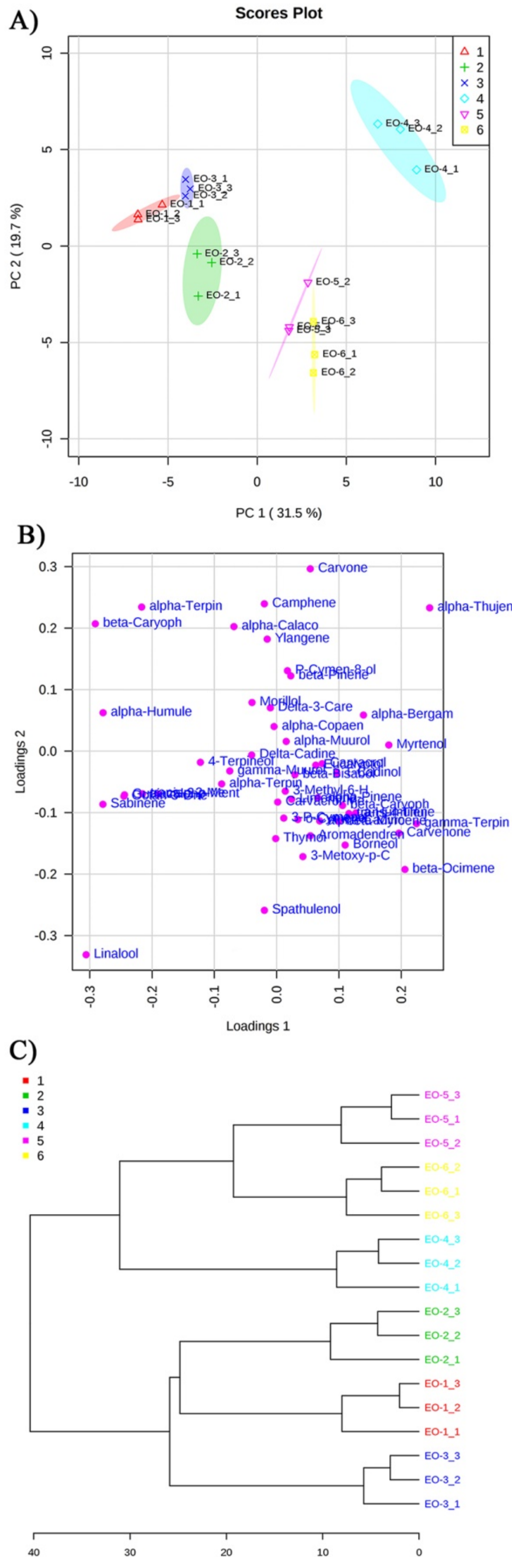

Figure 1: PCA and hierarchical cluster analysis carried on the metabolite identified and quantified in the EOs of 6 different populations of *O. heracleoticum* collected at two different altitudes in Calabria. A) Score plot of the Partial Least Square Discriminant Analysis (PLS-DA); B) loading plots; C) Hierarchical cluster analysis. N=3.

at about 720 m. above s.l., with an IC_{50} value of 32.77 µg/mL, followed by sample EO-3, with an IC_{50} value equal to 49.48 µg/mL (Table 4). Also at the lowest concentration tested, 25 µg/mL, these two last samples were effective, inducing more than 30% of inhibition.

Table 3: Antioxidant activity of *O. heracleoticum* EOs.

Sample	IC_{50} (µg/mL)		
	DPPH test	β-carotene bleaching test	
		30 min	60 min
EO-1	430.6 ± 6.2 [d]	> 100	> 100
EO-2	466.4 ± 0.6 [e]	55.12 ± 3.08 [h]	> 100
EO-3	390.3 ± 0.5 [c]	4.68 ± 0.03 [a,b]	15.39 ± 0.49 [c]
EO-4	320.9 ± 3.6 [b]	4.00 ± 0.03 [a,b]	6.91 ± 0.12 [b]
EO-5	454.3 ± 1.1 [e]	25.86 ± 1.00 [e]	33.88 ± 0.93 [f]
EO-6	403.3 ± 5.9 [c]	32.88 ± 0.92 [f]	40.91 ± 1.17 [g]
Ascorbic acid*	2.00 ± 0.01 [a]	-	-
Propyl gallate*	-	1.00 ± 0.02 [a]	1.00 ± 0.02 [a]

Data are expressed as mean ± SE (n = 3). Different letters along column (DPPH test) or between columns (β-carotene bleaching test) indicate statistically significant differences at $P < 0.05$ (Bonferroni test). * Positive controls.

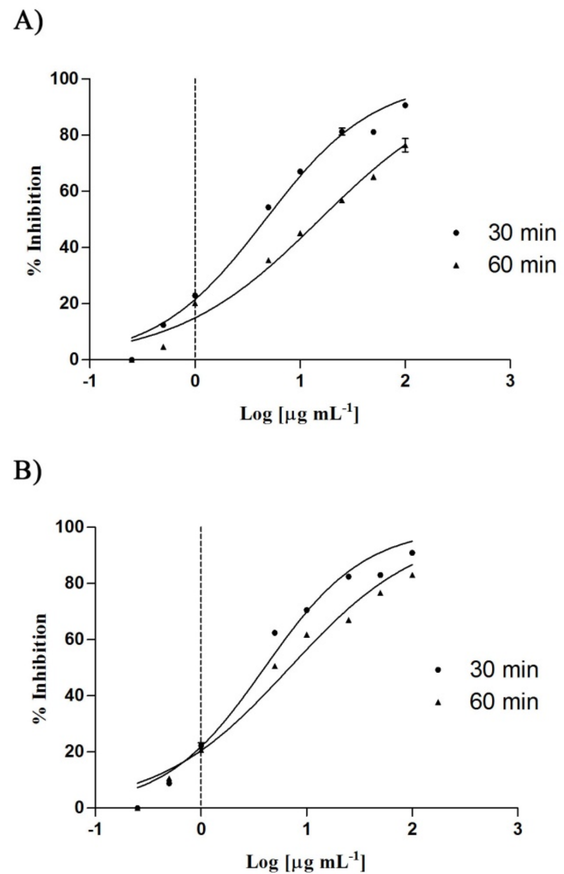

Figure 2: Inhibition of lipid peroxidation induced by *O. heracleoticum* essential oils EO-3 (A) and EO-4 (B).

Table 4: Inhibitory effects of *O. heracleoticum* EOs on the LPS-induced NO production in RAW 264.7 cells.

Sample	Locality	IC_{50} (µg/mL)
EO-1	Sant'Eufemia d'Aspromnte	128.7 ± 5.26 [d]
EO-2	Podargoni	78.62 ± 3.12 [c]
EO-3	Bagaladi	49.48 ± 0.62 [b]
EO-4	Longobucco	32.77 ± 0.40 [a]
EO-5	Rogliano	118.0 ± 2.82 [d]
EO-6	Acri	170.9 ± 2.90 [e]
Indomethacin*		58.00 ± 0.90 [b]
L-NAME*		45.86 ± 0.46 [b]

Data are expressed as mean ± SE (n = 4). Different letters indicate statistically significant differences at $P < 0.05$ (Bonferroni test). * Positive controls.

Among the other EOs an IC_{50} value of 78.62 μg/mL was obtained for sample EO-2, while the other samples showed a lower activity (IC_{50} values ranging from 118.0 to 170.9 μg/mL).

Besides their antioxidant activity, carvacrol and thymol could be responsible, at least in part, also for the *in vitro* anti-inflammatory activity observed, as it has been demonstrated a modest inhibition of NO production [17]. However, the effects observed on RAW cells could be more likely related to the major content of γ-terpinene and β-pinene found in essential oil EO-4. These compounds, as a matter of fact, were demonstrated to be effective inhibitors of NO production [18]. In conclusion, *O. heracleoticum* EO could be an interesting source of antioxidant and anti-inflammatory compounds. Therefore, due to these antioxidant and anti-inflammatory activities, this EO could be used in many applications in the future, including the use as a functional ingredient in health foods or as a drug for treating inflammatory related diseases.

Experimental

Chemicals: 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), ascorbic acid, β-carotene, linoleic acid, Tween 20, Dulbecco's modified Eagle's medium (DMEM), phosphate-buffered saline (PBS), fetal bovine serum (FBS), L-glutamine, penicillin/streptomycin, trypan blue and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich S.p.A. (Milan, Italy). Murine monocytic macrophage cell line RAW 264.7 was purchased from ATCC no. CRL-2278, UK. Ethanol, methanol and dimethyl sulfoxide (DMSO) were purchased from VWR International s.r.l. (Milan, Italy).

Plant collection and EO extraction: The study was carried out on the EOs isolated from six different Calabrian populations of *O. heracleoticum* Samples were collected between July and August (depending by the altitude) at the beginning of the flowering stage (balsamic period) in Calabria (southern Italy) (Table 1). Fresh plant material was immediately processed after the harvest. EOs were extracted using a semi-industrial stainless steel distilling apparatus. Samples were distilled for four hours and the obtained oils were separated from the hydrolates and dried with anhydrous sodium sulfate, and stored in dark vials at -20°C.

GC-MS analysis: The EOs were chemically characterized using a Thermo Fisher gas chromatograph apparatus (Trace 1310) equipped with a single quadrupole mass spectrometer (ISQ LT). The capillary column was a TG-5MS 30 m×0.25 mm×0.25μm the gas carrier was helium with a flow of 1 mL/min. Injector and source were settled at the temperature of 200°C and 260°C, respectively. Samples were solubilized in ethanol at the concentration of 1000 ppm and fenchone was added as internal standard (2000 ppm). Samples were injected in a split mode with a split ratio of 60. The programmed temperature was as follow: isocratic for 7 minutes 45°C, from 45°C to 80°C with a rate of 10°C×min, from 80°C to 200°C with a rate of 20°C×min then isocratic for 3 minutes 200°C. Mass spectra were recorded in electronic impact (EI) mode at 70 eV, scanning the 45–500 *m/z* range. Compounds identification was carried out comparing the relative retention time and mass spectra of the molecules with those of the libraries (NIST).

Free radical scavenging activity assay: Radical scavenging activity was determined using the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) as previously described [19]. Briefly, about 200 μl of different concentrations of test samples solutions (ranging from 5 to 1000 μg/mL) were added to 800 μL of a 10^{-4} M methanolic solution of DPPH. Absorbance was measured at 517 nm after 30 minutes in the dark using a Perkin Elmer Lambda 40 UV/VIS spectrophotometer. Experiments were run in triplicate and ascorbic acid was used as positive control.

β-carotene bleaching test: The ability of *O. heracleoticum* EOs to inhibit lipid peroxidation was assessed by the β-carotene bleaching test [20]. A β-carotene–linoleic acid emulsion was prepared by adding a 0.2 mg/mL β-carotene chloroformic solution (1 mL) to linoleic acid (0.02 mL) and 100% Tween 20 (0.2 mL). After the evaporation of chloroform 100 mL of water were added. Different concentrations (ranging from 0.25 to 100 μg/mL) of each samples (0.2 mL) were combined with prepared emulsion (5 mL). Mixtures were then placed in a water bath at 45°C for 60 min. Propyl gallate was used as positive control and absorbance values were measured at 470 nm using the Perkin Elmer Lambda 40 UV/VIS spectrophotometer. Measurements were carried out at initial time (t = 0) and after 30 and 60 min and antioxidant activity was evaluated by measuring the prevention of β-carotene bleaching.

Inhibition of NO production: The *in vitro* anti-inflammatory activity of *O. heracleoticum* EOs was verified by evaluating the ability to inhibit NO production in LPS-stimulated murine macrophage RAW 264.7 cells. Cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine and 1% antibiotic solution (penicillin/streptomycin) under 5% CO_2 at 37 °C. Cells were removed from culture flask by scraping. Cells counts and viability were performed using a standard trypan blue cell counting technique and cells were subcultured onto 96 well culture plates (1 $\times 10^5$ cells/well) used for experiments 24 h later. RAW 264.7 cells were then incubated with different concentrations of each EO (25–1000 μg/mL) in the presence of 1 μg/mL LPS for 24 h.

The Griess reagent (1% sulfanamide and 0.1% N-(1-naphtyl) ethylenediamine dihydrochloride in 2.5% H_3PO_4) was used to determine the presence in cell culture media of nitrite, a stable oxidized product of NO, as previously described [21]. Briefly, cell culture supernatant (100 μL) was combined with Griess reagent (100 μL) in a 96-well plates and absorbance was measured at 550 nm using a microplate reader (GDV DV 990 B/V, Roma, Italy).
The absence of cytotoxic effects was assessed by means of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, as previously reported [22]. MTT was solubilized in phosphate buffered saline (0.5% w/v), and 100 μL were added to each well. After 4 h of incubation, 100 μL of dimethyl sulfoxide were added to each well to dissolve the formazan crystals and absorbance values at 550 nm were measured.

Experimental design and statistical analysis: EOs extraction and chemical characterization were carried out in triplicate. After chemical characterization and quantitation raw data were analyzed through unsupervised principal component analysis (PCA) Moreover, Oregano populations were clusterized through hierarchical cluster analysis where the distance was measured with the Euclidean method using the Ward's algorithm for clustering. Both multivariate and cluster analyses were performed using MetaboAnalyst 3.0 [23].

Biological activity assays were carried out in triplicate, with the exception of NO inhibition assay, that was conducted in quadruplicate. Data were expressed as means ± S.E.M. Raw data were fitted through non-linear regression in order to calculate the IC_{50} values (concentration providing 50% inhibition). Data were checked for normality (D'Agostino-Pearson test) and tested for

homogeneity of variances (Levene's test). Statistical significance was assessed through one-way analysis of variance (ANOVA). Significant differences among means were analyzed using Bonferroni post hoc test with $P \leq 0.05$.

References

[1] Stefanaki A, Cook CM, Lanaras T, Kokkini S. (**2016**) The Oregano plants of Chios Island (Greece): Essential oils of *Origanum onites* L. growing wild in different habitats. *Industrial Crops and Products*, ***82***, 107-113.

[2] Carrasco A, Perez E, Cutillas AB, Martinez-Gutierrez R, Tomas V, Tudela J. (**2016**) *Origanum vulgare* and *Thymbra capitata* essential oils from Spain: Determination of aromatic profile and bioactivities. *Natural Product Communications*, ***11***, 113-120.

[3] Pesavento G, Maggini V, Maida I, Lo Nostro A, Calonico C, Sassoli C, Perrin E, Fondi M, Mengoni A, Chiellini C, Vannacci A, Gallo E, Gori L, Bogani P, Bilia AR, Campana S, Ravenni N, Dolce D, Firenzuoli F, Fani R. (**2016**) Essential oil from *Origanum vulgare* completely inhibits the growth of multidrug-resistant cystic fibrosis pathogens. *Natural Product Communications*, ***11***, 861-864.

[4] Stoilova I, Bail S, Buchbauer G, Krastanov A, Stoyanova A, Schmidt E, Jirovetz L. (**2008**) Chemical composition, olfactory evaluation and antioxidant effects of an essential oil of *Origanum vulgare* L. from Bosnia. *Natural Product Communications*, ***3***, 1043-1046.

[5] D'Antuono LF, Galletti GC, Bocchini, P. (**2000**) Variability of essential oil content and composition of *Origanum vulgare* L. populations from a North Mediterranean Area (Liguria Region, Northern Italy). *Annals of Botany*, ***86***, 471-478.

[6] De Martino L, De Feo V, Formisano C, Mignola E, Senatore F. (**2009**) Chemical composition and antimicrobial activity of the essential oils from three chemotypes of *Origanum vulgare* L. ssp. *hirtum* (Link) Ietswaart growing wild in Campania (Southern Italy). *Molecules*, ***14***, 2735-2746.

[7] Russo M, Galletti GC, Bocchini P, Carnacini A. (**1998**) Essential oil chemical composition of wild populations of Italian oregano spice (*Origanum vulgare* ssp. *hirtum* (Link) Ietswaart): A preliminary evaluation of their use in chemotaxonomy by cluster analysis. 1. Inflorescences. *Journal of Agricultural and Food Chemistry*, ***46***, 3741-3746.

[8] De Mastro G, Tarraf W, Verdini L, Brunetti G, Ruta C. (**2017**) Essential oil diversity of *Origanum vulgare* L. populations from Southern Italy. *Food Chemistry*, ***235***, 1-6.

[9] Čabarkapa I, Škrinjar M, Milovanović I, Plavšić D, Palić D, Kokić B, Arsić I. (**2012**) Antimicrobial activity of *Origanum heracleoticum* L. essential oil from Serbia. *AgroFOOD industry hi-tech*, ***5***, 55-58.

[10] Kulisic T, Radonic A, Katalinicn V, Milos M. (**2004**) Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chemistry*, ***85***, 633-640.

[11] Tsimogiannis D, Stavrakaki M, Oreopoulou V. (**2006**) Isolation and characterisation of antioxidant components from oregano (*Origanum heracleoticum*). *International Journal of Food Science & Technology*, ***41***, 39-48.

[12] Veres K, Varga E, Schelz Z, Molnar J, Bernath J, Mathe I. (**2007**) Chemical composition and antimicrobial activities of essential oils of four lines of *Origanum vulgare* subsp *hirtum* (Link) Ietswaart grown in Hungary. *Natural Product Communications*, ***2***, 1155-1158.

[13] Conforti F, Marrelli M, Menichini F, Tundis R, Statti GA, Solimene U, Menichini F. (**2011**) Chemical composition and protective effect of oregano (*Origanum heracleoticum* L.) ethanolic extract on oxidative damage and on inhibition of NO in LPS-stimulated RAW 264.7 macrophages. *Journal of Enzyme Inhibition and Medicinal Chemistry*, ***26***, 404-411.

[14] Aeschbach R, Löliger J, Scott BC, Murcia A, Butler J, Halliwell B, Aruoma OI. (**1994**) Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. *Food and Chemical Toxicology*, ***32***, 31-36.

[15] Yanishlieva NV, Marinova EM, Gordon MH, Raneva VG. (**1999**) Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. *Food Chemistry*, ***64***, 59-66.

[16] Bryan NS, Grisham MB. (**2007**) Methods to detect nitric oxide and its metabolites in biological samples. *Free Radical Biology and Medicine*, ***43***, 645-657.

[17] Kavoosi G, Teixeira da Silva JA, Saharkhiz MJ. (**2012**) Inhibitory effects of *Zataria multiflora* essential oil and its main components on nitric oxide and hydrogen peroxide production in lipopolysaccharide-stimulated macrophages. *Journal of Pharmacy and Pharmacology*, ***64***, 1491-1500.

[18] Bourgou S, Pichette A, Marzouk B, Legault J. (**2010**) Bioactivities of black cumin essential oil and its main terpenes from Tunisia. *South African Journal of Botany*, ***76***, 210-216.

[19] Conforti F, Marrelli M, Statti G, Menichini F, Uzunov D, Solimene U, Menichini F. (**2012**) Comparative chemical composition and antioxidant activity of *Calamintha nepeta* (L.) Savi subsp. *glandulosa* (Req.) Nyman and *Calamintha grandiflora* (L.) Moench (Labiatae). *Natural Product Research*, ***26***, 91-97.

[20] Marrelli M, Cristaldi B, Menichini F, Conforti F. (**2015**) Inhibitory effects of wild dietary plants on lipid peroxidation and on the proliferation of human cancer cells. *Food and Chemical Toxicology*, ***86***, 16-24.

[21] Marrelli M, Statti GA, Menichini F, Conforti F. (**2017**) *Echinophora tenuifolia* L. inflorescences: phytochemistry and *in vitro* antioxidant and anti-inflammatory properties in LPS-stimulated RAW 264.7 macrophages. *Plant Biosystems*, ***151***, 1073-1081.

[22] Marrelli M, Conforti F, Toniolo C, Nicoletti M, Statti G, Menichini F. (**2014**) *Hypericum perforatum*: Influences of the habitat on chemical composition, photo-induced cytotoxicity, and antiradical activity. *Pharmaceutical Biology*, ***52***, 909-918.

[23] Xia J, Sinelnikov IV, Han B, Wishart DS (**2015**) MetaboAnalyst 3.0—making metabolomics more meaningful. *Nucleic acids research*, ***43***, W251-W257.