Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 104 (2007) 889–895
Food Chemistry
www.elsevier.com/locate/foodchem

In vitro activity of the essential oils of *Origanum vulgare*, *Satureja montana* and their main constituents in peroxynitrite-induced oxidative processes

Josè M. Prieto [a,b,1], Patrizia Iacopini [b], Pierluigi Cioni [b], Silvio Chericoni [b,*]

[a] *Centre for Pharmacognosy and Phytotherapy, The School of Pharmacy, University of London, 29–39 Brunswick Sq., London WC1N 1AX, United Kingdom*
[b] *Dipartimento di Chimica Bioorganica e Biofarmacia, Università di Pisa, via Bonanno 33, 56126 Pisa, Italy*

Received 27 January 2006; received in revised form 18 October 2006; accepted 26 October 2006

Dedicated to the memory of Professor Ivano Morelli (1940–2005)

Abstract

The essential oil obtained from aerial parts of *Satureja montana* L. and *Origanum vulgare* L. (Labiatae) along with four of its main components, *p*-cymene, carvacrol, thymol and γ-terpinene were tested in models of *in vitro* peroxynitrite-induced formation of both 3-nitrotyrosine and malondialdehyde, two biomarkers of the oxidative stress of recognised pathological and toxicological significance. The essential oils showed a significant activity, thus decreasing 3-nitrotyrosine formation (IC_{50} values of 43.9 μg/ml for *S. montana* and 19.2 μg/ml for *O. vulgare*), and also inhibited the peroxynitrite induced malondialdehyde formation (IC_{50} values of 27.2 μg/ml and 17.0 μg/ml respectively). Thymol and carvacrol inhibited 3-nitrotyrosine formation (IC_{50} values of 81.3 μM and 106.3 μM; ascorbic acid $IC_{50} = 400$ μM) and reduced malondialdehyde formation (IC_{50} values of 43.9 μM and 70.1 μM respectively; trolox $IC_{50} = 240$ μM). On the contrary, *p*-cymene and γ-terpinene were completely inactive in both assays under the concentration of 300 μg/ml. These results support, in particular for origanum, the nutraceutical value of these spices and the potential of thymol and carvacrol in preventing the formation of toxic products by the action of reactive nitrogen species.

Keywords: Peroxynitrite; Malondialdehyde; 3-Nitrotyrosine; *Origanum vulgare*; *Satureja montana*; Essential oils; Carvacrol; Thymol

1. Introduction

Origanum vulgare (oregano, origanum or wild marjoram) and *Satureja montana* (winter savory) are strong aromatic herbs used in Mediterranean food. The former is present in combination with vegetables, pasta and pizza; also known as the "pizza herb", while the second is mainly used as meat and fish seasoning. These and other Mediterranean spices are present every day in the diet of millions of people after Italian style food has spread all over the world during the last decades.

Plant metabolites with known antioxidant properties have been implicated for their beneficial health effects among people consuming Mediterranean diet (Zeghichi, Kallithraka, Simopoulos, & Kypriotakis, 2003). Essential oils (EOs) are natural products with antibacterial, antifungal, antioxidant, and anti-carcinogenic properties while preserving and giving specific flavours to many Mediterranean foods (Teissedre & Waterhouse, 2000). This consideration has encouraged their use as natural additives either in food technology or in nutraceutical preparations. The worldwide production of origanum EO is 62 tonnes per year

Abbreviations: EO, essential oil; 3-NT, 3-nitrotyrosine; MDA, malondialdehyde.

* Corresponding author. Tel.: +39 3284764589/0502 219684.
E-mail address: chersil@hotmail.com (S. Chericoni).
[1] Fax: +44 2077535909.

0308-8146/$ - see front matter © 2006 Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2006.10.064

translating to approximately 1.2 million USD (Lawrence, 1993). Origanum EO is used as a food flavour and also as a fragrance component in soaps, detergents and perfumes (McGimpsey, 1993). Origanum has a good antioxidant capacity and presents antimicrobial activity against pathogenic microorganisms (Arcila-Lozano, Loarca-Pina, Lecona-Uribe, & Gonzalez de Mejia, 2004). The production of *Satureja montana* EO, with 1500 kg per year and a value of 90 000 USD is also considered to be of commercial significance (Lawrence, 1993).

Peroxynitrite, $ONOO^-$, is now considered one of the more relevant radicals involved in pathophysiological and toxicological processes. The importance of this radical in biological systems is due to its ability to react with almost all classes of biomolecules due to the radicals formed from its degradation, $NO_2^{\cdot}$ and $OH^{\cdot}$, under physiological conditions. These radicals induce lipid peroxidation, disruption of cellular structures, inactivation of enzymes and ion channels through protein oxidation and nitration, and DNA damages (Virág, Szabó, Gergely, & Szabó, 2003). All these actions contribute to the onset and maintenance of pathologies as chronic inflammation (Kaur & Halliwell, 1994), atherosclerosis (Beckman, Carson, Smith, & Koppenol, 1993), neurodegenerative diseases (Wang, Shum, Ho, & Wang, 2003), hepatopathies (Poli, 1993), and cardiovascular disorders (Wattanapitayakul, Weinstein, Holycross, & Bauer, 2000).

Origanum and winter savory EOs, along with their principal components, carvacrol, thymol, *p*-cymene, and γ-terpinene among others, exhibit excellent antioxidant activities when used in proper concentrations in a variety of *in vivo* and *in vitro* models but none of them dealing with the peroxynitrite-mediated processes, except for thymol that proved to be a scavenger of this radical (Balavoine & Gelettii, 1999; Braga et al., 2006). Thus, the aim of the present study was to evaluate the ability of the essential oils obtained from dried and powdered leaves of *O. vulgare* and *S. montana*, to prevent the *in vitro* peroxynitrite-induced formation of 3-nitrotyrosine (3-NT) and malondialdehyde (MDA), two biomarkers of the oxidative stress (Althaus et al., 2000; Del Rio & Pellegrini, 2005; Mohiuddin et al., 2006) and determine how its main constituents can contribute to this activity.

2. Materials and methods

2.1. Plant material

Dried leaves of *O. vulgare* were purchased from a local market (January 2004, Pisa, Italy) while aerial parts of *S. montana* were collected at Molina di Quosa (January 2004, Pisa, Italy). Both samples were checked for homogeneity and identity by the authors. The essential oils were obtained by hydrodistillation in a Clevenger apparatus for two hours as described in the Italian Pharmacopea XI Ed. (2002). Carvacrol, *p*-cymene, thymol and γ-terpinene were from Fluka Chemie (Buchs, Switzerland). The essential oil and pure compounds were diluted in HPLC grade methanol (JT Baker, Deventer, The Netherlands) to the required concentration just before use, and the maximum volume added was 100 μl.

2.2. Reagents and synthesis of HPLC standards

Peroxynitrite was synthesised from sodium nitrite/H_2O_2 acidified with HCl as described by Beckman, Chen, Ischiropoulos, and Crow (1994) and the residual H_2O_2 was removed by passing the solution through granular MnO_2. The yellowish stock solution was stored at −80 °C and its concentration was evaluated immediately before use by measuring the absorbance at 302 nm ($\varepsilon = 1670\ M^{-1}\ cm^{-1}$).

3-Nitrotyrosine was synthesised and purified according to the literature (Harington & Pitt Rivers, 1944; Keith & Powell, 1969). Standard solutions were prepared by successive dilutions with phosphate buffer (100 mM, pH 7.4).

Malondialdehyde was synthesised as reported by Fenaille, Mottier, Turesky, Ali, and Guy (2001) and its complex with 2-thiobarbituric acid (TBA) as previously described by Brannan and Decker (2001). Standard solutions of MDA-2TBA complex were obtained by dilutions with 100 mM Tris buffer (pH 7.3).

Ascorbic acid (Riedel de Haën, Seelze, Germany) and Trolox (Sigma–Aldrich, St. Louis, USA) were taken as reference compounds. All other chemicals were of analytical or higher grade and the aqueous solution were prepared by using freshly deionised, ultrafiltered water further purified by using a Milli-Q system (Molsheim, France).

2.3. Gas chromatography–mass spectrometry

GC/EIMS analyses were performed with a Varian system (Palo Alto, USA) consisting in a C*P*-3800 gas-chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm; coating thickness 0.25 μm) and a Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperatures 220 and 240 °C, respectively; oven temperature programmed from 60 to 240 °C at 3 °C/min, carrier gas helium at 1 ml/min, injection of 0.2 μl (10% hexane solution), split ratio 1:30. Identification of the constituents was based on the comparison of the retention times with those of authentic samples (Fluka Chemie, Buchs, Switzerland), comparing their linear retention indices relative to the series of *n*-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS) and home-made library mass spectra built from pure substances and components of known oils. Moreover the molecular weights of all the identified substances were confirmed by GC/CIMS, using methanol as the CI gas.

2.4. HPLC analyses

The HPLC system consisted of a 600E pump system coupled with a UV 486 detector and a 717 Plus autosampler (Waters, Milford, USA). Data were collected and processed

by using the software Millenium v.3.2 (Waters, Milford, USA). Column: Lichrosphere 100 RP-18 5 μm, 250 × 4.6 mm (Merck, Darmstadt, Germany).

Elution conditions for the detection of 3-NT: 20 mM phosphate buffer (pH 3.2)/methanol (92:8); flow 1 ml/min in isocratic mode (Goldstein, Czapski, Lind, & Merényi, 2000); UV detection 356 nm. For the detection and quantification of the MDA-2TBA complex we used 20 mM phosphate buffer (pH 6)/methanol (6:4); flow 1.1 ml/min in isocratic mode, UV detection 532 nm according to Fenaille et al. (2001).

2.5. Liver microsomal preparations

Wistar rat livers were kindly donated by the Department of Psychiatry, Neurobiology, Pharmacology and Biotechnology of the University of Pisa. Animal handling was according to the guidelines established by the European Union on Animal Care (CEE Council 86/609) as required by the Ethical Committee of the Faculty of Pharmacy, University of Pisa (Italy).

Liver microsomes were prepared by standard differential centrifugation techniques as described by (Slater & Sawyer (1971)). Briefly, under diethyl ether anaesthesia male Wistar rats (200–250 g) were killed by ex-sanguination from the abdominal aorta. The liver was exhaustively perfused with ice-cold saline through the portal vein until uniformly pale, immediately removed, and weighed. After being trimmed and minced, the pieces of liver were homogenised with 4 volumes of ice-cold 0.1 M potassium phosphate buffer (pH 7.4) containing 1.15% (w/v) KCl. The homogenate was spun at 10,000 × *g* for 30 min. The supernatant was collected and further centrifuged at 105,000*g* for 60 min. The resultant microsomal fraction was washed and resuspended in the same buffer as above. Aliquots of microsomal suspensions were stored at −80 °C for maximum 2 months. Protein contents were quantified as described by Lowry, Rosebrough, Farr, and Randal (1951) using bovine serum albumin as standard.

2.6. Tyrosine nitration induced by peroxynitrite

This method is based on the determination, by HPLC-UV analyses, of the quantity of 3-NT formed from the reaction between free tyrosine and peroxynitrite at the physiological pH of 7.4. The reaction was carried out adding, under vigorous vortexing, peroxynitrite (5–40 μl, 1 mM final concentration) to a solution containing the essential oil or pure compounds at the desired concentrations, tyrosine (2 mM) and HCO_3^- (50 mM) all dissolved in 50 mM phosphate buffer (pH 7.4). Test compounds were dissolved in methanol (final concentration 0.5%) and kept into ice till used. Blanks, with or without methanol, were always performed to detect any interference of the solvent with the tests. Quantitative determination of the formed 3-NT was performed by HPLC-UV using an external standard calibration curve ($r^2 = 0.999$).

2.7. Malondialdehyde formation induced by peroxynitrite

Peroxynitrite (5–30 μl, final concentration of 1 mM) was added to a solution of microsomes (5 mg/ml) in 50 mM phosphate buffer solution. Test compounds and essential oil were prepared and added as described above. The reaction mixtures were incubated at 37 °C; after 20 min 1 ml of TCA 10% (p/v) and 1 ml of TBA 0.7% (p/v) were added, and then the solutions were kept at 90 °C for 1 h to allow the formation of the complex MDA-2TBA. Selective elution of malondialdehyde, in the form of the MDA-2TBA complex, was accomplished by HPLC-UV and its quantification performed by using an external standard calibration curve obtained with different concentrations of the synthetic MDA-2TBA complex obtained as detailed above ($r^2 = 0.999$).

2.8. Statistics

The inhibitory concentration 50% (IC_{50}) was calculated from concentration/effect regression; an appropriate range of 4–5 concentrations was used.

3. Results

3.1. Chemical composition of the essential oils

The yield of the *O. vulgare* EO was 0.6% and 41 compounds, representing 99.6% of the total oil, were identified. The yield of the *S. montana* EO was 0.9% and 30 compounds, representing 99.7% were identified (Table 1).

3.2. Tyrosine nitration induced by peroxynitrite

The EO of *O. vulgare* and *S. montana* significantly inhibited the formation of 3-NT with an IC_{50} of 19.2 μg/ml and 43.9 μg/ml respectively ($r^2 = 0.996$). Thymol and carvacrol showed a value of IC_{50} of 12.1 and 15.9 μg/ml (81.3 μM and 106.3 μM) ($r^2 = 0.998$) respectively. The reference compound, ascorbic acid, exhibited an IC_{50} of 71.3 μg/ml (400 μM) ($r^2 = 0.998$). *p*-Cymene and γ-terpinene did not show any inhibitory activity in this model under the concentration of 300 μg/ml. Qualitative analyses were previously performed to avoid any interference of the tested products with the detection of 3-NT. No co-elution or other problems were detected.

3.3. Malondialdehyde formation induced by peroxynitrite

The EO of *O. vulgare* and *S. montana* inhibited significantly the formation of MDA measured as the MDA-2TBA complex with a IC_{50} of 17.0 μg/ml and 27.2 μg/ml, respectively ($r^2 = 0.996$). Carvacrol (IC_{50} 70.1 μM, 10.5 μg/ml) and thymol (IC_{50} 43.9 μM, 6.6 μg/ml) ($r^2 = 0.998$) also highly reduced its production. The reference compound, Trolox had an IC_{50} of 59.0 μg/ml or 240 μM ($r^2 = 0.997$). *p*-Cymene and γ-terpinene did not show any

Table 1
GC-EIMS analysis of the essential oils from the aerial parts of *S. montana* and *O. vulgare*

Compound	*O. vulgare*[a]	*S. montana*[a]	L.R.[b]
α-Thujene	0.3	0.9	933
α-Pinene	0.4	1.8	942
Camphene	tr	0.7	959
β-Pinene	0.2	2.0	984
3-Octanone	0.4	–	989
Myrcene	0.6	0.8	992
α-Phellandrene	tr	–	1010
3-Carene	tr	tr	1013
α-Terpinene	0.8	0.8	1022
p-Cymene	5.5	41.4	1030
Limonene	tr	0.7	1035
β-Phellandrene	–	tr	1037
1,8-Cineole	0.6	0.5	1039
γ-Terpinene	6.0	3.0	1064
cis-Sabinene-hydrate	0.3	0.8	1075
Terpinolene	tr	tr	1089
p-Cymenene	–	tr	1093
Linalool	1.1	tr	1101
trans-Sabinene hydrate	0.2	tr	1104
α-Thujone	tr	–	1111
β-Thujone	0.4	–	1122
Chrysanthenone	tr	–	1127
cis-*p*-Menth-2-en-1-ol	tr	–	1129
camphor	tr	–	1154
4-Terpineol	0.8	2.0	1184
p-Cymen-8-ol	tr	0.8	1190
α-Terpineol	0.5	2.6	1197
Methyl chavicol	0.3	–	1199
trans-Dihydrocarvone	tr	–	1206
Methyl thymol	0.3	–	1233
Methyl carvacrol	1.5	–	1242
Thymoquinone	–	0.8	1254
Isobornyl acetate	–	tr	1286
Thymol	22.1	tr	1292
Carvacrol	54.7	37.0	1300
α-Terpinyl acetate	–	tr	1349
Eugenol	tr	–	1356
Carvacrol acetate	tr	–	1367
α-Copaene	tr	–	1377
β-Bourbonene	tr	–	1384
Methyl-ugenol	–	tr	1402
Longifolene	–	tr	1410
β-Caryophyllene	1.5	1.6	1420
α-Humulene	tr	–	1457
γ-Muurolene	tr	–	1476
β-Bisabolene	0.6	0.5	1506
δ-Cadinene	0.2	–	1519
Caryophyllene oxide	0.3	1.0	1584
Total identified	99.6	99.7	

[a] Percentage of total.
[b] L.R.I. Kovak's Retention Index; tr = traces.

inhibitory activity in this model under the concentration of 300 μg/ml. Qualitative analyses were previously performed to avoid any interference of the tested products with the detection of MDA-2TBA. No co-elution or other problems were detected.

Results from the two biochemical assays are summarized in Table 2 and formulas are shown in Fig. 1.

Table 2
The antioxidative and anti-nitrosation activities of the essential oils and compounds studied. Values are the concentrations needed to decrease by 50% the amount of 3-NT or MDA-2TBA formed in the controls

	3-NT		MDA-2TBA	
	μg/ml	μM	μg/ml	μM
O. vulgare EO	19.2	–	17.0	–
S. montana EO	43.9	–	27.2	–
Thymol	12.1	81.3	6.6	43.9
Carvacrol	15.9	106.3	10.5	70.1
Cymene	>300	>2200	>300	>2200
γ-Terpinene	>300	>2200	>300	>2200
Trolox	–	–	59.0	240
Ascorbic Acid	71.3	400	–	–

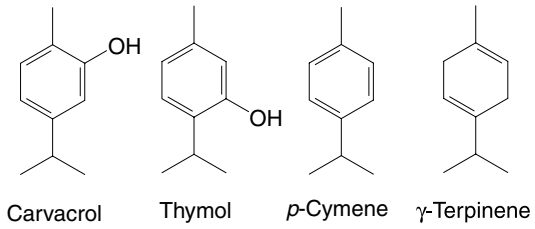

Fig. 1. Typical main compounds present in *Origanum* and *Satureja* essential oils.

4. Discussion

The origanum composition depends on the climate, altitude, time of recollection and the stage of growth (Arcila-Lozano et al., 2004). *O. vulgare* growing in a Mediterranean climate or a continental one contains a higher amount of phenols (Hristova, Ristc, Brkic, Stefkov, & Kulevanova, 1999; Montes, Wilkomirsky, & Bello, 1998) or terpenic alcohols, respectively (Tkachenko, Tkachev, & Koroljuk, 2002). However, the variability between commercial and wild plants inside the same climate remains high, as shown by (Hristova et al., 1999) in terms of yield and phenolic contain. In general, EO yields peak under hot summer conditions species producing oil containing 60–75% phenols, mostly carvacrol (McGimpsey, 1993). This compound together with thymol, *p*-cymene and γ-terpinene are commonly reported as the main components of origanum EOs (Arnold, Bellomaria, & Valentini, 2000; Daferera, Ziogas, & Polissiou, 2003; Hristova et al., 1999; Kulisica, Radonic, Katalinic, & Milos, 2004; Marino, Bersani, & Comi, 2001; Montes et al., 1998; Nostro et al., 2004).

The variability in the composition of *S. montana* EOs, leads to the definition of two chemotypes of *S. montana*, namely A and B, depending on the prevalence of phenolic compounds or terpenic alcohols, respectively (Palic, Salic, & Gasic, 1983). These chemotypes are subdivided in A (thymol), A (carvacrol), B (linalool), and B (*p*-cymene + α-terpineol). Thus, "typical" EOs from Italian species contain carvacrol, *p*-cymene and γ-terpinene as main components according to Piccaglia and Marotti (1991), and Chialva, Liddle, Ulian, and De Smedt (1980). Again

there is a positive correlation of the percentages in the composition of the EO and the dryness and high temperature. The content of carvacrol and γ-terpinene was higher in August, at the beginning of the flowering while the percentage of *p*-cymene and the yield of the EO was higher in September (Bilia et al., 1992).

These general considerations are corroborating that our oils are completely within the normal range of variability found in any EOs from these species as reported in the literature, that is, containing thymol, carvacrol, *p*-cymene, or γ-terpinene as their main compounds. Thus, the results obtained from these EOs, and the compounds used in this paper are significant. Furthermore, the study of the IC_{50} of the selected compounds allows evaluating their contribution in the activity of similar EOs beyond the particular composition of our EOs.

Previous work on EOs from origanum leaves has established that they are good scavengers of the DPPH and $ABTS^{+\cdot}$ radicals and this activity is mainly due to its content in carvacrol and thymol (Puertas-Mejia, Hillebrand, Stashnko, & Winterhalter, 2002). Both origanum and winter savory EOs are reported to inhibit both the β-carotene bleaching, and reduction of the thiobarbituric acid reactive substances (Kulisica et al., 2004; Radonic & Milos, 2003).

Carvacrol and thymol, the most characteristic compounds of the origanum EO, are effective inhibitors of the lipid peroxidation of microsomes induced by the Fe^{2+}/ascorbate system (Aeschbach et al., 1993) and the mechanism of this action has been explored by Yanishlieva, Marinova, Gordon, and Raneva (1999). They are good scavengers of the $CCl_3O_2^{\cdot}$ and DPPH radicals (Aeschbach et al., 1993; Vardar-Unlu et al., 2003) and inhibit the Cu^{2+}-induced oxidation of LDL (Teissedre & Waterhouse, 2000). Thymol is a direct peroxynitrite scavenger as measured by the pirogallol red method (Balavoine & Gelettii, 1999) and is able to reduce the Fenton's reagent-induced oxidation of horse blood plasma (Lee & Shibamoto, 2001). Furthermore, nutritional studies have highlighted that the dietary supplementation with this compound could provide beneficial effects by protecting the liver against radical-induced injures (Alam et al., 1999) and increasing the antioxidant status of the ageing rat brain (Youdim & Deans, 2000).

γ-Terpinene is also present as a major component of essential oils made from economically important species such thymus, citrus fruits and of Australian tea tree oil, and it is responsible, at least in part, for their antioxidant activity (Suzuki et al., 2004). In fact it is reported that γ-terpinene reduces DPPH (Choi, Song, Ukeda, & Sawamura, 2000), and inhibits the Cu^{2+}- and AAPH-induced oxidation of LDL (Takahashi, Inaba, Kuwahara, & Kuki, 2003). Moreover, it shows a very promising inhibitory activity in a model of peroxidation of linoleic acid, where a rapid chain termination via a very fast cross-reaction between hydroperoxyl radical and linoleylperoxyl radicals is induced, a mechanism completely different from the mechanism of the antioxidant action of vitamin E (Foti & Ingold, 2003). Regarding *p*-cymene, it is active in the DPPH assay (Dapkevicius et al., 2002). When tested in the two models described here, both γ-terpinene and *p*-cymene were ineffective, but we cannot exclude the possibility of a synergistic action with more hydrophilic antioxidants present in the EO as it has been previously shown in other models (Grassmann, Schneider, Weiser, & Elstner, 2001; Milde, Elstner, & Grassmann, 2004).

The *in vitro* anti-nitrosation activity of the crude EOs of origanum and winter savory in peroxinitrite-induced processes is unique among other EOs from Labiatae considered of relevance (Lawrence, 1993). Our previous work showed that lavender, rosemary, sage, thyme, and mint EOs fail to inhibit *in vitro* 3-NT formation when tested at 300 μg/ml (Chericoni, Prieto, Iacopini, & Morelli, 2005). The evaluation of the antioxidant power of different fractions or pure constituents of EOs often affords controversial results. For example, when Radonic and Milos (2003) isolated the hydrocarbon fraction of *S. montana* EO, containing γ-terpinene, α-terpinene, *p*-cymene and terpinolene which previously were identified as potential antioxidants, it showed the poorest effectiveness. On the other hand, thymol and carvacrol, the most active components of *Thymus pectinatus* EO were individually found to possess weaker antioxidant activity than the crude oil itself (Vardar-Unlu et al., 2003). These antecedents together with our results indicate that other constituents of the essential oil may contribute to the observed antioxidant activity. Synergistic actions between the components could take place and, regarding the complexity of EOs, it is virtually impossible to explain all of them. In the present work we cannot conclude a clear association between the activity and the concentration of the main compounds for the origanum EO. On the contrary, the activity of the winter savory EO in both models seems to be due to its content on carvacrol. In the 3-NT model the oil had an IC_{50} of 43.9 μg/ml, implying that the concentration of carvacrol was about 16 μg/ml, just the IC_{50} of this compound in this assay (15.9 μg/ml). In the model of lipid peroxidation, the EO inhibited the formation of malondialdehyde with an IC_{50} of 27.2 μg/ml corresponding to approximately 10 μg/ml of carvacrol, again the IC_{50} of this compound in this model (10.5 μg/ml).

There is a lack of information on the bioavailability of the compounds here assayed. Only thymol was studied (Kohlert et al., 2002) in healthy humans but in very low doses (1 mg) giving a plasma peak of only 90 ng/ml. Furthermore, the effect of the matrix used in the tablets was not ascertained so an absolute consideration on the bioavailability of thymol cannot be done. Ingestion of 1 g of dried origanum, as the one here studied, may afford circa 6 mg of essential oil, thus containing more than 4 mg of thymol + carvacrol.

Peroxynitrite can promote oxidative damage to blood vessels, skin, heart, lung, kidney and brain (Virág et al., 2003). 3-NT and MDA, two toxic compounds arising from the reactivity of peroxinitrite in *in vivo* systems, are nowadays considered biomarkers of pathological stress (Del Rio & Pellegrini, 2005; Mohiuddin et al., 2006). Our values

show that *O. vulgare* and *S. montana* essential oils are food ingredients able to prevent *in vitro* peroxynitrite-induced formation of both biomarkers better than antioxidants of reference such as ascorbic acid and Trolox. Thymol and carvacrol are active compounds responsible, at least in part, of these activities. Further studies on their preventive effect on deleterious processes involving reactive nitrogen species, should be encouraged to ascertain their full potential as nutraceuticals.

References

Aeschbach, R., Loliger, J., Scott, B. C., Murcia, A., Butler, J., Halliwell, B., et al. (1993). Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. *Food Chemistry and Toxicology, 32*, 31–36.

Alam, K., Nagi, M. N., Badary, O. A., Al-Shabanah, O. A., Al-Rikabi, A. C., & Al-Bekairi, A. M. (1999). The protective action of thymol against carbon tetrachloride hepatotoxicity in mice. *Pharmacology Research, 40*, 159–163.

Althaus, J. S., Schmidt, K. R., Fountain, S. T., Tseng, M. T., Carroll, T. R., Galatsis, P., et al. (2000). LC-MS/MS detection of peroxynitrite-derived 3-nitrotyrosine in rat microvessel. *Free Radicals in Biology and Medicine, 29*, 1085–1095.

Arcila-Lozano, C. C., Loarca-Pina, G., Lecona-Uribe, S., & Gonzalez de Mejia, E. (2004). Oregano: propiedades, composicion y actividad biologica. *Archivos Latinoamericanos de Nutricion, 54*, 100–111.

Arnold, N., Bellomaria, B., & Valentini, G. (2000). Composition of the essential oil of three different species of Origanum in the eastern Mediterranean. *Journal of Essential Oil Research, 12*, 192–196.

Balavoine, G. G., & Gelettii, Y. V. (1999). Peroxynitrite scavenging by different antioxidants Part I: convenient assay. *Nitric Oxide, 3*, 40–54.

Beckman, J. S., Carson, M., Smith, C. D., & Koppenol, W. H. (1993). ALS, SOD and peroxynitrite. *Nature, 364*, 584.

Beckman, J. S., Chen, J., Ischiropoulos, H., & Crow, J. P. (1994). Oxidative chemistry of peroxynitrite. *Methods in Enzymology, 233*, 229–240.

Bilia, A. R., Cioni, P. L., Morelli, I., Coppi, C., Lippi, A., & Tomei, P. E. (1992). Essential oil of *Satureia montana L ssp. montana*. Composition and yields of plants grown under different environmental conditions. *Journal of Essential Oil Research, 4*, 563–568.

Braga, P. C., Dal Sasso, M., Culici, M., Galastri, L., Marceca, M. T., & Guffanti, E. E. (2006). Antioxidant potential of thymol determined by chemiluminescence inhibition in human neutrophils and cell-free systems. *Pharmacology, 76*, 61–68.

Brannan, R. G., & Decker, E. A. (2001). Peroxynitrite-induced oxidation of lipids: implications for muscle foods. *Journal of Agricultural and Food Chemistry, 49*, 3074–3079.

Chericoni, S., Prieto, J. M., Iacopini, P., & Morelli, I. (2005). Essential oils of commonly used plants as inhibitors of peroxynitrite-induced tyrosine nitration. *Fitoterapia, 76*, 481–483.

Chialva, F., Liddle, P. A. P., Ulian, F., & De Smedt, P. (1980). Essential oil composition of *Satureja hortensis* L grown in Piedmont. Comparison with other oils of various origins. *Rivista Italiana EPPOS, 6*, 297–300.

Choi, H. S., Song, H. S., Ukeda, H., & Sawamura, M. (2000). Radical-scavenging activities of citrus essential oils and their components: detection using 1,1-diphenyl-2-picrylhydrazyl. *Journal of Agricultural and Food Chemistry, 48*, 4156–4161.

Daferera, D. J., Ziogas, B. N., & Polissiou, M. G. (2003). The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, *Fusarium* sp. and *Clavibacter michiganensis* subsp. *Michiganensis. Crop Protection, 22*, 39–44.

Dapkevicius, A., van Beek, T. A., Lelyveld, G. P., van Veldhuizen, A., de Groot, A., & Linssen, J. P. H. (2002). Isolation and structure elucidation of radical scavengers from *Thymus vulgaris* leaves. *Journal of Natural Products, 65*, 892–896.

Stewart, A. J., Del Rio, D., & Pellegrini, N. (2005). A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutrition, Metabolism & Cardiovascular Diseases, 15*, 316–328.

Fenaille, F., Mottier, P., Turesky, R. J., Ali, S., & Guy, P. A. (2001). Comparison of analytical techniques to quantify malondialdehyde in milk powders. *Journal of Chromatography A, 921*, 237–245.

Foti, M. C., & Ingold, K. U. (2003). Mechanism of inhibition of lipid peroxidation by gamma-terpinene, an unusual and potentially useful hydrocarbon antioxidant. *Journal of Agricultural and Food Chemistry, 51*, 2758–2765.

Goldstein, S., Czapski, G., Lind, J., & Merényi, G. (2000). Tyrosine nitration by simultaneous generation of $^{\bullet}NO$ and $O_2^{-\bullet}$ under physiological conditions. *Journal of Biological Chemistry, 275*(5), 3031–3036.

Grassmann, J., Schneider, D., Weiser, D., & Elstner, E. F. (2001). Antioxidative effects of lemon oil and its components on copper induced oxidation of low density lipoprotein. *Arzneimittelforschung, 51*, 799–805.

Harington, C. R., & Pitt Rivers, R. V. (1944). Mono-iodotyrosine. *Journal of the Chemical Society, 38*, 320–321.

Hristova, R., Ristc, M., Brkic, D., Stefkov, G., & Kulevanova, S. (1999). Comparative analysis of essential oil composition of *Origanum vulgare* from Macedonia and commercially available Origani herba. *Acta Pharmaceutica, 49*, 299–305.

Kaur, H., & Halliwell, B. (1994). Evidence for nitric oxide-mediated oxidative damage in chronic inflammation Nitrotyrosine in serum and synovial fluid from rheumatoid patients. *FEBS Letters, 350*, 9–12.

Keith, W. G., & Powell, R. E. (1969). Kinetics of decomposition of peroxynitrous acid. *Journal of the Chemical Society A: Inorganic, Physical, Theoretical* (1), 90.

Kohlert, C., Schindler, G., Marz, R. W., Abel, G., Brinkhaus, B., Derendorf, H., et al. (2002). Systemic availability and pharmacokinetics of thymol in humans. *Journal of Clinical Pharmacology, 42*, 731–747.

Kulisica, T., Radonic, A., Katalinic, V., & Milos, M. (2004). Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chemistry, 85*, 633–640.

Lawrence, B. M. (1993). A planning scheme to evaluate new aromatic plants for the flavour and fragrance industries. In J. Janick & J. E. Simon (Eds.), *New crops* (pp. 20–627). New York: J. Wiley & Sons.

Lee, K. G., & Shibamoto, T. (2001). Inhibition of malonaldehyde formation from blood plasma oxidation by aroma extracts and aroma components isolated from clove and eucalyptus. *Food Chemistry and Toxicology, 39*, 1199–1204.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randal, R. J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry, 193*, 265–275.

Marino, M., Bersani, C., & Comi, G. (2001). Impedance measurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositae. *International Journal of Food Microbiology, 67*, 187–195.

McGimpsey, J. (1993). Oregano – *Origanum vulgare*. *Crop & Food Research Broadsheet, 19*, 4.

Milde, J., Elstner, E. F., & Grassmann, J. (2004). Synergistic inhibition of low-density lipoprotein oxidation by rutin, gamma-terpinene, and ascorbic acid. *Phytomedicine, 11*, 105–113.

Mohiuddin, I., Chai, H., Lin, P. H., Lumsden, A. B., Yao, Q., & Chen, C. (2006). Nitrotyrosine and chlorotyrosine: clinical significance and biological functions in the vascular system. *Journal of Surgical Research, 133*, 143–149.

Montes, M. A., Wilkomirsky, T., & Bello, H. (1998). Antibacterial activity of essential oils from aromatic plants growing in Chile. *Fitoterapia, 69*, 170–172.

Nostro, A., Blanco, A. R., Cannatelli, M. A., Enea, V., Flamini, G., Morelli, I., et al. (2004). Susceptibility of methicillin-resistant staphylococci to oregano essential oil, carvacrol and thymol. *FEMS Microbiological Letters, 230*, 191–195.

Palic, R., Salic, C., & Gasic, M. S. (1983). Chemical composition of volatile oil from *Satureja montana* subsp montana. *Acta Pharmaceutica Jugoslavica, 33*, 227–231.

Piccaglia, R., & Marotti, M. (1991). Composition of the essential oil of an Italian *Thymus vulgaris* L ecotype. *Flavour and Fragrance Journal, 6*, 241–244.

Poli, G. (1993). Liver damage due to free radicals. *British Medical Bulletin, 49*, 604–620.

Puertas-Mejia, M., Hillebrand, S., Stashnko, E., & Winterhalter, P. (2002). In vitro radical scavenging activity of essential oils from Columbian plants and fractions from oregano (*Origanum vulgare* L.) essential oil. *Flavour and Fragrance Journal, 17*, 380–384.

Radonic, A., & Milos, M. (2003). Chemical composition and in vitro evaluation of antioxidant effect of free volatile compounds from *Satureja montana* L. *Free Radical Research, 37*, 673–679.

Slater, T. F., & Sawyer, B. C. (1971). The stimulatory effects of carbon tetrachloride and other halogenoalkanes on peroxidative reaction in rat liver fractions *in vitro*. *Biochemical Journal, 123*, 805–814.

Suzuki, Y., Sakai, H., Shimada, T., Omura, M., Kumazawa, S., & Nakayama, T. (2004). Characterization of gamma-terpinene synthase from *Citrus unshiu* (Satsuma mandarin). *Biofactors, 21*, 79–82.

Takahashi, Y., Inaba, N., Kuwahara, S., & Kuki, W. (2003). Antioxidative effect of citrus essential oil components on human low-density lipoprotein in vitro. *Bioscience, Biotechnology and Biochemistry, 67*, 195–197.

Teissedre, P. L., & Waterhouse, A. L. (2000). Inhibition of oxidation of human low-density lipoproteins by phenolic substances in different essential oils varieties. *Journal of Agricultural and Food Chemistry, 48*, 3801–3805.

Tkachenko, K. G., Tkachev, A. V., & Koroljuk, E. A. (2002). Component composition of essential oil from *Origanum vulgare* L, grown in Leningrad region. *Rastitel'nye Resursy, 38*, 97–101.

Vardar-Unlu, G., Candan, F., Sokmen, A., Daferera, D., Polissiou, M., Sokmen, M., et al. (2003). Antimicrobial and antioxidant activity of the essential oil and methanol extracts of Thymus pectinatus Fisch. et Mey. Var. pectinatus (Lamiaceae). *Journal of Agricultural and Food Chemistry, 51*, 63–67.

Virág, L., Szabó, É., Gergely, P., & Szabó, C. (2003). Peroxynitrite-induced cytotoxicity: mechanism and opportunities for intervention. *Toxicological Letters, 140-141*, 113–124.

Wang, J. Y., Shum, A. Y., Ho, Y. J., & Wang, J. Y. (2003). Oxidative neurotoxicity in rat cerebral cortex neurons: synergistic effects of H_2O_2 and NO on apoptosis involving activation of p38 mitogen-activated protein kinase and caspase-3. *Journal of Neurosciences Research, 72*, 508–519.

Wattanapitayakul, S. K., Weinstein, D. M., Holycross, B. J., & Bauer, J. A. (2000). Endothelial dysfunction and peroxynitrite formation are early events in angiotensin-induced cardiovascular disorders. *FASEB Journal, 14*, 271–278.

Yanishlieva, N. V., Marinova, E. M., Gordon, M. H., & Raneva, V. G. (1999). Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. *Food Chemistry, 64*, 59–66.

Youdim, K. A., & Deans, S. G. (2000). Effect of thyme oil and thymol dietary supplementation on the antioxidant status and fatty acid composition of the ageing rat brain. *British Journal of Nutrition, 83*, 87–93.

Zeghichi, S., Kallithraka, S., Simopoulos, A. P., & Kypriotakis, Z. (2003). Nutritional composition of selected wild plants in the diet of Crete. *World Review of Nutrition and Dietetics, 91*, 22–40.