Effect of Cultivar on the Protection of Cardiomyocytes from Oxidative Stress by Essential Oils and Aqueous Extracts of Basil (*Ocimum basilicum* L.)

Francesca Danesi,[†,§] Simona Elementi,[§,#] Roberta Neri,[§,#]
Magda Maranesi,[†] Luigi F. D'Antuono,[§,#] and Alessandra Bordoni*,[†,§]

Research Center on Nutrition and Vitamins, Department of Biochemistry "G. Moruzzi", University of Bologna, via Irnerio 48, 40126 Bologna, Italy, Department of Agroenvironmental Science and Technology, University of Bologna, via Fanin 40, 40138 Bologna, Italy, and Food Science University Campus, University of Bologna, Piazza Goidanich 60, 47023 Cesena (FC), Italy

Notwithstanding the wide range of biological and pharmacological activities reported for sweet basil (*Ocimum basilicum* L.), many discrepancies are still present in the evaluation of its health-promoting properties. These discordances could be at least in part due to insufficient details of qualitative and quantitative composition, connected to the ample variability of this species. Furthermore, many investigations have been carried out in vitro, with few data available on the effectiveness in biological systems. In this study, the protective effect of essential oils and water-soluble extracts derived from three different cultivars of sweet basil has been evaluated in cultured cardiomyocytes. To verify the effectiveness of supplemented oils/extracts in counteracting oxidative damage, cardiomyocytes were stressed by the addition of hydrogen peroxide. The results indicate that (a) in vitro antioxidant activity is not predictive of biological activity and (b) basil can yield extracts with substantially different protective effects, in relation to composition and extraction techniques. Variation among different cultivars has also been detected.

KEYWORDS: Basil (*Ocimum basilicum* L.); cultured cardiomyocytes; essential oil; aqueous extract; oxidative stress

INTRODUCTION

Natural antioxidants of plant origin belong to several chemical and functional categories, mostly vitamins, phenolic compounds, including flavonoids and phenolic acids, and volatile compounds. These natural antioxidants are becoming increasingly important not only in the food industry, because they retard oxidative degradation of lipids and thereby improve the quality and nutritive value of foods, but also in medicine, for the prevention and cure of diseases associated with oxidative damage.

Notwithstanding the increasing amount of research, many discrepancies are still present in the determination of the antioxidant potency of the different plant extracts and essential oils, possibly due to different reasons. Plant extracts are traditionally obtained by steam distillation (essential oils) or by extraction with polar or nonpolar solvents (water, ethanol, methanol, acetone, hexane) and consequent solvent removal by evaporation (*1*). The major components of the essential oil and solvent extracts of the same plant are often very different, with consequent variation in their bioactive properties.

Furthermore, the different hydrophilic and lipophilic systems used for the evaluation of the antioxidant activity are not always predictive of the antioxidant potential in mammalian cell systems. Generally, these model systems appear rather suitable for a rapid comparative evaluation of the antioxidant power of plant sources. On the other hand, their evaluation in real food systems or, to an even greater extent, as potential agents to alleviate diseases by preventing oxidative deterioration, requires different scientific approaches.

In this study we evaluated the biological activity of aqueous extracts and essential oils from three cultivars of sweet basil (*Ocimum basilicum* L.) using primary cultures of rat cardiomyocytes as model system. The heart is an aerobic organ, and most of the energy required for the contraction and maintenance of ion gradients comes from oxidative phosphorylation. Oxidative stress caused by free radicals plays a crucial role in the

* Address correspondence to this author at the Centro Ricerche sulla Nutrizione e Vitaminologia, Dip. di Biochimica "G. Moruzzi", Via Irnerio 48, 40126 Bologna, Italy (telephone +39.051.2091227; fax +39.051.2091235; e-mail alessandra.bordoni@unibo.it).
† Research Center on Nutrition and Vitamins, Department of Biochemistry "G. Moruzzi".
§ Food Science University Campus.
# Department of Agroenvironmental Science and Technology.

pathophysiology associated with cardiovascular diseases; there-fore, a great deal of attention has focused on the naturally occurring antioxidant phytochemicals as potential therapy.

The genus *Ocimum* L. includes approximately 150 species (*2*), with a great variation in plant morphology and biology, essential oil content, and overall chemical composition. Holy basil [*Ocimum sanctum* L. (syn. *Ocimum tenuiflorum* L.)] and sweet basil (*Ocimum basilicum* L.) are presently the two more considered species. *O. basilicum* is used as a culinary herb, but is also a well-known source of flavoring principles and scents. The leafy parts of basil have antimicrobial properties (*3*), are suitable for the treatment of pain and otitis (*4*), and have high antioxidant activity (*5*).

The essential oil derived from European basil, the major components of which are linalool and methylchavicol, according to the genotype (*6*), is considered to possess the finest aroma. Water-soluble extracts, which are known to contain phenolic compounds such as rosmarinic acid (*7*), are widely used as a preservative in the food industry due to the antioxidant activity of some of their constituents.

Many investigations on the antioxidant activity of basil oils and extracts have been carried out (*8, 9*), particularly regarding their antioxidant capacity in vitro. Tests in biological systems are, however, necessary to confirm their real effectiveness as protecting agents.

We investigated the effect of extract/oil supplementation in cultured cardiomyocytes in both normal conditions and after an oxidative stress, comparing it to the effect of 20 μM α-tocopherol (TC) supplementation. TC is considered to be the most important endogenous antioxidant in cardiac cells (*10*), and it is often used as positive control in studies regarding antioxidant protection in cultured cardiomyocytes.

The final aims of this study were to evaluate the biological activity of basil extracts, comparing it to the activity of a well-known antioxidant, and to verify if the different natures of extracts, as determined by the cultivar or extraction techniques, may affect their biological activities.

MATERIALS AND METHODS

Materials. Horse serum (HS), fetal calf serum (FCS), Ham F10 culture medium, 2,2′-azinobis(3-ethylbenzo-6-thiazoline-6-sulfonic acid) (ABTS), Trolox, α-tocopherol, and other biochemicals were purchased from Sigma Chemical Co. (St. Louis, MO). α-Tocopherol (TC) was dissolved in ethanol at a concentration of 5 mM and kept at −20 °C until use. All of the chemicals and solvents were of the highest analytical grade.

Basil seeds of three standard types:, Genovese, Lettuce leaf, and Purple, were obtained by a local seed company, currently using them as controls for breeding programs. Seeds were sown in alveolated plates with peat substrate at the end of March 2006. The plantlets were transplanted in the open field on a clay-loam soil, at the experimental farm of the University of Bologna, on May 19, in a two replication, randomized block design, as a part of a more extensive comparison of genotypes. Harvest took place on July 16, at preflowering stage. Mature leaves were removed from stems, separated into two subsamples, and immediately frozen. One subsample was lyophilized for extract preparation, and the other was used for essential oil extraction by distillation.

Methods. *Aqueous Extracts and Essential Oils Preparation.* For preparation of lyophilized water extracts, 25 g of lyophilized basil leaves, ground to a fine powder in a mill, were mixed by magnetic stirrer with 500 mL of boiling water and then filtered over Whatman no. 1 paper. The filtrates were frozen and lyophilized in a lyophilizer at 5 mmHg pressure and at −50 °C and then redissolved to solubilize the amount of lyophilized extract derived from 250 mg of fresh leaves in 1 mL of sterile bidistilled water. The obtained solutions were filtered

on 0.20 μM filters (Whatman Europe, U.K.) and then used for cardiomyocytes supplementation.

The essential oil was obtained from 250 g of fresh frozen leaves by means of hydrodistillation and simultaneous solvent extraction (SDE) in a modified Likens−Nickerson apparatus (*11*), using diethyl ether as extraction solvent. This method is being routinely used in our research (*12*), because it is known to produce better recovery of all components with respect to ordinary hydrodistillation (*13*). The solvent was removed under vacuum, at room temperature, and the amounts of essential oils were determined gravimetrically. The essential oils were then solubilized to dissolve the amount derived from 250 mg of fresh leaves in 1 mL of ethanol. The obtained solutions were filtered on 0.20 μM filters (Whatman Europe, U.K.) and then used for cardiomyocytes supplementation.

Aqueous Extracts and Essential Oils Analysis. Total phenol content of the aqueous extracts was determined according to ref *14*, with slight modifications: 100 mg of lyophilized material was extracted with 0.99 mL of 80% (v/v) ethanol + 0.01 mL of 36% (v/v) HCl.

Essential oil composition was evaluated by gas chromatography (Perkin-Elmer Autosystem XL) combined with flame ionization detection (FID) and by GC-MS (Agilent 5973 Network Mass Selective Detector), using a BPX-5 (SGE Australia Pty-Ltd.) capillary column (95% polydimethylsiloxane + 5% phenyl, 30 m, 0.25 mm i.d., 0.25 μm d.f.). GC conditions were as follows: injection volume, 1 μL of 1/1000 (v/v) diethyl ether diluted essential oil; injector temperature, 240 °C; split ratio, 15/1; oven temperature program, 60 °C, increasing by 3 °C/min to 240 °C, held for 5 min. FID temperature was held at 280 °C. MS conditions were as follows: acquisition, full-scan mode; ionization energy, 70 eV; transfer line temperature, 240 °C; ion trap temperature, 210 °C. Essential oil components were identified by comparison of their retention times with those of pure standards, by means of Wiley and NIST/NBS mass spectra libraries, and by literature data (*15*).

Total Antioxidant Activity (TAA) of Extracts/Oils. TAA was measured using the method of Re et al. (*16*), based on the capacity of antioxidant molecules to reduce the radical cation of ABTS, determined by the decoloration of ABTS$^{\bullet+}$, and measured as the quenching of the absorbance at 734 nm. Values obtained for each sample were compared to the concentration−response curve of the standard Trolox solution and expressed as micromoles of Trolox equivalent (TE).

Cell Cultures. Primary cultures of cardiomyocytes were obtained from the ventricles of newborn Wistar rats according to the method of Yagev et al. (*17*). The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health (NIH Publication 85-23, revised 1996). To obtain pure cultures avoiding the presence of fibroblasts, cells were preplated twice before the final seeding, and the absence of cells other than cardiomyocytes was verified microscopically. Cells were randomly divided in the control, grown in Ham F10 medium plus 10% fetal calf serum plus 10% horse serum, and supplemented cardiomyocytes, grown in the same medium but supplemented with different concentrations of extract solutions (1, 10, 20, or 40 μL mL^{-1} medium) or oil solutions (1 or 10 μL mL^{-1} medium), or with 20 μM TC. Media were changed every 48 h, the last medium change being 48 h before the experiments. At complete confluence in a monolayer, media were removed and substituted with Earl's Balanced Salt Solution (EBSS), pH 7.4 (116 mM NaCl, 5.4 mM KCl, 0.9 mM NaH$_2$PO$_4 \cdot$2H$_2$O, 26 mM NaHCO$_3$, 1.8 mM CaCl$_2$, 0.8 mM MgSO$_4 \cdot$7H$_2$O).

To cause oxidative stress, some cells received EBSS buffer added with 0.1 mM hydrogen peroxide. After 1 h, EBSS buffer was removed and collected for the determination of lactate dehydrogenase activity; cardiomyocytes were washed three times with 0.9% NaCl, and cell proliferative activity was assayed.

Lactate Dehydrogenase (LDH) Activity in Media. Cardiomyocyte plasma membrane integrity was assessed by measuring the release of LDH to the EBSS buffer. Enzyme activity was determined spectro-photometrically by measuring NADH levels at 340 nm (*18*).

Cell Proliferative Activity. Cell proliferative activity was measured using the 3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay (*19*). The test is based on the capacity of mitochondrial dehydrogenase in viable cells to convert MTT reagent

Biological Activity of Basil Extracts and Essential Oils

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Table 1. Extraction Yield of Aqueous Extracts and Essential Oils and Total Phenolic Content of Lyophilized Aqueous Extracts[a]

| | aqueous extracts | | essential oil |
type	sample amount (mg g^{-1} of fresh leaves)	total phenolic content (mg of GAE g^{-1} of lyophilized extract)	content (mg g^{-1} of fresh leaves)
Genovese	35.21 ± 3.56	10.46 ± 0.30 b	1.12 ± 0.06 a
Lettuce leaf	34.31 ± 4.99	10.04 ± 0.38 b	0.81 ± 0.09 b
Purple	36.37 ± 2.99	20.57 ± 0.34 a	0.69 ± 0.03 b

[a] Different letters indicate significant differences among types, according to Tukey's HSD, $p \leq 0.05$.

to a soluble blue formazan dye. Briefly, after a washing with 0.9% NaCl, 1 mL of MTT reagent diluted in RPMI-1640 medium modified without phenol red (1 mg mL^{-1}) was added to each dish, and the cell cultures were incubated for 3 h at 37 °C. After removal of the medium, the cells were lysed with isopropanol for 15–20 min. Formazan production, which is proportional to cell vitality, was determined spectrophotometrically at 560 nm.

Statistical Analysis. The reported data are the means of at least three samples obtained from different cell cultures. Statistical analysis was carried out by means of one-way and two-way ANOVA. The differences between means were detected by means of Tukey HSD or Dunnett's test, in the case of comparisons with the untreated controls.

RESULTS

Table 1 reports the extraction yield of aqueous extracts and essential oils, and the total phenolic contents of lyophilized aqueous extracts, expressed as milligrams of gallic acid equivalent (GAE) per gram. The three basil types did not significantly differ for the amount of aqueous extract, whereas Purple basil contained almost twice the amount of phenolic compounds of Genovese and Lettuce leaf, in accordance with other results (*20*). Genovese basil had significantly higher essential oil content than the two other types.

Table 2 lists the chemical components of essential oils. The main constituent of the three types was linalool, significantly more abundant in Purple basil. Besides that, the essential oil composition of the three types was however different. Lettuce leaf oil was rich in methylchavicol, not present in the other two types, but had a substantially lower amount of eugenol. This component also differentiated Genovese, with the highest content, from Purple. Significant differences among types were also detected in the relative amounts of several less abundant components. These results are in agreement with those in the literature (*21, 22*).

In vitro total antioxidant activity (TAA) was calculated for the solutions obtained from aqueous extracts and essential oils and used for cell supplementation (**Figure 1A**). TAA, expressed as micromoles of TE per milliliter, was significantly lower in Lettuce leaf oil solution than in other oil solutions, whereas the corresponding extract had the highest TAA. These differences can be accounted for not only by the different phenolic content or qualitative composition of extracts/oils but also by the different extraction yields of the three cultivars. In fact, when the TAA of unit weight of pure oils or aqueous extracts was calculated (**Figure 1B**), Purple basil had the highest value, in clear relation to the highest content of phenolic compounds, as reported in **Table 1**.

Regarding essential oils, Lettuce leaf had the lowest TAA, independent of the reference units, indicating that not only quantitative but also qualitative composition deeply influences in vitro TAA.

Cell proliferative activity in cardiomyocytes supplemented with basil oil and aqueous extract solutions at 1 and 10 μL mL^{-1}

Table 2. Average Composition of the Three Examined Basil Essential Oils[a]

| | content (% essential oil) | | |
compound	Genovese	Lettuce leaf	Purple
trans-2-hexenal	0.88 ± 0.03 a	0.64 ± 0.01 a	0.31 ± 0.10 b
cis-3-hexenol	0.22 ± 0.04 b	0.35 ± 0.01 a	0.23 ± 0.01 b
1*R*-α-pinene	0.41 ± 0.08	0.35 ± 0.05	0.48 ± 0.00
camphene	0.06 ± 0.01	0.03 ± 0.04	0.09 ± 0.01
sabinene	0.40 ± 0.04	0.31 ± 0.03	0.35 ± 0.01
β-pinene	1.33 ± 0.14 b	1.14 ± 0.11 b	1.67 ± 0.01 a
β-myrcene	0.99 ± 0.13 a	0.58 ± 0.06 b	1.17 ± 0.01 a
α-terpinene	0.02 ± 0.03	0.02 ± 0.03	0.06 ± 0.01
limonene	0.35 ± 0.05 b	0.30 ± 0.05 b	0.64 ± 0.01 a
1,8-cyneole (eucalyptol)	8.10 ± 0.26	6.64 ± 0.60	7.90 ± 0.02
cis-ocymene	0.07 ± 0.00	0.04 ± 0.05	0.00 ± 0.00
3,7-dimethyl-1,3,6-octatriene	1.60 ± 0.06 a	1.12 ± 0.07 b	0.06 ± 0.02 c
γ-terpinene	0.07 ± 0.01	0.10 ± 0.02	0.10 ± 0.01
cis-sabinene hydrate	0.09 ± 0.01 a	0.00 ± 0.00 c	0.07 ± 0.01 b
linalool	38.27 ± 3.65 b	35.97 ± 0.57 b	47.46 ± 0.43 a
camphor	0.19 ± 0.08 b	0.32 ± 0.04 b	1.08 ± 0.01 a
fenchyl alcohol	0.47 ± 0.01 a	0.19 ± 0.00 c	0.26 ± 0.01 b
1-terpene-4-ol	0.18 ± 0.01	0.26 ± 0.04	0.24 ± 0.00
α-terpineol	1.09 ± 0.06 a	0.80 ± 0.04 b	1.18 ± 0.05 a
methylchavicol (estragole)	0.00 ± 0.00 b	33.07 ± 2.75 a	0.00 ± 0.00 b
fenchyl acetate	0.00 ± 0.00 b	0.00 ± 0.00 b	0.19 ± 0.01 a
chavicol	0.02 ± 0.03 b	0.40 ± 0.06 a	0.00 ± 0.00 b
geraniol	0.08 ± 0.03 b	0.00 ± 0.00 b	0.95 ± 0.05 a
endobornyl acetate	0.36 ± 0.07 a	0.19 ± 0.02 b	0.02 ± 0.02 b
eugenol	35.01 ± 3.99 a	9.36 ± 0.53 c	23.34 ± 0.82 b
β-elemene	0.22 ± 0.03 b	0.15 ± 0.01 b	0.40 ± 0.02 a
methyleugenol	0.09 ± 0.00 b	0.14 ± 0.01 a	0.18 ± 0.03 a
β-caryophyllene	0.00 ± 0.00 b	0.00 ± 0.00 b	0.72 ± 0.03 a
α-bergamotene	2.49 ± 0.07 a	1.27 ± 0.07 b	0.26 ± 0.03 c
α-guaiene	0.09 ± 0.01 b	0.00 ± 0.00 c	0.18 ± 0.01 a
δ-cadinene	0.09 ± 0.02 b	0.09 ± 0.01 b	0.16 ± 0.01 a
α-caryophyllene	0.14 ± 0.03 b	0.21 ± 0.01 ab	0.23 ± 0.00 a
β-farnesene	0.07 ± 0.00 b	0.00 ± 0.00 c	0.22 ± 0.00 a
epi-bicyclo-sesquiphellandrene	0.18 ± 0.01 b	0.16 ± 0.01 b	0.29 ± 0.01 a
germacrene D	0.85 ± 0.10	0.76 ± 0.01	0.80 ± 0.01
bicyclo-germacrene	0.41 ± 0.06	0.45 ± 0.00	0.39 ± 0.00
α-cubebene	0.29 ± 0.04 b	0.26 ± 0.02 b	0.43 ± 0.01 a
α-cadinol	2.24 ± 0.24 b	1.90 ± 0.06 b	3.12 ± 0.00 a

[a] Only the identified components are reported. Within lines, different letters indicate among-varieties significant differences of compound relative content, according to Tukey's HSD, $p \leq 0.05$.

medium concentration is reported in **Figure 2**. Because basil oils were dissolved in ethanol, some control cells received the same amount of ethanol to exclude any interference due to the vehicle. Although ethanol addition did not modify either proliferative activity or LDH release (data not shown), supplemented cardiomyocytes were always compared to corresponding controls.

Cell proliferative activity was slightly decreased by Genovese aqueous extract supplementation, but the rather high data variability determined a significant deviation from the control only for the lower dose. Aqueous extracts from the other cultivars had no effect (**Figure 2A**). On the contrary, proliferative activity, although not affected by the lowest oil concentration, significantly increased in cardiomyocytes supplemented with the highest concentration, particularly of Purple basil oil (**Figure 2B**).

LDH release in cell medium is reported in **Figure 3**. Neither basil aqueous extracts nor basil oil supplementation caused modifications in LDH release, independent of the concentration.

To verify the biological protective effect of oils/extracts after an oxidative stress caused by the addition of hydrogen peroxide to the culture medium, cardiomyocytes were supplemented with solutions of basil oils and extracts at 10 μL mL^{-1} medium concentration. The results were compared to the protective effect obtained supplementing cells with a well-known antioxidant, 20 μM TC.
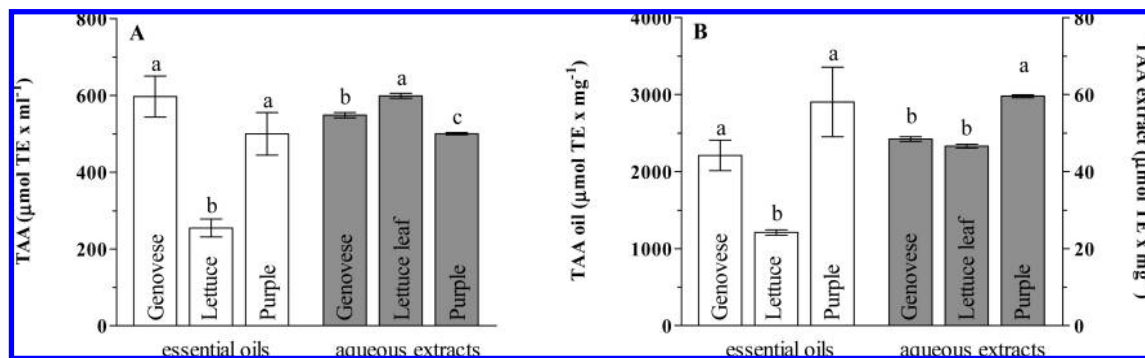
Figure 1. Total antioxidant activity (TAA) of basil essential oils and aqueous extracts. TAA is reported as micromoles of TE per milliliter (**A**) and micromoles of per milligram (**B**). Data are means ± SD of three replicates. Different letters indicate significant differences ($p \leq 0.01$) between cultivars, within type of extract (essential oil or aqueous extract), according to Tukey's HSD.
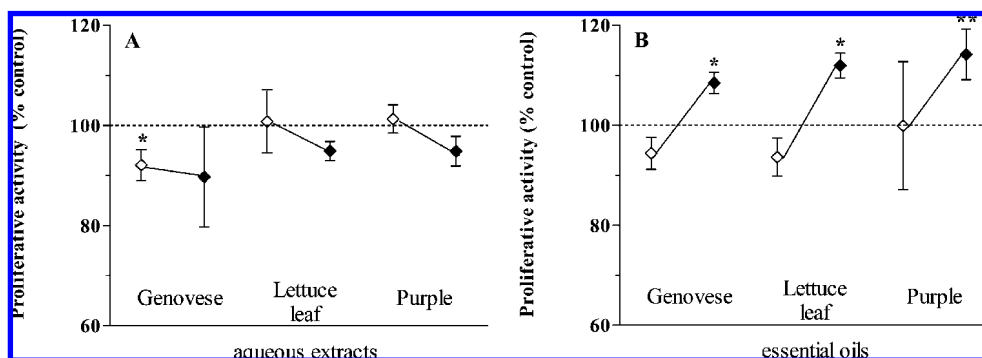
Figure 2. Proliferative activity in cardiomyocytes supplemented with basil aqueous extracts (**A**) and essential oils (**B**). Cardiomyocytes were supplemented with basil aqueous extracts and essential oils at two concentrations: 1 μL ($\diamond$) and 10 μL mL^{-1} of medium ($\blacklozenge$). Data are expressed as percent of value in the corresponding control cells. ∗, ∗∗, significant differences with respect to the unsupplemented corresponding control (dashed line), according to Dunnett's test ($p \leq 0.05$ and ≤ 0.01, respectively).
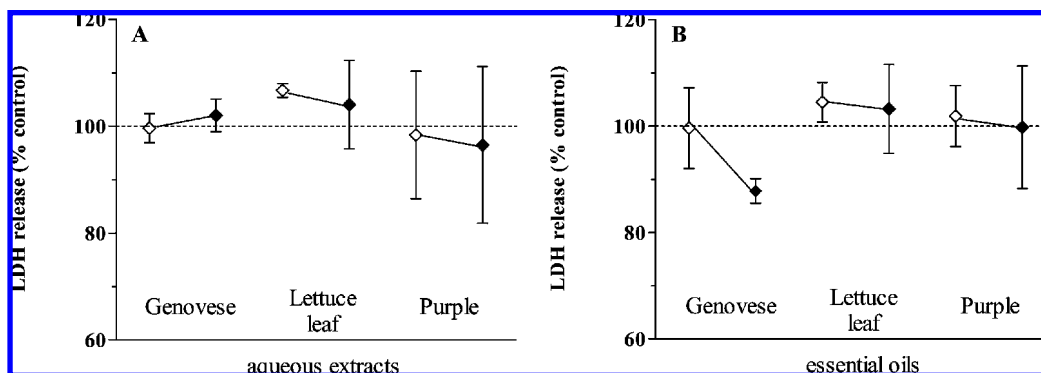
Figure 3. LDH release in cardiomyocytes supplemented with basil aqueous extracts (**A**) and essential oils (**B**). Cardiomyocytes were supplemented with basil aqueous extracts and essential oils at two concentrations: 1 μL ($\diamond$) and 10 μL mL^{-1} of medium ($\blacklozenge$). Data are expressed as percent of value in the corresponding control cells. No significant differences were detected with respect to the unsupplemented corresponding control (dashed line), according to Dunnett's test.

The addition of hydrogen peroxide to unsupplemented cells appeared to be cytotoxic, causing a significant decrease of cell proliferative activity, and a significant increase of LDH release with respect to nonstressed controls (**Figures 4** and **5**). Tocopherol supplementation exerted a complete protection against oxidative stress, as indicated by the increase in proliferative activity, which was even higher than in nonstressed controls, and by the prevention of LDH release.

The oxidative stress-induced reduction of proliferative activity was not counteracted by basil aqueous extract supplementation at 10 μL mL^{-1} medium (**Figure 4A**). Other experiments were then performed using higher concentrations (20 and 40 μL mL^{-1} medium); notwithstanding, basil aqueous extracts were unable to preserve proliferative activity in stressed cardiomyocytes.

Conversely, basil essential oils at 10 μL mL^{-1} medium concentration showed a protective effect in stressed cells, proliferative activity being similar to that in nonstressed controls (**Figure 4B**).

In stressed cardiomyocytes the increase of LDH release was not prevented either by basil aqueous extracts or by oils (panels **A** and **B**, respectively, of **Figure 5**).

DISCUSSION

It is well-known that most spices, especially those belonging to the Lamiaceae family, possess a wide range of biological and pharmacological activities. In addition to providing taste and flavor to foods, the antioxidant function of spices has been
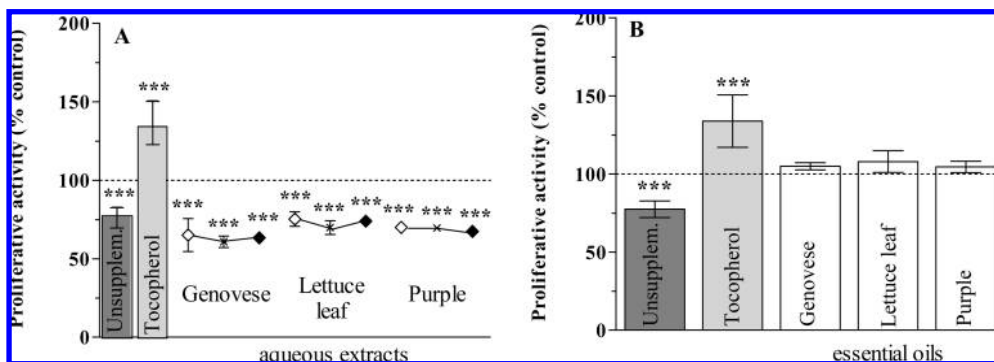
Figure 4. Proliferative activity in stressed cardiomyocytes supplemented with tocopherol, basil aqueous extracts (**A**) or basil oils (**B**). Cardiomyocytes were supplemented with basil aqueous extracts at three concentrations, 10 μL ($\diamond$), 20 μL ($\times$), and 40 μL mL^{-1} of medium ($\blacklozenge$) and with basil essential oils at 10 μL mL^{-1} of medium concentration. Data are expressed as percent of value in nonstressed unsupplemented cells. $\ast\ast\ast$, significant difference with respect to nonstressed unsupplemented controls (dashed line), according to Dunnett's test ($p \leq 0.001$).
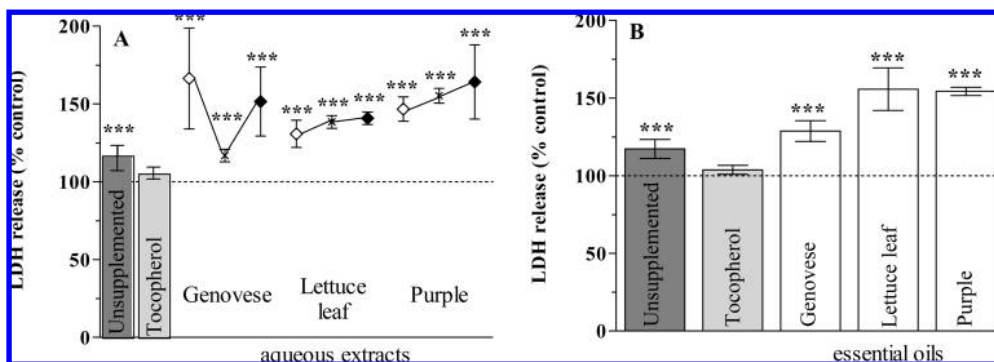
Figure 5. LDH release in stressed cardiomyocytes supplemented with tocopherol, basil aqueous extracts (**A**) or basil essential oils (**B**). Cardiomyocytes were supplemented with basil aqueous extracts at three concentrations, 10 μL ($\diamond$), 20 μL ($\times$), and 40 μL mL^{-1} of medium ($\blacklozenge$) and with basil essential oils at 10 μL mL^{-1} medium concentration. Data are expressed as percent of value in nonstressed unsupplemented cells. $\ast\ast\ast$, significant difference with respect to the nonstressed unsupplemented controls (dashed line), according to Dunnett's test ($p \leq 0.001$).

documented (*23*). Dragland et al. (*24*) speculated that culinary herbs and spices contribute significantly to the total intake of antioxidants in a normal diet, even more effectively than a number of other food groups such as fruits, cereals, and vegetables.

In this study, the antioxidant activity of essential oils and water-soluble extracts from three cultivars of *O. basilicum* have been first evaluated in an in vitro test, and then their protective effect has been evaluated in cultured cardiomyocytes. To verify the effectiveness of supplemented oils/extracts in counteracting oxidative damage, cardiomyocytes were stressed by the addition of hydrogen peroxide.

As previously reported (*25*), we observed significant differences in the extraction yield of essential oil among the different cultivars, Genovese having the highest one. Because plants were grown in the same period and under the same conditions, these differences, as well as differences in essential oil composition, cannot be accounted for by environmental variations. According to ref *26*, the linalool content characterized all cultivar oils, which differed in their content of eugenol (lower in Lettuce leaf) and methylchavicol (absent in Genovese and Purple).

The extraction yields of aqueous extracts were similar among cultivars, but total phenolic content of Purple was almost twice the content of the other cultivars.

To compare results, extracts and oils were solubilized to dissolve the amount of extract/oil derived from 250 mg of fresh leaves in 1 mL of water/ethanol, respectively, and then these solutions were used to supplement cardiomyocytes. Apart from Lettuce leaf oil, all solutions showed an in vitro TAA in the range of 500−600 μmol of TE mL^{-1}, Genovese appearing to

be the most antioxidant among oils and Lettuce leaf among aqueous extracts. On the basis of the in vitro analysis, we could therefore suppose a similar biological activity for the different oils/extracts, apart Lettuce leaf oil.

When basil extracts/oils were supplemented to cardiomyocytes in basal condition, no cytotoxic effect was evidenced, in agreement with Zheljazkov et al. (*27*); on the contrary, essential oil supplementation at the highest concentration significantly increased cell proliferative activity.

An antiproliferative activity for basil oils has been reported by Manosroi et al. (*28*) in a murine leukemia (P388) cell line, with an IC$_{50}$ value of 0.0362 mg mL^{-1}. In our study, basil oil concentration ranged from 0.0017 (Purple) to 0.0028 (Genovese) mg mL^{-1} medium, being well below the toxic value. It is interesting to note that pro-proliferative activity was not present in cells supplemented with aqueous extracts and was not related to in vitro TAA, confirming that antioxidant activity in vitro does not predict biological effectiveness.

Differences among oils and extracts appeared to be even more evident in oxidative condition. Essential oil supplementation completely reversed the effect of hydrogen peroxide on proliferative activity, independent of the cultivar considered. On the contrary, extracts had no effect on this character, even at 4 times higher concentrations than essential oils.

With regard to LDH release, used to evaluate cell membrane integrity, neither oils nor extracts evidenced protective effect. Sharma et al. (*29*) reported a reduction of LDH release in the heart of infarcted rats after subcutaneous administration of a hydroalcoholic extract of *O. sanctum*, but it is difficult to compare the two studies. Although a comparison with data

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obtained in other studies is out of the scope of the present work, which aimed at evidencing differences among oils and extracts derived from different cultivars of basil, the work of Sharma et al. (*29*) represents an important confirmation of the bioavailability of basil extracts and of the possibility of extract components to protect the heart.

Considering the proliferative activity, after oxidative stress basil oils appeared to be protective, whereas aqueous extracts had no effect. Major components of the essential oil and water-soluble extract are very different, and it is conceivable that they have different effects. It is interesting to note that, when the in vitro TAA of the solutions obtained from oils and extracts, which represented the solutions added to cardiomyocyte medium, was measured, extracts revealed similar or higher activity than oils, whereas they had no effect in cells. Our results underscore the need for biological measurement before protective activity is ascribed to plant material.

The highest protective activity of TC is not surprising because we used the most effective dose of the vitamin as positive control. The 20 μM dose has been reported to increase cellular TC content about 2−3-fold (*30*), to reduce membrane lipid alteration, and to actively protect cardiomyocytes from oxidative damage (*31*). In a previous study the same dose of TC appeared to be more protective than extracts of green tea (*31*).

Although extraction techniques seem to be the main determinant of biological activity, with regard to oils, differences can be attributed even to cultivar. In fact, in basal condition, the Purple one was the most effective in enhancing proliferative activity. Basil cultivars differ in chemical composition, and it is conceivable that individual compounds may act differently, modulating different pathways, also in relation to specific experimental conditions.

The results obtained in this study clearly show that essential oils derived from *O. basilicum* have powerful protective activity against the antiproliferative activity due to oxidative stress, whereas water-soluble extracts are ineffective. Moreover, differences ascribable to cultivars have been detected. Notably, these results were not predictable simply by measuring in vitro TAA.

As far as we know, this is the first paper describing and comparing the biological effect of extracts/oils of different basil cultivars; our data may be useful for the consideration of basil both as a possible food ingredient or food supplement or in pharmaceutical applications. Further studies comparing the effects of basil extract to other antioxidants are needed, particularly considering that the quite recent knowledge that many botanical compounds can affect gene expression at the transcriptional level, thus regulating cell function (*32*), has widened the range of possible mechanisms of action of these compounds, which cannot be considered as mere antioxidants.

ABBREVIATIONS USED

ABTS, 2,2′-azinobis(3-ethylbenzo-6-thiazoline-6-sulfonic acid); EBSS, Earl's balanced salt solution; FCS, fetal calf serum; FID, flame ionization detector; GAE, gallic acid equivalent; GC-MS, gas chromatography−mass spectrometry; HS, horse serum; LDH, lactate dehydrogenase; MTT, 3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyltetrazolium bromide; NADH, nicotinamide adenine dinucleotide (reduced form); SDE, simultaneous solvent extraction; TAA, total antioxidant activity; TC, α-tocopherol; TE, Trolox equivalent.

LITERATURE CITED

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