

Antioxidant Potential in Lipophilic and Hydrophilic Extracts from Medicinal Herbs (*Salvia officinalis* and *Echinacea angustifolia*). A Comparison Between Assays Based on Electron Paramagnetic Resonance and Spectrophotometry

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ABSTRACT

Recently, detection of antioxidant activity of plant extracts has caught the attention of researchers. Determination of antioxidant activity is usually performed using stable free radicals. A number of assays is available for screening the antioxidant activity of food constituents, but few of these methods are applicable to lipophilic substances. EPR is the analytical technique that directly measures free radicals making possible the analysis of turbid or highly colored samples. The aim of this study was to determine how the detection technique, plant species and the different growing conditions influenced the antioxidative activity of aqueous and lipid extracts from leaves. Sage (*Salvia officinalis* L.) and coneflower (*Echinacea angustifolia* DC.) grown in hydroponics and in soil, were analyzed. Radical decay kinetics, due to plant extracts, were monitored by an EPR spectrometer, equipped with a data acquisition system and a software package designed for analysis and simulation of spectra. Parameters obtained by the fitting of the curves allowed the calculation of the antioxidant potential. The radicals Fremy's salt (hydrophilic) and 1,1-diphenyl-2-picrylhydrazyl (DPPH, lipophilic) were employed. Comparisons with data obtained by a spectrophotometric detection with the radical cation ABTS⁺ [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] were also made. Fremy's salt and DPPH gave 1st and 2nd order kinetics, respectively. EPR can give more reliable measurements of the antioxidant potential than spectrophotometric assay. The reduced sensitivity of spectrophotometric detection was showed both for sage and coneflower extracts. Antioxidant potential of sage resulted extraordinary high compared to coneflower and a different ratio of aqueous to lipid antioxidant potential in the two species could be monitored. Antioxidant potentials were also higher for coneflower grown in hydroponics than in soil. In addition to the technique adopted, the detection of antioxidant potential was influenced by the growing conditions of plants and hydroponics showed to be able to increase antioxidant potential of both coneflower lipophilic and hydrophilic extracts. By registration of EPR kinetics we can get reliable measurements of the antioxidant potential of plant extracts, avoiding the artifacts due to the appearance of other radicals resulting from oxidative processes.

Keywords: Coneflower, 1,1-Diphenyl-2-Picrylhydrazyl (DPPH), Peroxylamine Disulphonate (Fremy's Salt), Sage

1. INTRODUCTION

Traditional systems of medicine have been used throughout the world for centuries. Certain ancient

systems, such as traditional Chinese and Tibetan medicines, are still used extensively, particularly in their country of origin. Anyway, in the United States and Europe the interest for therapies belonging to such

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systems, particularly for the treatment of chronic illnesses, is growing. These therapies, usually known as alternative medicine, include the use of medicinal herbs which generally refer to plant parts-sometimes ground, extracted, or otherwise prepared-used for health benefits.

Both sage (*Salvia officinalis* L.) and coneflower (*Echinacea angustifolia* DC.) have been used as medicines by different cultures throughout the centuries. Together with rosemary, sage is one of the most potent spices as natural antioxidants. In fact, these spices can substitute synthetic antioxidants such as butylated hydroxytoluene (BHT), which is believed to possess carcinogenic activity, in retarding lipid oxidation and thus in preserving food from deterioration (Lindberg Madsen and Bertelsen, 1995). It is well-documented that sage is able to scavenge hydroxyl radicals and to slightly reduce the rate of oxygen consumption (Lindberg Madsen *et al.*, 1996). In the scientific world, interest has been growing in the recent years for *Echinacea* spp. in consideration of its immunostimulatory properties. These properties can be ascribed above all to roots and for this reason its cultivation by hydroponic systems is an interesting alternative to conventional soil culture (Pacifi *et al.*, 2007; Zheng *et al.*, 2006). Echinacoside is the main phenolic compound found in *E. angustifolia* and *E. pallida*, both of which have been used to treat infections and traumatic diseases for long time (Bauer, 2000). Recently, several studies reported that echinacoside might possess antioxidant and anti-inflammatory properties (Pellati *et al.*, 2003).

The most commonly alternative therapy used is represented by dietary supplements, which include medicinal herbs and nutraceuticals. A dietary supplement could be defined as any product that contains a vitamin, mineral or amino acid, of which plants are rich. Tomato berries constitute an example of healthy benefit plant product containing considerable amounts of vitamins C and E, lipoic acid, phenols and minerals (Sgherri *et al.*, 2007). It is a common opinion that the origin of most illnesses resides in the generation of free radicals, natural products of the aerobic metabolism which can increase following pathological events such as inflammation and that antioxidants can help in contrasting their progress. The interest in vitamins C, E and phenols is due to their antioxidant properties, which strongly determine their biological functions in both plant and animal metabolism. In fact, they can interact enzymatically and non-enzymatically with damaging oxygen free radicals and their derivatives, thus protecting plants from oxidative stress and mammals from oxidative stress-related diseases such as cancer, cardiovascular pathologies and aging (Calucci *et al.*, 2003).

Antioxidative properties are the result of the capacity of antioxidants to inhibit the initiation of free radical processes or to interrupt the chain reactions in the propagation of oxidation.

In plant cells two different types of antioxidants can be distinguished: hydrophilic such as vitamin C and lipophilic such as vitamin E and carotenoids. In the last years the detection of the antioxidant activity of plant extracts or food has caught the attention of researchers. Determination of antioxidant activity is usually performed by using stable free radicals. These are chromophores that disappear in the presence of an antioxidant, the decolorization of which can be followed spectrophotometrically. A number of assays is available for screening the antioxidant activity of pure compounds, food constituents and body fluids, but few of these methods are applicable to lipophilic substances (Pellegrini *et al.*, 1999). The 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS⁺) has been used to screen the relative radical-scavenging abilities of both lipophilic and aqueous systems (Pellegrini *et al.*, 1999). The method is carried out as a decolorization assay in which the antioxidant is added to the preformed radical cation produced by the one-electron oxidation of ABTS.

Electron Paramagnetic Resonance (EPR) spectroscopy is the analytical technique that directly measures free radicals. Owing to the unpaired electron in the outer orbital, free radicals are paramagnetic species and, when present in sufficient amount, are directly detectable by EPR spectroscopy. EPR technique has been employed to determine antioxidant activities of plant extracts (Canadianovic-Brunet *et al.*, 2006) using stable free radicals such as peroxyamine disulphonate (Fremy's salt). This technique is very sensitive allowing the detection at the sub-micromolar level and presents many advantages over spectrophotometry as the possibility to analyze turbid or highly colored samples (Gardner *et al.*, 1998). Recently, EPR studies performed with standard antioxidants allowed to distinguish between fast and slow antioxidants in lipid extracts from basil, identifying the contribution of the different molecules in plant extract to the fast and slow antioxidant activity (Sgherri *et al.*, 2011b). The role of chlorophyll as antioxidant was also demonstrated (Sgherri *et al.*, 2011a).

The task of this study was to determine the antioxidative activity of aqueous and lipid extracts from leaves of sage and coneflower. In particular, for coneflower antioxidative activity was determined on extracts obtained from plants cultivated both in soil and hydroponics. A comparison between assays based on spectrophotometric detection with the radical cation ABTS⁺ and EPR detection by using two stable radicals Fremy's salt (hydrophilic) and 1,1-Diphenyl-2-Picrylhydrazyl (DPPH, lipophilic) was also made.

2. MATERIALS AND METHODS

2.1. Chemicals

ABTS, DPPH and Fremy's salt were purchased from Sigma (Milan, Italy).

2.2. Plant Material

Experiments were performed during spring 2006. Sage plants were taken from the collection of the Botanical Garden of Pisa, Italy. Coneflower seeds (Gold Nugget Seed®), pre-treated by the producer in order to break dormancy, were purchased from Jelitto Staudensamen GmbH, Schwarmstedt (Germany) and sowed in rockwool tray plugs in a climatic cell at 25°C, with a light intensity of 0.92 mmol m⁻² sec⁻¹ (from fluorescent lamps) and 14 h photoperiod. After emergence, seedlings were moved to a climate-controlled glasshouse and four weeks later, when the first two-three leaves were 4-5 cm long, the plants were transferred at a density of approximately 100 plant m⁻² into 12 cm-diameter plastic pots filled with a peat-perlite mixture or into a floating raft growing system consisting of polystyrene plug trays floating on 300 L m⁻² stagnant, aerated, full-strength nutrient solution. The nutrient solution supplied to pot-grown plants (via drip or bottom irrigation) or used in floating systems was prepared by dissolving appropriate amounts of NH₄NO₃, CaCl₂, KNO₃ and K₂SO₄ in tap water containing approximately 5.0 mol m⁻³ NaCl. The composition was the following: 4.0 mol m⁻³ N-NO₃⁻, 0.5 mol m⁻³ P-H₂PO₄⁻, 3.0 mol m⁻³ S-SO₄²⁻, 9.0 mol m⁻³ Cl⁻, 4.0 mol m⁻³ N-NH₄⁺, 4.5 mol m⁻³ K⁺, 2.0 mol m⁻³ Ca²⁺, 1.0 mol m⁻³ Mg²⁺, 5.0 mol m⁻³ Na⁺. Trace elements were added as EDTA chelates. The Electrical Conductivity (EC) ranged between 1.5 and 2.0 dS m⁻¹ and the pH was maintained in the range 5.5-6.5 by frequent adjustments with sulfuric acid. Oxygen content was maintained above 6.5-7.0 mg L⁻¹. Minimum (heating) and ventilation air temperatures inside the glasshouse were 16 and 27°C, respectively. Photosynthetic Photon Flux Density (PPFD) inside the greenhouse ranged from 500-700 mmol m⁻² s⁻¹.

2.3. Sample Preparation

Fresh leaves were pulverized with liquid nitrogen using a mortar. The aqueous extract was obtained using Milli Q water accurately degassed. After centrifugation at 12100 g for 15 min, the pellet was discarded and the supernatant was used for determination of the antioxidant activity. Lipid extraction was carried out in the dark and under a continuous flux of nitrogen using chloroform/methanol (2:1, v/v). The extract was washed three times with KCl 0.88% (w/v) in order to eliminate

salts (Sgherri *et al.*, 2011b). The chloroform phase was taken to dryness with a rotavapor and resuspended in chloroform/ethanol (1:5, v/v).

2.4. EPR Analysis

EPR spectra were recorded by a Varian E112 (X-band) spectrometer equipped with a Varian variable temperature accessory. The spectrometer was interfaced to a AST Premium 486/25 EISA computer by means of an acquisition board (Ambrosetti and Ricci, 1991) and a software package designed for EPR measurements (Sgherri *et al.*, 2010). Computer-based simulations of EPR spectra were performed using the Winsim programme (Duling, 1994). Spectra were recorded at 25°C in a 1 mm quartz sample tube sealed at one end inserted into the microwave cavity of the spectrometer. The EPR parameters used were: microwave power 10 mW, microwave frequency 9.16 GHz, modulation amplitude 1 Gauss, central magnetic field 3265 Gauss. Fremy's salt and DPPH were employed to determine the antioxidant potential in aqueous and lipid extracts, respectively. In the incubation mixture the final concentration of Fremy's salt was 0.5 mM and that of DPPH was 3.3 mM. The above concentrations were chosen because allowed to monitor adequate and well resolved signals over time in order to get decay kinetics. To know the decay of the radical before measuring the antioxidant activity, decay kinetics of Fremy's salt or DPPH with and without the extract were recorded. The amplitude of the central line of Fremy and DPPH spectra was taken for registration of kinetics. Parameters obtained by the fitting of the curves (Winsim programme) allowed the calculation of the antioxidant potential which was expressed as the number of DPPH or Fremy's radicals reduced by 1 g of fresh plant material.

2.5. Spectrophotometric Analysis

The radical cation ABTS⁺ was generated as described by Pellegrini *et al.* (1999). A stable stock solution of ABTS⁺ radical was produced by reacting a 7 mM aqueous solution of ABTS with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 16 h before use. The radical solution was diluted in ethanol or water for lipophilic or aqueous extracts, respectively, in order to obtain an absorbance at 734 nm of 0.70 ± 0.05. After addition of the extract, the decrease in absorbance was monitored and the antioxidant potential was referred as the number of ABTS⁺ radicals reduced by 1 g of fresh plant material. Absorbance was recorded by a Varian Cary 1E UV-visible spectrophotometer.

2.6. Statistical Analysis

Results are the means of four replicates from four separate plants ($n = 4$). Data in the graphs are reported as mean values \pm Standard Errors (SE). Calculations were carried out with the Costat 6.4 program (CoHort software).

3. RESULTS

EPR spectra of DPPH and Fremy's salt are reported in **Fig. 1**. They are characterized by 5 and 3 lines, respectively, which are narrow enough, compared to the spectrum acquisition time, to be used for registration of kinetics with many close points. Kinetics could not be registered for the radical cation $ABTS^+$ due to a very large EPR line (data not shown) and thus this radical could not be used for determination of the antioxidant potential by EPR.

Decay kinetics of Fremy's salt with and without sage aqueous extract are shown in **Fig. 2A**. Both of them were 1st order kinetics. The decay kinetics of Fremy's salt in the presence of sage was 10-fold higher than the constant rate of decay of the radical alone (**Fig. 2A**).

The decay kinetics of DPPH in the presence of lipid extract of sage was a 2nd order kinetics, whereas the decay of DPPH itself followed a 1st order kinetics with a rate constant 100-fold lower than in the presence of sage lipid extract (**Fig. 2B**).

In **Fig. 3 and 4** the antioxidant potential was expressed as the ability to reduce radicals by 1 g of leaves of sage or coneflower, respectively. In sage the antioxidant potential detected by EPR was 62- and 10-fold higher for hydrophilic and lipophilic extracts, respectively, compared to the one detected spectrophotometrically (**Fig. 3A and 3B**). When measured by EPR, the ratio of aqueous to lipid antioxidant potential of sage was about 1:1, but it was 1:4 when detected by spectrophotometer.

Using EPR technique, aqueous and lipid extracts of sage showed an ability to reduce radicals 6- and 40-fold higher than coneflower leaves (**Fig. 3 and 4**). In addition, coneflower grown in hydroponics showed a higher antioxidant potential in comparison with coneflower grown in pot (**Fig. 4 and 5**). The trend was the same when the antioxidant potential was detected spectrophotometrically, but with values from 8 to 20-fold lower than those obtained by EPR (**Fig. 4**). In contrast to sage, in coneflower grown in hydroponics and in pot the EPR-analyzed hydrophilic extract showed an antioxidant activity ca. 6-fold higher than that of the lipophilic one (**Fig. 4**).

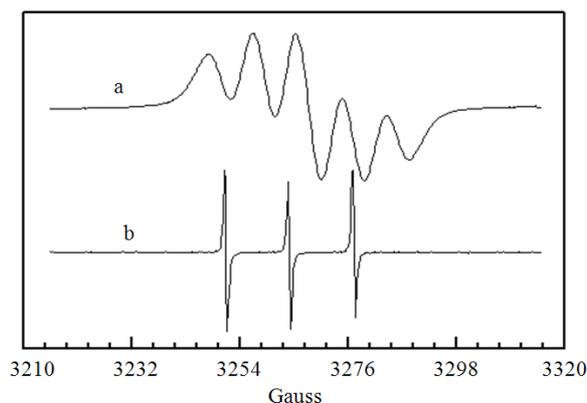


Fig. 1. EPR spectra of 1, 1-diphenyl-2-picrylhydrazyl (DPPH, a) and of peroxyamine disulphonate (Fremy's salt, b)

4. DISCUSSION

Even if radicals used to measure antioxidant activity are stable, they have a characteristic half-life and spontaneously decay with a 1st order kinetics (**Fig. 2A and 2B**) following radical disproportionation. In the presence of antioxidants from aqueous and lipophilic extracts of sage leaves, the decay kinetics of Fremy's salt and DPPH had 1st and 2nd order fittings, respectively (**Fig. 2A and 2B**) depending on the relative proportion of the molecules of antioxidants and of the radical. It can be hypothesized that in the first case the incubation mixture contained antioxidants in excess, whereas in the second case the number of antioxidant molecules was similar to that of the molecules of the radical. The fact that the decay kinetic constants due to the addition of aqueous and lipophilic extracts of sage were 10- and 100-fold higher when compared to those of Fremy's salt and DPPH, respectively and that kinetics showed 1st and 2nd order fittings gives a warrant about the proportion between EPR amplitude and antioxidant amounts. These observations suggest that the radical (Fremy's salt or DPPH) reacts with the antioxidant with a 1:1 stoichiometry (Sgherri *et al.*, 2011b).

The results obtained with sage showed that spectrophotometric technique was less sensitive than EPR. In addition, the spectrophotometric technique determined changes in the ratio between the antioxidant potential of aqueous and lipid extracts (**Fig. 3**). This could be due to the presence of chatechins which are known to scavenge the $ABTS$ radical cation in the aqueous phase (Salah *et al.*, 1995). Another reason could be ascribed to the changes in the reduction potentials of the different radicals and $ABTS^+$ in the various solvents used for determination of the antioxidant activity in aqueous and lipid extracts. Indeed, the reduction potentials of the various hydroxyl groups of polyphenols may change in different solvent systems depending on their state of protonation or deprotonation.

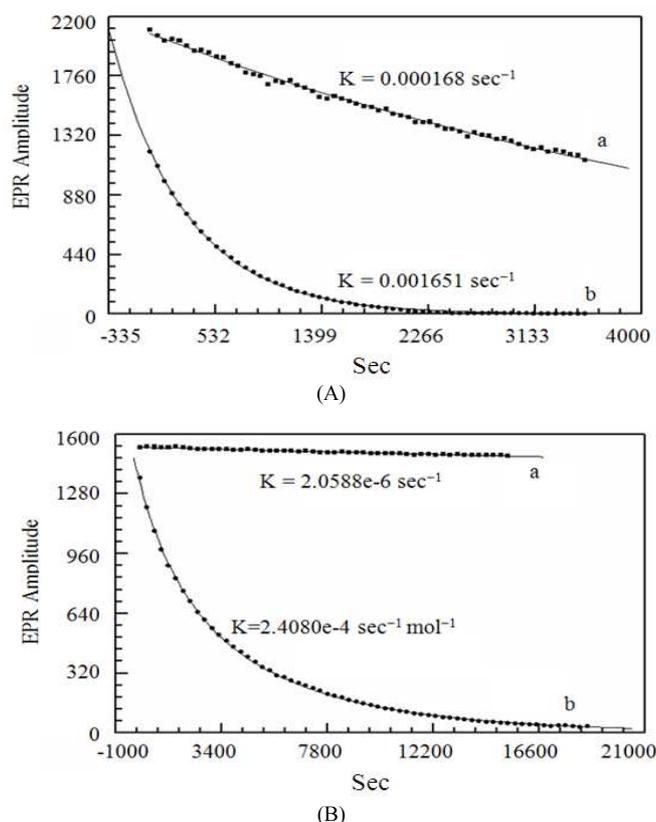


Fig. 2. EPR decay kinetics of 0.5 mM Fremy's salt (A) without (a) or with (b) the addition of aqueous extract of sage leaves and of 3.3 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH, B) without (a) or with (b) the addition of lipophilic extract of sage leaves. K, decay kinetic constant. Continuous lines are the fittings of experimental points (squares and dots)

The electrostatic repulsion between the Fremy's radical anion and the deprotonated moieties on the phenolics may result in the different antioxidant activity of the systems under investigation (Gardner *et al.*, 1998). The reduction potential of antioxidants can be altered by solvent conditions that change polarities and thus influence the pK_a values and molecular orbital energies (Gardner *et al.*, 1998). Moreover, at pH below 7 (in the present experiment it was 6.45) thiol groups do not react with the $ABTS^+$ radical and the antioxidant capacity of these compounds is lost (Arnao *et al.*, 1996).

A disadvantage of the spectrophotometric technique is that the sensitivity changes when readings are recorded at different absorbance values. In fact, when measurements are made at 734 nm, such as in the present experiment, the molar extinction coefficient is half of that obtained at 414 nm ($\epsilon_{414} = 31, 1 \text{ M}^{-1} \text{ cm}^{-1}$) when ABTS is oxidized in the presence of H_2O_2 in a typical peroxidative reaction which generates a metastable radical with a characteristic absorption spectrum and an absorption maximum at 414 nm (Arnao *et al.*, 1996).

Confirmation of the reduced sensitivity of the spectrophotometric detection over EPR was obtained with the extracts from coneflower leaves (**Fig. 4**). With respect to sage, this species showed a lower antioxidant activity to which hydrophilic compounds contributed mostly (**Fig. 3 and 4**). A different lipid to aqueous ratio means a different antioxidant composition and different synergism or antagonisms among antioxidants, with different effects on the antioxidant activity. Vitamin C (ascorbate) is the most powerful antioxidant in aqueous extract participating to the well-known ascorbate-glutathione cycle (Sgherri and Navari-Izzo, 1995) which detoxifies the cell from activated oxygen species causing oxidative stress-related diseases. Lipid extract (**Fig. 3-5**) is supposed to contain noticeable amounts of vitamin E represented mainly by α - and γ -tocopherol (Sgherri *et al.*, 2007) as well as by carotenoids. Antioxidants from aqueous and lipid extracts are related to each other. In fact, reduced ascorbate regenerates tocopherol from its oxidized form, the chromanoxyl radical.

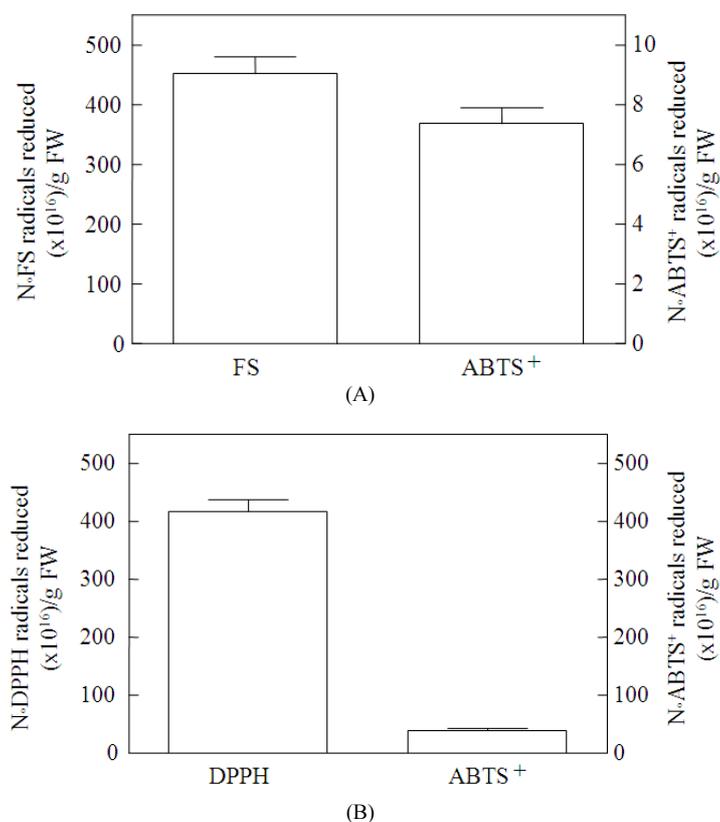


Fig. 3. Antioxidant activity of aqueous (A) or lipophilic (B) extract from sage leaves. FS, Fremy's salt; DPPH, 1,1-diphenyl-2-picrylhydrazyl; ABTS⁺, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation; FW, fresh weight

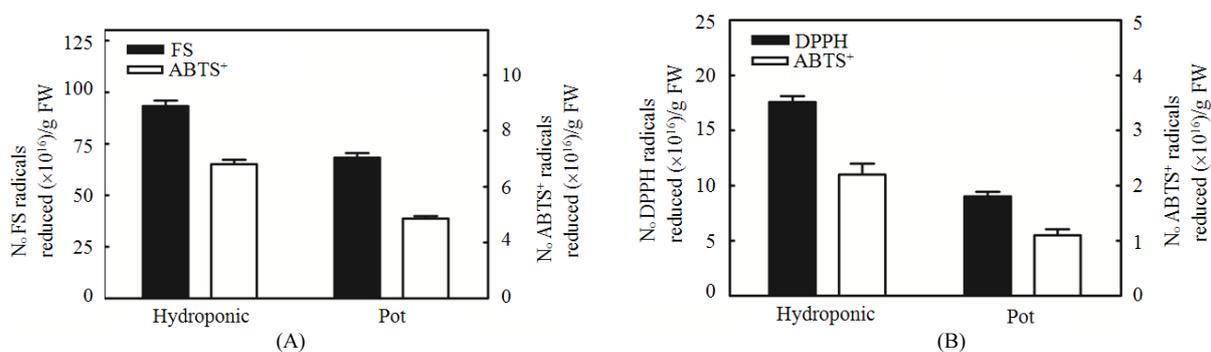


Fig. 4. Antioxidant activity of aqueous (A) or lipophilic (B) extract from leaves of coneflower grown in hydroponics or in pot. FS, Fremy's salt; DPPH, 1,1-diphenyl-2-picrylhydrazyl; ABTS⁺, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation; FW, fresh weight

A synergistic effect in cell protection by β -carotene and vitamins E and C has been observed (Bohm *et al.*, 1997) and may be related to the fact that β -carotene does not only quench oxy-radicals but also repairs the α -tocopheroxyl radical.

The extraordinary high antioxidant potential of the aqueous extract of sage, compared to coneflower, is in agreement with the fact that sage showed the highest content of ascorbate in comparison with other herbs and spices (Calucci *et al.*, 2003).

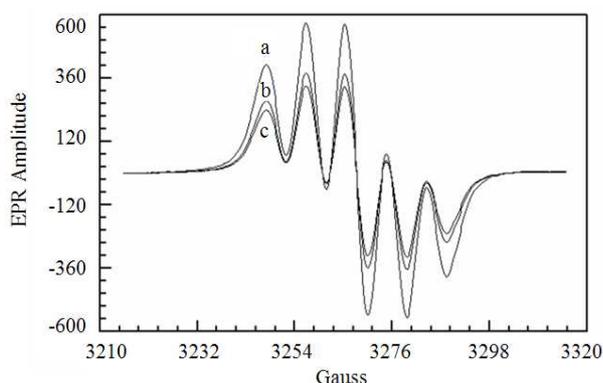


Fig. 5. EPR spectra after 5 min of reaction of 1,1-diphenyl-2-picrylhydrazyl (DPPH) in the absence (a) or in the presence of lipophilic extract from leaves of coneflower grown in pot (b) or in hydroponics (c)

A higher content of antioxidants in both aqueous and lipid extracts from coneflower grown in hydroponics compared to coneflower grown in pot was evident. An indication of this was already clear after 5 min from the addition of lipid extract to the DPPH solution (**Fig. 5**) and was confirmed by the data obtained both for lipid and aqueous extracts at the end point of the reactions (**Fig. 4**). Synthesis of antioxidants can be stimulated under a moderate level of oxidative stress (Sgherri and Navari-Izzo, 1995) and hydroponics could represent a stress situation where redox metals, such as copper, are in direct contact with roots (Sgherri *et al.*, 2002). Thus, cultivation of coneflower by hydroponic system, besides allowing the production of roots that can be easily processed by the industry, could improve the antioxidant properties of leaves.

5. CONCLUSION

Besides plant species and the technique adopted, the detection of antioxidant potential was influenced by the growing conditions of plants and hydroponics showed to be able to increase antioxidant potential of both coneflower lipophilic and hydrophilic extracts. An EPR spectrometer, equipped with a data acquisition system and a software package designed for analysis and simulation of spectra, gives reliable measurements of the antioxidant potential of plant extracts, allowing to follow the kinetics of reactions and the appearance of other radical species resulting from oxidative processes. EPR results to be a more sensitive technique than spectrophotometry and the use of hydrophilic and lipophilic radicals for aqueous and lipid extracts,

respectively, appears more reliable than the non-specific use of $ABTS^+$ radical.

6. ACKNOWLEDGEMENT

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7. REFERENCES

- Ambrosetti, R. and D. Ricci, 1991. A fast time averaging data acquisition system for the PC-AT bus. *Rev. Sci. Instrum.*, 62: 2281-2287. DOI: 10.1063/1.1142350
- Arnao, M.B., A. Cano, J. Hernandez-Ruiz, F. Garcia-Cánovas and M. Acosta, 1996. Inhibition by L-ascorbic acid and other antioxidants of the 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) oxidation catalyzed by peroxidase: A novel approach for determining total antioxidant status of foods. *Anal. Biochem.*, 236: 255-261. DOI: 10.1006/abio.1996.0164
- Bauer, R., 2000. Chemistry, Pharmacology and Clinical Applications of Echinacea Products. In: Herbs, Botanicals and Teas. Functional Foods and Nutraceuticals Series, Mazza, L.G. and B. Oomah (Eds.), Technomic Press, ISBN-10: 15667685, pp: 45-74.
- Bohm, F., R. Edge, E.J. Land, D.J. McGarvey and T.G. Truscott, 1997. Carotenoids enhance vitamin E antioxidant efficiency. *J. Am. Chem. Soc.*, 119: 621-622. DOI: 10.1021/ja962512c
- Calucci, L., C. Pinzino, M. Zandomenighi, A. Capocchi and S. Ghiringhelli *et al.*, 2003. Effects of γ -irradiation on the free radical and antioxidant contents in nine aromatic herbs and spices. *J. Agric. Food Chem.*, 51: 927-934. DOI: 10.1021/jf020739n
- Canadanovic-Brunet, J.M., S.M. Djilas, G.S. Cetkovic, V.T. Tumbas and A.I. Mandic *et al.*, 2006. Antioxidant activities of different *Teucrium montanum* L. Extracts. *Int. J. Food Sci. Technol.*, 41: 667-673. DOI: 10.1111/j.1365-2621.2006.01133.x
- Duling, D.R., 1994. Simulation of multiple isotropic spin trap EPR spectra. *J. Magn. Reson. B.*, 104: 105-110. DOI: 10.1006/jmrb.1994.1062
- Gardner, P.T., D.B. McPhail and G.G. Duthie, 1998. Electron Spin Resonance spectroscopic assessment of the antioxidant potential of teas in aqueous and organic medi., *J. Sci. Food Agric.*, 76: 257-262. DOI: 10.1002/(SICI)1097-0010(199802)76:2<257::AID-JSFA944>3.0.CO;2-B

- Madsen, L.H. and G. Bertelsen, 1995. Spices as antioxidants. *Trends Food Sci. Technol.*, 6: 271-277. DOI: 10.1016/S0924-2244(00)89112-8
- Madsen, L.H., B. Rud Nielsen, G. Bertelsen and L.H. Skibsted, 1996. Screening of antioxidative activity of spices. A comparison between assays based on ESR spin trapping and electrochemical measurement of oxygen consumption. *Food Chem.*, 57: 331-337. DOI: 10.1016/0308-8146(95)00248-0
- Pacifici, S., L. Tozzini, R. Maggini, A. Pardossi and F. Tognoni, 2007. La Coltivazione Idroponica Delle Piante Medicinali: Il Caso Dell'echinacea Angustifolia. In: *Colture Artificiali di Piante Medicinali*, Rome, Pardossi, A., F. Tognoni and A. Mensuali, (Eds.), Arance Editrice Srl, ISBN: 9788854812451, pp: 21-36.
- Pellati, F., S. Benvenuti, L. Magro, M. Melegari and F., Soragni, 2003. Analysis of phenolic compounds and radical scavenging activity of Echinacea. *J. Pharm. Biomed. Anal.*, 35: 289-301. DOI: 10.1016/S0731-7085(03)00645-9
- Pellegrini, N., R. Re, M. Yang and C. Rice-Evans, 1999. Screening of dietary carotenoids and carotenoid-rich fruit extracts for antioxidant activities applying 2,2'-Azinobis (3-ethylenebenzothiazoline-6-sulfonic acid radical cation decolorization assay. *Methods Enzymol.*, 299: 379-389. DOI: 10.1016/S0076-6879(99)99037-7
- Salah, N., N.J. Miller, G. Paganga, L. Tijburg, G.P. Bolwell and C. Rice-Evans, 1995. Polyphenolic flavanols as scavengers of aqueous-phase radicals and as chain-breaking antioxidants. *Arch. Biochem. Biophys.*, 322: 339-346. DOI: 10.1006/abbi.1995.1473
- Sgherri C., F. Navari-Izzo, A. Pardossi, G.P. Soressi and R. Izzo, 2007. The influence of diluted seawater and ripening stage on the content of antioxidants in fruits of different tomato genotypes. *J. Agric. Food Chem.*, 55: 2452-2458. DOI: 10.1021/jf0634451
- Sgherri, C., C. Pinzino and R. Izzo, 2011a. The role of dietary chlorophylls: An EPR study on the antioxidant activities of tomato lipid extracts. *Agrochimica*, 55: 249-260.
- Sgherri, C., C. Pinzino, F. Navari-Izzo and R. Izzo, 2011b. Contribution of major lipophilic antioxidants to the antioxidant activity of basil extracts: An EPR study. *J. Sci. Food Agric.*, 91: 1128-1134. DOI: 10.1002/jsfa.4296
- Sgherri, C., M.F. Quartacci, R. Izzo and F. Navari-Izzo, 2002. Relation between lipoic acid and cell redox status in wheat grown in excess copper. *Plant Physiol. Biochem.*, 40: 591-597. DOI: 10.1016/S0981-9428(02)01421-3
- Sgherri, C., S. Cecconami, C. Pinzino, F. Navari-Izzo and R. Izzo, 2010. Levels of antioxidants and nutraceuticals in basil grown in hydroponics and soil. *Food Chem.*, 123: 416-422. DOI: 10.1016/j.foodchem.2010.04.058
- Sgherri, C.L.M. and F. Navari-Izzo, 1995. Sunflower seedlings subjected to increasing water deficit stress: Oxidative stress and defence mechanisms. *Physiol. Plant.*, 93: 25-30. DOI: 10.1034/j.1399-3054.1995.930105.x
- Zheng, Y., M. Dixon and P.K. Saxena, 2006. Greenhouse production of *Echinacea purpurea* (L.) and *E. angustifolia* using different growing media, NO₃⁻/NH₄⁺ ratios and watering regimes. *Can. J. Plant Sci.*, 86: 809-815. DOI: 10.4141/P05-16