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Use of an electrochemical method to evaluate the antioxidant activity of herb extracts from the Labiatae family

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Abstract

This paper describes a rapid method for the evaluation of the antioxidant activity of herb extracts from the Labiatae family. Herb methanolic extracts were analysed by using a flow injection (FI) system with an electrochemical detector equipped with a glassy carbon working electrode operating amperometrically at a potential of 0.5 V (vs. Ag/AgCl).

Results obtained on extracts were compared to those obtained by using 2,2-diphenyl-1-picrylhydrazyl (DPPH·) test. A good correlation between the two methods was found ($y = 1.3726x - 0.2266$, $r = 0.9856$). The samples were also submitted to screening for their total phenol content.

Keywords: Herb extracts; Antioxidant activity; FI; Electrochemical method; Voltammetric experiment; DPPH

1. Introduction

Oxidation is one of the most important processes of food deterioration since it may affect food safety, colour, flavour and texture. Antioxidants may protect food quality by preventing oxidative deterioration. In recent years, the restriction in the use of synthetic antioxidants, such as BHA and BHT, has caused an increased interest towards natural antioxidant substances (Ames, 1983; Baardseth, 1989; Branen, 1975). The antioxidants contained in foods, especially vegetables, are phenolic compounds (phenolic acids and flavonoids) that are important protective agents for human health (Block, Patterson, & Subar, 1992; Gillman et al., 1995;Vinson, Yong, Su, & Zubik, 1998). Their antioxidant property is related to the chemical structure, in particular to the electronic delocalisation on the aromatic nucleus. During the reaction of these compounds with free radicals, new radical species are generated and stabilised by resonance.

Herbs, notably those from the Labiatae family, are well-known for their antioxidant properties and in recent years their extracts have been used as antioxidants in food industry (Chevalleau, Mallet, Gamisans, & Gruber, 1992; Chipault, Mizumo, & Lundberg, 1956; Cuvelier, Richard, & Berset, 1996; Gerhardt & Scröter, 1983; Kim, Kim, Kim, Kim, & Jung, 1994; Löliger, 1989; Schuler, 1990).

The total antioxidant activity of herbs has been evaluated by various assays, but a comparison of the results is often impossible because of the different experimental methods adopted. Antioxidant activities of pure compounds and plant extracts have been determined by an accelerated test (Bendini, Toschi, & Lercher, 2001; Kahkonen et al., 1999), by using radical species such as ABTS· (Cano, Hernàndez-Ruiz, Garcia-Canovas, Acosta, & Arnao, 1998) and DPPH· (Brand-Williams, Cuvelier, & Berset, 1995; Sanchez-Moreno, Larrueri, & Saura-Calixto, 1998), by ESR spin trapping technique and by measuring the oxygen consumption in a

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heterogeneous lipid/water emulsion with lipid oxidation initiated by metamyoglobin (Madsen, Nielsen, Bertelsen, & Skibsted, 1996). However, all these procedures present some drawbacks since they require the use of specific reagents and tedious and time consuming sample preparation.

In previous works, an electrochemical method for the determination of the antioxidant power of wines and oils was reported (Mannino, Brenna, Buratti, & Cosio, 1998; Mannino, Buratti, Cosio, & Pellegrini, 1999).

In this paper, we introduced a similar scheme for the determination of the antioxidant activity of herbs used in food formulations. The proposed procedure offers distinct advantages over existing methods since it is rapid, simple and based on the chemico-physical properties of the molecules. The results of the proposed procedure were compared with those obtained by the DPPH· test and with the phenolic content.

2. Materials and methods

2.1. Herb materials

Dried herbs, purchased at local supermarkets, were ground and stored at 20 °C. The herbs included rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.), thyme (*Thyme vulgaris* L.), oregano (*Origanum vulgaris* L.), basil (*Ocinum basilicum* L.), peppermint (*Mentha piperita*), laurel (*Laurus nobilis* L.).

2.1.1. Preparation of the herb methanolic extracts

About 0.25 g of dried herbs were introduced into a centrifuge tube with 10 ml of 99% methanol from BDH (Poole, England) and stirred slightly. The suspension was sonicated for 5 min and centrifugated at 1500 rpm for 10 min; then the supernatant was removed. Herbs were extracted twice and supernatant was evaporated under vacuum at room temperature. These concentrated extracts were weighed, diluted with methanol and stored at −20 °C prior to analysis.

2.2. Electrochemical method

2.2.1. Standard preparation

All standard compounds were purchased from commercial sources: rosmarinic acid and carnosic acid from Chromadex (Santa Ana, USA), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, 97%), thymol (98%), caffeic acid, quercetin and hesperitin, from Sigma–Aldrich (Chemie GmbH, Steinheim, Germany), galangine and kaempferol from Extrasynthese (Genay, France), ferulic and *p*-cumaric acid (98%) from Fluka Chemie GmbH CH –9471 Buchs.

All standards, accurately weighed, were dissolved in 10 ml of methanol and stored at −20 °C. Prior to analysis, standards were adequately diluted with carrier solution.

2.2.2. Sample preparation

Before analysis, methanol extracts were simply diluted with carrier solution. Results are expressed as trolox equivalent (mg of trolox/g of dried weight).

2.2.3. Operating procedure

The flow injection (FI) equipment consisted of a Jasco model 880 PU pump (Tokio, Japan) and an EG&G Princeton Applied Research model 400 thin layer electrochemical detector (Princeton, NJ, USA) equipped with a single glassy carbon electrode (surface area 8 mm^2) operating at a potential of +0.5 V, a reference (Ag/AgCl, satured) electrode and a platinum counter electrode. Data were recorded using a Philips (Eindhoven, Netherlands) PM 8252 recorder. The connecting tubes were of peek (1.5 mm od × 0.5 mm id). The detailed description of the system configuration has been reported elsewhere (Mannino et al., 1999). The FI experiments were performed at room temperature using a carrier solution constituted by methanol 70%, sodium acetate–acetic acid buffer (0.1 M, pH 4) 28%, sodium perchlorate monohydrate 2% Merck (Darsmstadt, Germany). A flow rate of 1 ml/min was employed.

Hydrodynamic voltammograms were carried out in order to investigate the electrochemical behaviour of phenolic compounds identified in herbs. The hydrodynamic voltammograms were obtained by running a series of FI experiments in which the potential was stepped incrementally from +0.1 to +0.8 V (vs. Ag/AgCl) and the current was measured.

2.3. Voltammetric experiments

Cyclic voltammetric experiments were performed in methanol 70%, sodium acetate–acetic acid buffer (0.1 M, pH 4) 28%, sodium perchlorate monohydrate 2%. Standard solutions of phenolic compounds (3.2×10^{-5} M) identified in herbs were submitted to analysis immediately after their preparation. Voltammetric measurements were carried out using a conventional three electrode system consisting of a glassy carbon working electrode, platinum auxiliary electrode and Ag/AgCl reference electrode. Cyclic voltammograms were acquired with a Model 270 Electrochemical Analysis System from PAR at a scan rate of 100 mV s^{-1}.

2.4. Antioxidant activity (DPPH· radical-scavenging activity)

The antioxidant activity of herb methanolic extracts was determined using DPPH· (2,2-diphenyl-1-picrylhydrazyl, free radical, 95%, Sigma–Aldrich) according to Brand-Williams et al. (1995). Different

concentrations of herb methanolic extracts were added to 3.9 mL of a 6.1×10^{-5} DPPH· methanolic solution. The initial DPPH· concentration ($C_{DPPH\cdot}$) in the reaction medium was calculated by a calibration curve ($Abs_{515} = 11771 \times (C_{DPPH}) + 0.01$). The bleaching of DPPH· was monitored at 515 nm (Spectrophotometer Uvidec-610, Jasco, Tokyo, Japan) for 30 min. Results were reported as $1/IC_{50}$, where IC_{50} was defined as the extract concentration (g dried weight) necessary to decrease the initial DPPH· concentration of 50% and was extrapolated from a dose–response curve. Tests were carried out in triplicate.

2.5. Determinations of phenolics

Total phenolics of herb extracts were determined by the Folin-Ciocolteau reagent according to Shingleton and Rossi (1965), using gallic acid as standard. Results were expressed as mg of gallic acid equivalents (GAE)/g of dried weight (dw).

3. Results and discussion

The antioxidant activity of natural compounds depends not only on the amount of specific molecules present in the matrix but also on structural factors, such as the number and positions of phenolic hydroxyl or methoxyl groups, etc. (Larson, 1988; Shahidi & Wanasundara, 1992). Considering that the electrochemical behaviour of these compounds depends on their structural features, useful information on their antioxidant functionality can be drawn.

For this purpose cyclic voltammetry (CV) experiments for each analyte of interest (carnosic acid, caffeic acid, rosmarinic acid, thymol, galangine, quercetin, kaempferol and hesperitin) were performed. These compounds were chosen on the basis of the data reported in literature about their presence in the herbs under study. In Fig. 1, the chemical structures and the cyclic voltammograms of all these standard compounds are shown. Some phenolics typical of aromatic plants, such as carnosic acid, caffeic acid, rosmarinic acid, quercetin, kaempferol and hesperitin, are reversible species and show a very low oxidation potential. This behaviour, which is in relation with their molecular structure, clearly shows their good antioxidant property. Redox potentials of the species considered are summarised in Table 1. Concerning the flavonols quercetin and kaempferol, the –OH groups present on the B ring are mainly responsible for their antioxidant capacity. In kaempferol and quercetin, the –OH groups present on the B ring are responsible for the first oxidation peak. On the contrary galangine does not present any redox system on the B ring and the first oxidation peak is due to the conjugate system present in the C ring (formed by –OH and –C=O groups). The oxidation peak at +530 mV of quercetin is probably due to the formation of an intermediate radical produced during the oxidation process. In all flavonols, the highest oxidation peak is due to the –OH groups present in the meta position of the A ring. In flavanones, such as hesperitin, the absence of –OH group and double bond in the C ring caused a decrease of their antioxidant power and the peak at +560 mV can be attributed only to the –OH groups present on A ring. In caffeic and rosmarinic acids the oxidation peaks at low potentials (+540 and +620 mV, respectively) is due to the presence of –OH groups in *ortho* position. All these results are in accordance with pioneering works, that studied the structure–activity relationship of natural antioxidants (Bors, Heller, Michel, & Saran, 1990; Rice-Evans, Miller, & Paganga, 1996).

In order to determine the optimal operating potential used in the FI system, hydrodinamic voltammograms experiments were generated using a flow cell amperometric detector. Hydrodynamic voltammograms, obtained by plotting the detector response (peak current) versus the applied potential, are reported in Fig. 2, where it can be seen no compounds were detected at a potential equal or lower than +0.2 V (vs. Ag/AgCl). The exception is carnosic acid, that can be classified as the compound with the highest antioxidant activity. Carnosic acid is a diterpene with two orthophenolic functions and one isopropyl group on the adjacent carbon. Rosmarinic acid and quercetin can be detected at +0.3 V, while thymol, hesperitin and ferulic acid are oxidisable at a potential greater than +0.6 V. Consequently the last three compounds can be classified as having the lowest antioxidant activity.

On the basis of the hydrodynamic voltammogram results and considering that the oxidation potential of a compound provides an estimate of its antioxidant activity, an operating potential of +0.5 V (vs. Ag/AgCl) was selected. It appears to be selective discriminating only the compounds having high reducing capacity hence with effective antioxidant activity. Fig. 3 shows the antioxidant activity, which decreases in the order carnosic acid > quercetin > rosmarinic acid > kaempferol > caffeic acid > galangin. Ferulic acid, thymol, *p*-cumaric acid and hesperitin have very little or no antioxidant effect.

Fig. 4(a) displays typical flow injection peaks, obtained for increasing concentration of trolox used as standard. As shown, the detector responds rapidly to the dynamic changes in the trolox concentration, allowing about 60 determination/h. These measurements are part of a calibration experiment over the 0.5 and 8 mg/l concentration. The resulting calibration plot is highly linear (slope 0.4725 μA/mg/l, intercept 0.0478 μA). The relative standard deviation at the

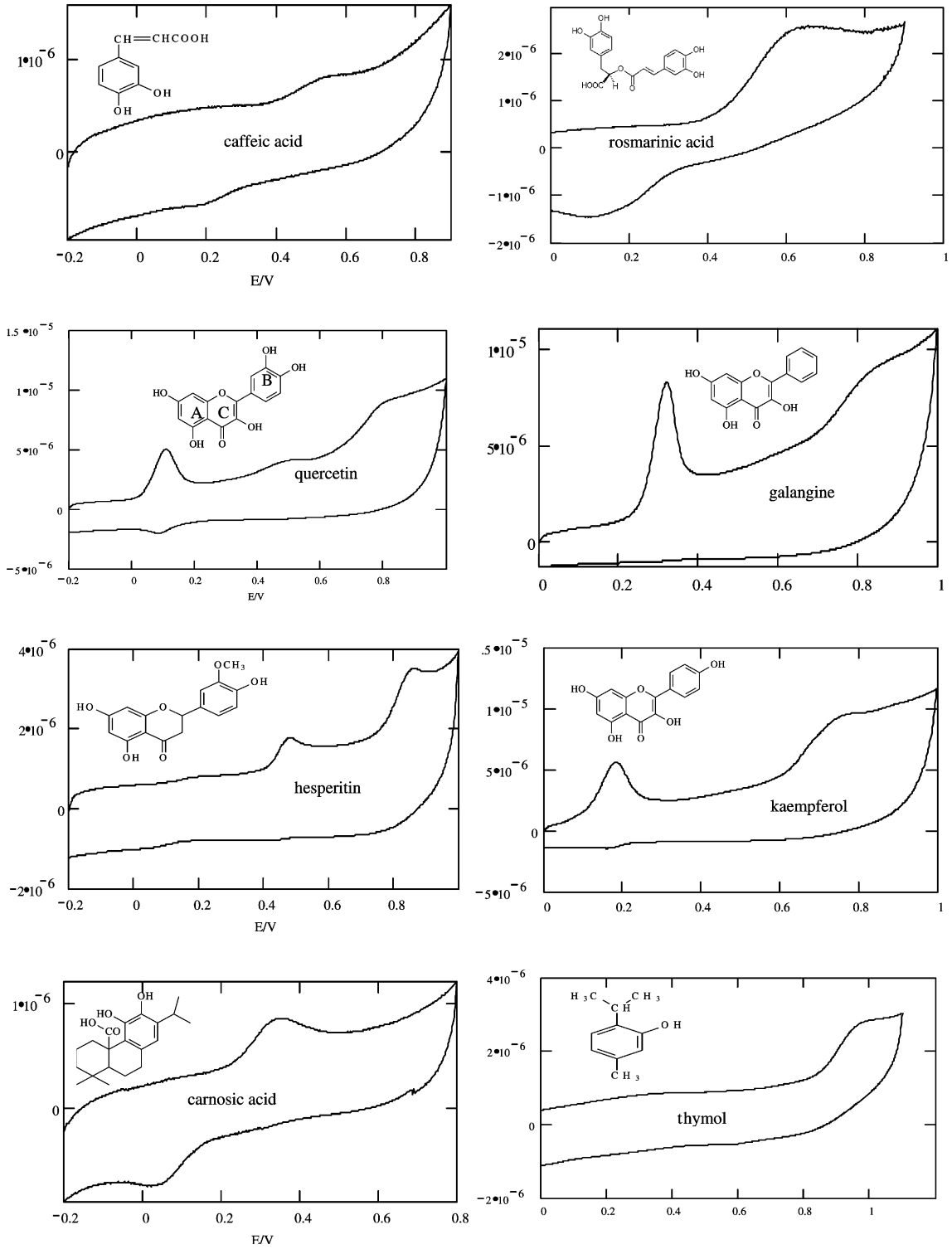

Fig. 1. Cyclic voltammograms of standard compounds. Operative conditions: concentration of each compound 3.2×10^{-5} M; solution, methanol 70%, sodium acetate–acetic acid buffer (0.1 M pH 4.5) 28% sodium perchlorate monohydrate 2%; Scan rate 100 mV s^{-1}.

concentration level of 4 mg regression technique is 0.14 mg/l (Miller & Miller, 1988).

To evaluate the accuracy of the proposed method, a series of recovery experiments, in which caffeic acid or quercetin was added directly to samples, was performed. Recovery results were satisfactory and ranged from 100% to 102%.

Herb methanolic extracts were analysed with the proposed method and the results were expressed as mg of trolox equivalents/g of dried weight. Typical ampero-

Table 1
Redox potentials of various standard compounds analysed by cyclic voltammetry (CV) experiments

Compounds	Oxidation potential (mV)	Reduction potential (mV)
Caffeic acid	540	180
Ferulic acid	600	–
p-Cumaric acid	850	–
Quercetin	108, 530, 806	88
Kaempferol	192, 770	158
Galangine	318, 840	–
Hesperitin	478, 860	–
Thymol	970	–
Rosmarinic acid	620	130
Carnosic acid	350	60

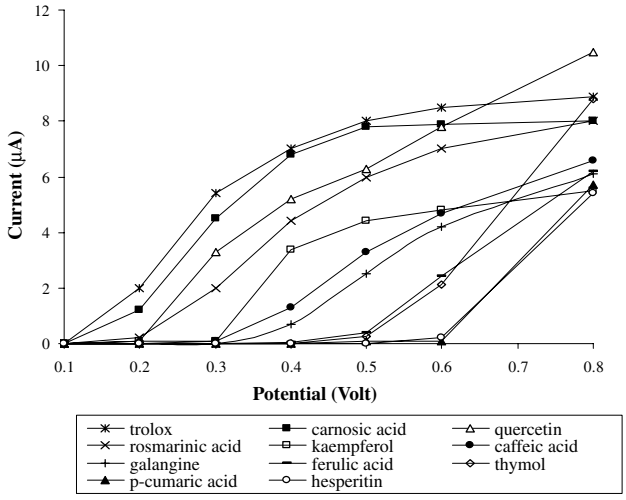

Fig. 2. Hydrodinamic voltammetric profiles of phenolic compounds. Operative conditions: standard concentrations, 3.2×10^{-5} M; carrier solution, methanol 70%, sodium acetate–acetic acid buffer (0.1 M pH 4.5) 28% sodium perchlorate monohydrate 2%; injection volume, 20 μl; flow rate 1.0 ml min^{-1}.

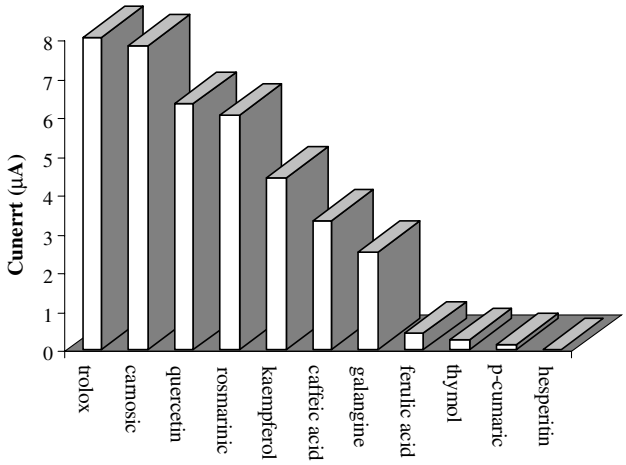

Fig. 3. Current responses of phenolic compounds injected in the FI system with the electrochemical detector operating with at the established potential of +0.5 V (vs. Ag/AgCl). Operative conditions as in Fig. 2.

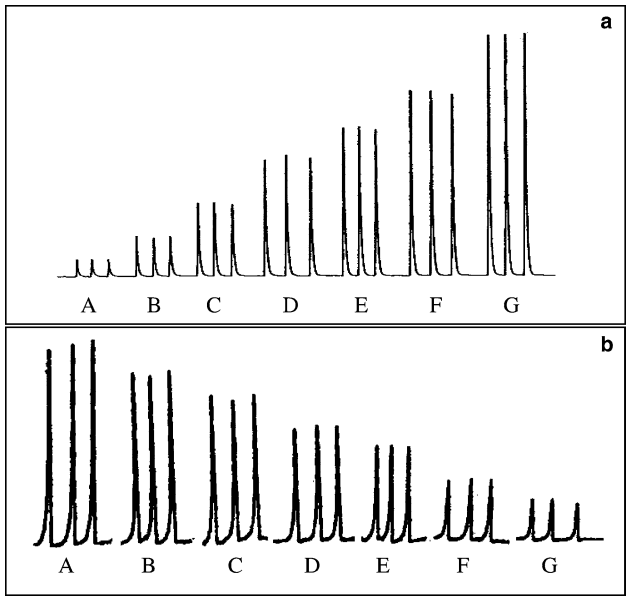

Fig. 4. (a) Flow injections peaks for increasing concentrations of trolox. Concentration (mg equivalents of trolox/l): A (0.5), B (1), C (2), D (3), E (4), F (5), G (6). (b) Flow injection responses to dried herbs from rosemary (A), sage (B), oregano (3), thyme (4), laurel (5), peppermint (6), basil (7). Methanolic extracts were diluted to 20 μg/ml in carrier solution prior to injection. Conditions: operating potential, 0.5 V; carrier solution, methanol 70%, sodium acetate–acetic acid buffer (0.1 M pH 4.5) 28% sodium perchlorate monohydrate 2%; injection volume, 20 μl; flow rate 1.0 ml min^{-1}.

metric responses at the established potential of +0.5 V of herb extracts are shown in Fig. 4(b). In Table 2, the antioxidant activity of herb extracts, determined electrochemically and by DPPH· assay, are reported. The data obtained by the electrochemical method were substantially confirmed by the DPPH· method. The two methods are highly correlated ($P > 99.9\%$), as shown in Fig. 5. Among the analysed herbs, rosemary and sage exhibit the highest antioxidant power as shown in Table 2; this was probably due to high content of rosmarinic acid, carnosic acid and its derivatives (Nakatani & Inatani, 1984). The antioxidant activity of the other herbs decreases in the following order: oregano > thyme > peppermint > laurel > basil. Other studies confirmed that many leafy herbs, especially those of the Labiatae family such as sage, rosemary, oregano and thyme, show strong antioxidant activity (Schwarz & Ternes, 1992a, 1992b). In Table 2 the total phenolic content of herbs is also reported, the content varying from 10.2 to 35.1 mg GAE/g of dry weight. The results showed that the antioxidant activity was not necessarily correlated with phenolic content in fact, rosemary and sage have a high total phenolic content and a high antioxidant power, while other herbs, such as laurel and basil, have a high phenolic content and a low antioxidant power. However, the relationship between the total phenolic content and antioxidant activity evaluated by the proposed method show a good correlation ($P > 99.9\%$) (Fig. 6).

Table 2
Antioxidant activity (average ± SD) of herb extracts using the electrochemical method (EM) and the DPPH· method; phenol content with Folin method

Dried herbs	EM (mg trolox equivalents/g dw)[a,b]	Folin (mg gallic acid equivalents/g dw)[a]	DPPH· test $1/IC_{50}$ (mol DPPH·/g dw)[a]
Rosemary 1	18.0 ± 1.4	32.0 ± 1.4	13.8 ± 2.5
Rosemary 2	16.1 ± 1.2	27.5 ± 1.9	11.0 ± 2.3
Rosemary 3	19.2 ± 1.5	35.1 ± 1.7	14.1 ± 2.6
Rosemary 4	16.4 ± 1.2	29.5 ± 1.8	12.8 ± 2.5
Sage 1	15.0 ± 1.1	26.0 ± 0.8	11.5 ± 2.1
Sage 2	14.0 ± 0.8	23.2 ± 0.5	10.0 ± 2.0
Sage 3	14.3 ± 1.2	24.0 ± 0.6	9.9 ± 2.0
Sage 4	16.1 ± 1.1	25.6 ± 0.6	12.0 ± 2.4
Oregano 1	12.1 ± 0.5	24.0 ± 0.2	8.0 ± 1.9
Oregano 2	10.4 ± 0.4	22.1 ± 0.1	6.9 ± 1.8
Oregano 3	10.1 ± 0.3	18.4 ± 0.1	7.9 ± 1.6
Thyme 1	5.5 ± 0.8	12.1 ± 1.1	4.8 ± 1.2
Thyme 2	8.0 ± 0.6	14.1 ± 1.2	6.1 ± 1.1
Thyme 3	7.0 ± 0.4	11.6 ± 0.9	4.8 ± 1.0
Laurel 1	5.0 ± 0.7	18.2 ± 1.1	4.4 ± 0.9
Laurel 2	4.9 ± 0.6	16.0 ± 0.9	4.6 ± 0.5
Laurel 3	4.7 ± 0.6	19.1 ± 1.3	4.1 ± 0.6
Laurel 4	4.9 ± 0.6	20.1 ± 1.5	5.0 ± 0.8
Sweet Basil 1	2.6 ± 0.5	12.0 ± 1.1	2.5 ± 0.5
Sweet Basil 2	2.9 ± 0.4	11.0 ± 0.9	2.4 ± 0.6
Sweet Basil 3	2.6 ± 0.5	12.2 ± 0.8	2.1 ± 0.3
Peppermint 1	5.2 ± 0.3	12.3 ± 1.2	3.6 ± 0.9
Peppermint 2	5.7 ± 0.2	13.3 ± 1.1	3.1 ± 1.2
Peppermint 3	6.1 ± 0.2	14.3 ± 1.4	3.8 ± 1.3

[a] dw, dry weight.
[b] EM, electrochemical method.

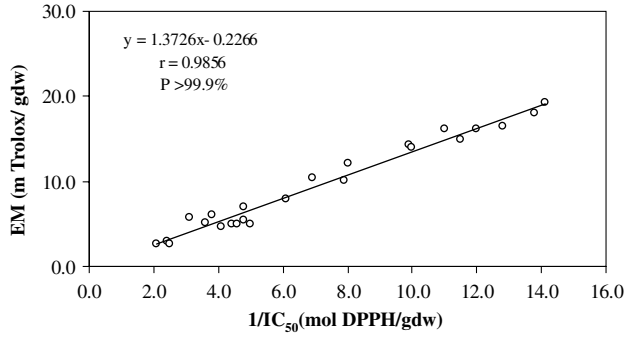

Fig. 5. Correlation between DPPH· ($1/IC_{50}$) and electrochemical methods (EM) for the analysis of antioxidant activity.

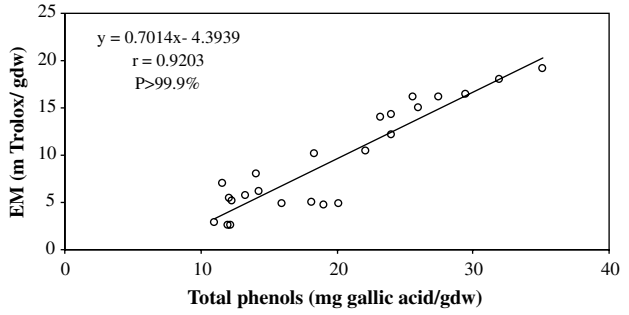

Fig. 6. Correlation between total phenols (Folin) and antioxidant activity (EM).

4. Conclusions

Results suggest that the electrochemical method can be successfully employed for the direct, rapid and reliable monitoring of antioxidant activity in herb extracts. The rapidity of the analysis (60 determination h^{-1}), which makes the flow system an attractive alternative over other reported methods, is of particular interest. Furthermore, the proposed method is based only on the chemical–physical properties of the molecules and does not require the use of reactive species. The capability of the method to rapidly evaluate the antioxidant activity of herbs could be useful for industry in the formulation of herb mixtures to be used in product development. In addition, considering that our results are in good agreement with those obtained by the mostly used DPPH· test, the proposed method can be adopted confidently with a minimum sample pretreatment.

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