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Original article

New insights into the antioxidant activity of hydroxycinnamic acids: Synthesis and physicochemical characterization of novel halogenated derivatives

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ABSTRACT

An interdisciplinary research project was developed combining the synthesis of a series of hydroxycinnamic acid derivatives and the evaluation of their physicochemical parameters (namely redox potentials and partition coefficients), along with the corresponding antioxidant activity. A structure–property–activity relationship (SPAR) approach was then applied aiming at establishing a putative relation between the physicochemical parameters of the compounds under study and their antioxidant activity.

The results gathered allow concluding that the redox potentials could contribute to the understanding of the antioxidant activity and that the presence of an electron withdrawing group (EWG) of halogen type, namely a bromo atom, in an *ortho* position to a phenolic group of the cinnamic scaffold does not influence the antioxidant activity. On the other hand after the introduction of this type of substituent a significant increase on the lipophilicity of cinnamic derivatives was observed, which is a feature of extreme importance in the development of novel lipophilic antioxidants.

The SPAR results revealed a relation between the redox potentials and the antioxidant activity of hydroxycinnamic acids and derivatives. The data obtained operate as a positive reinforce of the tendency to use redox properties as a guideline of the rational design of this type of compounds.

1. Introduction

Antioxidants, used to prevent or inhibit the natural phenomena of oxidation, have a broad application in diverse industrial fields as they have a huge importance either as industrial additives or health agents [1,2]. Research data have revealed that they could be suitable for preventive and/or therapeutic purposes in several diseases related with oxidative stress (*e.g.* atherosclerosis, inflammatory injury, cancer and cardiovascular diseases) [3–7]. Despite the fact that many natural and synthetic compounds display antioxidant activity, the list of authorized antioxidant additives still correspond to an incredibly restricted one, due mainly to solubility or toxicity problems [8]. Although the quest for new phenolic antioxidants is emergent many questions regarding primarily the mechanism underlying their protective action are not completely understood yet.

Phenolic acids, particularly hydroxycinnamic acids (HCA) such as caffeic, ferulic and sinapic acids and flavonoids have been considered as attractive potential antioxidants due to their natural origin [9–11]. Some results evidence that their antioxidant activity is strongly dependent on their structural features and intrinsically related to the presence of hydroxyl function(s) in the aromatic structure [12–16]. This type of phenolic compounds often acts through its radical-scavenging activity that is linked to their hydrogen- or electron-donating ability and to the stability of the resulting phenoxyl radicals [17–19]. However, other mechanisms of action have been suggested such as chelation of transition metals, like copper or iron, which are well-known catalysts of oxidative stress [20,21]. The knowledge gathered from this type of mechanistic studies could lead in a near future to the rational design of new and more effective antioxidants. Generally, phenolic acids work well in aqueous media being their hydrophilic nature a restriction for lipophilic systems protection (*e.g.* food or cosmetic

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matrix or biological membranes) due mainly to the difficulty of their incorporation into fat and oil matrices [22].

An interdisciplinary project was developed aiming a more reliable understanding of the structure–property–activity relationships (SPAR) that underlie the antioxidant activity of several hydroxycinnamic acids (Table 1). With this aim, halogenated derivatives of hydroxycinnamic acids, such as 5-bromoferulic acid (*trans*-3-(5-bromo-4-hydroxy-3-methoxyphenyl)-2-propenoic acid) (2) and 5-bromocaffeic acid (*trans*-3-(5-bromo-3,4-dihydroxyphenyl)-2-propenoic acid) (3) and the corresponding ethyl esters, ethyl 5-bromoferulate (*trans*-ethyl 3-(5-bromo-4-hydroxy-3-methoxyphenyl)propenoate) (4); ethyl 5-bromocaffeate (*trans*-ethyl 3-(5-bromo-3,4-dihydroxyphenyl)propenoate) (5) were synthesized and some biological relevant physicochemical parameters, such as redox potentials (E_p) and partition coefficients (Log *P*), were evaluated.

In addition, the antioxidant activity of the synthesized compounds was evaluated using total antioxidant capacity radical assays (DPPH• - 2,2'-diphenyl-1-picrylhydrazyl radical; ABTS - 2-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid). Among the different published methodologies for determining the antiradical activity, both isolated compounds and complex mixtures, these radical assays have been widely used due to their simplicity and rapidity [23,24]. The antiradical properties obtained for the halogenated hydroxycinnamic derivatives were compared with those acquired for the lead natural compounds (caffeic and ferulic acids) and their corresponding ethyl esters.

2. Results and discussion

2.1. Chemistry

Cinnamic acid derivatives were synthesized straightforward either by a Knoevenagel-type condensation or by Fisher acid catalysis esterification [6,9,12–14]. The hydroxycinnamic acids and esters were synthesized by reaction between the corresponding benzaldehydes, with the same aromatic substitution pattern of the final product, and malonic acid or monoethylmalonate, using aniline as catalyst. The appropriate substituted benzaldehydes were obtained by bromination of vaniline (Br_2 solution in acetic acid) [25] followed by *O*-demethylation with boron tribromide (BBr_3) (Scheme 1) [26]. These reactions led to moderate/high yields of the desired compounds, which were identified by spectroscopic techniques: NMR (^{1}H NMR, ^{13}C NMR), FT-IR and MS-EI. The synthetic strategies of the synthesized compounds are depicted in Scheme 1.

2.2. Electrochemical measurements

Voltammetric methods have been often applied to characterize a diversity of natural phenolic antioxidants, including flavonoids, and synthetic antioxidants mainly to get insight their mechanism [27,28].

Accordingly, in this work the redox potentials of the novel synthesized compounds were measured and their electrochemical behaviour compared with that obtained for the lead compounds (caffeic and ferulic acids). The redox potentials of the synthesized halogenated hydroxycinnamic derivatives at physiological pH 7.3 were obtained, at a glassy carbon working electrode, using differential pulse and cyclic voltammetry.

For caffeic and 5-bromocaffeic (3) acids and the corresponding ethyl esters (dihydroxylated cinnamic series – see Table 1) one well-defined anodic peak was observed at physiological pH using differential pulse voltammetry (Fig. 1). The oxidation peaks of these cinnamics compounds occurred at $E_p = +0.183$ V, $E_p = +0.182$ V for the acids and $E_p = +0.175$ V and $E_p = +0.174$ V for the esters. These waves are intrinsically related with the oxidation of catechol group.

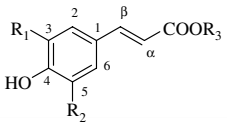
Cyclic voltammograms were also recorded at different sweep rates. The cyclic voltammograms obtained are characteristic of an electrochemical reversible reaction showing only one anodic peak and one cathodic peak on the reverse scan (Fig. 2). Linear plots of peak current (I_p) as a function of the square root of scan rate (ν) were obtained indicating that the oxidation processes are diffusion controlled [29]. The ratio of the anodic to cathodic peak heights raises gradually with increasing potential scan rate from 10 to 100 mV s^{-1} and the calculated current functions ($I_p\nu^{-1/2}$) diminish gradually with the potential scan rate, showing the classical behaviour of an oxidation process coupled with a slow subsequent chemical reaction. All the results are in agreement with the literature which concerns the oxidative behaviour of the lead compounds [30,31]. In fact, electrochemical studies on the caffeic acid oxidation mechanism have shown that the first oxidation step involves two electrons *per* molecule which likely correspond to the formation of the caffeic acid *o*-quinone, an intermediate that is quickly decomposed at pH higher than 7.4 [30–32].

The differential pulse voltammetric study of monohydroxycinnamic acids, ferulic and 5-bromoferulic (2) acids (Table 1), revealed the presence of two convolved anodic peaks at physiological pH (Fig. 3). These oxidation peaks at $E_p = +0.335$ V, $E'_p = +0.447$ V and $E_p = +0.335$ V, $E'_p = +0.442$ V, respectively, are related with the oxidation of the phenolic group present in the structures. The results may be interpreted by assuming that the oxidation of cinnamic acids takes place by electron transfer for both free and adsorbed forms. The free form corresponds to the first peak, E_p, while the strongly adsorbed form, which is consequently stabilized, is oxidized at a more anodic potential, E'_p. The appearance of an adsorption peak, E'_p, is also pointed out by other authors, namely in the electrochemical studies involving ferulic acid and phenol in gold and platinum electrodes [33,34].

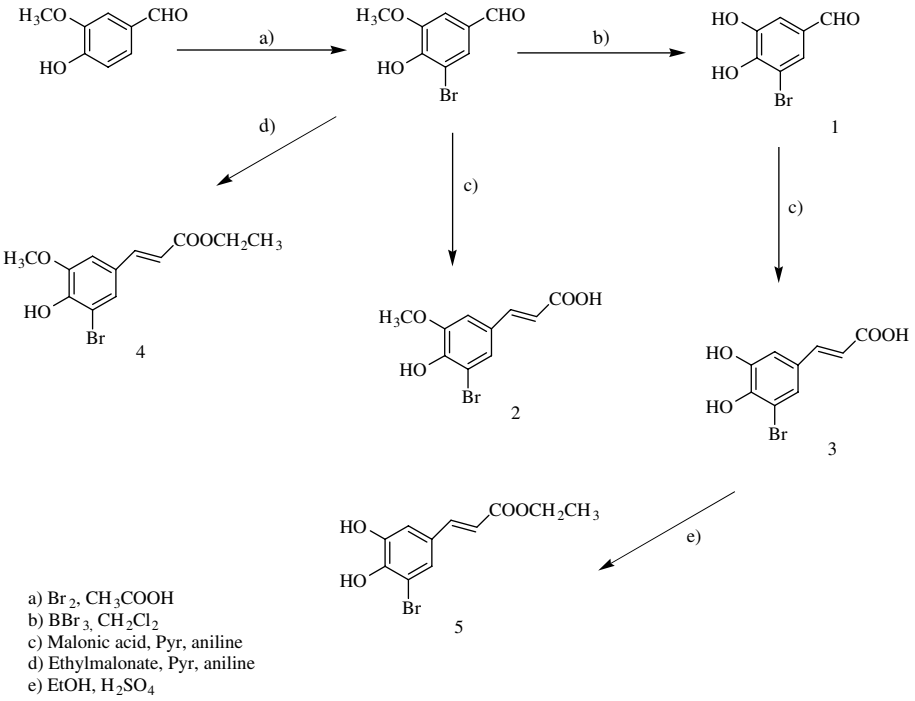
For ethyl ferulate and 5-bromoferulate (4) only one anodic wave can be observed at physiological pH using differential pulse voltammetry (Fig. 3). These oxidation peaks at $E_p = +0.368$ V and $E_p = +0.365$ V, respectively, are related with the oxidation of the phenolic group present in the structure. The large wave shape observed could be a sign of a higher superimposition of the peaks from both free and adsorbed forms or might indicate a lesser adsorption propensity of this molecules. From the cyclic voltammetric studies we can conclude that the former is more likely.

Cyclic voltammograms were recorded at different sweep rates. The cyclic voltammograms obtained for ferulic and 5-bromoferulic (2) acids have also shown two convolved anodic peaks. For ethyl ferulate and 5-bromoferulate (4) only one anodic wave is seen. All these anodic peaks appear to correspond to irreversible processes, as no wave is observed in the reverse scan (Fig. 2). Plots of peak current (I_p) as a function of the square root of scan rate (ν) gave a straight line indicating that the oxidation processes are diffusion controlled [29]. The assembled data corroborate the results found

Table 1
Hydroxycinnamic acids and their alkyl esters under study.



Hydroxycinnamic compounds	R_1	R_2	R_3
Caffeic acid	OH	H	H
Ferulic acid	OCH_3	H	H
5-Bromocaffeic acid (3)	OH	Br	H
5-Bromoferulic acid (2)	OCH_3	Br	H
Ethyl caffeate	OH	H	CH_2CH_3
Ethyl ferulate	OCH_3	H	CH_2CH_3
Ethyl 5-bromocaffeate (5)	OH	Br	CH_2CH_3
Ethyl 5-bromoferulate (4)	OCH_3	Br	CH_2CH_3
Sinapic acid	OCH_3	OCH_3	H



Scheme 1. Synthetic strategies used for the obtention of bromocinnamic acids and derivatives.

in literature regarding ferulic acid oxidative behaviour. The proposed mechanism involves a one-electron transfer from the phenolate ion followed by one irreversible dimerisation process due to a radical–radical coupling reaction between two phenoxyl radicals [30,33].

The voltammetric data obtained for hydroxycinnamic acids under study appear to be in agreement with the general mechanism proposed for the oxidation of a phenolic group [35]. The results indicated that the higher the number of hydroxyl substituents on the aromatic ring, the lower the electrochemical potential. The shift of redox potential values indicates an increase in the nucleophilicity of the compound and that its antioxidant activity is thermodynamically favored.

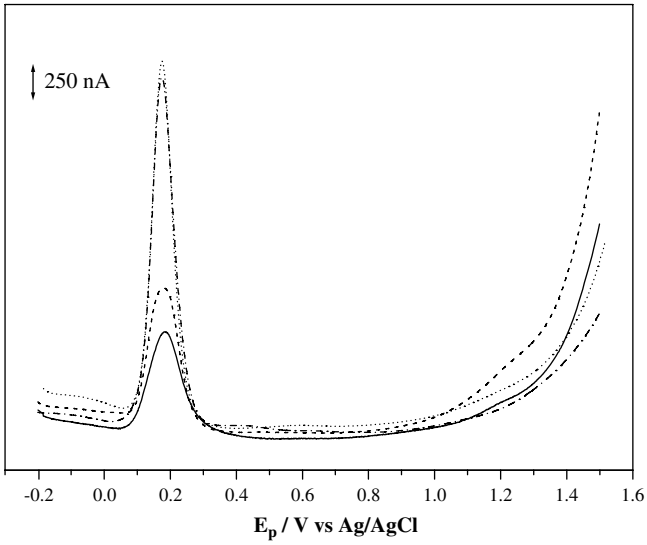


Fig. 1. Differential pulse voltammograms for 0.1 mM solutions of (—) caffeic acid, (——) 5-bromocaffeic acid, (…) ethyl caffeate and (-·-·-) ethyl 5-bromocaffeate, in physiological pH 7.3 supporting electrolyte. Scan rate: 5 mV s^{-1}.

The presence of a catechol moiety in cinnamic derivatives is known to cause a decrease of the redox potential due to additional resonance stabilization and *o*-quinone formation. This fact seems to explain that redox potential of caffeic acid derivatives are lower (and consequently antioxidative efficiency are greater) than those of ferulic acid derivatives. Actually, the methoxylation of the *meta*-phenolic group of the cinnamic derivatives (ferulic acid derivatives) increases twice the peak potential.

The introduction of an alkyl ester group in the conjugated side chain or a bromide group in position-5 did not significantly change the oxidation potentials, when compared to the lead compounds. The data obtained strongly suggest that alkyl ester group have modest or even no effect on the electron density of the phenol or catechol ring. Hence, the introduction of an alkyl group did not significantly influence the energetic of the electron transfer as can be ascribed from the similarity of the E_p values for these derivatives. With regard to the aromatic substitution it's generally accepted that the introduction of groups that could have a positive influence on the formation and lifetime, through a stabilizing effect, of the corresponding phenoxyl radical would significantly increase the antioxidant activity of the compounds. Therefore, it would be expected that the introduction of a bromide group in position-5 of the phenol or catechol group could affect the formation and/or stabilization of the radical intermediate causing a decrease of the oxidation potential of the lead compounds. The results had showed that this tendency had not occurred, either in free acids or their corresponding esters.

In order to verify this assumption, the voltammetric behaviour of sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid) was also studied at a glassy carbon working electrode using differential pulse voltammetry. Sinapic acid presents two convolved anodic peaks, $E_p = +0.188$ V, $E'_p = +0.295$ V, at physiological pH, using differential pulse voltammetry (data not shown). As expected, the presence of a methoxyl group in position-5 causes a cathodic shift on the oxidation potential, with respect to ferulic acid. This lower potential value results from the increased stabilization of the phenoxyl radical originated by the electron-donating effect of the methoxyl group. So, the similarity of the anodic potentials between

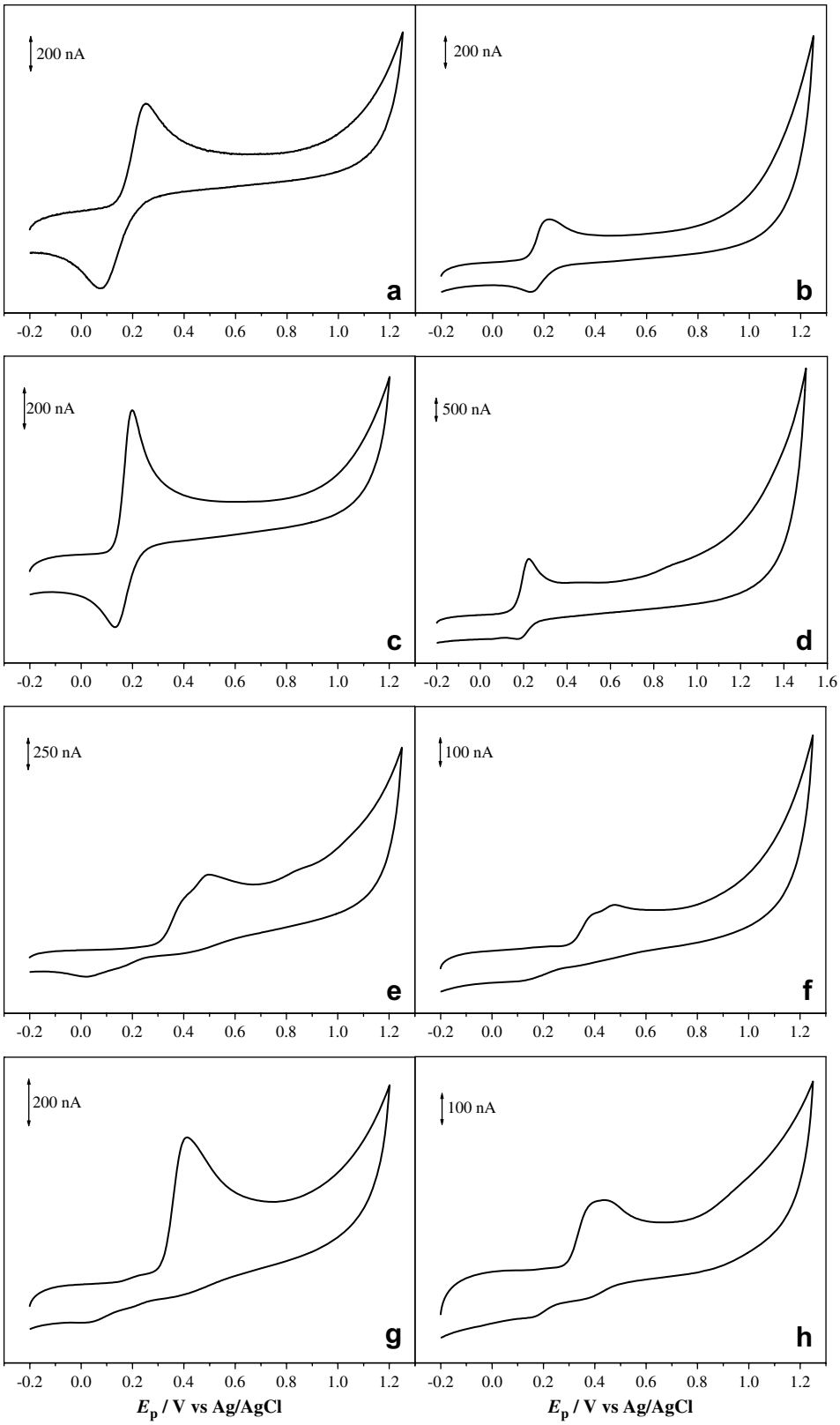


Fig. 2. Cyclic voltammograms for 0.1 mM solutions of (a) caffeic acid, (b) 5-bromocaffeic acid, (c) ethyl caffeate, (d) ethyl 5-bromocaffeate, (e) ferulic acid, (f) 5-bromoferulic acid, (g) ethyl ferulate and (h) ethyl 5-bromoferulate, in physiological pH 7.3 supporting electrolyte. Scan rate: 50 mV s^{-1}.

5-bromoferulic acid (2) and ferulic acid could be interpreted considering the electronic nature differences between the bromide and methoxyl groups. In fact, although these two substituents are σ-acceptors, bromide group is a weaker π-donor than methoxyl group. This small change in substitution pattern could be responsible for the diminishing of the stabilization of the phenoxyl radical and as a result to attain comparable anodic potentials. Further studies were designed using other halogen substituents in order to

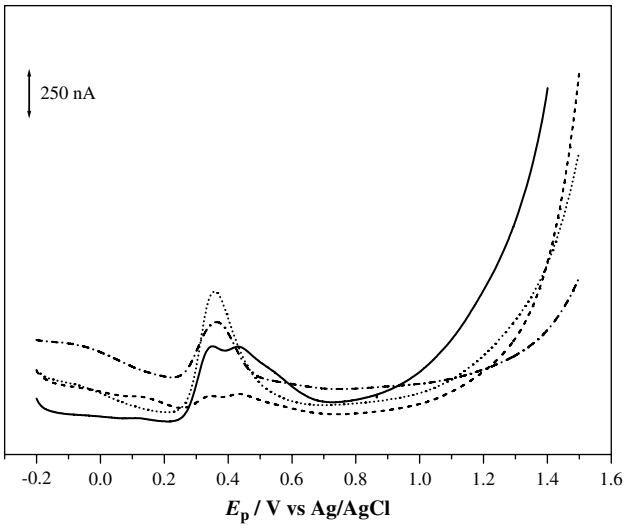


Fig. 3. Differential pulse voltammograms for 0.1 mM solutions of (—) ferulic acid, (——) 5-bromoferulic acid, (…) ethyl ferulate and (-·-·-) ethyl 5-bromoferulate, in physiological pH 7.3 supporting electrolyte. Scan rate: 5 mV s^{-1}.

evaluate their ability to stabilize the phenoxyl radical. Nevertheless, Diniz et al. [36] have shown through quantum chemistry calculations at the B3LYP theory level, together with the 6-31G* basis set, that the O–H bond dissociation energies (BDEO–H) for homolytic cleavage of an hydroxyl group are not significantly affected by the presence in an *ortho* position of any type of halogen (chloro, bromo or fluor). Really, this BDEO–H energy is similar to that one obtained for a phenolic group without substituents. Hence, the same results would be expected for other halogen derivatives. Nitration of aromatic compounds has been extensively reported in recent years due to its valuable biological activity [37]. Although the introduction of a nitro group on the cinnamic scaffold was a plausible hypothesis, literature data show that the O–H bond dissociation energy found for nitro substituted phenols is comparable to that obtained for halogen derivatives [38]. Moreover the Log *P* value obtained for the nitro cinnamic analogues is quite similar to that found for the parent compounds. Hence, little valuable contribution in terms of lipophilicity would be expected for this type of compounds.

From the electrochemical results one can conclude that the structural principles governing the redox potentials of the cinnamic acids and derivatives under study were found to be the presence of a phenolic group, preferentially a catechol, and additional resonance-effective substituents in the aromatic ring.

2.3. Antioxidant activity

In order to evaluate the radical-scavenging ability of the phenolic acids and its ester derivatives (Table 1) total antioxidant capacity assays (DPPH• - 2,2'-diphenyl-1-picrylhydrazyl radical; ABTS - 2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) were used. The DPPH method was carried out in a homogeneous phase and has the advantage of establish a real ranking hierarchy of antioxidant activity of electron- or H-donating agents, since it was not affected by some factors which interfere in other model systems, such as metal chelation or partitioning abilities [9,11]. The generation of the ABTS radical cation form is the basis of other spectrophotometric method that is currently used for the measurement of the total antioxidant activity of solutions of pure substances, aqueous mixtures and beverages [39]. This assay is based in a decolorization technique in which the radical is generated directly in a stable form prior to reaction with putative antioxidants. In this work the technique is based on the direct production of the blue/green $ABTS^{\bullet+}$ chromophore through the reaction between ABTS and potassium persulfate. Trolox, a water-soluble vitamin E analogue, was used as reference standard in both assays.

Table 2 shows the scavenging effects of hydroxycinnamic acids and derivatives on DPPH and ABTS radicals (expressed as Trolox Equivalents (TE) at the concentration of 50 μM. The data obtained with DPPH radical revealed that all the caffeates and ferulates scavenged about 80 and 60% of DPPH radical, respectively. Although few noteworthy differences among each series were observed, the activity decreased in the following order: trolox 4 caffeic acid (4) ≈ 5-bromocaffeic acid (3) > ethyl 5-bromocaffeate (5) ≈ ethyl caffeate > ferulic acid ≈ 5-bromoferulic acid (2) > ethyl 5-bromoferulate (4) ≈ ethyl ferulate. No significant differences among halogenated and non-halogenated counterparts were observed. The scavenging activity order was consistent with the antioxidant efficacy described in the literature for the parent compounds [9,40,41]. From the results it can be also concluded that ethyl bromocinnamates and ethyl cinnamates present almost the same scavenging activity that is, in both series, lower than that displayed by their parent compounds (bromocaffeic and bromoferulic acids and their non-halogenated counterparts). This tendency is also described in the literature for related esters and could be due to steric hindrance caused by the bulkiness of alkyl groups [42].

The radical-scavenging ability data obtained for hydroxycinnamic acids and derivatives against DPPH radical were in good agreement with the expected activities of this type of phenolic systems: higher when a catechol group is present (caffeic series) and with a decrease of activity when the *meta*-hydroxyl function is substituted by a methoxyl group (ferulic series). Important to notice that the introduction of another methoxyl group in a *ortho* position to a hydroxyl group (*e.g.* sinapic acid) lead to an increase of the antioxidant activity relatively to ferulic acid (see Table 1).

On the other hand, it was demonstrated that the presence of an electron withdrawing group (EWG) of halogen type (σ-acceptor and a weak π-donor) in a *ortho* position to a hydroxyl group does not influence appreciably the activity. This type of substituent appears not to disturb the stability of the phenoxyl radical formed as well as its stabilization through intramolecular interactions.

In which concerns to the $ABTS^{\bullet+}$ radical-scavenging activity one can conclude that all the phenolic compounds were found to be active toward $ABTS^{\bullet+}$ and better antioxidants than trolox. However, structure–activity relationships (SAR) could not be established since they exhibited the same antioxidant profile. The results are in accordance with Nenadis et al. [43] that concluded that $ABTS^{\bullet+}$ radical assay may give an indication for the presence of antioxidants in a certain system but SARs cannot be readily inferred.

Table 2
Antioxidant activity (DPPH and ABTS), redox potentials (Ep) and partition coefficients (Log *P*) values acquired for the hydroxycinnamic acids and their derivatives.

Compounds	DPPH (TE)[a]	ABTS (TE)[a]	Ep (V)	Log *P*
Caffeic acid	0.978	2.538	+0.183	1.15
Ferulic acid	0.781	2.534	+0.335; +0.447	1.42
5-Bromocaffeic acid (3)	1.007	2.496	+0.182	1.98
5-Bromoferulic acid (2)	0.808	2.554	+0.335; +0.442	2.25
Ethyl caffeate	0.877	2.558	+0.175	1.75
Ethyl ferulate	0.593	2.538	+0.368	2.02
Ethyl 5-bromocaffeate (5)	0.938	2.527	+0.174	2.58
Ethyl 5-bromoferulate (4)	0.558	2.558	+0.365	2.85
Sinapic acid	0.862	2.538	+0.188; +0.295	–
Trolox	1.000	1.000	–	–

[a] TE - Trolox Equivalents (procedure is given in Section 4).

2.4. Estimation of partition coefficients (Log P)

In view of better understanding the overall properties of the compounds the lipophilicity, expressed as the octanol–water partition coefficient and herein called Log *P*, was calculated according to Crippen's fragmentation method [44].

The examination of the data obtained allow to conclude that the introduction of a halogen group in the position-5, that is in an *ortho* position to a phenolic group, lead in both series to a significant increase of the Log *P* of the cinnamic acids and derivatives (Table 2). It must be stressed that the partition coefficients calculated for the hydroxycinnamic derivatives are related with their structural features.

Higher lipophilicity is often required in this research field since it could modify the ADME (absorption, distribution, metabolism, and excretion) properties of hydroxycinnamic antioxidants, increasing their ability to interact with the polar head groups of the membrane and local concentration at the water–lipid interface. They can prevent the initial reaction between aqueous radicals and membrane phospholipids.

3. Concluding remarks

Taken together, the results obtained in the present work reveal the existence of a relation between redox potentials and antioxidant activity either for hydroxycinnamic acids or their alkyl ester derivatives. The antioxidant efficacy is higher when the redox potential is lower (as seen in the caffeic series). The presence of a catechol group leads to an increase of the activity due mainly to resonance stabilization of the phenoxyl radical intermediate with subsequent *ortho*-quinone formation [45]. On the other hand the methoxylation of the *meta*-phenolic group (ferulic series) lead to an increase of redox potential and a decrease of the antioxidant activity, as shown throughout the TAC assay. In addition, it was shown that the introduction of a substituent of halogen type, bromo atom, in an *ortho* position to a phenolic group of hydroxycinnamic acids or their linear monoalkyl esters does not affect neither the redox potential nor the antioxidant activity. SPAR's were found to rule the antioxidant activities of these compounds, being an effective approach for the design of new antioxidant agents as well as for the understanding of their mechanism of action [46,47].

In conclusion, these results confirmed the importance of exploring the phenolic cinnamic systems as templates to obtain new antioxidant candidates.

4. Experimental section

4.1. Chemicals

Caffeic, ferulic and sinapic acids, 4-hydroxy-3-methoxybenzaldehyde, malonic acid and ethylmalonate were purchased from Sigma-Aldrich Química S.A. (Sintra, Portugal). All other reagents and solvents were *pro analysis* grade and were acquired from Merck (Lisbon, Portugal) and used without additional purification. Deionised water (conductivity $< 0.1\ \mu S\ cm^{-1}$) was used throughout all the experiments.

4.2. Apparatus

^1H and ^{13}C NMR data were acquired, at room temperature, on a Brüker AMX 300 spectrometer operating at 300.13 and 75.47 MHz, respectively. Dimethylsulfoxide-d_6 was used as a solvent; chemical shifts are expressed in δ (ppm) values relative to tetramethylsilane (TMS) as internal reference; coupling constants (*J*) are given in Hz. Assignments were also made from DEPT (distortionless enhancement by polarization transfer) (underlined values). Electron impact mass spectra (EI-MS) were carried out on a VG AutoSpec instrument; the data are reported as *m/z* (% of relative intensity of the most important fragments). Melting points were obtained on a Köfler microscope (Reichert Thermovar) and are uncorrected.

Voltammetric studies were performed using an Autolab PGSTAT 12 potentiostat/galvanostat (Eco-Chemie, Netherlands) and a one-compartment glass electrochemical cell. Voltammetric curves were recorded at room temperature using a three-electrode system. A glassy carbon working electrode (GCE) ($d = 2$ mm), a platinum wire counter electrode and an Ag/AgCl saturated KCl reference electrode were used. A Crison pH-meter with glass electrode was used for the pH measurements (Crison, Spain).

Spectrophotometric measurements were carried out with a Varian Cary 1E spectrophotometer and on a Bio-Tek Synergy HT multiplate reader.

4.3. Synthesis

4.3.1. Synthesis of 3-bromo-4,5-dihydroxybenzaldehyde (1)

5-Bromovanillin (3-bromo-4-hydroxy-5-methoxybenzaldehyde) (8.7 mmol, 2 g) was suspended in anhydrous dichloromethane (50 mL) in an inert atmosphere (N_2). To this suspension, at −60 °C, boron tribromide (15 mL of 1 M solution in dichloromethane) was added and the reaction was then allowed to reach room temperature with additional stirring for 6 h. After this period, the reaction was quenched by cautious addition of methanol (15 mL). The solvent was partially evaporated and the compound was extracted between diclorometane/water. The organic layer was then dried over Na_2SO_4, filtered, and the solvent was evaporated to a residue which was recrystallized from water as a white solid.

Yield 86%. IR: 3426, 3060, 2974, 1659, 1579, 1441, 1404, 1361, 1307, 1252, 1180, 862, 685, 631, 588, 543, 401. ^1H NMR δ: 7.26 (1H, *d*, J = 1.6, H(2)), 7.58 (1H, *d*, J = 1.6, H(6)), 9.71 (1H, *s*, CHO), 10.45 (1H, *s*, 3-OH), 10.55 (1H, *s*, 4-OH). ^{13}C NMR δ: 109.4 (C5), 112.7 (C2), 127.4 (C6), 129.0 (C1), 146.5 (C–OH), 149.3 (C–OH), 190.6 (C=O). mp 219–221 °C.

4.4. Synthesis of bromocinnamic acids

The synthetic procedure was a modification of the process described by Freudenberg and Hubner [48]: the corresponding benzaldehyde (13 mmol, 3 g) and malonic acid (35 mmol, 3.6 g) were dissolved in pyridine (15 mL) using aniline (1 mL) as catalyst. The reaction occurred at room temperature during 24 h and was followed by thin layer chromatography (hexane/ethyl acetate 6:4). The reaction was then diluted with HCl 5 M and extracted with 3×25 mL of dichloromethane. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated until a residue was obtained. Compound 2 was purified by flash chromatography (from ethyl acetate/petroleum ether 5:5 until ethyl acetate), while compound 3 was recrystallized from diethyl ether.

4.4.1. trans-3-(5-Bromo-4-hydroxy-3-methoxyphenyl)-2-propenoic acid (2)

Yield 50%. IR: 3378, 2967, 2932, 1655, 1619, 1499, 1423, 1384, 1274, 1172, 1038, 970, 836, 589, 541. ^1H NMR δ: 3.94 (3H, *t*, CH_3), 6.37 (1H, *d*, J = 15.8, H(β)), 7.19 (1H, *d*, J = 1.8 H(2)), 7.34 (1H, *d*, J = 1.8 H(6)), 7.55 (1H, *d*, J = 15.9, H(α)). ^{13}C NMR δ: 56.5 (OCH_3), 109.5 (C5), 110.0 (C2), 117.6 (Cα), 125.8 (C6), 126.8 (C1), 143.3 (Cβ), 146.0 (C–OH), 148.7 (C–OCH_3), 168.0 (COOH). EI-MS *m/z* (%): 274 (M + 2, 97), 272 ($M^{+\bullet}$, 100), 178 (33), 150 (22), 133 (25), 105 (28), 97 (22), 85 (23), 76 (23), 75 (28), 71 (38), 65 (27), 63 (22), 58 (72), 57 (67). mp 233–235 °C (lit. 246 °C (dec.). [49]; 243–244 °C [50]; 257–258 °C (dec.) [51]).

4.4.2. trans-*3-(5-Bromo-3,4-dihydroxyphenyl)-2-propenoic acid (3)*

Yield 90%. IR: 3409, 2928, 1678, 1626, 1497, 1430, 1365, 1283, 1182, 1127, 1006, 979, 841, 591. ^{1}H NMR δ: 6.21 (1H, *d*, J = 15.9, H(β)), 7.01 (1H, *d*, J = 1.9 H(2)), 7.30 (1H, *d*, J = 1.9 H(6)), 7.38 (1H, *d*, J = 15.9, H(α)), 9.71 (1H, *s*, 3-OH), 10.06 (1H, *s*, 4-OH), 12.25 (1H, *s*, COOH). ^{13}C NMR δ: 110.0 (C5), 113.4 (C2), 116.8 (C α), 123.9 (C6), 126.5 (C1), 143.1 (Cβ), 145.4 (C–OH), 146.3 (C–OH), 167.7 (COOH). EI-MS *m/z* (%): 260 (M + 2, 83), 258 ($M^{+\bullet}$, 86), 214 (31), 162 (70), 134 (53), 133 (39), 105 (41), 79 (30), 77 (67), 71 (54), 69 (56), 62 (31), 58 (100), 57 (91), 55 (69), 53 (60), 51 (78). mp 177–178 °C.

4.5. Synthesis of trans-*ethyl 3-(5-bromo-4-hydroxy-3-methoxyphenyl)propenoate (4)*

Compound 4 was synthesized by a Knoevenagel-type condensation between the corresponding benzaldehyde (4.6 mmol, 1 g) and monoethylmalonate (9.0 mmol, 1.2 g) in pyridine (5 mL) and using aniline (1 mL) as catalyst. The reaction occurred at 50 °C during 20 h and was followed by thin layer chromatography (chloroform/methanol 9:1). The solvent was then partially evaporated, diluted with diethyl ether and washed twice with HCl 2 M and water. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The remaining residue was recrystallized from dichloromethane/*n*-hexane.

Yield 70%. IR: 3293, 3098, 3069, 3006, 2970, 2938, 2845, 2740, 1673, 1585, 1499, 1461, 1424, 1401, 1351, 1289, 1155, 1042, 968, 851, 826, 789, 676. ^{1}H NMR δ: 1.24 (3H, *t*, CH_3), 3.87 (3H, *s*, OCH_3) 4.16 (2H, *m*, CH_2), 6.59 (1H, *d*, J = 16.0, H(α)), 7.39 (1H, *d*, J = 1.4, H(2)), 7.47 (1H, *d*, J = 1.4, H(6)), 7.53 (1H, *d*, J = 16.0, H(β)), 10.05 (1H, *s*, 4-OH). ^{13}C NMR δ: 14.2 (CH_3), 56.4 (OCH_3), 59.9 (CH_2), 109.3 (C5), 110.0 (C2), 116.4 (Cα), 125.9 (C6), 126.51 (C1), 143.6 (Cβ), 146.0 (C–OH), 148.5 (C–OCH_3), 166.4 (COOH). EI-MS *m/z* (%): 302 (M + 2, 57), 300 ($M^{+\bullet}$, 58), 257 (32), 252 (31), 230 (26), 228 (32), 176 (58), 133 (32), 105 (34), 77 (27), 58 (100), 53 (27), 51 (31). mp 134–136 °C.

4.6. Synthesis of trans-*ethyl 3-(5-bromo-3,4-dihydroxyphenyl)propenoate (5)*

The ethyl ester was synthesized by a Fischer esterification, following the procedure described by Borges and Pinto [52]: 5-bromocaffeic acid (3.9 mmol, 1.0 g) was heated under reflux for *ca.* 5 h, in ethanol (150 mL) containing H_2SO_4 (2 mL). The reaction was followed by thin layer chromatography (chloroform/methanol 9:1) until no 5-bromocaffeic acid was detected. After cooling, the solvent was partially evaporated under reduced pressure and the solution was neutralised with 10% Na_2CO_3. The mixture was then extracted with diethyl ether. The organic phases were combined, washed twice with water, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, petroleum ether/ethyl ether).

Yield 70%. IR: 3694, 3303, 3079, 2977, 2929, 2860, 1685, 1630, 1595, 1519, 1429, 1370, 1351, 1303, 1281, 1177, 1117, 1030, 973, 875, 846, 808, 722, 619, 587, 508, 446, 466. ^{1}H NMR δ: 1.23 (3H, *t*, CH_3), 4.15 (2H, *m*, CH_2), 6.32 (1H, *d*, J = 16.0, H(α)), 7.05 (1H, *s*, H(6)), 7.36 (1H, *s*, H(2)), 7.45 (1H, *d*, J = 16.0, H(β)), 9.77 (1H, *s*, 3-OH), 10.10 (1H, *s*, 4-OH). ^{13}C NMR δ: 14.3 (CH_3), 59.9 (CH_2), 110.0 (C5), 113.7 (C2), 115.8 (Cα), 124.1 (C6), 126.3 (C1), 143.6 (Cβ), 145.6 (C–OH), 146.3 (C–OH), 166.3 (COOH). EI-MS *m/z* (%): 288 (M + 2, 43), 286 ($M^{+\bullet}$, 45), 243 (34), 241(35), 216 (22), 214 (32) 162 (100) 134 (49) 133 (18), 105(30), 77 (35), 53 (30), 51 (50). mp 159–161 °C (dec.).

4.7. Electrochemical measurements

The stock solutions of the cinnamic acids and derivatives (10 mM) were prepared by dissolving an appropriate amount in ethanol. The voltammetric working solutions were prepared, in the electrochemical cell, by diluting 0.1 mL of the stock solution in 10 mL of supporting electrolyte to get a final concentration of 0.1 mM.

The pH 7.3 supporting electrolyte used in the voltammetric determinations was prepared by dilution to 100 mL of 6.2 mL of 0.2 M dipotassium hydrogen phosphate and 43.8 mL of 0.2 M potassium dihydrogen phosphate.

4.8. Total antioxidant capacity (TAC) assays

4.8.1. DPPH radical assay

A total antioxidant capacity assay was carried out using DPPH as radical. The experimental procedure was adapted from the literature, only with slight modifications [53,54].

Briefly, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical in ethanol (250 μM, 2 mL) was added to 2 mL of an ethanolic solution of the test compounds. The final concentration of the test compounds in the reaction mixtures was 50 μM. Each mixture was then shaken vigorously and held for 40 min at room temperature in the dark. The decrease in absorbance of DPPH at 517 nm was then measured. Ethanol was used as a blank and a DPPH solution (2 mL) in ethanol (2 mL) as the control solution. All tests were performed in triplicate.

4.8.2. ABTS radical cation assay

The spectrophotometric analysis of $ABTS^{\bullet+}$ radical-scavenging activity was determined according to the method of Re et al. [55]. The $ABTS^{\bullet+}$ cation radical was produced by the reaction between 7 mM ABTS in H_2O and 2.45 mM potassium persulfate, stored in the dark at room temperature for 12–16 h. Before usage, the $ABTS^{\bullet+}$ solution was diluted to get an absorbance of 0.450 ± 0.001 at 734 nm with water.

Different ethanolic solutions of each cinnamic acids and derivatives (with concentration of 50 μM) were prepared. A total of 20 μL of each were added to 180 μL of radical solution (in triplicate) and absorbances were recorded for $ABTS^{\bullet+}$, every 5 min during a 20 min period. The absorbance of a blank control (20 μL ethanol and 180 μL of radical) was set as 100% of radical (0% bleaching).

The radical-scavenging activity of the samples was expressed as Trolox Equivalents (TE) and calculated according to the following equation:

$$TE = (A_{control} - A_{test})/(A_{control} - A_{trolox})$$

where $A_{control}$ is the absorbance of the control solution (DPPH or ABTS solution without test sample) and A_{test} is the absorbance of the test sample (DPPH or ABTS solution plus compound). Trolox was used as reference compound in both assays.

4.9. Calculation of partition coefficient (Log P*)*

Calculation of the Log *P* values, simulating partitioning of phenols in an *n*-octanol/water (1:1, v/v) system, was based on Crippen's fragmentation method [44] and was accomplished using the ChemDraw software (ChemDraw Ultra 11.0, Cambridge Soft Corp.).

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