Antioxidant potential of chestnut, *Castanea sativa* L., and almond, *Prunus dulcis* L., by-products

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ABSTRACT

The antioxidant properties of almond green husks (Cvs. *Duro Italiano*, *Ferraduel*, *Ferranhês*, *Ferrastar* and *Orelha de Mula)*, chestnut skins and chestnut leaves (Cvs. *Aveleira*, *Boa Ventura*, *Judia* and *Longal*) were evaluated through several chemical and biochemical assays in order to provide a novel strategy to stimulate the application of waste products as new suppliers of useful bioactive compounds, namely antioxidants. All the assayed by-products revealed good antioxidant properties, with very low EC_{50} values (lower than 380 μg/mL), particularly for lipid peroxidation inhibition (lower than 140 μg/mL). The total phenols and flavonoid contents were also obtained. The correlation between these bioactive compounds and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, reducing power, inhibition of β-carotene bleaching and inhibition of lipid peroxidation in pig brain tissue through formation of thiobarbituric acid reactive substances (TBARS), was also obtained. Although, all the assayed by-products proved to have a high potential of application in new antioxidants formulations, chestnut skins and leaves demonstrated better results.

KEYWORDS: Chestnut, Almond, By-products, Antioxidant activity, total phenols.

INTRODUCTION

The interest in polyphenolic antioxidants has increased remarkably in the last decade because of their elevated capacity in scavenging free radicals associated with various diseases (Silva et al., 2007). Some studies indicate that dietary polyphenols have a protective effect against coronary heart disease (Weisburger, 1999; Engler & Engler, 2006), cancer (Fang et al., 2002; Nichenametla et al., 2006), neurodegenerative diseases (Lau et al., 2005) and osteoporosis (Weaver & Cheong, 2005).

Chestnut and almond are important sources of phenolic compounds. Particularly chestnut fruits (Ribeiro et al., 2007), chestnut leaves (Calliste et al., 2005), almond hulls (Sang et al., 2002; Takeoka & Dao, 2003), almond skins (Sang et al., 2002), almond shells (Pinelo et al., 2004), and almond fruits (Milbury et al., 2006) contain those compounds.

Portugal is one of the most important chestnut producers, with nearly 25% of European production. Trás-os-Montes region represent 75.8% of Portuguese chestnut crops and 84.9% of chestnut orchards area (23338 ha). The best development conditions are found at altitudes higher than 500 m and winter low temperatures, as in the "Terra Fria Transmontana" region (Northeast of Portugal) in which **12500 ha** are used for chestnut cultivation (Ribeiro et al., 2007). Almond is also an important product, with **24522** crops spread trough **36530 ha**. This culture is mainly located in Algarve and "Terra Quente Transmontana" (http://portal.min-agricultura.pt/portal/page/portal/MADRP/PT; Cordeiro & Monteiro, 2001; Martins et al., 2003). Accordingly, it would be very important to perform a complete characterization of the antioxidant potential of different by-products originated in these Portuguese crops or by their industrial applications. Due to the multifunctional characteristics of phytochemicals, the antioxidant efficacy of a plant extract is best evaluated based on results obtained by commonly accepted assays, taking into account different oxidative conditions, system compositions, and antioxidant mechanisms (Weisburger, 1999).

In the present work, the antioxidant properties of almond green husks (Cvs. *Duro Italiano*, *Ferraduel*, *Ferranhês*, *Ferrastar* and *Orelha de Mula)*, chestnut skins and chestnut leaves (Cvs. *Aveleira*, *Boa Ventura*, *Judia* and *Longal*) were evaluated through several chemical and biochemical assays: DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, reducing power, inhibition of β-carotene bleaching and thiobarbituric acid reactive substances (TBARS) formation in brain cells. The whole extracts were used since they contain different compounds that can act synergistically, constituting a benefit in comparison to individual compounds (Pellegrini et al., 2006; Pereira et al., 2006).

The evaluation of the antioxidant properties stands as an interesting and valuable task, particularly for finding new sources for natural antioxidants and nutraceuticals, providing a novel strategy to stimulate the application of these by-products as new suppliers of useful bioactive compounds.

MATERIALS AND METHODS

Standards and Reagents

Standards BHA (2-*tert*-butyl-4-methoxyphenol), TBHQ (tert-butylhydroquinone), L-ascorbic acid, α-tocopherol, gallic acid and (+)-catechin were purchase from Sigma (St. Louis, MO, USA). 2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Methanol was obtained from Pronalab (Lisbon, Portugal). Water was treated in a Mili-Q water purification system (TGI Pure Water Systems, USA).

Samples and sample preparation

89 Chestnut tree leaves and chestnut skins were obtained from four different cultivars (Cvs.
90 *Aveleira*, *Boa Ventura*, *Judia* and *Longal*) and collected from orchards located in Vinhais
91 (*Trás-os-Montes*), in the Northeast side of Portugal. Leaves were collected monthly from June
92 to October and used miscellaneously (equal number of leaves for each month), and fruits were
93 collected in October and November. These samples were obtained during the crop year of
94 2006. Almond husks were obtained from five different cultivars (*Duro Italiano*, *Ferraduel*,
95 *Ferranhês*, *Ferrastar* and *Orelha de Mula*) and collected in August-September 2006 in
96 orchards located in Southwest Trás-os-Montes, Northeast Portugal. Selected plants are not
97 irrigated and no phytosanitary treatments were applied.
98 Chestnut leaves and almond husks were dried at 65 ºC until constant weight was achieved and
99 kept at -20 ºC until further use. Outer and inner skins were removed from chestnuts and
100 submitted to a roasting process conducted at 250 ºC in a muffle furnace (ECF 12/22, Lenton
101 Thermal Designs Limited) for 15 minutes, to mimetize industrial practices. Inner and outer
102 skins were assayed together maintaining the individual proportion found for each variety
103 (outer skins represent a higher chestnut weight percentage, when compared with inner skins).
104 For antioxidant compounds extraction, a fine dried powder (20 mesh) of sample was extracted
105 using water, under magnetic stirring (150 rpm) at room temperature during 1h. The extracts
106 were filtered through Whatman nº 4 paper under reduced pressure, frozen at – 80 ºC and then
107 lyophilized (Ly-8-FM-ULE, Snijders) at -80 to -90 ºC under a reduced pressure of ~0.045
108 mbar. All the samples were redissolved in water at a concentration of 50 mg/mL, diluted to
109 final concentrations and analysed for their contents in polyphenols and flavonoids, DPPH
110 radical scavenging activity, reducing power, inhibition of β-carotene bleaching and inhibition
111 of lipid peroxidation.
112
113 **Determination of antioxidants content**

Content of total phenols in the extracts was estimated by a colorimetric assay based on procedures described by Singleton and Rossi (Singleton & Rossi, 1965) with some modifications. Basically, 1 mL of sample was mixed with 1 mL of Folin and Ciocalteu's phenol reagent. After 3 min, 1 mL of saturated sodium carbonate solution was added to the mixture and adjusted to 10 mL with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm (Analytik Jena 200-2004 spectrophotometer). Gallic acid was used for constructing the standard curve (0.01-0.4 mM, y = 2.94848x – 0.09211, R^2 = 0.99914) and the results were expressed as mg of gallic acid equivalents/g of extract (GAEs).

Flavonoid contents in the extracts were determined by a colorimetric method described by Jia *et al*. (1999) with some modifications. The extract (250 µL) was mixed with 1.25 mL of distilled water and 75 µL of a 5% $NaNO_2$ solution. After 5 min, 150 µL of a 10% $AlCl_3.H_2O$ solution was added. After 6 min, 500 µL of 1M NaOH and 275 µL of distilled water were added to prepare the mixture. The solution was mixed well and the absorbance was read at 380 nm, 425 nm and 510 nm, in order to compare the results. (+)-Catechin (0.250-2.500 mM) was used to calculate the standard curves, (y=2.4553x – 0.1796, R^2=0.997, at 340 nm, y=0.7376x – 0.0131, R^2=0.997, at 425 nm, y=0.5579x – 0.0494, R^2=0.992, at 510 nm, and the results were expressed as mg of (+)-catechin equivalents (CEs) per g of extract.

DPPH radical-scavenging activity

Various concentrations of extracts (0.3 mL) were mixed with 2.7 mL of methanolic solution containing DPPH radicals ($6x10^{-5}$ mol/L). The mixture was shaken vigorously and left to stand for 60 min in the dark (until stable absorbance values were obtained). The reduction of the DPPH radical was determined by reading the absorbance at 517 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discolouration using the

equation: % RSA = $[(A_{DPPH}-A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution (Barreira et al., 2008). The extract concentration providing 50% of radicals scavenging activity (EC_{50}) was calculated from the graph of RSA percentage against extract concentration. BHA and α-tocopherol were used as standards.

Reducing power

Several concentrations of extracts (2.5 mL) were mixed with 2.5 mL of 200 mmol/L sodium phosphate buffer and 2.5 mL of potassium ferricyanide (1%). The mixture was incubated at 50 ºC for 20 min. After 2.5 mL of trichloroacetic acid (10% w/v) were added, and the mixture was centrifuged at 1000 rpm for 8 min (Centorion K24OR- 2003 refrigerated centrifuge). The upper layer (5 mL) was mixed with 5 mL of deionised water and 1mL of ferric chloride (0.1%), and the absorbance was measured spectrophotometrically at 700 nm (Barreira et al., 2008). The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 700 nm against extract concentration. BHA and α-tocopherol were used as standards.

Inhibition of β-carotene bleaching

The antioxidant activity of aqueous extracts was evaluated by the β-carotene linoleate model system. A solution of β-carotene was prepared by dissolving 2 mg of β-carotene in 10 mL of chloroform. 2 mL of this solution were pipetted into a 100 mL round-bottom flask. After the removal of the chloroform at 40ºC under vacuum, 40 mg of linoleic acid, 400 mg of Tween 80 emulsifier, and 100 mL of distilled water were added to the flask with vigorous shaking. Aliquots (4.8 mL) of this emulsion were transferred into different test tubes containing 0.2 mL of different concentrations of chestnut extracts. The tubes were shaken and incubated at

50ºC in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm. Absorbance readings were then recorded at 20-min intervals until the control sample had changed colour. A blank, devoid of β-carotene, was prepared for background subtraction. Lipid peroxidation (LPO) inhibition was calculated using the following equation: LPO inhibition = (β-carotene content after 2h of assay/initial β-carotene content) × 100 (Barreira et al., 2008). The extract concentration providing 50% antioxidant activity (EC_{50}) was calculated from the graph of antioxidant activity percentage against extract concentration. TBHQ was used as standard.

Inhibition of lipid peroxidation using thiobarbituric acid reactive substances (TBARS)

Brains were obtained from pig (*Sus scrofa*) of body weight ~150 kg, dissected and homogenized with a Polytron in ice-cold Tris–HCl buffer (20 mM, pH 7.4) to produce a 1:2 (w/v) brain tissue homogenate which was centrifuged at 3000g for 10 min. An aliquot (0.1 mL) of the supernatant was incubated with the extracts (0.2 mL) in the presence of $FeSO_4$ (10 µM, 0.1 mL) and ascorbic acid (0.1 mM, 0.1 mL) at 37ºC for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28% w/v, 0.5 mL), followed by thiobarbituric acid (TBA, 2%, w/v, 0.38 mL), and the mixture was then heated at 80 ºC for 20 min. After centrifugation at 3000g for 10 min to remove the precipitated protein, the colour intensity of the TBARS in the supernatant was measured by its absorbance at 532 nm. The inhibition ratio (%) was calculated using the following formula: Inhibition ratio (%) = [(A – B)/A] x 100%, where A and B were the absorbance of the control and the compound solution, respectively (Barreira et al., 2008). The extract concentration providing 50% lipid peroxidation inhibition (EC_{50}) was calculated from the graph of antioxidant activity percentage against extract concentration. BHA was used as standard.

189 **Statistical analysis**

190 For all the experiments three samples were analysed and all the assays were carried out in
191 triplicate. The results are expressed as mean values and standard error or standard deviation
192 (SD). The differences between the different extracts were analyzed using one-way analysis of
193 variance (ANOVA) followed by Tukey's honestly significant difference post hoc test with α =
194 0.05, coupled with Welch's statistic. The regression analysis between total phenols or
195 flavonoid contents, and EC_{50} values for antioxidant activity used the same statistical package.
196 These treatments were carried out using SPSS v. 16.0 program.

197

198 **RESULTS AND DISCUSSION**

199 **Table 1** presents extraction yields (expressed as w/w percentages), total phenols and
200 flavonoids content (mg/g of extract) obtained for chestnut and almond by-products. The
201 results are presented for each single variety in order to analyse possible differences. However,
202 and regarding the aim of this work, the results obtained for each by-product, as presented in
203 the bottom of the table, are the most significant, once it would be difficult to obtain supplies
204 of these by-products selected by variety. Among all of the extracts analyzed, an interesting
205 content of total phenols (from 228 to 859 mg/g) was detected with mean values of 592 mg/g
206 for almond husk, 413 mg/g for chestnut leaf and 710 mg/g for chestnut skins. The marked
207 differences of the results obtained for Longal leaf when compared with our previous study
208 (Barreira et al., 2008) can be explained on the basis of three different factors. First, the leaves
209 used in our previous work presented a higher ripeness state, second, they were utilized in
210 fresh (a drying step was not conducted), and finally the extraction procedure was conducted at
211 water boiling temperature. These results revealed the high potential of the assayed by-
212 products as new sources of antioxidant compounds. Extraction yields were generally low, but
213 their bioactivity indicates that the extraction procedure was effective, considering that the

objective was to achieve a clean extract. Despite this consideration, not all cases revealed a relationship between extracted mass and total phenols content. Actually, extracts obtained with chestnut skins proved to be the most uncontaminated, promoting it as the more adequate by-products, considering the posterior purifying processes. Likewise, this observation could probably be explained by a higher amount of other polar compounds in chestnut leaves and almond husks.

Figures 1 to 4 show the antioxidant activity of the extracts examined as a function of their concentration. Several biochemical assays were used to screen the antioxidant properties: inhibition of β-carotene bleaching (by neutralizing the linoleate-free radical and other free radicals formed in the system which attack the highly unsaturated β-carotene models), inhibition of lipid peroxidation in brain tissue (measured by the colour intensity of MDA-TBA complex), scavenging activity on DPPH radicals (measuring the decrease in DPPH radical absorption after exposure to radical scavengers) and reducing power (measuring the conversion of a Fe^{3+}/ferricyanide complex to the ferrous form). The assays were carried out using whole extracts instead of individual compounds, once additive and synergistic effects of phytochemicals in fruits and vegetables are responsible for their potent bioactive properties and the benefit of a diet rich in fruits and vegetables is attributed to the complex mixture of phytochemicals present in whole foods (Liu, 2003). This enhances the advantages of natural phytochemicals over single antioxidants when they are used to achieve health benefits. Analysis of **figures 1 to 4** revealed that antioxidant activity increased with the concentration, being obtained very good results even at low extract concentrations, especially for TBARS assay.

The bleaching inhibition, measured by the peroxidation of β-carotene, is presented in **figure 1**. The linoleic acid free radical attacks the highly unsaturated β-carotene model. The presence

239 of different antioxidants can hinder the extent of β-carotene-bleaching by neutralizing the
240 linoleate-free radical and other free radicals formed in the system (Jayaprakasha et al., 2001).
241 Hence, the absorbance diminishes fast in samples without antioxidant, whereas in the
242 presence of an antioxidant, they maintain their colour, and thus absorbance, for a longer time.
243 Bleaching inhibition in the presence of different extracts increased with concentration and
244 proved to be very good. At 500 µg/mL, all the extracts presented inhibition percentages
245 superior to 65%, except in the cases of Orelha de Mula husk, a very good result once that the
246 antioxidant activity of TBHQ standard reached 82.2% only at 2 mg/ml. It is expectable that
247 the antioxidative components in the chestnut extracts reduce the extent of β-carotene
248 destruction by neutralizing the linoleate free radical and other free radicals formed in the
249 system. It became clear that chestnut derived by-products revealed higher efficiency in this
250 antioxidant activity biochemical assay when compared with almond by-products.
251 Inhibition of lipid peroxidation was evaluated using thiobarbituric acid reactive substances
252 (TBARS). When oxidation processes occur, a pinkish solution is formed. If antioxidant
253 compounds are present in the system, the formation of the substances responsible for the
254 coloration is prevented. As it can be easily understood after **figure 2** observation, the capacity
255 of inhibition of lipid peroxidation is proportional to the extract concentration. This method
256 revealed very high inhibition percentages at extremely low concentrations. All extracts
257 showed inhibition percentages superior to 60% at concentrations of 100 µg/mL, except for
258 Ferraduel husk and Judia leaf. Generally, chestnut skins and almond husks extracts proved to
259 be better inhibitors in this model.
260 The radical scavenging activity (RSA) values were expressed as the ratio percentage of
261 sample absorbance decrease and the absorbance of DPPH solution in the absence of extract at
262 517 nm. From the analysis of **figure 3**, we can conclude that the scavenging effects of all
263 extracts on DPPH radicals increased with the concentration increase and were remarkably

264 good, with RSA percentages superior to 90% at 500 µg/mL for almost all the extracts, except
265 for Aveleira and Judia leaves and Ferraduel and Ferranhês husks, again better than the
266 scavenging effects of some usual standards like BHA (96% at 3.6 mg/ml) and α-tocopherol
267 (95% at 8.6 mg/ml).
268 Like in the other assays previously referred, the reducing power increased with concentration,
269 and the values obtained for all the extracts were very good (**figure 4**). At 250 µg/mL, the
270 absorbance values were higher than 0.5 for all extracts, with the exception of Judia leaf and
271 Ferraduel and Orelha de Mula husks, proving once more to have much more high antioxidant
272 activity than some common standards (reducing powers of BHA at 3.6 mg/ml and a-
273 tocopherol at 8.6 mg/ml were only 0.12 and 0.13, respectively).The extracts obtained with
274 chestnut skins revealed better reducing properties. This difference could be explained by the
275 presence of high amounts of reductones, which have been associated with antioxidant action
276 due to breaking the free radical chain by donating a hydrogen atom (Shimada et al., 1992).
277 **Table 2** shows antioxidant activity EC_{50} values of chestnut and almond by-products extracts
278 measured by different biochemical assays. In the lower part of the table these results are
279 represented for each one of the by-products. Overall, chestnut skins revealed better
280 antioxidant properties (significantly lower EC_{50} values, $p < 0.05$). The EC_{50} values obtained
281 for these extracts were excellent (less than 110 µg/mL, average value), particularly for LPO
282 inhibition (less than 40 µg/mL, average value). However, chestnut leaves (less than 220
283 µg/mL in average, for all assays) and almond husks (less than 260 µg/mL in average, for all
284 assays) also revealed very good antioxidant activity.
285 The obtained results are generally in agreement with the total phenol and flavonoid contents
286 determined for each sample and showed in **table 1**. The EC_{50} values obtained for lipid
287 peroxidation inhibition were better than for reducing power, scavenging effects on DPPH

radicals and β-carotene bleaching inhibition caused by linoleate free radical, which were similar.

Other tree nuts had demonstrate their potential antioxidant activity namely walnuts (Anderson et al., 2001; Fukuda et al., 2004) and hazelnuts (Alasalvar et al., 2006; Sivakumar & Bacchetta, 2005). Nevertheless, those studies were carried out with extracts from the fruits.

In previous works (Barreira et al., 2008; Barros et al., 2007; Sousa et al., 2008) we observed a significantly negative linear correlation between the total phenols content and EC_{50} antioxidant activity values. This negative linear correlation proves that the samples with highest total phenols content show lower EC_{50} values, confirming that phenols are likely to contribute to the antioxidant activity of the extracts, as it has been reported in other species (Velioglu et al., 1998). The flavonoids contents were also correlated with EC_{50} scavenging capacity values with similar correlation coefficients values. Furthermore, approximately half of the results showed statistical significance, as it can be seen in **table 3**. This may represent an important tool to predict this kind of bioactivity just by quantifying phenols.

In conclusion, all the assayed by-products revealed good antioxidant properties, with very low EC_{50} values, particularly for lipid peroxidation inhibition, and might provide a novel strategy to stimulate the application of waste products as new suppliers of useful bioactive compounds, particularly antioxidants. This represents an additional advantage since almond and chestnut are important products, with high economic value, which originate high amounts of the studied by-products.

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393 **Fig. 1.** Inhibition of β-carotene bleaching as a function of extracts concentration.

394

395 **Fig. 2.** Lipid peroxidation (LPO) inhibition as a function of extracts concentration.

396

397 **Fig. 3.** Radical Scavenging Activity (RSA) as a function of extracts concentration.

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399 **Fig. 4.** Reducing power as a function of extracts concentration.

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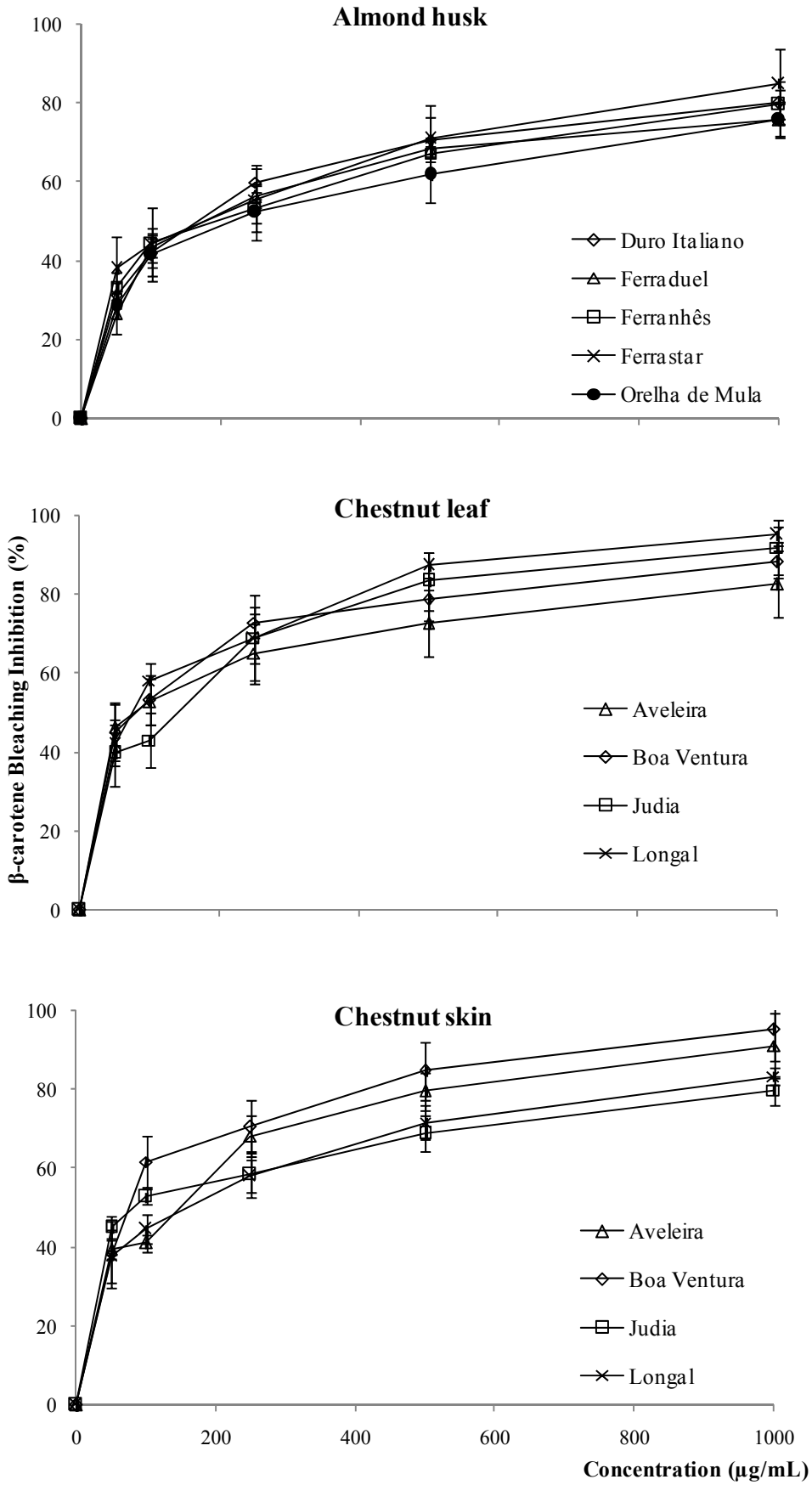
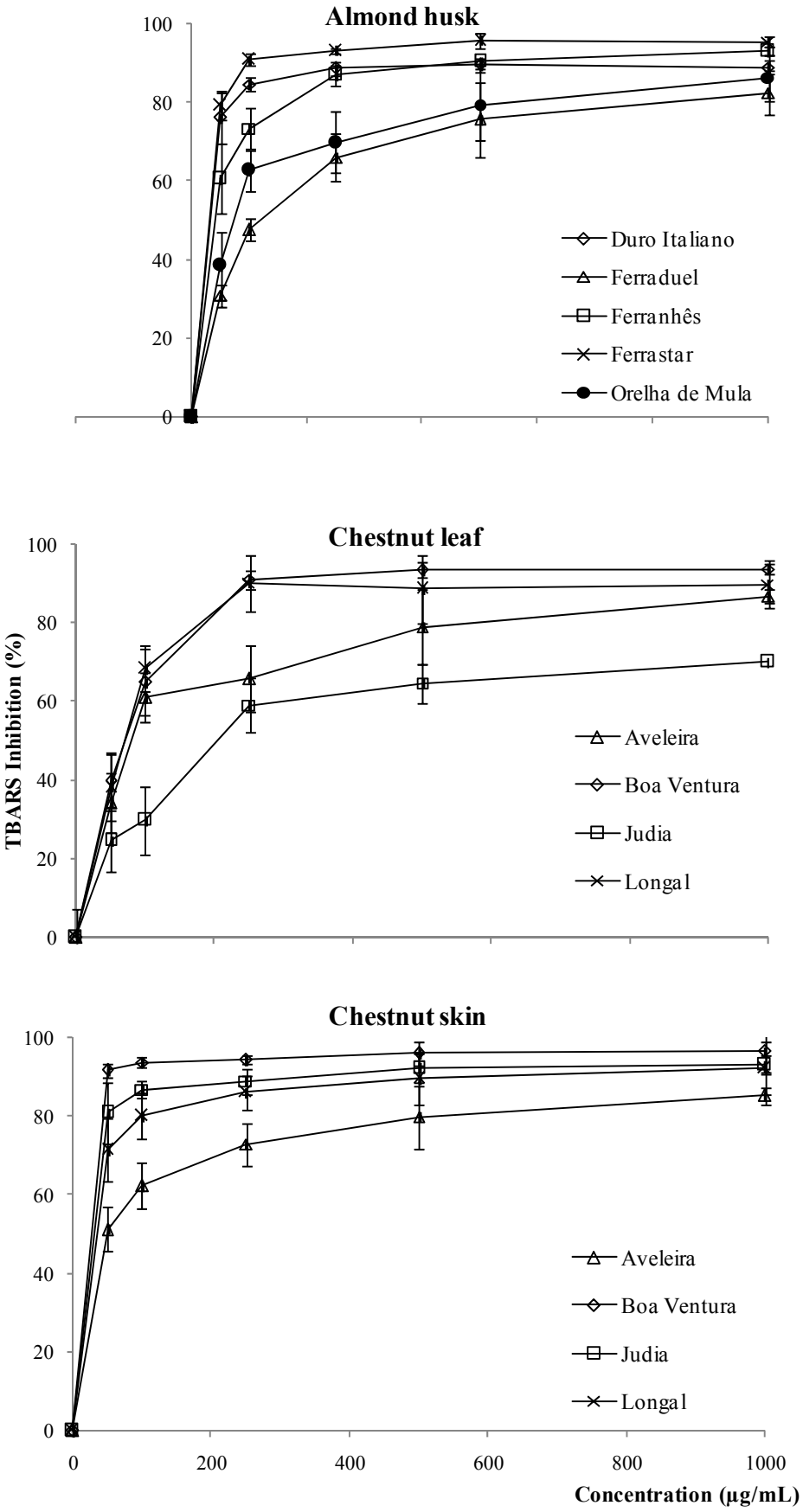


403

404 **Figure 1.**



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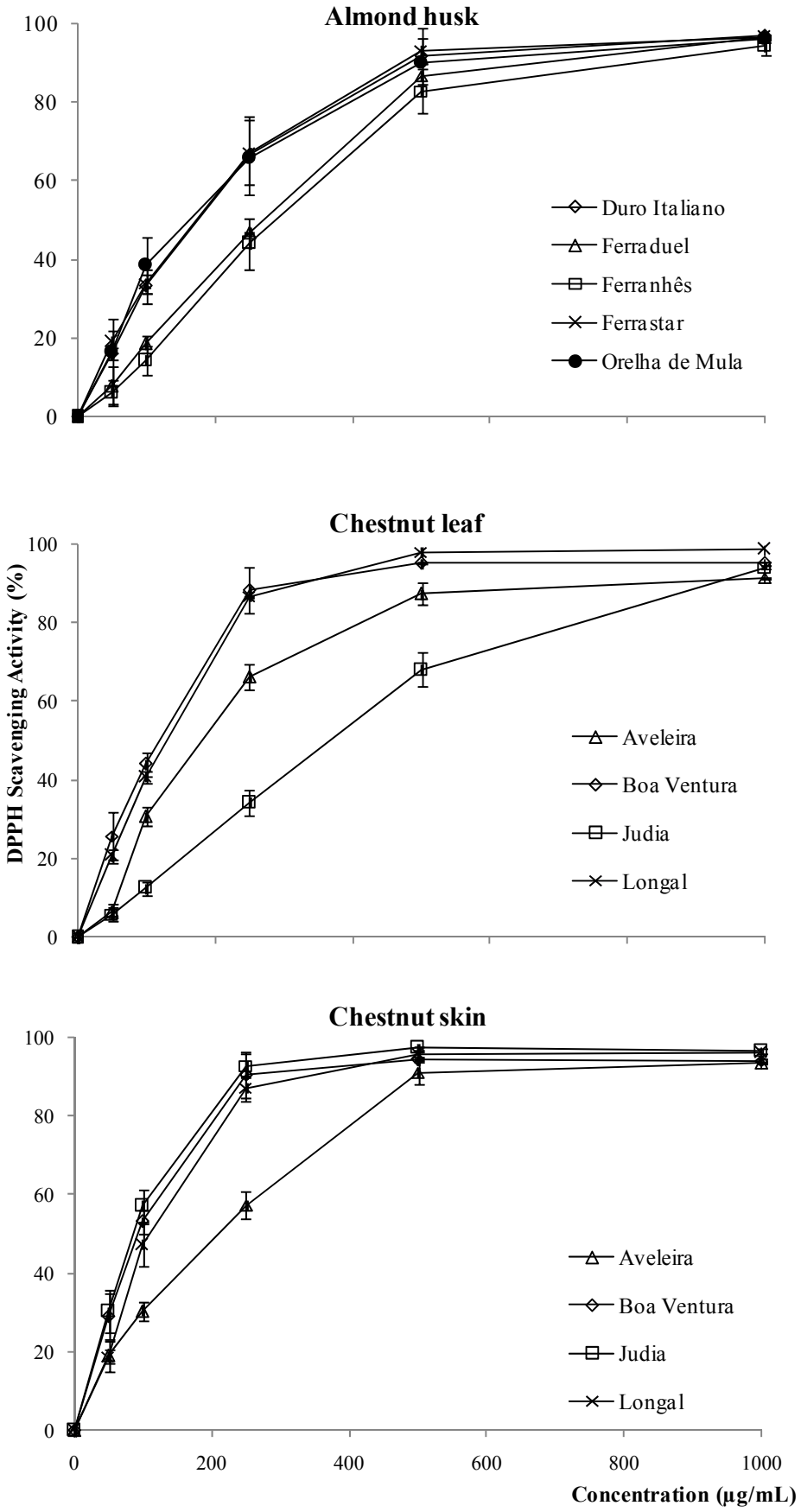
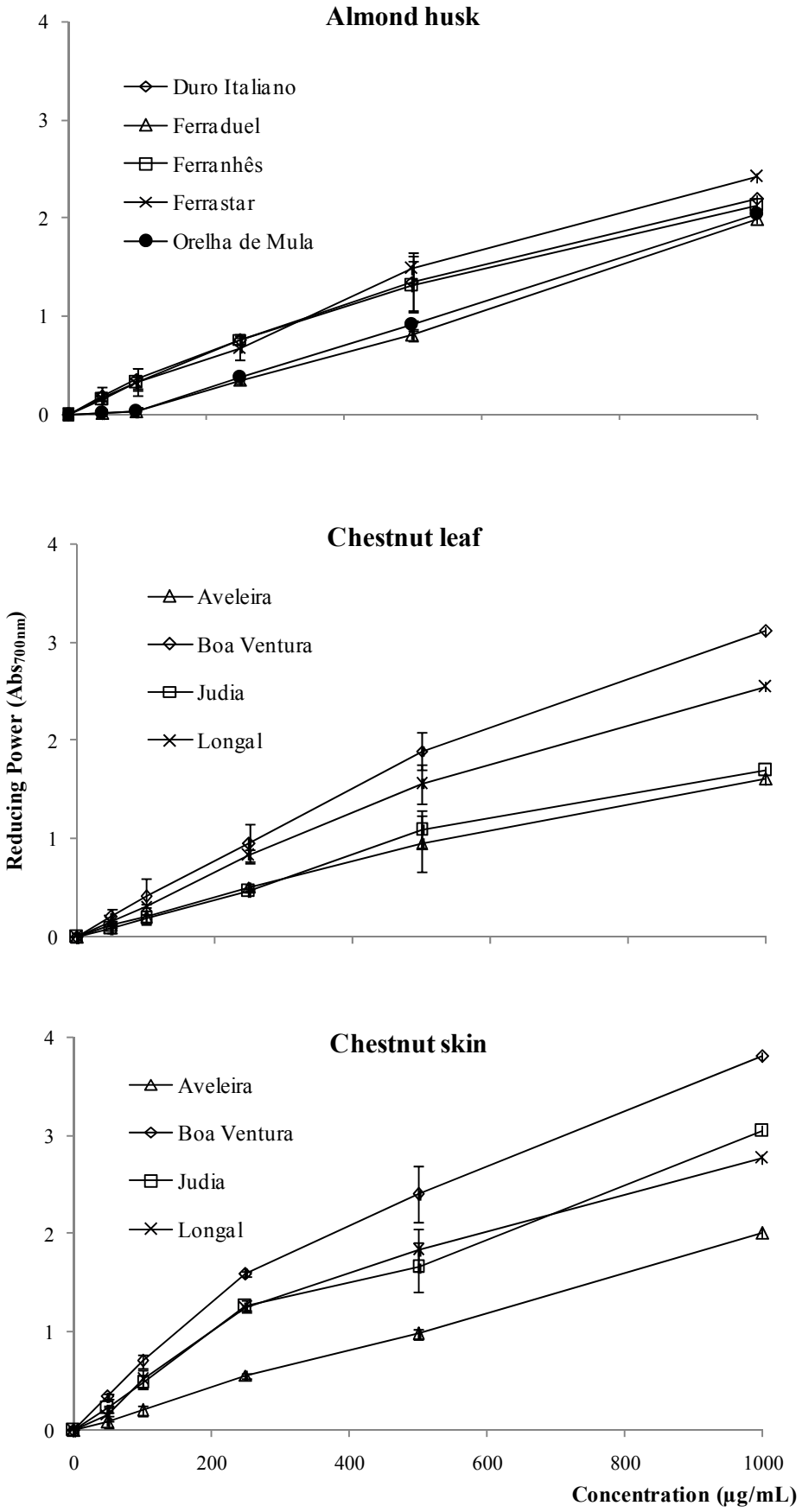
406 **Figure 2.**



407

408 **Figure 3.**



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410 **Figure 4.**

411 **Table 1.** Extraction yields, content of total phenols and flavonoids in the extracts of chestnut
412 and almond by-products. In each column and for each by product, different letters mean
413 significant differences (p<0.05).

		Extraction yield (%)	Total phenols (mg/g)	Flavonoids (mg/g)
Almond husk (AH)	Duro Italiano	17.65±1.02 c	777.21±18.78 b	237.20±2.52 b
	Ferraduel	14.14±0.60 c	304.79±22.06 e	70.48±3.61 e
	Ferranhês	27.49±2.11 a	378.70±9.42 d	130.68±5.91 c
	Ferrastar	22.58±1.18 b	859.07±74.50 a	284.61±12.06 a
	Orelha de Mula	22.81±1.55 b	639.75±33.91 c	116.88±19.49 d
Chestnut leaf (CL)	Aveleira	17.67±0.94 a	468.34±25.47 b	84.68±3.72 b
	Boa Ventura	15.62±0.93 bc	432.16±37.59 c	83.09±6.82 b
	Judia	17.08±0.62 ab	228.37±13.99 d	73.31±4.89 c
	Longal	13.73±0.49 c	522.98±23.82 a	90.39±5.57 a
Chestnut skin (CS)	Aveleira	7.17±0.29 b	533.81±30.90 c	49.92±1.93 d
	Boa Ventura	6.43±0.32 b	805.74±74.31 a	146.08±4.19 a
	Judia	12.59±0.84 a	757.95±67.51 b	98.10±6.62 b
	Longal	6.47±0.43 b	742.33±37.46 b	72.27±3.78 c
AH	$\bar{x}$	20.93±4.91 a	591.90±221.39 b	167.97±80.88 a
CL	$\bar{x}$	16.02±1.72 b	412.96±114.91 c	82.87±8.13 b
CS	$\bar{x}$	8.16±2.72 c	709.96±118.38 a	91.59±36.21 b

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Table 2. EC_{50} values (μg/mL) obtained in the antioxidant assays for chestnut and almond by-products and corresponding coefficients of variation (%).In each column and for each by product, different letters mean significant differences (p<0.05).

		Bleaching inhibition	LPO inhibition	RSA	Reducing Power
Almond husk (AH)	Duro Italiano	227.37±18.44 c	29.20±2.65 d	175.03±11.42 c	206.96±20.63 c
	Ferraduel	284.91±17.52 a	103.52±6.78 a	216.37±14.15 a	376.30±27.67 a
	Ferranhês	250.23±18.83 b	39.95±3.63 c	209.22±14.61 a	218.11±21.06 c
	Ferrastar	211.37±9.25 d	28.11±1.15 d	176.82±12.34 c	169.85±4.53 d
	Orelha de Mula	276.77±10.53 a	74.15±3.61 b	190.33±4.53 b	306.46±22.13 b
Chestnut leaf (CL)	Aveleira	99.47±5.33 b	78.32±6.01 b	182.97±8.23 b	210.09±18.92 b
	Boa Ventura	99.09±5.37 b	71.54±5.86 c	161.34±9.08 c	215.62±8.87 b
	Judia	160.04±15.17 a	133.52±5.60 a	367.06±27.89 a	267.00±26.54 a
	Longal	64.14±3.76 c	69.04±3.53 c	129.91±5.02 d	152.38±2.39 c
Chestnut skin (CS)	Aveleira	151.27±15.55 a	49.07±4.83 a	159.99±15.37 a	117.58±12.71 a
	Boa Ventura	74.62±8.92 d	27.29±0.48 d	82.41±5.52 c	79.25±6.39 d
	Judia	86.07±7.16 c	30.47±2.05 c	86.52±7.77 c	104.61±8.22 b
	Longal	120.84±7.84 b	34.53±3.21 b	108.87±6.73 b	94.55±6.31 c
AH	$\bar{x}$	250.13±32.03 a	54.98±29.82 b	193.56±20.52 a	255.53±78.19 a
CL	$\bar{x}$	105.68±35.71 b	88.10±27.08 a	210.32±94.11 a	211.27±44.08 b
CS	$\bar{x}$	108.20±31.97 b	35.34±8.90 c	109.45±32.44 b	99.00±16.54 c

Table 3. Correlations established between total phenols and flavonoids with antioxidant activity EC_{50} values.

		Total phenols			Flavonoids		
		Equation R^2	*F*	*Sign.*	Equation R^2	*F*	*Sign.*
Almond husk	Bleaching inhibition	y = -0.0001x + 0.3086 0.584	4.218	n.s.	y = -0.0003x + 0.3073 0.937	44.610	**
	LPO inhibition	y = -0.0001x + 0.1096 0.463	2.590	n.s.	y = -0.0003x + 0.1080 0.733	18.238	n.s.
	RSA	y = -0.0001x + 0.2386 0.976	120.893	**	y = -0.0001x + 0.2245 0.774	10.269	*
	Reducing Power	y = -0.0002x + 0.3964 0.473	2.6886	n.s.	y = -0.0008x + 0.3942 0.769	9.979	n.s.
Chestnut leaf	Bleaching inhibition	y = -0.0003x + 0.2312 0.962	50.278	*	y = -0.0056x + 0.5686 0.990	208.436	*
	LPO inhibition	y = -0.0002x + 0.1825 0.927	25.419	*	y = -0.0040x + 0.4162 0.848	11.133	n.s.
	RSA	y = -0.0008x + 0.5452 0.955	42.044	*	y = -0.0143x + 1.3957 0.905	19.055	*
	Reducing Power	y = -0.0003x + 0.3507 0.857	12.020	n.s.	y = -0.065x + 0.7466 0.957	44.141	*
Chestnut skin	Bleaching inhibition	y = -0.0003x + 0.2949 0.830	9.738	n.s.	y = -0.0008x + 0.1800 0.866	12.890	n.s.
	LPO inhibition	y = -0.0001x + 0.0916 0.984	121.371	**	y = -0.0002x + 0.0537 0.742	5.736	n.s.
	RSA	y = -0.0003x + 0.3150 0.958	45.713	*	y = -0.0007x + 0.1759 0.708	4.851	n.s.
	Reducing Power	y = -0.0001x + 0.1811 0.741	5.731	n.s.	y = -0.0003x + 0.1299 0.741	5.727	n.s.

*, $p \leq 0.05$ (significant correlation), **, $p \leq 0.01$ (very significant correlation),***, $p \leq 0.001$ (extremely significant correlation), n.s., not significant correlation.