Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 101 (2007) 524–531
Food Chemistry
www.elsevier.com/locate/foodchem

Dose-effect study on the antioxidant properties of leaves and outer bracts of extracts obtained from *Violetto di Toscana* artichoke

Rita Coinu [a], Stefania Carta [a], Pier Paolo Urgeghe [a], Nadia Mulinacci [b], Patrizia Pinelli [b], Flavia Franconi [a], Annalisa Romani [b,*]

[a] *Centre for Biotechnology Development and Biodiversity Research, University of Sassari, 07100 Sassari, Italy*
[b] *Department of Pharmaceutical Science, University of Florence, Via U. Schiff, 6, 50019 Sesto F.no Florence, Italy*

Received 23 May 2005; received in revised form 2 February 2006; accepted 12 February 2006

Abstract

Artichoke (*Cynara scolymus* L.) is an edible vegetable largely used in the Mediterranean diet and in folk medicine. The present paper discusses the analysis of the polyphenol content of leaves and outer bracts of *Violetto di Toscana* artichoke using different extraction procedures with the aim of establishing a correlation between polyphenol subclasses and antioxidant activity measured on human LDL oxidized by copper ions. HPLC/DAD and HPLC/MS analyses revealed that both the matrixes contain identical polyphenol subclasses, with mainly quantitative differences. The antioxidant effect of four artichoke extracts decreases in the following order when the sum of total phenolic compounds was considered: ethanolic extract from leaves ($IC_{50} = 2.92 \pm 0.46$ μM); ethanolic extract from outer bracts ($IC_{50} = 4.04 \pm 0.21$ μM); ethyl acetate extract from leaves ($IC_{50} = 4.91 \pm 0.11$ μM); ethyl acetate extract from outer bracts ($IC_{50} = 10.18 \pm 1.6$ μM). IC_{50} were also calculated considering the concentrations of single polyphenol subclasses. In both cases, the potency of antioxidant properties was not related to the amount of total polyphenols or the single subclasses.

Keywords: *Cynara scolymus* L.; Caffeoylquinic acids; Flavonoids; Antioxidant activity

1. Introduction

Epidemiological and animal studies and in vitro experiments reveal that the polyphenols present in certain kinds of fruits and vegetables possess antioxidant properties and it has been suggested that they may exert anticangerogenic, antimutagenic, antibacterial, antiviral, anti-inflammatory and anti-arteriosclerotic effects (Halliwell, 1994; Halliwell, 1996). A major class of polyphenols is the caffeic acid derivatives (Tapiero, Tew, Ngugen Ba, & Mathè, 2002) and in edible vegetables they mainly occur as esters with quinic acid; the leaves of *Cynara scolymus* L. are very rich (Chen & Ho, 1997; Schutz, Kammerer, Carle, & Schieber, 2004) in mono and dicaffeoylquinic compounds (Llorach, Espin, Tomas-Barberan, & Ferreres, 2002; Wang et al., 2003). The artichoke has been traditionally used for dyspeptic disorders (Anonymous, 1990; Ernst, 1995; Holtmann et al., 2003), while some recent studies have suggested other potential health-promoting effects of this vegetable (Adzet, Camarasa, & Laguna, 1987; Clifford, 2000; Englisch, Beckers, Ruepp, & Zinserling, 2000) including a hypocholesterolemic one (Wojcicki, Samochowiec, & Kosmider, 1981) probably due to an inhibition of cholesterol synthesis (Gebhardt, 1998). In addition, antioxidant properties of artichoke have been reported by various authors (Brown & Rice-Evans, 1998; Gebhardt, 1997; Gebhardt & Fausel, 1997; Jimenez-Escrig, Dragsted, Daneshvar, Pulido, & Saura-Calixto, 2003; Perez Garcia, Adzet, & Canigueral, 2000; Wang et al., 2003; Zapolska-Downar et al., 2002) as well as some reports concerning

Abbreviations: LDL, low density lipoproteins; LDLox, oxidized low density lipoproteins; HDL, high density lipoproteins; MDA, malonaldehyde; EtOAc, ethyl acetate; LLE, liquid–liquid extraction; HPLC, high performance liquid chromatography; MS, mass spectrometry; DAD, diode array detector; IC_{50}, concentration that gives 50% of inhibition; Viot, *Violetto di Toscana*.

* Corresponding author. Tel.: +39 55 4573775; fax: +39 55 4573737.
E-mail address: annalisa.romani@unifi.it (A. Romani).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.02.009

its antioxidant properties toward LDL oxidation (Brown & Rice-Evans, 1998; Jimenez-Escrig et al., 2003). In one case, a commercial artichoke leaf extract was compared with those obtained with luteolin and luteolin-7-*O*-glucoside (Brown & Rice-Evans, 1998), which are not the main compounds, as they are representative of a single subclass of artichoke polyphenols (Schutz et al., 2004; Wang et al., 2003). Furthermore, the antioxidant activity of artichoke extract has been evidenced in the LDL obtained from only one hypercholesterolemic patient (Jimenez-Escrig et al., 2003). Until now, leaves have been mainly used as raw material (Brown & Rice-Evans, 1998; Gebhardt, 1998; Perez Garcia et al., 2000; Zapolska-Downar et al., 2002), thus their chemical composition and biological properties are better known than those of the edible parts and other byproducts (Llorach et al., 2002; Wang et al., 2003). However, recently studies have been carried out on the edible parts of artichoke (Jimenez-Escrig et al., 2003; Schutz et al., 2004; Wang et al., 2003) and a byproduct containing outer bracts, receptacles and stems (Llorach et al., 2002). To our knowledge the chemical composition and antioxidant activity of the outer bracts have not yet been studied alone, but rather have only be considered in extracts containing also receptacles and stems (Llorach et al., 2002). Up to now, the true values of overall antioxidant properties of artichoke extracts have been difficult to estimate due to the application of some artificial radicals, whose actual reaction with antioxidants remains uncertain, and for the lack of chemical characterization of extracts which may limit the validity of published structure-activity interpretations. Conversely, it has been evidenced (Mulinacci et al., 2004) that commercial extracts have different quantitative amounts of caffeoylquinic acid derivatives and flavonoids, which ultimately may affect the biological activity. Indeed, chemical composition depends on cultivar, tissues and kind of extraction (Wang et al., 2003). The absence of chemical characterization is also a problem for safety of extracts because many polyphenols may interact with cytochrome P450 enzymes (Bailey & Dresser, 2004; Harris, 2003; Ho & Saville, 2001) which are heavily implicated in the metabolism of many drugs (Dahan & Altman, 1992), therefore it is important to select artichoke extracts which do not contain polyphenols that interact with cytochrome P450 enzymes. For these reasons, we decided to test a specific cultivar of artichoke, *Violetto di Toscana* (Viot) traditionally used for its peculiar organoleptic properties, to determine its chemical characterization in order to ascertain the importance of single polyphenol subclasses on antioxidant activities in a model relevant for human health, such as LDL oxidation (Chisolm & Steinberg, 2000).

2. Materials and methods

2.1. Chemicals

5-*O*-caffeoyl quinic acid (chlorogenic acid), 1,3-*O*-dicaffeoyl quinic acid (cynarin) and apigenin 7-*O*-glucoside were from Roth (Karlsrube, Germany); luteolin 7-*O*-glucoside (cynaroside), luteolin 7-*O*-rutinoside (scolymoside), narirutin, naringin, and naringenin were purchased from Extrasynthese S.A. (Lyon, Nord-Genay, France); finally, caffeic acid was obtained from Fluka (Busch, Switzerland). All solvents were HPLC grade and were from E. Merck (Darmstadt, Germany). All other reagents were of analytical grade and were purchased from Sigma (St Louis, Mo, USA).

2.2. Preparation of artichoke extracts

Leaves and outer bracts of artichoke cultivar Viot, which is typical of southern Tuscany (Italy) were processed as follows. The lyophilised and homogenised artichoke tissues (10–30 g) were used for extraction with 3×100 ml of 70% ethanol (pH 2); the extract, after a complete defatting with *n*-hexane (4×20 ml) was then concentrated under vacuum (Rotavapor 144 R, Büchi, Switzerland) to a final volume of 5 ml (hydro-alcoholic extract). A part of the hydro-alcoholic extract was fractionated by LLE with EtOAC. The ethyl acetate fraction was dried under vacuum and dissolved in ethanol 70%. An aqueous residue was also obtained after LLE extraction with EtOAc. The extracts were lyophilised and then stored at −20 °C until use; they remained stable for at least 12 months.

2.3. HPLC/DAD and HPLC/MS analysis

Analysis was carried out using a HP-1100 liquid chromatograph equipped with a DAD detector and a HP 1100 MSD API-electrospray (Agilent Technologies, Palo Alto, USA) operating in positive and negative ionisation mode. Analytical conditions have previously been described (Mulinacci et al., 2004). Identification of individual polyphenols was carried out using their retention times, and both spectroscopic and spectrometric data. Quantitation of the single polyphenol was directly performed by HPLC-DAD using a four-point regression curve built with the available standards. Curves with a $r^2 > 0.9998$ were considered. Calibration was performed at the wavelength of maximum UV–Vis absorbance applying the correction for molecular weight. In particular, caffeoylquinic mono and di-ester amounts were calculated at 330 nm using chlorogenic acid and cynarin as reference, respectively. Luteolin 7-*O*-malonilglucoside was calibrated at 350 nm using cynaroside as reference, luteolin (aglycone) was calibrated at 350 nm using pure standard. Finally, apigenin 7-*O*-glucuronide was calibrated at 350 nm using apigenin 7-*O*-glucoside as reference.

2.4. Subjects

Thirty healthy volunteers (49.9 ± 9.8 years old), well matched for sex, gave informed consent to participate in the study. Individuals who reported to be non-smokers

were included. None of the subjects had a family history of diabetes, hypertension, or dyslipidemia. They remained free of drugs, vitamins, amino acids, hormones, dietary supplements and botanical remedies for 14 days, including the day before the experiment. All the subjects consumed a typical Mediterranean diet. Fresh whole blood was obtained only from normolipidemic subjects.

2.5. LDL isolation and preparation

In the morning, following 12 h of fasting, 40 ml of blood were collected by venepuncture, put into vials containing EDTA (1 g/l) and immediately centrifuged for 10 min at $2000 \times g$ at 4 ° C. LDL was isolated from plasma by using discontinuous ultracentrifugation method in a TL-100 tabletop ultracentrifuge (Beckman Palo Alto, California, USA) according to a previous work (Himber, Bulher, Moll, & Moser, 1995) with minor revisions. To protect the LDL from oxidative alterations during ultracentrifugation, each density solution contained EDTA. The samples were then exhaustively dialyzed in PD-10 desalting columns (Amersham Pharmacia Biotech Uppsala, Sweden) to remove the excess salt and most of the EDTA. The LDL were used immediately.

2.6. Biochemical determinations

Total cholesterol, LDL, HDL and triglycerides were measured as previously described (Franconi et al., 2001). The protein concentration of LDL was determined using bovine serum albumin as standard and LDL was diluted to 50 μg protein/ml (Bradford, 1976). LDL oxidation was performed using a previously described method (Romani et al., 2004). Oxidation was initiated by adding freshly prepared 5 μM $CuSO_4$ (final concentration). The oxidation of LDL was quantified in triplicate by thiobarbituric acid method (Esterbauer & Cheeseman, 1990), using malonaldehyde-bisdiacetale as standard. The MDA was measured in basal condition and after 8 h of exposure to copper ions, and then in presence of different concentrations of artichoke extracts or pure compounds which were added before the copper ions. Conjugated diene formation was determined at 37 °C, monitoring the absorbance at 234 nm, as previously described (Esterbauer, Striegl, & Rothender, 1989). The results are expressed as time-course curve and as lag-time, defined as the intercept at abscissa in the diene versus time plot.

2.7. Statistical analysis

Results are expressed as means ± SE. IC_{50} was calculated using Sigmaplot 8.0. The analysis of tendency was performed using logarithmic function with Microsoft Excel using data from the dose–response curve. Comparison of means using a significance level of $P < 0.05$ was performed with ANOVA followed by Turkey's multiple range test IC_{50}.

3. Results

3.1. Identification and characterisation of bioflavonoids in artichoke extracts

In the leaves, we identified and calibrated, by HPLC/DAD and MS, the following compounds: 1-*O*-caffeoylquinic acid, 3-*O*-caffeoylquinic acid, chlorogenic acid, caffeic acid, luteolin 7-*O*-rutinoside, luteolin 7-*O*-glucoside, 1,5-*O*-dicaffeoylquinic acid, luteolin 7-*O*-malonilglucoside and luteolin (aglycone). The principal compound was the 1,5-*O*-dicaffeoylquinic acid followed by chlorogenic acid and luteolin glycosides. In the outer bracts, the following compounds were identified and calibrated: 1-*O*-caffeoylquinic acid, 3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, chlorogenic acid and apigenin 7-*O*-glucuronide, 1,5-*O*-dicaffeoylquinic acid, other dicaffeoylquinic acids derivatives and luteolin (aglycone) is undetectable. In the outer bracts, the main compound was 1,5-*O*-dicaffeoylquinic acid followed by chlorogenic acid, and, among flavonoids the presence of apigenin 7-*O*-glucuronide (Fig. 1), which is absent from the leaves, was observed. The chromatograms of EtOAC extracts were omitted because they were very similar to the ethanolic ones. In both matrixes, EtOAC had a major extraction capacity followed by ethanol and water (Table 1) which mainly influenced the quantitative composition (Table 1). In particular, dicaffeoylquinic acids were the main compounds in the outer bract extracts and in the aqueous residues of the leaves. Monocaffeoylquinic acids were the principal compounds either in ethanolic extract or in EtOAC extract of the leaves (Table 1). Due to the low concentrations in total polyphenols of aqueous residues (Table 1) they were not used for biological tests.

3.2. LDL test

The exposure of LDL to copper ions produced a dramatic increase in MDA from 6.2 ± 0.8 to 107.9 ± 4.0 nmoles/mg protein ($P < 0.001$) ($N = 30$). Pilot experiments performed with extracts of both matrixes (leaves and outer bracts) and pure standards showed that they did not influence the MDA content in basal LDL (data not shown). The dose–response curves performed on either artichoke extracts or standards clearly showed that artichoke samples and standards were able to reduce the MDA formation in a dose-dependent manner. The potency of extracts and standards was measured as the dose that induced the 50% inhibition (IC_{50} expressed in μM) of oxidation of LDL (Tables 2 and 3). IC_{50}values were calculated either considering the total polyphenol amounts or considering the amount of polyphenol subclasses as indicated in Table 1. From the table it emerges that the IC_{50} values were not related to total polyphenols. Paradoxically, the EtOAc extract of outer bracts, which was the richest in total polyphenols, had the higher IC_{50}; while the lowest IC_{50} was obtained with ethanolic extract from leaves, which had the lowest content of polyphenols. Again the IC_{50}(expressed in μM)

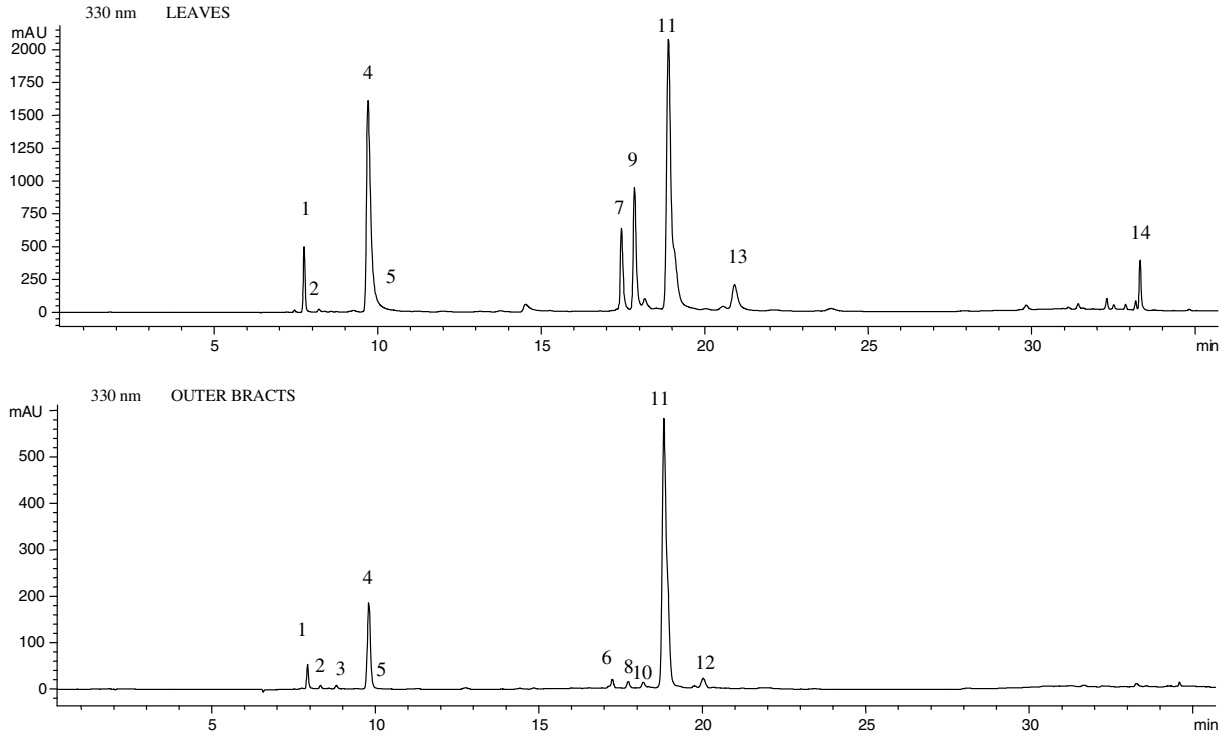

Fig. 1. Chromatographic profiles registered at 330 nm of ethanolic extracts (EtOH:H_2O pH 2 by HCOOH 70:30) of leaves and outer bracts, respectively (cv. *Viot*). *Compounds*: 1. 1-*O*-caffeoylquinic acid; 2. 3-*O*-caffeoylquinic acid; 3. 4-*O*-caffeoylquinic acid; 4. Chlorogenic acid; 5. Caffeic acid; 6. Dicaffeoylquinic acid; 7. Luteolin 7-*O*-rutinoside; 8. Luteolin 7-*O*-glucuronide; 9. Luteolin 7-*O*-glucoside; 10. Dicaffeoylquinic acid; 11. 1,5-*O*-dicaffeoylquinic acid; 12. Apigenin 7-*O*-glucuronide; 13. Luteolin 7-*O*-malonilglucoside; 14. Luteolin (aglycone).

Table 1
Polyphenolic subclasses in different artichoke extracts from leaves and outer bract tissues

Class of compounds (mM)	Outer bract ethanolic extract	Outer bract EtOAc extract	Outer bract aqueous residue	Leaf ethanolic extract	Leaf EtOAc extract	Leaf aqueous residue
Monocaffeoylquinic acid	1.022	3.210	0.111	2.103	8.767	0.109
Dicaffeoylquinic acid	3.780	25.300	0.408	0.166	0.988	0.335
Flavonoids	0.215	2.040	0.123	0.271	1.176	0.125
Total polyphenols	5.017	30.550	0.642	2.540	10.931	0.569

Data reported are the mean of three determinations, each one performed in triplicate. SE was in the range 1–3%.

Table 2
IC_{50} (μM) of artichoke extracts calculated on the generation of MDA induced by copper ions in human LDL using total polyphenol amounts and polyphenol subclass concentrations (see Table 1)

Samples	Total polyphenols	Mono caffeoylquinic acid	Dicaffeoylquinic acid	Flavonoids
Ethanolic extract from outer bracts	4.04 ± 0.21	0.90 ± 0.44	3.35 ± 0.16	0.19 ± 0.001
EtOAc extract from outer bracts	10.18 ± 1.6	1.02 ± 0.16	8.34 ± 1.31	0.66 ± 0.10
Ethanolic extract from leaves	2.92 ± 0.46	0.32 ± 0.05	0.19 ± 0.03	2.46 ± 0.39
EtOAc extract from leaves	4.91 ± 0.11	3.93 ± 0.09	0.44 ± 0.01	0.49 ± 0.10

IC_{50} (μM) values are means ± SE of at least four independent experiments, each one performed in duplicate.

of single polyphenol subclasses were not related to the amount of single subclasses. The IC_{50} of standards are shown in Table 3, although the differences were relatively small; caffeic acid appeared to be the most active compound followed by chlorogenic acid, luteolin 7-*O*-glucoside, cynarin, luteolin, and apigenin 7-*O*-glucoside, this latter being inactive up to 100 μM. Generally the IC_{50}, calculated using total concentrations of flavonoids, were lower than those obtained with standard compounds luteolin 7-*O*-glucoside and apigenin 7-*O*-glucoside (Tables 2 and 3), this latter being present at significant levels only in outer bracts (Fig. 1). The $IC_{50}$of chlorogenic acid and cynarin, reference compounds respectively for monocaffeoyl acid and dicaffeoyl acid subclasses, were 6.32 ± 1.04 and 8.58 ± 0.97 μM. In the extracts, the IC_{50}calculated considering the concentrations of total

Table 3
IC_{50} (μM) of different standards calculated on MDA generation induced by copper in human LDL

Standards	ED_{50} (μM)
Caffeic acid	4.89 ± 0.04
Chlorogenic acid	6.32 ± 1.04
Luteolin 7-*O*-glucoside	6.94 ± 1.71
Cynarin	8.58 ± 0.97
Luteolin (aglycone)	22.11 ± 5.30
Apigenin 7-*O*-glucoside	>100

IC_{50} values are means ± SE of at least four independent experiments, each one performed in duplicate.

monocaffeoyl derivatives and the total dicaffeoyl derivatives were decreased in comparison with those obtained with the standards (Table 3), with the exception of the IC_{50} (8.4 ± 1.31 μM) for dicaffeoyl acid subclasses present in the EtOAc extract of outer bracts whose was practically identical to that of pure standard (8.58 ± 0.97 μM). The dose–response curves of extracts and standards were also analysed for their tendency (Table 4). They evidenced that chlorogenic acid and luteolin-7-*O*-glycosides had slopes of −62.783 and −62.258, respectively; while the slopes of caffeic acid and cynarin were −70.122 and −72.583, respectively. Regarding the extracts, ethanolic and EtOAc extract from outer bracts had practically the same slope, while the extracts from leaves had a different slope, suggesting a different efficiency in antioxidant activity (Table 4). The measurable LDL conjugated-diene formation did not occur in the absence of copper ions (data not shown). The lag-phase was increased in the presences of plant extract in a dose-dependent manner (Table 5). The typical profile in conjugate dienes formation with all extracts in the presence of copper ions is shown in (Fig. 2).

4. Discussion

Leaves and outer bracts of Viot contain mono- and dicaffeoylquinic derivatives and flavonoids, with the outer bracts being the richest. The two tissues present mainly quantitative differences, although apigenin derivatives are present at detectable levels only in outer bracts. Comparison with literature findings shows that the subclasses present in the leaves of Viot are the same as those found in leaves coming from other artichoke cultivars of the Violet variety (Hausler, Ganzera, Popp, & Stuppner, 2002; Sanchez-Rabaneda et al., 2003; Schutz et al., 2004; Wang et al., 2003). It is not to possible to compare the chemical composition of outer bracts because, to our knowledge, this is the first time that it has been fully characterized. Indeed, it is similar to that found in the extract, which utilizes as raw material outer bracts, receptacles and stems (Llorach et al., 2002). Differently from other extracts coming from other artichoke varieties (Schutz et al., 2004; Wang et al., 2003), in Viot naringenin, naringin and narirutin are undetectable using extract ions ($[M–H]^-$ 271, 433, 579 *m*/*z*, respectively) by HPLC/MS. This is an important point because they seem to have inhibitory effects on CYP enzymes, reducing the metabolism of ethic drugs, such as statins and cyclosporine (Ho & Saville, 2001) to the point of requiring a dosage adjustment. In human LDL, all extracts inhibit MDA generation and diene-formation induced by copper ions as this is a dose-dependent effect within a very low concentration range. Using DPPH (2,2-diphenyl-1-picrylhydrazyl) test, which is intrinsically different from the test used here, other authors (Wang et al., 2003) have found that antioxidant activity of leaves and head artichoke extracts correlate to their total polyphenolic content. Paradoxically, here, the potency is not related to the total polyphenol amounts or to the amounts of single polyphenol subclasses, suggesting that antioxidant activity is not simply a function of polyphenol concentrations. Indeed, the analysis of tendency reveals that the most potent extracts had the lowest slope and, vice versa for the least potent. Other factors, such as interactions among different molecules, water solubility etc. could explain the lack of correlation between potency and amount of polyphenols. Interactions are plausible because the IC_{50} of single subclasses are generally different in the single extracts and they are also generally different from those obtained with the representative standards. The lower IC_{50} is found in ethanolic extract of leaves and the higher one in the EtOAc extract of the outer bracts, which have more or less the same concentration of flavonoids. Regarding the monocaffeoyl quinic acid, the $IC_{50}$of chlorogenic acid is 6.32 ± 1.04 μM. In ethanolic and EtOAc extracts from

Table 4
Tendency analysis of dose–response curves on the antioxidant activity of standards and artichoke extracts measured on MDA formation in human LDL oxidized by copper ions

Samples and standards	Regression line	r^2
Cynarin	$y = -72.583\ln(x) + 147.03$	0.64
Chlorogenic acid	$y = -62.783\ln(x) + 125.12$	0.77
Luteolin-7-*O*-glycosides	$y = -62.258\ln(x) + 131.01$	0.66
Caffeic acid	$y = -70.122\ln(x) + 137.94$	0.71
Luteolin (aglycone)	$y = -0.3918\ln(x) + 27.604$	0.69
Ethanolic extract from outer bracts	$y = -58.844\ln(x) + 117.97$	0.83
EtOAc extract from outer bracts	$y = -58.249\ln(x) + 120.01$	0.78
Ethanolic extract from leaves	$y = -51.931\ln(x) + 119.38$	0.77
EtOAc extract from leaves	$y = -65.489\ln(x) + 120.45$	0.75

Table 5
Dose–response-curve of artichoke extracts (μM, calculated as total polyphenols) on conjugate-diene generation induced by cupric ions measured as lag-time (min) at 234 nm in isolated human LDL

	0	5 μM	7.5 μM	10 μM	20 μM
Copper ions	40 ± 11				
Ethanolic extract from outer bracts		50 ± 5	90 ± 15	>300	>300
EtOAc extract from outer bracts		43 ± 8	60 ± 12	97	>300
Ethanolic extract from leaves		60 ± 5	120 ± 14	>300	>300
EtOAc extract from leaves		50 ± 7	65 ± 5	>300	>300

Values are means ± SE of at least four independent experiments, each one performed in duplicate.

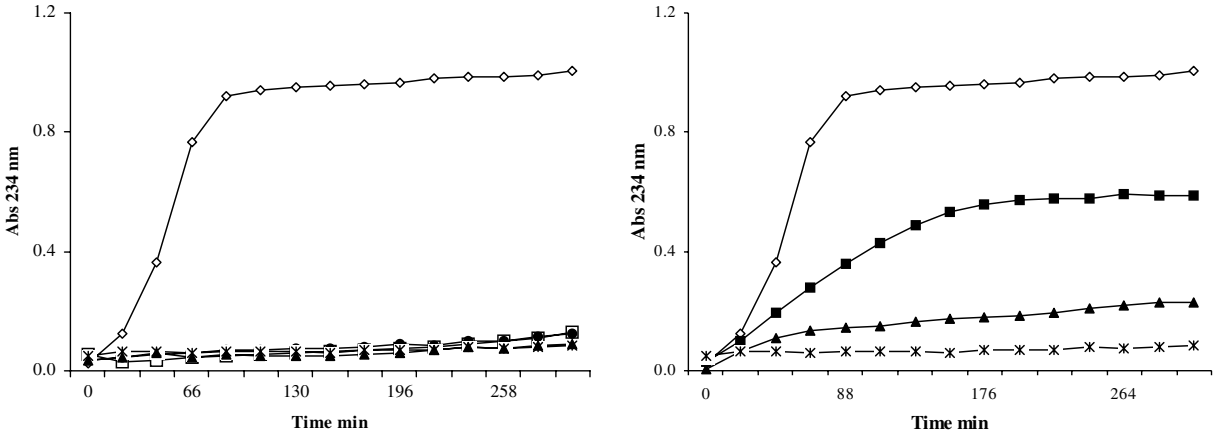

Fig. 2. Representative curves (left side) of the maximum effects obtained with different extracts (ethanolic (⁎), EtOAc (▲) extract of outer bracts, ethanolic (○) and EtOAc (□) extracts from the leaves) at concentration of 20 μM (calculated as total polyphenols) on diene formation induced by cupric ions (◇) measured at 234 nm in isolated human LDL. A representative dose-effect response (right side) performed with different concentrations of EtOAc from outer bracts at following concentrations calculated as total polyphenols: (■) 5 μM; (▲) 7.5 μM; (⁎) 20 μM.

outer bracts, the IC_{50} (μM) of monocaffeoyl quinic acids are 0.90 ± 0.44 and 1.02 ± 0.16, respectively; and ethanolic and EtOAc extracts from the leaves are 0.32 ± 0.05, 3.93 ± 0.09, respectively. The lowest IC_{50} was obtained in ethanolic extract of leaves, and has the lowest concentration of dicaffeoyl quinic acids, an intermediate concentration of monocaffeoyl quinic acids and flavonoids, while the highest IC_{50} was calculated in EtOAc of leaves, characterized by the highest level of monocaffeoyl quinic acids, and an intermediate concentration of flavonoids and dicaffeoylquinic acids. In this latter case, the IC_{50} is approximately similar to that found with pure standard. The calculated $IC_{50}$for cynarin is 8.58 ± 0.97 μM, while the IC_{50}(μM) of dicaffeoylquinic acids are 3.35 ± 0.16, 8.34 ± 1.31 in ethanolic and EtOAc extracts from outer bracts, and 0.19 ± 0.03, 0.44 ± 0.01 in ethanolic and EtOAc extracts from the leaves, respectively. The lowest IC_{50} was obtained in ethanolic extract of leaves, with the lowest concentration of dicaffeoyl quinic acids, an intermediate concentration of monocaffeoyl quinic acids and flavonoids, while the highest IC_{50} was calculated in EtOAc of outer bracts, characterized by the highest level of dicaffeoyl quinic acids and flavonoids and by the lowest levels of monocaffeoylquinic acids. In this latter case, the IC_{50} is practically identical to the standard one. However, interactions are not only important for the potency but also for the slope, because in all extracts the slope is lower than that of the standard. Among the standards it has been shown that caffeic acid and cynarin have the same slope and it is higher than those obtained with chlorogenic acid and luteolin-7-*O*-glycosides, suggesting that it is not dependent on the number of substitutions on caffeic acid. Moreover, the partition coefficient of the different molecules could influence the accessibility of antioxidants to the lipid peroxyl radicals (Castelluccio, Bolwell, Gerrish, & Rice-Evans, 1996) and ultimately their ability to scavenge reactive oxygen species, as the location of the phenol at lipid-water interface is important. The difference of IC_{50} of pure standards confirms that this point is critical for caffeic acid and its derivatives as previously described (Esterbauer, Gebicki, Puhl, & Jurgens, 1992). It is possible to make some general conclusions: (1) considering standard effects, it emerges that apigenin 7-*O*-glucoside is not important for the antioxidant activity because it is inactive up to100 μM; (2) the IC_{50} of the main compounds in the class of quinic acids is less influenced by the presence of other polyphenols while the IC_{50} of flavonoids, which are the least represented subclass, is reduced in a substantial and differential way when compared with standard; (3) tendency analysis shows that the extract with the most potency has the lowest slope and this could explain the lack of correlation between potency and amount of polyphenols; and finally (4) the overall antioxidant effect is a complex process that depends on several modes of polyphenol interaction which may participate to differing degrees due to their different ratios among subclasses or single molecules. On the other hand, it is also necessary to consider that antioxidant properties could be ascribed to various mechanisms between molecules, such as chelation, etc (Brown & Rice-Evans, 1998). In conclusion, extracts of Viot are able to reduce lipid peroxidation in LDL obtained from healthy individuals in a dose-dependent manner. This is in line with previous results in a single subject with hypercholesterolemia (Jimenez-Escrig et al., 2003) and with a study focused on artichoke flavonoids (Brown & Rice-Evans, 1998) performed using a commercial extract. The present paper aims at correlating the antioxidant activity with chemical structure of polyphenol artichoke subclasses and from our results it emerges that the antioxidant activity is not merely dependent on total amount of polyphenols, implying the need of detailed characterization of an extract before it is put on the market. In addition, this study clearly shows that also the outer bracts of artichoke are a good raw material to obtain antioxidants. The antioxidant activity here described could synergize with the hypocholesterolemic of artichoke previously described (Brown & Rice-Evans,

1998; Englisch et al., 2000; Gebhardt, 1998; Kraft, 2001; Pittler, Thompson, & Ernst, 2002; Thompson Coon & Ernst, 2003) in inhibiting the risk for cardiovascular diseases, in agreement with the oxidative hypothesis of atherosclerosis (Chisolm & Steinberg, 2000). Obviously before introducing artichoke extracts in therapy more rigorous clinical trials are need to assess larger patient samples over longer intervention periods to establish whether artichoke extracts are an effective and safe treatment option for patients with hypercholesterolaemia, either alone or in association with other drugs. In this regard, it is important to know the composition of extracts in order to eliminate samples which contain polyphenols that interfere with CYP enzyme, thus avoiding drug interaction as occurs with many herbal remedies (Bailey & Dresser, 2004). An accurate selection of raw material could meet this requirement. Leaves and outer bracts of Viot are naringenin-, narigin- and naritutin-free while these compounds can be present in artichoke extracts from other cultivars or tissues. Based on these findings, Viot could be a good variety for preparation of botanical remedies.

Acknowledgements

This work was partly supported by grant from the Provincia di Grosseto and Centre for Biotechnology Development and Biodiversity Research; S.C. is supported by a fellowship from GIO.I.A Foundation onlus.

References

Adzet, T., Camarasa, J., & Laguna, C. J. (1987). Hepatoprotective activity of polyphenolic compounds from *Cynara scolymus* against CCl_4 toxicity in isolated rat hepatocytes. *Journal of Natural Products, 50*, 612–617.

Anonymous (Korrektur 1.9. 1990). Monographie Cynarae folium. Artischockenblatter. BGA-Aufbereitungsmonographie, Bundesanzeiger 6.7. 1988.

Bailey, D. G., & Dresser, G. K. (2004). Natural products and adverse drug interactions. *Canadian Medical Association Journal, 170*, 1531–1532.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248–254.

Brown, J. E., & Rice-Evans, C. A. (1998). Luteolin-rich artichoke extract protects low density lipoprotein from oxidation in vitro. *Free Radical Research, 29*, 247–255.

Castelluccio, C., Bolwell, G. P., Gerrish, C., & Rice-Evans, C. (1996). Differential distribution of ferulic acid to the major plasma constituents in relation to its potential as an antioxidant. *Biochemical Journal, 1*, 691–694.

Chen, J. H., & Ho, C. T. (1997). Antioxidant activities of caffeic acid and its related hydroxycinamic acid compounds. *Journal of Agricultural and Food Chemistry, 45*, 2374–2378.

Chisolm, G. M., & Steinberg, D. (2000). The oxidative modification hypothesis of atherogenesis: an overview. *Free Radical Biology and Medicine, 28*, 1815–1826.

Clifford, M. (2000). Chlorogenic acid and other cinnamates-nature occurance, dietary burden, absorption and metabolism. *Journal of the Science of Food and Agriculture, 80*, 1033–1043.

Dahan, A., & Altman, H. (1992). Food-drug interaction: grapefruit juice augments drug bioavailability – mechanism, extent and relevance. *European Journal of Clinical Nutrition, 58*, 1–9.

Englisch, W., Beckers, C., Ruepp, M., & Zinserling, V. (2000). Efficacy of artichoke dry extract in patients with hyperlipoproteiniemia. *Arneimittel-Forschung/Drug Research, 50*, 260–265.

Ernst, E. (1995). Die Artischocke: eine Heipflanze mit Geschichte und Zukunftsperspektive. *Naturmed, 10*, 1–4.

Esterbauer, H., & Cheeseman, K. H. (1990). Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydrononenal. *Methods in Enzymology, 186*, 407–421.

Esterbauer, H., Gebicki, J., Puhl, H., & Jurgens, G. (1992). The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radical Biology and Medicine, 13*, 341–390.

Esterbauer, H., Striegl, G., & Rothender, M. (1989). Continuous monitoring of in vitro oxidation of human low density lipoprotein. *Free Radical Research, 6*, 67–75.

Franconi, F., Miceli, M., Alberti, L., Boatto, G., Coinu, R., De Montis, M. G., et al. (2001). Effect of gamma-hydroxybutyric acid on human platelet aggregation in vitro. *Thrombosis Research, 102*, 255–260.

Gebhardt, R. (1997). Antioxidant and protective properties of extract from the leaves of artichoke (*Cynara scolymus* L.) against hydroperoxide-induced oxidative stress in cultured rat hepatocytes. *Toxicology and Applied Pharmacology, 144*, 286–293.

Gebhardt, R. (1998). Inhibition of cholesterol biosynthesis in primary cultured rat hepatocytes by artichoke (*Cynara scolymus* L.) extracts. *Journal of Pharmacology and Experimental Therapeutics, 287*, 1122–1128.

Gebhardt, R., & Fausel, M. (1997). Antioxidant and hepatoprotective effects of artichoke extracts and constituents in cultured rat hepatocytes. *Toxicology in vitro, 144*, 279–286.

Halliwell, B. (1994). Free radicals, antioxidants and human disease: curiosity, cause or consequence? *Lancet, 344*, 721–724.

Halliwell, B. (1996). Oxidative stress, nutrition and health. Experimental strategies for optimization of nutritional antioxidant intake in humans. *Free Radical Research, 25*, 57–74.

Harris, R. Z. (2003). Dietary effects on drug metabolism and transport. *Clinical Pharmacokinetics, 42*, 1071–1088.

Hausler, M., Ganzera, M., Popp, M., & Stuppner, H. (2002). Determination of caffeoylquinic acids and flavonoids *Cynara scolymus* L. by high performance liquid chromatography. *Chromatographia, 56*, 407–411.

Himber, J., Bulher, E., Moll, D., & Moser, U. K. (1995). Low density lipoproteins for oxidation and metabolic studies. Isolation from small volumes of plasma using a tabletop ultracentrifuge. *International Journal for Vitamin and Nutrition Research, 65*, 137–142.

Ho, P. C., & Saville, D. J. (2001). Inhibition of human CYP3A4 activity by grapefruit flavonoids, furanocoumarins and related compounds. *Journal of Pharmaceutical Sciences, 4*, 217–227.

Holtmann, G., Adam, B., Haag, S., Collet, W., Grunewald, E., & Windeck, T. (2003). Efficacy of artichoke leaf extract in the treatment of patients with functional dyspepsia: a six-week placebo-controlled, double-blind, multicentre trial. *Alimentary Pharmacology and Therapeutics, 18*, 1099–1105.

Jimenez-Escrig, A., Dragsted, L. O., Daneshvar, B., Pulido, R., & Saura-Calixto, F. (2003). In vitro antioxidant activities of edile artichoke (*Cynara scolymus* L.) and effect on biomarkers of antioxidants in rats. *Journal of Agricultural and Food Chemistry, 51*, 5540–5545.

Kraft, K. (2001). Therapy of cardiovascular diseases with phytopharmaceuticals – New findings from clinical studies with common hawthorn, artichoke leaf and garlic. *Biologische Medizin, 30*, 56–60.

Llorach, R., Espin, J. C., Tomas-Barberan, F. A., & Ferreres, F. (2002). Artichoke (*Cynara scolymus* L.) byproducts as a potential source of health-promoting antioxidant phenolics. *Journal of Agricultural and Food Chemistry, 50*, 3458–3464.

Mulinacci, N., Prucher, D., Romani, A., Peruzzi, M., Pinelli, P., Giaccherini, C., et al. (2004). Phenols determination of laboratory

and commercial herbal products from artichoke leaves. *Journal of Pharmaceutical and Biomedical Analysis, 34*, 349–357.

Perez Garcia, F., Adzet, T., & Canigueral, S. (2000). Activity of artichoke leaf extract on reactive oxygen species in human leukocytes. *Free Radical Research, 33*, 661–665.

Pittler, M. H., Thompson, C. O., & Ernst, E. (2002). Artichoke leaf extract for treating hypercholesterolaemia. *Cochrane Database Syst Rev, 2003*(1), CD003383.

Romani, A., Coinu, R., Carta, S., Pinelli, P., Galardi, C., Vincieri, F. F., et al. (2004). Evaluation of antioxidant effect of different extracts of *Myrtus communis* L.. *Free Radical Research, 38*, 97–103.

Sanchez-Rabaneda, F., Jauregui, O., Lamuela-Raventos, R. M., Bastida, J., Viladomat, F., & Codina, C. (2003). Identification of phenolic compounds in artichoke waste by high-performance liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A, 1008*, 57–72.

Schutz, K., Kammerer, D., Carle, R., & Schieber, A. (2004). Identification and quantification of caffeoylquinic acids and flavonoids from artichoke (*Cynara scolymus* L.) heads, juice, and pomace by HPLC-DAD-ESI/MS(n). *Journal of Agricultural and Food Chemistry, 52*, 4090–4096.

Tapiero, H., Tew, K. D., Ngugen Ba, G., & Mathè, G. (2002). Polyphenols: do play a role in the prevention of human pathologies. *Biomedicine Pharmacotherapy, 56*, 200–207.

Thompson Coon, J. S., & Ernst, E. (2003). Herbs for serum cholesterol reduction: a systematic view. *Journal of Family Practice, 52*, 468–478.

Wang, M., Simon, J. E., Aviles, I. F., He, K., Zheng, Q., & Tadmor, Y. (2003). Analysis of antioxidative phenolic compounds in artichoke (*Cynara scolymus* L.). *Journal of Agricultural and Food Chemistry, 51*, 601–608.

Wojcicki, J., Samochowiec, L., & Kosmider, K. (1981). Influence of an extract from artichoke (*Cynara scolymus* L.) on the level of lipids in serum aged men. *Herba Pol, 27*, 265–268.

Zapolska-Downar, D., Zapolski-Downar, A., Naruszewicz, M., Siennicka, A., Krasnodebska, B., & Kolodziej, B. (2002). Protective properties of artichoke (*Cynara scolymus*) against oxidative stress induced in cultured endothelial cells and monocytes. *Life Science, 71*, 2897–2908.