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

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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*.

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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