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In vitro antioxidant activities and phenolic content in crop residues of Tunisian globe artichoke

Dabbou Sihem[a], Dabbou Samia[a,b], Pandino Gaetano[c,*], Lombardo Sara[c], Mauromicale Giovanni[c], Chahdoura Hassiba[a], Gasco Laura[d], Helal Ahmed Noureddine[a]

[a] *Laboratory of Bioressources, Integrative Biology and Valorisation, Higher Institute of Biotechnology of Monastir, Av. Tahar Hadded, BP 74, 5000 Monastir, Tunisia*
[b] *Dentistry Faculty, Avicenne Street, 5019 Monastir, Tunisia*
[c] *Dipartimento di Agricoltura, Alimentazione e Ambiente (Di3A), Università degli Studi di Catania, via Valdisavoia 5, 95123 Catania, Italy*
[d] *Dipartimento di Scienze Agrarie Forestali e Alimentari (DISAFA), Via Leonardo Da Vinci, 44, 10095 Grugliasco, Torino, Italy*

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ABSTRACT

The phenolic content and *in vitro* antioxidant activity of the bracts, leaves and floral stems of two Tunisian globe artichoke cultivars ('Violet d'Hyéres' and 'Blanc d'Oran') were assessed; the tests used to assay antioxidant activity were based on ABTS [2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid] and DPPH [2,2-diphenyl-l-picrylhydrazyl]. In addition reducing power was measured and the phosphomolybdenum total antioxidant activity assay was included. The bracts of 'Violet d'Hyéres' possessed, on average, twice the quantity of phenolic compounds than those of 'Blanc d'Oran'. They also had a higher content of 3,5-*O*-dicaffeoylquinic acid. The bracts and floral stems contained high levels of total caffeoylquinic acid, whereas the leaves provided a potentially exploitable source of luteolin. Ethanolic extracts of both cultivars exhibited a high level of antioxidant activity. The ABTS EC_{50} value was high in extracts from the leaves of both cultivars, whereas the DPPH test showed no variation between the bracts, floral stems and leaves. The bracts were associated with strong reducing power and total antioxidant activity. Overall, the leaves were associated with more antioxidant activity than the bracts or the floral stems. The implication is that globe artichoke crop residues could provide a useful source of antioxidant compounds.

1. Introduction

The increasing demand for high-quality bio-products, economically and environmentally friendly technologies, as well as restrictive legislative actions, has stimulated scientific research on the extraction, purification and identification of bioactive compounds from natural sources. Indeed, the growing interest in the substitution of synthetic food antioxidants by natural ones has fostered research on vegetable sources and, as consequence, on the screening of raw materials in order to identify new antioxidants (Moure et al., 2001). Replacing synthetic antioxidants with natural ones may have benefits for health implications and industrial applications, such as the solubility in both oil and water. Phenolic compounds, ubiquitous in plants, are an essential part of the human diet and are of considerable interest due to their antioxidant properties (Balasundram et al., 2006). In particular, several researchers have investigated the possibility of extracting natural antioxidants from agricultural and industrial residues such as potato peel waste (Rodriguez de Sotillo et al., 1994), olive oil waste waters (Visioli et al., 1999), grape seeds (Yamaguchi et al., 1999), mango peels (Berardini et al., 2005) and apple pomace (Schieber et al., 2003).

The globe artichoke [*Cynara cardunculus* L. var. *scolymus* (L.) Fiori] is native to the Mediterranean area, where its commercial production makes a significant contribution to the agricultural economy (Mauromicale and Ierna, 2000). About 65% of its global production is concentrated in the Mediterranean Basin (FAOstat, 2012), a region where autochthonous landraces have been grown (Mauro et al., 2009). This crop is grown for its immature inflorescence (also referred as capitulum or head), which is widely consumed as fresh or conserved vegetable, due to its good sensory properties and content of health-promoting compounds (Lombardo et al., 2010, 2012, 2013; Schütz et al., 2006). Globe artichoke is commonly used in traditional medicine due

* Corresponding author. Tel.: +39 095234421.
E-mail address: g.pandino@unict.it (P. Gaetano).

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to its pharmacological properties such as its anti-inflammatory, antihepatotoxicity, antioxidative, anti-lipoperoxidation, antidiabetic, anti-cancer, glycaemia reduction and antimicrobial activities (Coinu et al., 2007; Fantini et al., 2011; Rondanelli et al., 2011; Saénz-Rodriguez et al., 2002). The use of residues (mostly external bracts, leaves and floral stem) as sources of bioactive compounds has emerged as an economically viable solution to the problem of solid waste treatment. These crop residues represent 80–85% of the above-ground biomass and have a high content of flavones luteolin and apigenin and caffeoylquinic acids (Llorach et al., 2002; Pandino et al., 2013a, 2013b; Romani et al., 2006; Wang et al., 2003). The two main phenolic compounds are 5-*O*-caffeoylquinic acid and 1,5-di-*O*-caffeoylquinic acid (Schütz et al., 2004; Wang et al., 2003; Yoo et al., 2012), which have a strong antioxidant capacity (Vinson et al., 1995).

A comparison of the phenolic content of globe artichoke part of plants showed an extremely low content of hydroxycinnamic acids and flavonoids in the leaves versus the content in receptacle and flower bracts (Fratianni et al., 2007). The high phenolic content in globe artichoke crop residues may vary according to plant parts and cultivars (Farag et al., 2013; Pandino et al., 2012a), head maturity, storage and processing techniques (Lutz et al., 2011; Pandino et al., 2012b; Wang et al., 2003).

In Tunisia, even if the globe artichoke production has increased during last years, only papers on the characterization of wild cardoon (*C. cardunculus* var. *sylvestris*) have been published (Falleh et al., 2008; Khaldi et al., 2013). To the best of our knowledge, this is the first study to report the phenolic profile and antioxidant activities from waste material (bracts, leaves and stems) of Tunisian cultivars of globe artichoke. The aim of this research was to quantify the extracted phenolic contents in the capitula residues (bracts, floral stem and leaves) of the two Tunisian globe artichoke cultivars and to evaluate the antioxidant activities of the extracts, so as to identify the plant's materials of major interest for pharmaceutical and related industries.

2. Materials and methods

2.1. Plant material, experimental field and management practices

Two cultivars ('Violet d'Hyéres' and 'Blanc d'Oran'), native in northern Tunisia and producing green heads from April to May, were grown in 2011–2012 season at the experimental field of Technical Center of Potato and Artichoke of Tunisia located in Jdaida-Mannouba (latitude 36°49′25.24″ N, longitude 9°57′55.09″W, altitude 595 m). This is a typical area for globe artichoke cultivation in the Mediterranean Basin, with mild winter and hot rainless summer. Plants were spaced by 1.2 m × 0.6 m with 13.888 plants/ha for 'Blanc d'Oran' and by 1.2 m × 0.8 m with 10.416 plants/ha for 'Violet d'Hyéres'. Crop management practices (irrigation, fertilization, pest management, weeds control, etc.) were subsequently performed according to local practices.

2.2. Reagents and solvents

Reagents and solvents were purchased from VWR (Leighton Buzzard, UK) and were of analytical or HPLC grade. Apigenin-7-*O*-glucoside, apigenin, luteolin-7-*O*-glucoside, luteolin, 5-*O*-caffeoylquinic acid (chlorogenic acid) were obtained from Extrasynthese (Lyon, France), 1,3-*O*-di caffeoylquinic acid (cynarin) was from Roth (Karlsruhe, Germany).

2.3. Extraction procedure

Plant residue parts of globe artichoke (bracts, leaves and floral stem) were collected separately at the usual marketing stage regardless of their size when the central global flower buds of the capitula were shorter than 2 mm (this is the normal marketing stage, Mauromicale and Ierna, 2005). They were washed with running water to remove impurities, air-dried in an oven at a temperature of 37 °C and, finally, crushed and sieved through mesh cloth to get the fine powder. About 50 g of the powdered plant materials (bracts, leaves and floral stem) were extracted with 500 mL of 95% ethanol by maceration under stirring at room temperature for 7 days (Harikrishnan and Balasundaram, 2005). After filtration, the solvent was evaporated under vacuum and all the resulting extracts obtained were then transferred to vials and stored in the dark at 4 °C to preserve them from photo-oxidation.

2.4. HPLC analysis

The extraction procedure, performed for samples under study, was carried out as described previously by Pandino et al. (2010). Each extract was analysed using a series 1200 HPLC instrument (Agilent Technologies, Palo Alto, CA) equipped with ChemStation software (B.03.01) and a diode array detection system. Separations were achieved on a Zorbax Eclipse XDB-C18 (4.6 mm × 150 mm; 5.0 μm particle size), operated at 30 °C, with a 0.2 μm stainless steel inline filter. The method was adapted from Pandino et al. (2010): the mobile phase was 1% formic acid in water (solvent A) and in acetonitrile (solvent B) at a flow rate of 0.5 mL/min. The gradient started with 5% B to reach 10% B at 10 min, 40% B at 30 min, 90% B at 50 min, 90% B at 58 min. Chromatograms were recorded at 280, 310, and 350 nm from diode array and data collected between 200 and 600 nm. Each compound was identified based on retention time, UV spectrum and already published identification on compounds from globe artichoke (Schütz et al., 2004; Wang et al., 2003). Quantification was performed by calibration curve using the available standards. In particular, mono- and dicaffeoylquinic acids were calculated using 5-*O*-caffeoylquinic acid and 1,3-*O*-dicaffeoylquinic acid as reference, respectively. Here, the caffeoylquinic acids are presented according to Lattanzio et al. (2009). Apigenin and luteolin conjugates were quantified as apigenin-7-*O*-glucoside and luteolin-7-*O*-glucoside, respectively. All data presented are mean values ± standard deviation of three independent experiments ($n = 3$) and expressed as mg g^{-1} of dry matter (DM).

2.5. Antioxidant activity

To determine antioxidant activity, different concentrations of ethanolic extracts were prepared in methanol. Four common tests for measuring antioxidant activity were applied to globe artichoke waste material extracts: 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid antioxidant capacity assay (ABTS assay), 2,2-diphenyl-l-picrylhydrazyl assay (DPPH assay), reducing power assay and total antioxidant activity (Phosphomolybdenum assay). The four methods presented can be divided into two groups depending on the oxidizing reagent. Two methods use organic radical producers (ABTS and DPPH assays) and the others work with metal ions for oxidation (Phosphomolybdenum and reducing power assays). The ABTS and DPPH tests acting by radical reduction use preformed radicals and determine the decrease in absorbance, while the reducing power and phosphomolybdenum assays measure the formed ferrous ions by increased absorbance.

2.6. DPPH radical scavenging assay

An aliquot (20 μL) from the stock solution of each extract was dissolved in absolute ethanol to a final volume of 1 mL and then added to 1 mL DPPH (0.1 mM, in absolute ethanol) (Kontogiorgis