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ORIGINAL ARTICLE

Wild grown red and yellow hawthorn fruits from Tunisia as source of antioxidants

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Abstract Hawthorn fruits (*Crataegus* spp.), may be a good source of antioxidants if is consumed as fresh fruit since we know that it produce a numerous beneficial effects for human health. In this study, two species of hawthorn fruit, *Crataegus monogyna* and *Crataegus azarolus* were analyzed by HPLC–DAD–MS and compared with respect to their phytochemical composition. Phenolic profiles of studied fruits showed some similarities and differences in terms of polyphenols between the two species. Twenty phenolics compounds distributed into four subclasses were identified: four phenolic acids including three hydroxycinnamic acids and one hydroxybenzoic acid, eight flavonoids representing the most abundant subclass including six glucosylated flavonols and two flavones, two anthocyanins are present as glycosides of cyanidin, with cyanidin-3-*O*-glucoside is the most abundant, only in *monogyna* peel fraction and four flavanols divided into a monomer (−)-epicatechin identified in all fruit parts of both species, a dimer B2 and two trimers (C1 and C2). These phenolic compounds are concentrated especially in peel fraction. These results indicate that hawthorn fruits should be recommended in dietary habits as a potential source of antioxidant and anticarcinogenic phenolic compounds.

1. Introduction

The genus *Crataegus* (Hawthorn), belonging to the Rosaceae family, is a genus of spiny trees or shrubs present in the northern hemisphere (Verma et al., 2007). They are usually multibranched shrubby trees that can reach a height of up to 10 m. The color of the ripe fruit ranges from yellow, through

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green to red and on to dark purple. Most of the species ripen their fruit in early to mid-autumn (Brown, 1995).

Beneficial effects of hawthorn fruit extracts have been confirmed by various studies, pharmacological data show that hawthorn fruits and its preparations enhance myocardial contraction and conductivity, protect against ischemia (Veveris et al., 2004). They have a sedative action, a protective effect against arrhythmia and increase of coronary vessel flow (Zhang et al., 2001). They have also positive effects on the cardiovascular system (Caliskan et al., 2012). Recent studies have focused on the health benefits of hawthorn fruits such as antioxidant, antimicrobial, antiproliferative and mutagen properties (Froehlicher et al., 2009; Caliskan et al., 2012; Rodrigues et al., 2012 and Mraihi et al., 2013). These pharmacological properties are the consequence of the benefic effect of active phenolic compounds of hawthorn fruits that modulate a variety of biological events.

Polyphenols are secondary compounds widely distributed in the plant kingdom. They are divided into several classes, phenolic acids (hydroxybenzoic and hydroxycinnamic acids) (Fig. 1), which is distributed in plants and foods of plant origin (Manach et al., 2005). Additionally, phenolics act as metal chelators, antimutagens or anticarcinogens antimicrobial and clarifying agents (Proestos et al., 2005).

The flavonoid family is divided into a number of subgroups. The six main classes are flavonols, flavones, flavan-3-ols, isoflavones, flavanones and anthocyanidins with similar structure having a C6–C3–C6 flavone skeleton (Fig. 1). Flavonoids are one of the most important bioactive polyphenols, showing a diverse structure and a broad range of biological activities (Naczk and Shahidi, 2004; De Rijke et al., 2006).

Flavonols and flavones are synthesized in plant tissues from a branch of the phenylpropanoid pathway. The major flavonol aglycones found in plant foods are quercetin, myricetin and kaempferol, while a more limited number of fruits and vegetables contain the structurally-related flavones, apigenin and luteolin (Fig. 1). In plant tissues, flavonols and flavones are found conjugated to sugars such as glucose, galactose, rhamnose, and rutinose (Herrmann, 1988). Most conjugations occur at the 3 position of the B ring, although it can also occur frequently at the 7 and 4′ positions.

Flavan-3-ols are a complex subclass of flavonoids encompassing the simple monomers (+)-catechin, its isomer (−)-epicatechin, oligomeric and polymeric procyanidins, commonly known as condensed tannins (Catherine et al., 2005) (Fig. 1). In particular, condensed tannins are usually associated with astringent perception (Porter, 1988).

Anthocyanins are the strong antioxidants, which may be related to the health benefits. Anthocyanidins are flavylium (2-phenylbenzopyrylium) structures with varying hydroxyl or methoxyl substitutions. The anthocyanin forms found in foods are glycosides and acylglycosides of six common aglycon anthocyanidins: pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin (Nicoue et al., 2007). Anthocyanin pigments are responsible for the reddish blue and purple color of many fruit (Martinelli et al., 1992).

The aim of the present study was to identify the main phenolic compounds and to provide an overview of the phytochemical composition of hawthorn fruit extracts using HPLC–DAD–MS. Additionally, it was established the compositional differences between the two *Crataegus* species very distributed in Tunisian flora.

2. Materials and methods

2.1. Samples

2 kg of *Crataegus azarolus* and *Crataegus monogyna* fruit samples was recollected in September 2009 from Kef en Nsour (Jendouba) northwestern Tunisia, located at 36° 33′ 2″ N latitude, 8° 25′ 29″ E longitude and 606 m altitude. Fruits were immediately transported after recollection to our laboratory. Fruits were peeled with a sharp knife, and the three parts: peel, pulp and seeds were lyophilized and stored at −20 °C until analyzed.

2.2. Standards and reagents

Analytical grade phenolic standards: phloroglucinol, gallic, protocatechuic, OH-benzoic, vanillic, caffeic, syringic, *p*-coumaric, sinapic, 3-(2′,5′-dimethoxybenzoyl) propionic (DMB propionic), homovanillic, homogentisic, ferulic and chlorogenic acids; phloridzin, catechin, epicatechin, quercetin, quercetin-3-*O*-glucoside, kaempferol, were purchased from Sigma–Aldrich Química S. A. (Madrid, Spain). Solvents used were HPLC-grade and were obtained from Merck (Darmstadt, Germany). Methanol, ethanol, chloroform and chlorhydric acid were supplied by Prolabo (Madrid, Spain).

2.3. Preparation of the phenolic extracts

The lyophilized peel, pulp and seed were processed separately; Approximately 2 g of dried parts from each *Crataegus* species was extracted three times by 15 ml of methanol/acidified water HCl 1.5 N, during 30 min in an ultrasonic bath (FALC Instruments, Italy) (Khanizadeh et al., 2008). The extracts were centrifuged and the three methanolic supernatants were combined and the methanol was removed *in vacuo*. The aqua resultant extracts were lyophilized until dried. Finally, 5 ml of ultrapure water (Millipore Milli-Q water purification system) was added into the dried extracts and filtered through a 0.45 μm membrane-filter.

2.4. HPLC–UV Analysis of Phenolics

A sample of 20 μL of the different supernatants above-obtained was analyzed using an Agilent 1200 Series liquid chromatography with a quaternary pump and a photodiode array detector (DAD) and an Ultrabase C18 column (5 μm; 4.6 mm × 150 mm) which was set thermostatically at 25 °C. Solvents used to analysis were acetic acid 2.5% (A), HPLC-grade acetonitrile (B), ultra-pure water (C) and acetic acid 2.5% HPLC-grade acetonitrile (90:10) (D) at a flow rate of 0.5 mL min^{-1}. Elution was performed as previously described by Pallaufa et al. (2008).

2.5. HPLC–MS analysis

In order to confirm the identity of the phenolic compounds that could not be done by HPLC–UV, additional analysis was carried out using HPLC with mass spectrometry detection Agilent 1100 Series liquid chromatography equipped with an API source and employing an ESI (electrospray ionization)