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Ficus carica L.: Metabolic and biological screening

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ABSTRACT

Ficus carica L. is one of the earliest cultivated fruit trees. In this work, metabolite profiling was performed on the leaves, pulps and peels of two Portuguese white varieties of *F. carica* (Pingo de Mel and Branca Tradicional). Phenolics and organic acids profiles were determined by HPLC/DAD and HPLC/UV, respectively. All samples presented a similar phenolic profile composed by 3-*O*- and 5-*O*-caffeoylquinic acids, ferulic acid, quercetin-3-*O*-glucoside, quercetin-3-*O*-rutinoside, psoralen and bergapten. 3-*O*-Caffeoylquinic acid and quercetin-3-*O*-glucoside are described for the first time in this species. Leaves' organic acids profile presented oxalic, citric, malic, quinic, shikimic and fumaric acids, while in pulps and peels quinic acid was absent. The antioxidant potential of the different plant parts was checked. All materials exhibited activity against DPPH and nitric oxide radicals in a concentration-dependent way. However, only the leaves presented capacity to scavenge superoxide radical. Leaves were always the most effective part, which seems to be related with phenolics compounds. Additionally, acetylcholinesterase inhibitory capacity was evaluated, but no effect was observed. Antimicrobial potential was also assessed against several bacterial species, although no activity was noticed. This is the first study comparing the chemical composition and biological potential of *F. carica* pulps, peels and leaves.

1. Introduction

Ficus carica L., a deciduous tree belonging to the Moraceae family, is one of the earliest cultivated fruit trees. Mediterranean diets are characterized by abundant intake of this fruit (Solomon et al., 2006), which can be eaten fresh, dried or used as jam. Figs are an excellent source of minerals, vitamins and dietary fibre; they are fat and cholesterol-free and contain a high number of amino acids (Veberic et al., 2008; Solomon et al., 2006). Figs have been traditionally used for its medicinal benefits as laxative, cardiovascular, respiratory, antispasmodic and anti-inflammatory remedies (Guarrera, 2005).

Some studies have described the presence of several phenolic compounds in this species (Teixeira et al., 2006; Vaya and Mahmood, 2006; Guarrera, 2005), phytosterols and fatty acids in fruits and branches of fig trees (Jeong and Lachance, 2001) and its antioxidant activity (Solomon et al., 2006). However, leaf, pulp and peel's metabolic profile and biological activity have not been compared.

Biomolecules from plants have attracted a great deal of attention, mainly concentrated on their role in preventing diseases. Epidemiological studies have consistently shown that there is a clear significant positive association between intake of these natural products and reduced rate of heart disease mortalities, common cancers and other degenerative diseases. Free radicals present in human organism cause oxidative damage to various molecules, such as lipids, proteins and nucleic acids, being involved in the initiation of those diseases (García-Alonso et al., 2004). Antioxidant compounds, such as phenolics, organic acids, vitamin E and carotenoids, scavenge free radicals, thus inhibiting the oxidative mechanisms that lead to degenerative illnesses (du Toit et al., 2001; Silva et al., 2004). Additionally, growing interest has been devoted to natural antimicrobial agents, in order to avoid chemical preservatives and because of increasing antibiotics resistance.

Phenolic compounds are secondary metabolites that are quite widespread in nature. These compounds play many physiological roles in plants and some of them are also favourable to human health, since they are able to act as antioxidants by different ways: as reducing agents, hydrogen donators, free radicals scavengers, and singlet oxygen quenchers and, therefore, as cell saviours (Merken and Beecher, 2000; Costa et al., 2009; Fattouch et al., 2007).

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Organic acids are primary metabolites, which can be found in great amounts in all plants, especially in fruits. These compounds also have antioxidant properties (Silva et al., 2004; Valentão et al., 2005a, 2005b). Citric, malic and tartaric acids are commonly found in fruits and berries, while oxalic acid is present in higher amounts in green leaves (Oliveira et al., 2008). Ascorbic acid is probably the most widely distributed water soluble antioxidant in vegetables (Naidu, 2003; Seabra et al., 2006; Sousa et al., 2009).

Recently, several studies have been developed to assess the ability of natural compounds inhibition of acetylcholinesterases, since it is directly related with the treatment of neurological disorders, such as Alzheimer's disease, senile dementia, ataxia and *myasthenia gravis* (Mukherjee et al., 2007). Acetylcholinesterase breaks down the ester bond in the molecule of acetylcholine, thus leading to the loss of stimulatory activity. Inhibition of acetylcholinesterases will, as far as nervous transmission is concerned, result in a prolongation of the existence, and therefore of the activity, of acetylcholine (Houghton et al., 2006).

The aim of the present work was to characterise the phenolics and organic acids composition of the material obtained from two Portuguese *F. carica* varieties (Pingo de Mel and Branca Tradicional), using HPLC coupled to diode array and UV detectors, respectively, and to assess their biological potential, namely as antioxidant, acetylcholinesterase inhibitor and antimicrobial agent. To our knowledge this is the first report comparing chemical composition and bioactivity of several materials from *F. carica*. In addition, no previous study involved the above mentioned varieties.

2. Materials and methods

2.1. Standards and reagents

Quercetin 3-*O*-rutinoside, bergapten, psoralen, oxalic, malic, fumaric, shikimic, quinic and citric acids, ferulic acid, dimethyl sulfoxide (DMSO) and carbachol were from Sigma–Aldrich (St. Louis, MO, USA) and 5-*O*-caffeoylquinic acid was from Extrasynthése (Genay, France). Sodium nitroprussiate dihydrate was from Riedel-de Haën (St. Louis, MO). N-(1-Naphthyl)ethylene-diamine dihydrochloride, phosphoric acid and methanol were from Merck. Sulfanilamide, β-nicotinamide adenine dinucleotide (NADH), nitroblue tetrazolium chloride (NBT), phenazine methosulfate (PMS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), acetylthiocoline iodide and acetylcholinesterase were obtained from Sigma–Aldrich. The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA, USA). The Chromabond C18 SPE columns (70 mL/10,000 mg) were purchased from Macherey–Nagel (Duren, Germany).

2.2. Plant material

F. carica (var. Pingo de Mel and var. Branca Tradicional) leaves and fruits were collected in Mirandela region (Northeast Portugal), in August 2008. Fruit peel and pulp were separated. All materials were frozen and lyophilized. Voucher specimens were deposited at Department of Pharmacognosy, Faculty of Pharmacy, Porto University.

2.3. Extracts preparation

Fig leaves, peels and pulps (2 g) were boiled for 15 min in 500 ml of water. The resulting extracts were filtered over a filtration funnel and then lyophilized in a Labconco Freezone 4.5 apparatus (Kansas City, MO, US). The lyophilized extracts were kept in a desiccator, in the dark, until analysis.

2.4. HPLC/DAD for phenolic compounds analysis

Each lyophilized extract was redissolved in water, filtered and 20 μL were analysed on an analytical HPLC unit (Gilson), using a Spherisorb ODS2 (25.0 × 0.46 cm; 5 μm, particle size) column, according to a described procedure (Oliveira et al., 2007). The solvent system used was a gradient of water-formic acid (19:1) (A) and methanol (B), starting with 5% methanol and installing a gradient to obtain 15% B at 3 min, 25% B at 13 min, 30% B at 25 min, 35% B at 35 min, 45% B at 39 min, 45% B at 42 min, 50% B at 44 min, 55% B at 47 min, 70% B at 50 min, 75% B at 56 min and 80% B at 60 min, at a solvent flow rate of 0.9 mL/min. Detection was achieved with a Gilson Diode Array Detector (DAD). Spectral data from all peaks were accumulated in the range 200–400 nm, and chromatograms were recorded at 320 nm and 350 nm. The data were processed on an Unipoint® System software (Gilson Medical Electronics, Villiers le Bel, France).

The compounds in each extract were identified by comparing their retention times and UV–vis spectra in the 200–400 nm range with authentic standards and with the library of spectra previously compiled by the authors.

Phenolic compounds quantification was achieved by the absorbance recorded in the chromatograms relative to external standards. Phenolic acids and furanocoumarins were determined at 320 nm and flavonoids at 350 nm. 3-*O*-Caffeoylquinic acid was quantified as 5-*O*-caffeoylquinic acid and the other compounds were quantified as themselves.

2.5. HPLC/UV for organic acids analysis

Before HPLC analysis, each lyophilized extract was dissolved in acid water (pH 2 with HCl). The solution obtained was passed through an SPE C18 column, previously conditioned with 30 mL of methanol and 70 mL of acid water. The aqueous solution was then evaporated to dryness under reduced pressure (40 °C), redissolved in sulphuric acid 0.01 N (1 mL) and 20 μL were analysed on an analytical HPLC unit (Gilson), using an ion exclusion column Nucleogel® Ion 300 OA (300 × 7.7 mm), in conjunction with a column heating device at 30 °C. Elution was carried out at a solvent flow rate of 0.2 mL/min, isocratically, with sulphuric acid 0.01 N as the mobile phase. Detection was performed with a Gilson UV detector at 214 nm. Organic acids quantification was achieved by the absorbance recorded in the chromatograms relative to external standards.

2.6. Antioxidant activity

2.6.1. DPPH scavenging activity

The aqueous lyophilized extract of each sample was dissolved in water (five different concentrations). Antiradical activity was determined spectrophotometrically in a Multiskan Ascent plate reader (Thermo; electron corporation), by monitoring the disappearance of DPPH at 515 nm, according to a described procedure (Costa et al., 2009). The reaction mixtures in the sample wells consisted of extract and 150 μM DPPH. The plate was incubated for 30 min at room temperature after the addition of DPPH. Three experiments were performed in triplicate.

2.6.2. Nitric oxide scavenging activity

The aqueous lyophilized extract of each sample was dissolved in 0.1 M phosphate buffer (pH 7.4). The antiradical activity was determined in a Multiskan Ascent plate reader (Thermo; electron corporation), according to a described procedure (Sousa et al., 2008). Sodium nitroprusside (20 mM) was incubated with each extract (five different concentrations) for 60 min, at room temperature, under light. All solutions were prepared in phosphate buffer. After incubation, Griess reagent, containing 1% sulphanilamide and 0.1% naphthylethyldiamine in 2% phosphoric acid, was added to each well. The mixture was incubated at room temperature for 10 min, and the absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine was read at 562 nm. Three experiments were performed in triplicate.

2.6.3. Superoxide radical scavenging activity

The aqueous lyophilized extract of each sample was dissolved in 19 mM phosphate buffer (pH 7.4). Superoxide radicals were generated by NADH/PMS system according to a described procedure (Valentão et al., 2001). All components were dissolved in buffer. In each well, sample (five different concentrations), NADH, NBT and PMS were added. The absorbances were read at 560 nm. Three experiments were performed in triplicate.

2.7. Acetylcholinesterases inhibitory activity

2.7.1. Buffers

The following buffers were used. Buffer A: 50 mM Tris–HCl, pH 8; buffer B: 50 mM Tris–HCl, pH 8, containing 0.1% bovine serum albumin (BSA); buffer C: 50 mM Tris–HCl, pH 8, containing 0.1 M NaCl and 0.02 M $MgCl.6H_2O$.

2.7.2. Enzyme

Acetylcholinesterase was from *Electrophorus electricus* – electric eel – (type VI-s, lyophilized powder, 425 U/mg, 687 mg/protein). Lyophilized enzyme was dissolved in buffer A to make 1000 U/mL stock solution, and further diluted with buffer B to get 0.44 U/mL enzyme for the microplate assay.

2.7.3. Microplate assay

Acetylcholinesterase inhibitory activity was determined spectrophotometrically in a Multiskan Ascent plate reader (Thermo; electron corporation) based on Ellman's method, according to a described procedure (Pereira et al., 2009). In each well the mixture consisted of acetyltiocholine in water, DTNB in buffer C, buffer B and sample dissolved in a solution of 10% methanol in buffer A. The absorbance was read at 405 nm. After this step acetylcholinesterase was added and the