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Phenolic composition, antioxidant and anti-inflammatory activities of extracts from Moroccan *Opuntia ficus-indica* flowers obtained by different extraction methods

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ABSTRACT

The objectives of this study were to compare the phenolic extraction efficiency of four extraction procedures, to explore the extract phenolic profile by HPLC-DAD-ESI-MS and to evaluate the antioxidant and anti-inflammatory activities of cactus flowers from Morocco. accelerated solvent extraction (ASE) with 80% acetone in water at 80 °C gave rise to extracts with higher yields of total polyphenols, procyanidins and flavonoids. The qualitative analysis of the phenolic composition showed that these extracts were composed mainly by flavonol glycosides in addition to hydroxycinnamic acids which were detected in the extracts for first time. Our study also revealed the high antioxidant and anti-inflammatory potential of cactus pear flower extracts. In light of the obtained results, cactus flowers are a good source of polyphenols that could be used in foods, cosmetics or pharmaceutical products, thus contributing to diminish the environmental impact of cactus by-products and to its valorization.

1. Introduction

Phenolic compounds are secondary metabolites found ubiquitously in plants. They display a large range of structures and are responsible for the major organoleptic characteristics of plant-derived-foods and beverages, particularly color and taste properties. In addition, many studies have reported that their regular consumption impact positively on health reducing the risk of cardiovascular diseases, neurodegenerative disorders and cancer (Vauzour et al., 2010). Among polyphenol compounds, flavonoids have been strongly linked with beneficial effects in many human and animal studies (Vauzour et al., 2010). In vitro and animal studies have shown that flavonoids can exert multiple activities such as anti-inflammatory (Larrosa et al., 2010), anti-hypertensive (Hodgson and Croft, 2006), anti-oxidant (Jeong et al., 2005), anti-atherosclerotic (Fuhrman et al., 2005), anti-proliferative (Mantena et al., 2005) and anti-angiogenic (Piao et al., 2006) among others.

Cactus (*Opuntia* spp.) belongs to *Cactaceae* family, which is cultivated in both hemispheres and all continents. Among all species identified *Opuntia ficus-indica* L., also known as cactus pear, is the most common. Native to Mexico, this plant is widely distributed in arid and semi-arid regions of South and Central America, Africa and the Mediterranean area (Mohamed-Yasseen et al., 1995). Cactus pear has a rapid growth, good adaptation to poor soils and low water requirement.

Different parts of *O. ficus-indica* have increased its economic importance being exploited in food and pharmaceutical areas (Feugang et al., 2006). Cactus fruits (prickly pear) have been used for the manufacture of food products such as juices, jams, jellies alcoholic beverages, etc., while cladodes are commercialized mainly as minimally processed fresh products (Stintzing and Carle, 2005). Cactus pear cladodes, fruits and infusions of cactus pear flowers have also been used in traditional folk medicine for treatment of a number of diseases and pathological conditions, including inflammatory conditions, diabetes, stomach ulcers, renal diseases, etc. (Feugang et al., 2006; Kaur et al., 2012).

Abbreviations: ASE, accelerated solvent extraction; CE, catechin equivalent; CCE, cyanidin chloride equivalent; GAE, gallic acid equivalent; TE, Trolox equivalent.

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Table 1
Extraction conditions and yields of obtained extracts per 100 g of dried cactus pear flower.

Extract code	Extraction method	Solvent	Solvent ratio (%)	Temperature (°C)	Yield (g/100 g dm)[a]
A	Maceration	Acetone/HCl/water	79:1:20	25	18.00 ± 1.63[a]
B	Maceration	Methanol//HCl/water	49:1:50	25	14.00 ± 2.82[a]
C	ASE	Acetone/water	80:20	80	18.00 ± 0.01[a]
D	ASE	Methanol/water	50:50	80	34.00 ± 4.89[b]

*Data represent the mean ± standard deviation of three independent experiments.
Different uppercase letter in yield column are significantly different ($P < 0.05$, Duncant's test); ASE: accelerated solvent extraction.

More recently, cactus pear has attracted the interest of nutraceutical and healthy food areas. Cactus pear fruits and their by-products (peel and seeds) as well as cladodes are valuable sources of polyphenols (Moussa-Ayoub et al., 2011) which make them perfect candidates for the production of health-promoting foods and food supplements. Cactus pear fruit is a unique source of isorhamnetin glycosides, especially isorhamnetin-3-*O*-rutinoside and isorhamnetin tryglicosides and phenolic acids such as fukic acid, piscidic acid and eucomic acid (Moussa-Ayoub et al., 2011; Serra et al., 2013). The occurrence of these polyphenols in cactus pear fruits depends on many factors such as stage of maturity, growing region and post-harvest practices affecting consequently to their biological activity (Coria Cayupán et al., 2011). Moreover, quercetin, (+)-dihydroquercetin, and quercetin 3-methyl ether were isolated from cactus pear plant and reported as active neuroprotectants against oxidative stress (Dok-Go et al., 2003). More recently, polyphenolic-rich extracts from cactus pear by-products have reported to display antioxidant and antiproliferative activities in human colon carcinoma (Serra et al., 2013; Yeddes et al., 2014a,b).

The *O. ficus-indica* flowers constitute one of the by-products in cactus industry. The flowers are generally discarded by separation of the fruit. To the best of our knowledge, the polyphenolic profile and biological activity of *O. ficus-indica* flowers are very scarcely documented. Previous studies identified isorhamnetin glycosides being the major phenolic compounds followed by quercetin and kaempferol glycosides in methanolic extracts of cactus pear flowers grown in Italy and Tunisia obtained by maceration (De Leo et al., 2010; Yeddes et al., 2014a,b). Regarding health-promoting potential of flower extracts, an animal study has demonstrated their antioxidant and antiulcerogenic activity (Alimi et al., 2010).

The aim of this study was to identify the main phenolic compounds of *O. ficus-indica* flowers from Moroccan region, in order to evaluate the valorization of these flowers as raw materials for infusions or nutraceuticals. To provide accurate data on phenolic composition of cactus pear flower extract and its biological activity (antioxidant and anti-inflammatory) we have compared different organic solvents as well as conventional (maceration) and an advanced green extraction technology (accelerated solvent extraction, ASE). ASE has attracted increasing interest due to its economic, fast and automatic features which besides improving extraction yield, decreases time and solvent consumption (Hossain et al., 2011). Furthermore, the set up in ASE equipment provides protection for oxygen and light sensitive compounds, which is prominent for extraction of bioactive compounds such as polyphenols.

2. Materials and methods

2.1. Chemicals

Unless otherwise stated, all chemicals and reagents were obtained from Sigma–Aldrich (St. Louis, MO, USA). (+)-catechin (≥99%, HPLC grade) and gallic acid (≥95%, tritation), standards were purchased from Sigma–Aldrich and cyanidine chloride from Extrashynthese (Lyon, France). HPLC grade methanol and acetic acid were supplied from Panreac (Barcelona, Spain). Water was deionized by using a Milli-Q system (Millipore, Beldford, MA, USA). Macrophage RAW 264.7 cell line was obtained from American Type Culture Collection (Rockville, MD, USA). Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), trypsin-EDTA (1×), penicillin (105 U/L), streptomycin (0.1 g/L) were from GIBCO (Madison, WI, USA).

2.2. Plant material

The yellow colored *O. ficus-indica* flowers were collected by the Moroccan ADAGUEN cooperative (Tangier, Morocco) in the Rif region located in the north of Morocco during summer 2010. The fresh flowers were then air-dried at 25 °C, powdered and stored at −20 °C until use. A voucher specimen of the used plant material (MDFW) was deposited in the ICTAN-CSIC laboratory.

2.3. Phenolics extraction

Phenolic compounds of the *O. ficus-indica* flowers were extracted using four different procedures summarized in Table 1 and described below.

2.3.1. Maceration

First, 0.5 g of dried flowers was defatted by vigourosly agitation with *n*-hexane twice at 25 °C. Phenolic compounds were then extracted from the defatted residue using 10 mL of two different solvents: acetone/HCl/water (79/1/20 v/v/v) (extract A), and methanol/HCl/water (49/1/50 v/v/v) (extract B). The extractions were performed in an orbital shaker for 24 h at 25 °C. Samples were filtered through a Whatman No. 1 filter paper and the filtrate was evaporated using a rotary evaporator. The obtained residue was dissolved in 2 mL of methanol/water (50/50, v/v), filtered through a 0.45 μm PVDF membrane and finally kept at −20 °C until further analysis.

2.3.2. Accelerated solvent extraction (ASE)

An accelerated solvent extraction unit ASE 200 (Dionex Co., Sunnyvale, CA, USA) was also used for phenolics extraction. The unit was an automated system with temperature and pressure limits of 200 °C and 10,342 kPa, respectively. The ASE consists of stainless steel extraction cells (11 mL) with electronically controlled heaters, a pump and a solvent controller. The system included and autosampler carousel and a collection bottle tray holding 12 collection bottles and one rinse bottle. Extraction was performed in extraction cells containing 0.5 g of powdered cactus flowers and using the conditions reported on the US EPA 3545 method (EPA, 1998). Hexane was used first to remove lipids. The further used solvents were: acetone/water (80/20, v/v) (extract C), and methanol/water (50/50, v/v) (extract D), the temperature was 80 °C and the pressure was 1500 psi (10.3 MPa). The extraction was performed during three cycles including 5 min (heating) and 8 min (static time). The extraction cell was flushed with solvent (60% cell volume) and purged with nitrogen gas (120 s). Three replicate extractions for each experimental condition were performed ($n = 3$). At the end