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ScienceDirect

Food Chemistry 109 (2008) 484–492

FOOD CHEMISTRY

www.elsevier.com/locate/foodchem

Antioxidant potential of crocins and ethanol extracts of *Gardenia jasminoides* ELLIS and *Crocus sativus* L.: A relationship investigation between antioxidant activity and crocin contents

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Received 2 May 2007; received in revised form 9 August 2007; accepted 5 September 2007

Abstract

Crocins are water-soluble carotenoids responsible for the colour of saffron and gardenia. In this study, we isolated and identified three major crocins from gardenia, and then evaluated their antioxidant potential using four *in vitro* antioxidant tests in comparison with saffron ethanol extract (SE), gardenia ethanol extract (GE) and gardenia resin fraction (GRF). The relationship between total crocin contents and antioxidant activity of ethanol extracts of two herbs was investigated and the antioxidant potentials of three different polar crocins were compared. The crocins appeared to possess antioxidant activity when tested by four *in vitro* antioxidant models. However, in anti-hemolysis, DPPH radical-scavenging and lipid peroxidation assays, GRF exhibited significantly stronger antioxidant activity than crocins and no correlation between total crocin contents and antioxidative function was revealed, which implied that ingredients other than crocins in gardenia gave markedly strong antioxidant activity. In the phosphomolybdenum assay, antioxidant capacities of fractions and extracts correlated with total crocin contents ($R = 0.93$). Moreover, comparison of results indicated that sugars attached to the crocetin moiety seemed to be beneficial for the antioxidant activity of these water-soluble pigments.

Keywords: Gardenia; Saffron; Crocin; Antioxidant

1. Introduction

The constituents of gardenia fruits, *Gardenia jasminoides* ELLIS, are known as herb medicines and natural dyes in China. The yellow pigments of this herb have been used as a natural food colourant for a long time in Japan, mainly in coloured juice, jelly, candy and noodles, because of their water solubility (Watanabe & Terabe, 2000). Saffron, another herb containing the same yellow pigments of gardenia, is typically used as a spice with colouring properties in a wide range of culinary, bakery and confectionery preparations, as well as in alcoholic and non-alcoholic beverages (Selim, Tsimidou, & Biliaderis, 2000). Phytochemical studies of saffron and gardenia have shown that the main chemicals responsible for their colour are crocins, which are a series of mono and di-glucosyl esters of crocetin, a polyene dicarboxylic acid (8,8-diapocarotene-8, 8-dioic acid) (Pfister, Meyer, Steck, & Pfander, 1996; Van Calsteren et al., 1997). In contrast to most families of carotenoids, these compounds are known for their colouring properties owing to their unique water-soluble behaviour, which is the reason for their great application as a food colourants (Van Calsteren et al., 1997).

Numerous studies have shown crocins to be capable of a variety of pharmacological effects, such as protection against cardiovascular diseases (He et al., 2005; Shen & Qian, 2006; Xiang et al., 2006), inhibition of tumor cell proliferation (Magesh, Singh, Selvendiran, Ekambaram, & Sakthisekaran, 2006), neuroprotection (Ahmad et al.,

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0308-8146/$ - see front matter
doi:10.1016/j.foodchem.2007.09.080

2005; Ochiai et al., 2004) and protection of hepatocytes (Tseng, Chu, Huang, Shiow, & Wang, 1995). Among the mechanisms underlying their various protective actions, the antioxidant activity was hypothesised to be responsible for various pharmacological effects of crocins. The following evidence supports the notion that one of the important mechanisms by which crocetin or crocins exert their biological effects is their ability to modulate redox status of organisms. Growing evidence indicates that chronic or acute overproduction of reactive oxygen species (ROS) plays an important causal or contributing role in the development of the various above mentioned diseases (Cerutti, 1994; Ferrari et al., 1998; Smythies, 1999; Wattanapitayakul & Bauer, 2001) and the protective effects of crocin or crocetin against these diseases have been repeatedly demonstrated in various studies (Ahmad et al., 2005; He et al., 2005; Magesh et al., 2006; Ochiai et al., 2004; Shen & Qian, 2006; Tseng et al., 1995; Xiang et al., 2006), which may be attributed to antioxidant capacities of crocetin or crocins.

In the course of our previous study aimed to screen for antioxidants from gardenia fruits, we observed significant antioxidant capacity of saffron ethanol extract (SE), gardenia ethanol extract (GE) and gardenia resin fraction (GRF), tested in several *in vitro* antioxidant models. In order to study whether the antioxidant activity of the fraction and extracts was consistent with the contents of crocins present in the fractions and ethanol extracts of two spices from gardenia, we isolated, characterised and identified the major crocins (named crocin-1, crocin-2 and crocin-3) on the basis of NMR, ESI-MS, UV–visible and TLC data, and then quantified these three most abundant crocins in the fraction and extracts using RP-HPLC and UV–visible methods. In addition, antioxidant activity and radical-scavenging ability of crocins, in comparison with fractions and extracts of these two herbs, were assayed using anti-hemolysis, DPPH radical-scavenging, lipid peroxidation and phosphomolybdenum assays, and correlations between total crocin contents and antioxidative function of fractions and extracts were investigated. The present study might give insight into potencies of crocins and two herbs as biological antioxidants, and show if crocins are major contributors to the antioxidant properties of these two traditional medicines.

2. Materials and methods

2.1. Plant material

The dried gardenia (*Gardenia jasminoides* ELLIS) fruits and saffron (*Crocus sativus* L.) stigmas were purchased from Chengdu, Sichuan Province, in August 2004, and identified by Hao Zhang at West China School of Pharmacy, Sichuan University, China. The voucher specimens are deposited in West China School of Pharmacy, Sichuan University, China.

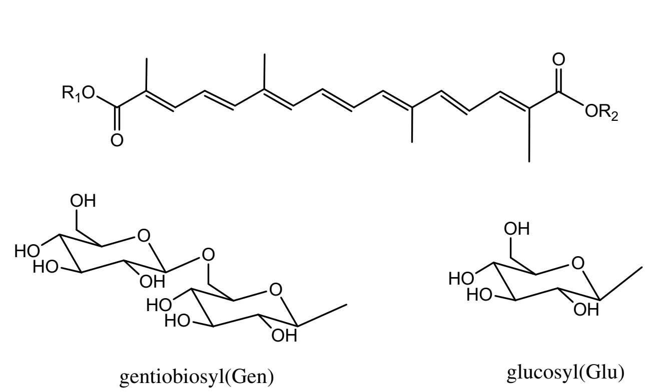
2.2. Chemicals and reagents

Methanol (Sigma, USA) was of chromatographic purity and water was double distilled for HPLC. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical (Sigma, USA) was used for testing the radical-scavenging activity of crocins and extracts. 2,2′-Azo-bis(2-amidinopropane) dihydrochloride (AAPH) was used for anti-hemolysis assay and purchased from Wako (Japan). α-Tocopherol (Sigma, USA) and ascorbic acid (Sigma, USA) were used as references. Assay kits for malondialdehyde (MDA) were purchased from Nanjing Jiancheng Bioengineering Institute, China. All other regents and solvents were analytically pure and purchased from local firms.

2.3. Extraction and isolation

The dried gardenia fruits (40 kg) were ground to a coarse powder and extracted with ethanol–water (40%) by cold percolation (4 × 40 l). The alcohol extract was concentrated, suspended in water, and then partitioned with ethyl acetate. The ethyl acetate layer extract was subjected to CC (column chromatography) on silica gel (2000 g) eluting with CH_2Cl_2 containing increasing amounts (3%, 5%, 7%, 10%) of methanol. Upon concentration of the fraction eluted with MeOH–CH_2Cl_2 (3%), crocetin (**4**, 40 mg) (Compound No. **4** in Fig. 1 yield) crystallized. The water layer, further diluted with water, was subjected to HPD-100 macroporous resin (15 kg, Cangzhou bon, Hebei, China), and eluted with water containing increasing amounts (0%, 25%, 60%) of ethanol.

The ethanol–water (60%) fractions (gardenia resin fraction, GRF) were combined and evaporated to dryness and separated by CC on silica gel, eluting with ethyl acetate containing increasing amounts (5%, 10%, 15%, 20%) of methanol–water (16:13); the methanol–water, (16:13)–ethyl acetate (5%) was further purified by a preparative ODS column to yield crocin-3 (**3**, 3 g). Similar treatment of the



crocin-1: R_1=R_2=Gen (1)
crocin-2: R_1=Gen, R_2=Glu (2)
crocin-3: R_1=Gen, R_2=H (3)
crocetin: R_1=R_2=H (4)

Fig. 1. Structures of crocins and crocetin.