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SHORT COMMUNICATION

Radical Scavenging Activity of *Crocus sativus* L. Extract and its Bioactive Constituents

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Radical scavenging activity is involved in aging processes, antiinflammatory, anticancer and wound healing activity. Hence, in the present study the DPPH radical scavenging activity of a natural product that possesses biological properties, an extract of *Crocus sativus* L. (saffron), grown in Crocos, Kozani (Greece), and some of its bioactive constituents (crocin, safranal) was studied.

It was shown that a methanol extract of *Crocus sativus* exhibited high antioxidant activity, although it contains several active and inactive constituents. In trying to approximate a structure-activity relationship, two bioactive constituents of saffron extract were tested, namely crocin and safranal. Crocin showed high radical scavenging activity (50% and 65% for 500 and 1000 ppm solution in methanol, respectively), followed by safranal (34% for 500 ppm solution). All the tested samples showed high radical scavenging activity, probably due to the ability to donate a hydrogen atom to the DPPH radical.

Thus, saffron grown in Greece can be used promisingly in functional foods, drinks with antioxidant activity, in pharmaceutical and cosmetic preparations for their antioxidant activity and probably for their antiaging activity. Saffron can also be used internally in the form of powder or other pharmacotechnical formulae as a food supplement with antioxidant properties.

Keywords: Crocus sativus; saffron; crocin; DPPH; radical scavenging activity; cancer.

INTRODUCTION

Reactive species initiate reactions that damage organic molecules of biological importance and are considered to cause cancer, heart diseases and the process of aging itself (Gutteridge and Halliwell, 1994). Radical scavenging activity is considered to be involved in aging processes, antiinflammatory, anticancer and wound healing activity. The development of antioxidants that scavenge reactive oxygen species (ROS) would support biological resistance to free radicals, retard the process of aging and decrease the risk of age-associated degenerative diseases.

Saffron harvested from the dried stigmas of *Crocus sativus* L. (Iridaceae) flowers is used mainly as a spice for flavouring and colouring food, in alcoholic and non-alcoholic beverages, as a perfume and as a herbal medicine. Since ancient times saffron has been considered to have a number of therapeutic properties, it has been used as a sedative, a tonic, a stimulant of the stomach and an expectorant in traditional medicine. It has also been used to treat several human health conditions, including ailments such as dysentery, measles, enlargement of the liver and gall bladder, urological infections, cough, stomach disorders, asthma and cardiovascular disorders (Abdullaev, 2003a; Abdullaev, 2003b). Saffron is the world's most expensive spice and, apart from its traditional value as a food additive and traditional herbal medicine, recent studies indicate its potential as an anticancer, antitumoural, cytotoxic, hypolipidaemic, antiinflammatory and oxygenation enhancement agent (Nair *et al.*, 1995; Rvos *et al.*, 1996; Escribano *et al.*, 1996; Hosseinzadeh and Younesi, 2002). It was also demonstrated that the saffron extract inhibited cellular nucleic acid synthesis (Abdullaev *et al.*, 2003). *Crocus sativus* is exclusively grown in Greece, India, Iran and Spain and thus the study tried to exploit its possible biological activities to extend its uses from a food additive to a functional food or a food supplement.

Characteristic compounds of saffron include crocins (1–4, Fig. 1) (Li *et al.*, 1999), crocetin, picrocrocin and β-carotene (Tarantilis *et al.*, 1994), while the main biologically active metabolites of saffron are crocins, a family of red-coloured water-soluble carotenoids, which are the glycosides of *trans*-crocetin and are responsible for the colour. Safranal (Fig. 1) is the main component of the essential oil and is responsible for the characteristic saffron aroma. Picrocrocin is responsible for the bitter taste of saffron and is the glycoside of the aglycone 4-hydroxy-2,6,6-trimethyl-1-carboxaldehyde-1-cyclohexene (HTCC), and thus a glycosidic precursor of safranal (Loskutov *et al.*, 2000; Lozano *et al.*, 1999; Tarantilis *et al.*, 1995). Crocins and specifically crocin 1 has been shown to be the major constituent of saffron, followed by picrocrocin. But picrocrocin under basic conditions and after the drying process is converted to

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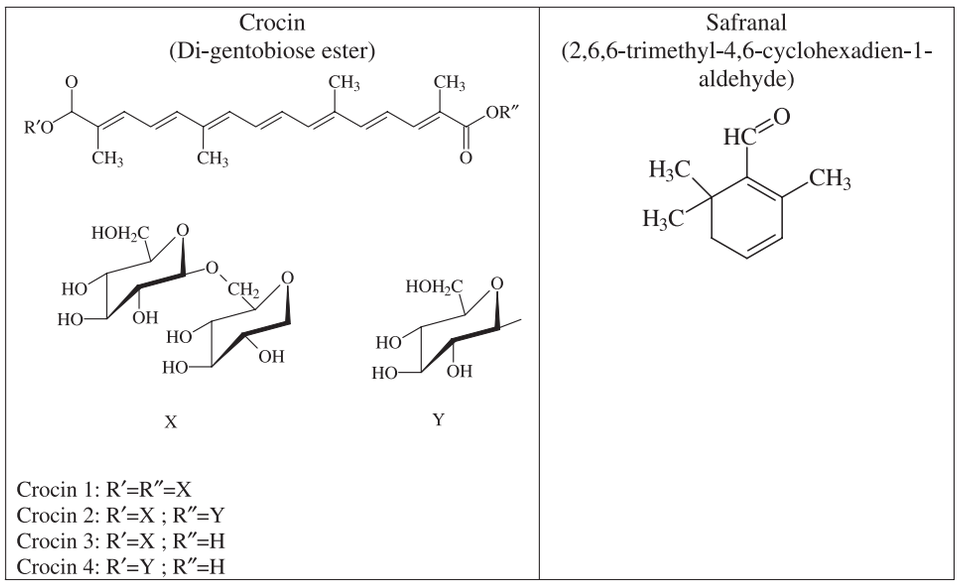

Figure 1. Chemical structures of the tested pure compounds contained in *Crocus sativus* L.

safranal (Loskutov *et al.*, 2000). Crocin derivatives and mainly crocin (1) suppress tumour growth, while safranal and crocetin were proved to possess antileukaemic activity (Sinakos *et al.*, 1999). Crocetin has been characterized as an antioxidant (Tseng *et al.*, 1995) and showed protective action against ROS-induced hepatotoxicity and genotoxicity (Tseng *et al.*, 1995). Saffron was only tested for its antioxidant properties *in vivo* on lipoprotein oxidation (Verma and Bordia, 1998), showing the potential of saffron as an antioxidant.

In this study the radical scavenging activity of saffron was tested. In addition, a structure-activity relationship was approximated for the DPPH radical scavenging activity of its main bioactive constituents crocin and safranal. This study is a continuation of efforts to evaluate natural products for possible radical scavenging activity that can be used as food supplements, functional foods, in pharmaceutical and cosmetic formulations (mainly antiaging).

MATERIALS AND METHODS

Chemicals. DPPH radical ($C_{18}H_{12}N_5O_6$, 1,1-diphenyl-2-picryl-hydrazyl, MW 394.3, Sigma) was used for testing the radical scavenging activity of saffron and its main constituents. Caffeic acid (3,4-dihydroxy-cinnamic acid, Riedel de Haen) was used as a control. Crocin (crocetin digentibiose ester, Fluka) and safranal (donated by Dr Z. Sinakos) were also tested for their possible DPPH radical scavenging activity.

Plant material. Stigmas of dried *Crocus sativus* flowers were kindly provided by the Association of Crocus Producers (Crocos, Kozani, Greece).

Preparation of samples. A methanol extract of *Crocus sativus* was prepared by soxhlet apparatus (3 h extraction; 40 g dried stigmas of *Crocus sativus* in 250 mL methanol), followed by solvent evaporation of methanol. All samples tested were dissolved in methanol at concentrations of 500, 1000, 1500, 2000 and 2500 ppm (g of extract/mL methanol), in order to discover the concentration with the best DPPH radical scavenging activity. Crocin was tested at two typical concentrations (500 and 1000 ppm solution), while safranal was tested in a 500 ppm solution.

DPPH radical scavenging test. A solution of DPPH 10^{-4} M was prepared by dissolving 0.0039 g of the radical DPPH in 100 mL methanol. The vial was wrapped in foil and stirred in a vortex apparatus. The solution obtained had a deep purple colour and was left in the refrigerator for 2 h in order for the absorbance to be stabilized.

A calibration curve of concentration versus absorbance of DPPH was prepared as follows: The 10^{-4} M solution of DPPH was diluted with the addition of methanol. These solutions were vortexed, left in darkness and their absorbance was measured in a UV-Vis spectrophotometer (Shimadzu type UV-160A) at λ_{max} of 517 nm. The calibration curve of absorbance (y) versus concentration (x) of DPPH is expressed by the following equation

$$y = 11222x - 0.0156;\ R = 0.9995$$

For the antioxidant test, methanol was used as the blank. 2.9 g of the DPPH 10^{-4} M solution was placed in a cuvette and the absorbance of the DPPH radical was measured at $t = 0$ (A_0). Subsequently, 0.1 mL solution of each of the tested pure compound/extract was added to the above volume of DPPH and the absorbance was measured at regular time periods, until the value reached a plateau (steady state, A_T). The concentration of the mixture of DPPH with each extract/pure compound was estimated by the above described calibration curve.

For each of the samples tested for its radical scavenging activity the following parameters were estimated, in order to evaluate the results of the antioxidant tests of several samples examined

- % decrease in DPPH absorbance = $\dfrac{A_o - A_T}{A_o} 100$
- % decrease in DPPH absorbance compared with caffeic acid

$$= \frac{\%\textit{decrease in DPPH absorbance}}{\%\textit{decrease in absorbance of caffeic acid}} 100$$

- % decrease in DPPH concentration = $\frac{C_o - C_T}{C_o} 100$
- % decrease in DPPH concentration compared with caffeic acid
$$= \frac{\%\textit{decrease in DPPH concentration}}{\%\textit{decrease in caffeic acid concentration}} 100$$
- EC_{50} = concentration of the sample required for a 50% decrease in DPPH concentration, which is estimated from graphs of the % decrease in DPPH concentration versus concentrations (mg compound or extract/mg DPPH).
- AE = $\frac{1}{EC_{50}}$, antiradical efficiency

where A_o is the initial absorbance, A_T is the final absorbance (steady state), C_o, initial DPPH concentration and C_T, final DPPH concentration.

All experiments were repeated in duplicate. The values in the Tables are expressed as the mean values of at least two determinations. The SD was estimated to be below 10% for all experiments.

RESULTS AND DISCUSSION

In the present study the possible radical scavenging activity of saffron (dried stigmas of *Crocus sativus* L.) grown in Crocos, Kozani (Greece), which exhibits biological properties, was examined. Additionally, two main bioactive constituents that comprise the active ingredients of saffron were tested for their possible antioxidant activity, in order to establish a structure-activity relationship. All results were expressed as the % activity of each sample related to caffeic acid, which is a strong DPPH radical scavenger.

Table 1 presents the results of the radical scavenging activity of *Crocus sativus* methanol extract and its bioactive constituents, crocin and safranal. Specifically, Table 1 presents for each sample tested the absorbance of DPPH before the addition of each sample (A_o), the final absorbance of the mixture of the DPPH + each sample (A_T), the decrease (%) in DPPH concentration and finally the decrease (%) in DPPH concentration related to caffeic acid or (%) activity compared with caffeic acid.

As shown, the methanol extact of saffron (*Crocus sativus*) grown in Kozani (Greece) at a concentration above 2000 ppm exhibited significant radical scavenging activity of about 40%–50%. This activity is high, since the saffron extract consists of several compounds, both active and inactive. In the saffron extract examined, a concentration increase was followed by an increase in radical scavenging activity.

In the present study an effort was made to examine the bioactive constituents of saffron, crocin and safranal, in order to ascribe the radical scavenging activity of saffron to its constituents. As shown in Table 1, crocin showed significant radical scavenging activity, since at concentrations of 500 and 1000 ppm the methanol solutions had radical scavenging activity of 48% and 64%, respectively. Safranal also showed radical scavenging activity that was lower than that of crocin at the same concentrations. In all the samples examined, radical scavenging activity proved to be concentration dependent.

Thus, the significant antioxidant activity of the saffron methanol extract should probably be attributed to a synergistic action of the main bioactive constituents: mainly crocin but also safranal, which is the major constituent of the essential oil and is obtained by picrocrocin degradation. Although safranal exhibited antioxidant activity, the toxicity of this substance (lethal dose is approximately 20 g) should be considered.

The parameters EC_{50} and antiradical efficiency (AE) estimated for the saffron methanol extract and crocin are presented in Table 2. As established, the EC_{50} for saffron methanol extract is 2.5 μg/mL which is a satisfactory value for a natural product and a mixture of products, whereas the EC_{50} for crocin was established to be 0.5 μg/mL, a very good value for a pure compound. As shown, at a concentration of 500 ppm solution crocin presented about 49% activity compared with caffeic acid, and scavenged about 50% of radicals, which is a very satisfactory activity. It was shown that the radical scavenging activity of the saffron methanol extract and its constituents, crocin and safranal, is

Table 1. Results of DPPH absorbance before and after the addition of *Crocus sativus* and its bioactive constituents

Sample	Concentration of samples: ppm (mg of sample/mL methanol)	Concentration of samples: mg of sample/mg DPPH	A_o	A_T	Reduction in DPPH absorbance (%)	Reduction in DPPH absorbance comparing to caffeic acid (%)
Caffeic acid	500	0.439	1.190	0.026	97.82	100
Crocus sativus methanol extract in methanol	500	0.439	1.062	0.882	16.95	17.33
	1000	0.877	1.062	0.785	26.08	26.67
	1500	1.316	1.055	0.689	34.69	35.47
	2000	1.754	1.063	0.618	41.86	42.80
	2500	2.193	1.129	0.553	51.02	52.16
Crocin	500	0.439	0.987	0.513	48.02	49.10
	1000	0.877	1.051	0.381	63.75	65.17
Safranal	500	0.439	1.054	0.696	33.97	34.72

A_o, initial DPPH absorbance.
A_T, final DPPH absorbance in the reaction medium (steady state).

Table 2. Results of the parameters EC_{50} and $1/EC_{50}$ used for evaluation of the DPPH test for *Crocus sativus* methanol extract and its bioactive constituent, crocin

Sample	EC_{50}		$AE = 1/EC_{50}$
	mg sample/mg DPPH	ppm	(mg DPPH/mg sample)
Crocus sativus extract in methanol	2.183	2482	0.458
Crocin	0.516	587	1.938

significant, probably because these donate hydrogen atoms for DPPH radical stabilization (Kurechi *et al*., 1980).

In summary, this study examined the antioxidant activity of saffron (dried stigmas of *Crocus sativus* L.) grown in Kozani (Greece) that exhibit several biological properties. It was shown that the saffron methanol extract solutions exhibited high antioxidant activity above 2000 ppm. Crocin, a carotene compound, which is a bioactive constituent of *Crocus sativus*, exhibited significant radical scavenging activity and thus antioxidant activity. Safranal, a monoterpene aldehyde, which is the major constituent of the essential oil of saffron, also showed good antioxidant activity. Thus, the saffron extract is a promising natural product with antioxidant activity and it is proposed to use it for its antioxidant activity as a food supplement, in functional foods, beverages, drinks, in pharmaceutical preparations and cosmetic formulations, since radical scavenging activity is strongly related to antiaging.

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REFERENCES

Abdullaev FI. 2003a. Saffron (*Crocus sativus* L.) and its possible role in the prevention of cancer. In *Recent Progress in Medicinal Plants*, Vol. 8, Majumdar DK, Govil JN, Sing VK (eds). SCI Tech Publishing LLC: Houston, Texas, USA, 53–67.

Abdullaev FI. 2003b. *Crocus sativus* against cancer. *Arch Med Res* **34**: 354.

Abdullaev FI, Riveron-Negrete L, Caballero-Ortega H *et al.* 2003. Use of *in vitro* assays to assess the potential antigenotoxic and cytotoxic effects of saffron (*Crocus sativus* L). *Toxicol In Vitro* **17**: 731–736.

Escribano J, Alonso GL, Coca-Prados M, Fernandez JA. 1996. Crocin, safranal and picrocrocin from saffron (*Crocus sativus* L.) inhibit the growth of human cancer cells *in vitro*. *Cancer Lett* **100**: 23–30.

Gutteridge JMC, Halliwell B. 1994. *Antioxidants in Nutrition, Health and Disease*. Oxford University Press: Oxford, New York, Tokyo.

Hosseinzadeh H, Younesi HM. 2002. Antinociceptive and anti-inflammatory effects of *Crocus sativus* L. stigma and petal extracts in mice. *BMC Pharmacol* **2**: 7.

Kurechi T, Kikugawa K, Kato T, Numasato T. 1980. Studies on the antioxidants.13. Hydrogen donating capability of antioxidants to 2,2-diphenyl-1-picrylhydrazyl. *Chem Pharm Bull* **28**: 2089–2093.

Li N, Lin G, Kwan YW, Min ZD. 1999. Simultaneous quantification of five major biologically active ingredients of saffron by high-performance liquid chromatography. *J Chromatogr A* **849**: 349–355.

Loskutov AV, Beninger CW, Hosfield GL, Sink KC. 2000. Development of an improved procedure for extraction and quantitation of safranal in stigmas of *Crocus sativus* L. using high performance liquid chromatography. *Food Chem* **69**: 87–95.

Lozano P, Castellar MR, Simancas MJ, Iborra JL. 1999. Quantitative high-performance liquid chromatographic method to analyse commercial saffron (*Crocus sativus* L.) products. *J Chromatogr A* **830**: 477–483.

Nair SC, Kurumboor SK, Hasegawa JH. 1995. Saffron chemoprevention in biology and medicine: a review. *Cancer Biother* **10**: 257–264.

Rvos JL, Recio MC, RM Giner, Manez. S. 1996. An update review of saffron and its active constituents. *Phytother Res* **10**: 189–193.

Sinakos Z, Papageorgiou A, Chrisogelou E, Alexides A. 1999. Effect of safranal and crocetin on rats with P 388 leukemia. *10th Panhellenic Haematological Conference* Book of Abstracts, Athens: Hellenic Society of Haematology, 297.

Tarantilis PA, Morjani H, Polissiou M, Manfait M. 1994. Inhibition of growth and induction of differentiation promyelocytic leukemia (HL-60) by carotenoids from *Crocus sativus* L. *Anticancer Res* **14**: 1913–1918.

Tarantilis PA, Tsoupras G, Polissiou M. 1995. Determination of saffron (*Crocus sativus* L.) components in crude plant extract using high-performance liquid chromatography-UV-visible photodiode-array detection-mass spectrometry. *J Chromatogr A* **699**: 107–118.

Tseng TH, Chu CY, Huang JM, Shiow SJ, Wang CJ. 1995. Crocetin protects against oxidative damage in rat primary hepatocytes. *Cancer Lett* **97**: 61–67.

Verma SK, Bordia A. 1998. Antioxidant property of saffron in man. *Indian J Med Sci* **52**: 205–207.

